Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Evaluation of phytoremediation efficiency of shooting range soil using the bioaccumulation potential and sensitivity of different plant species



Jūratė Mankė^a, Marius Praspaliauskas^b, Nerijus Pedišius^b, Gintarė Sujetovienė^{a,*}

^a Department of Environmental Sciences, Vytautas Magnus University, Universiteto 10, Akademija, Kaunas, Lithuania

^b Laboratory of Heat Equipment Research and Testing, Lithuanian Energy Institute, Breslaujos 3, LT44404 Kaunas, Lithuania

ARTICLE INFO

Keywords: Lead Shooting range Medicago sativa Festuca arundinacea Trifolium pratense Bioaccumulation

ABSTRACT

Shooting range soil is one of the most heavily contaminated sites in the world with heavy metals from shooting activities. The aim of the study was to evaluate remediation efficiency of contaminated shooting range soil using three plant species - Festuca arundinacea Schreb., Trifolium pratense L., and Medicago sativa L. assessing the impact of contamination on the development of plant species and the capacity of plants to accumulate potentially toxic elements from polluted soil. The field study was conducted in a Lithuanian civilian shooting range operating 65 years where lead (Pb) concentration in the shooting range soil was extremely high $-17,890 \text{ mg kg}^{-1}$ and the elevated levels of antimony (Sb) was found in soil of berm. F. arundinacea had the highest biomass. The shoot height of all species grown in the contaminated shooting range soil was significantly lower than that of those grown in the uncontaminated soil. The shoot height of M. sativa and T. pratense was 30% lower compared to the control group, while the height reduction of F. arundinacea was only 10%. The same trend of the effect of contaminated soil on root length was observed - the roots were statistically significantly shorter than the control, while the mean root length of F. arundinacea did not differ from the control plants. The content of photosynthetic pigments in all three species tested was also significantly lower as that of the control plants due to contaminated shooting range soil. Shoots of T. pratense plants grown in the shooting range soil accumulated 10 times higher concentrations of Pb and 3 times higher concentrations of Sb in M. sativa compared to the control. Among all studied plants, F. arundinacea was the plant capable of accumulating significantly higher amounts of Pb, nickel (Ni) and Sb in roots than in shoots. The roots of all treated plants accumulated significantly higher concentrations of Pb compared to the control. The highest concentrations of Pb were determined in the shoots of F. arundinacea and T. pratense. Only the roots of M. sativa and F. arundinacea, accumulated higher concentrations of Ni and Sb, compared to controls. The significantly higher bioconcentration factor of Pb (BCF_{Pb}) was determined in F. arundinacea but it still was lower than 1.0. The Pb accumulation capacity of the different plant species was in the following order: F. arundinacea > T. pratense > M. sativa showing phytostabilization potential of plants. T. pratense had the highest translocation factor (TF) values, being able to translocate a high proportion of Pb and Sb from roots to shoots, while F. arundinacea had the lowest efficiency.

1. Introduction

Although trace elements are a natural component of the Earth's crust and are found in small amounts in the soil, their accumulation in the soil is increasing due to anthropogenic activities such as industry, mining, agriculture, and others. Contamination of soil with these elements poses a potential risk to the environment and human health (Motuzova et al., 2014). The release and deposition of potentially toxic elements into the environment is also a result of shooting activities, which is of increasing concern (Sanderson et al., 2018). The environment of shooting ranges is contaminated with lead and other metals/metalloids at levels well above human and ecological health protection levels (Peddicord and LaKind, 2000). The deposited bullets and other materials associated with shooting activities enter the soil as gunshot residues (Lach et al., 2015) and usually remain in the top layer of the soil (Rodríguez-Seijo et al., 2016). Lead is the main component of bullets, accounting for about 80% of the bullet's mass, but smaller amounts of Sb, arsenic (As), Ni, cooper (Cu), manganese (Mn), zinc (Zn), and mercury (Hg) can also be found in the ammunition (Barker et al., 2020; Sanderson et al., 2018; Urrutia-Goyes et al., 2017).

* Corresponding author. E-mail address: gintare.sujetoviene@vdu.lt (G. Sujetovienė).

https://doi.org/10.1016/j.ecoleng.2023.107134

Received 20 January 2023; Received in revised form 24 October 2023; Accepted 30 October 2023 Available online 15 November 2023 0925-8574/© 2023 Elsevier B.V. All rights reserved.



Weathering of these materials leads to the accumulation of elemental lead and other metals. Lead concentrations in the shooting range soils range from 11 to 171,000 mg kg⁻¹ (Barker et al., 2021) and reach >500,000 mg kg⁻¹ (Scheinost et al., 2006). Soil contamination persists for a long period of time even after the cessation of the activity: it was found that, although the shooting activity had ceased many years ago, the Pb content in the soil of the abandoned shooting range exceeded the general reference levels (Reigosa-Alonso et al., 2021; Rodríguez-Seijo et al., 2016).

The accumulation of potentially toxic elements pose a serious environmental risk (Bai and Zhao, 2020; Dinake et al., 2018; Skalny et al., 2021). The toxicity of accumulated elements depends on a number of factors, including concentration, chemical species and sensitivity of the organism. Some metals such as arsenic, cadmium, chromium, lead, and mercury are considered to be a priority hazard affecting the environment and human well-being due to their high toxicity. These metallic elements cause damage to living organisms even when present at low concentrations. In the case of shooting ranges, health risks from exposure to heavy metals have been identified in the risk assessment at the outdoor (Dinake et al., 2018; Evangelou et al., 2012; Islam et al., 2016; Peddicord and LaKind, 2000; Sorvari, 2007) and indoor shooting ranges (Clarke et al., 2022; Grandahl et al., 2012; Orru et al., 2018; Sujetovienė and Česynaitė, 2021).

Even though contamination of firing ranges can pose a risk to the health of workers and shooters, there are also negative environmental impacts. The ecological risk from pollutants will depend on several factors, such as the level of contamination, the characteristics of the site and the sensitivity of ecological receptor. The impact of contamination on the biota also depends on the bioavailability of heavy metals, and high levels of heavy metals can lead to significant accumulation and even transfer to higher levels of the food chain (Hui, 2002). Due to the low mobility and leachability of heavy metals, the exposure to the contaminated soil has not always resulted in acute toxicity (Porfido et al., 2022) or in minor adverse effects on aquatic organisms (Reigosa-Alonso et al., 2021; Rodríguez-Seijo et al., 2017). Recent ecotoxicological studies have shown that high concentrations of heavy metals are taken up and bioaccumulated in organism's tissues, causing a toxic response (Česynaitė et al., 2021; Christou et al., 2022; Rodríguez-Seijo et al., 2017). The field study has also shown that the soil fauna recovered in the abandoned shooting range, compared to the active one, where community structure was altered, reduced in abundance and absence of some species (Selonen et al., 2014).

The specific nature of the shooting range pollution is related to the different shooting activities and means used, and the environmental conditions that lead to different levels of pollution and management options. One of the best management practices to reduce the mobility of metals in shooting ranges is the use of lead-free bullets, which can reduce or prevent environmental pollution in shooting ranges (Fayiga and Saha, 2016). Various soil remediation techniques can be used to reduce heavy metal contamination at the shooting range sites. The choice of the most commonly used technology depends on site specifics and cost (Mench et al., 2009; Mulligan et al., 2001). Methods of remediation of heavy metal contaminated shooting range soils include reducing bioavailability of heavy metals (e.g., application of phosphates, lime) and biological methods (Bandara and Vithanage, 2016; Hashimoto et al., 2009; Pedersen et al., 2018; Rodríguez-Seijo et al., 2020; Sanderson et al., 2016). Phytoremediation has emerged as a relatively inexpensive and sustainable technique to remediate soil contaminated with heavy metals. Plant characteristics favorable for phytoremediation include tolerance to heavy metals, fast growth rate, high biomass, and a well-developed root system with high absorption and accumulation capacity for heavy metals (Bandara and Vithanage, 2016). Although contamination from shooting ranges relates to a wide range heavy metal, soil is mainly contaminated with lead and antimony, and much of the research is concerned with remediation options for elements. When assessing the phytoremediation potential of metalcontaminated soil in the firing range, Pisum sativum L. had the highest lead removal efficiency of 96% (Tariq and Ashraf, 2016). Studies conducted on Pb-contaminated soil showed that F. arundinacea is a potential plant species for phytoremediation of Pb (Begonia et al., 2005; Hu et al., 2015). It is argued that even in the case of a mixture of different metals in the soil, as in the case of shooting range contamination, it is preferable to use a single plant species rather than a mixture of several plant (Lee et al., 2007). Although some species can accumulate the highest levels of Pb, it is important that high levels of contamination do not cause any harmful effects on plants (Khan et al., 2021; Midhat et al., 2018). It is therefore necessary to carry out research suitable for practical application in the field of shooting ranges. Effective remediation and management of shooting range soils should be considered in the future (Moon et al., 2021). The aim of the study was to determine the phytoremediation efficiency of a contaminated shooting range soil using different plant species - Festuca arundinacea Schreb., Trifolium pratense L., and Medicago sativa L. - by assessing the effect of contamination on the development of plant species, the potential of bioaccumulation of trace elements, and the ability to transfer elements to shoots.

2. Material and methods

2.1. Site description

The sampling area is in the civilian shooting range in Alytus, Lithuania ($54^{\circ}23'48.1^{\circ}$ N, $24^{\circ}2'41.3^{\circ}$ E). The shooting range is operating since 1957. The total area of the shooting range is about 400 m². The firing range is designed for target shooting at different distances, with two target lines of different lengths - 25 and 50 m from the firing line. According to the regulations of this shooting range, only 22 long rifle ammunition can be used, which means that this shooting range can only use small barrels (rifle and pistol) according to safety regulations. Shooting activities are most active in the warmer month of the year, especially in April – September. Detailed soil physicochemical properties are presented in previous work (Česynaitė et al., 2021). The most contaminated area of the shooting range with Pb and Sb ($53,022 \text{ mgkg}^{-1}$ and 599 mgkg^{-1} , respectively) is located 45 m from the firing line. The soil of the shooting range is also more acidic and denser due to the high mass of bullets.

2.2. Experimental design and plant cultivation

In order to assess the phytoremediation potential of the shooting range soil, three plant species were selected in this field study - Festuca arundinacea Schreb., Trifolium pratense L., and Medicago sativa L. The high phytoremediation potential of these plant species is demonstrated by their tolerance to pollutants and their ability to accumulate (Begonia et al., 2005; Hu et al., 2015; Liu et al., 2018; Midhat et al., 2018; Steliga and Kluk, 2020). F. arundinacea has high tolerance to contaminants, a strong root system and the ability to transfer large amounts of Pb to the shoots, as well as the ability to absorb more trace elements (e.g., Pb, Cd, Ni, Zn) than other plants (Begonia et al., 2005; Steliga and Kluk, 2020). M. sativa is characterised as a resilient and tolerant plant, able to withstand extreme conditions such as drought, high pollution, low temperatures, and has been tested as a stabiliser plant for acid mine tailings (Midhat et al., 2018). The nitrogen-fixing capacity of M. sativa can contribute to a more efficient use of nutrients and water. Trifolium spp. is suitable for phytoextraction of inorganic compounds, and its ability to fix nitrogen is often used to enhance the productivity of other plants (Lachapelle et al., 2020). T. pratense induces microbial activity in the rhizosphere, followed by increased uptake of inorganic metals from the soil (Delorme et al., 2001).

Three shooting sections of approximately 1 m width were selected at different distances from the firing line - 5, 30 and 45 m (Fig. S1). At each distance, plants of each species were grown in three random plots of 0.8 \times 1.3 m. The number of seeds was calculated according to the

recommended sowing rate: *F. arundinacea* and *T. pratense* – 1.5 g seeds per 1 m², *M. sativa* – 1.0 g seeds per 1 m². The field experiment lasted from June to September 2021. An area close to the shooting range was chosen as a control plot, and the sampling and experimental design was identical to that used at the shooting range, with three replications of the plots.

Before sowing, soil samples (0-20 cm) were collected from each of the plot where plants were grown (5, 30 and 45 m from the firing line) from the three sampling points. After air drying the soil samples were sieved using a 2 mm stainless steel sieve to remove bullets, stones, and organic particles. Soil samples were oven dried at 60 °C for 48 h.

2.3. Sample analysis

After 90 days three samples of plant material were collected from each plot, gently washed by deionised water to remove extraneous substances. Shoot height and root length were measured. Dry shoot and root biomass was determined after drying at 60 °C until constant weight. The concentrations of chlorophylls a + b in tissue extracts with acetone were determined by measuring absorbance at wavelengths of 648, 664, and 470 nm.

Dried plant samples (shoots and roots) were digested in HNO₃ (65%) and H₂O₂ (30%) using a high-pressure microwave digestion system (Milestone ETHOS One, Italy). Multi-element inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer, Optima 8000) was used to determine the concentrations of heavy metals in plants. Calibration of trace elements (Pb, Ni, Sb) was performed at four calibration points by analysing standard (Multi-Element Quality Control Standard, 21 Elements, Perkin Elmer) solutions. The precision of the analysis was also assessed by a linear correlation coefficient of at least 0.999 for all elements. To ensure the reliability of the results, a QC (2 mg L⁻¹) test was performed on every twenty samples. Calibration was performed each time the selected value exceeded the set limits in 10% range.

Soil samples were digested in 8 mL HCl (37%), 5 mL HNO₃ (65%), 5 mL aqueous H_3BO_3 solution (5%) and 3 mL HF (48%) in the Teflon vessels of a microwave digestion system (Milestone Ethos One, Italy) in triplicate. The total concentrations (mg kg⁻¹) of Pb, Ni, Sb were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 8000, Perkin-Elmer, USA). Calibrations of trace elements were made by analysing standard (Multi-Element Quality Control Standard, 21 Elements, Perkin Elmer) solutions in four replicates. The analysis of the certified reference material (CRM Metals in Soil (SQC001), Sigma–Aldrich) showed that the recovery of all elements varied within $\pm 10\%$ range of the certified values.

2.4. Data analysis

The BCF was calculated as a ratio of the metal concentration in the plant tissue (roots) and the metal concentration in the soil. The TF was used to evaluate plant's ability to transfer a metal from roots to shoots. Each treatment was performed in 9 replicates. Normal distribution of the data was tested by the Kolmogorov-Smirnov test. To evaluate the significance of differences between treatments comparing plants morphometric parameters and concentrations of trace elements at different distances and between plant species, a nonparametric Mann-Whitney two sample *U* test was used (p < 0.05). Data were analyzed with Statistics software.

3. Results

3.1. Total metal concentration in soil

The initial heavy metal concentrations (Pb, Ni and Sb) in the shooting range soil (SR) were substantially higher than in the control site soil (p < 0.05, Fig. 1). The mean lead concentrations in the shooting range soil were extremely high – 17,890 mg kg⁻¹. Antimony concentration was 599 mg kg⁻¹ and was found only in the soil of the berm. The elevated levels of Pb along with Sb in shooting range soil exceeded the background (15 mg Pb kg⁻¹ and 1.0–1.5 mg Sb kg⁻¹) and maximum permitted (100 mg Pb kg⁻¹ and 10 mg Sb kg⁻¹) concentrations in Lithuanian soils (HN 60-2004 (2004)). The concentration of Ni did not exceed the established maximum permitted level, but it was 2.2 times higher in the soil of the shooting range than in the control.

3.2. Plant growth

When comparing different plant species, *F. arundinacea* had the highest biomass and it was statistically significantly higher than



Fig. 2. Dry weight of shoot biomass following 3 months field study. Mean values (mean \pm SE, n_{control} = 5, n_{shooting range} = 15) followed by the same letter are insignificantly different (U test, p > 0.05).



Fig. 1. Concentration of Pb, Ni, and Sb in the soil of the shooting range (SR) and control (mean \pm SE, n = 6). Different letters above the columns indicate significant differences (*U* test, p < 0.05).

T. pratense (p < 0.05, Fig. 2). The dry biomass of any plant species grown in the contaminated soil did not differ from that of the control plant group (p > 0.05).

The shoot height of all species grown in the contaminated shooting range soil was significantly lower than that of those grown in the uncontaminated soil (p < 0.05, Fig. 3). The shoot height of *M. sativa* and *T. pratense* was 30% lower compared to the control group, while the height reduction of *F. arundinacea* was only 10%. In *M. sativa* and *T. pratense*, the same trend of the effect of contaminated soil on root length was observed - the roots were statistically significantly shorter than the control, while the mean root length of *F. arundinacea* did not differ from the root length of the control plants (Fig. 3).

The content of photosynthetic pigments in all three species tested was also significantly lower as that of the control plants due to contaminated shooting range soil – chlorophyll a + b content in *M. sativa, F. arundinacea* and *T. pratense* was 49%, 53% and 48% lower compared to control, respectively (p < 0.05, Fig. 4).

3.3. Assessment of trace element concentrations in plants

Content of Pb was higher in all treated plants compared to the control (Fig. 5). Shoots of *T. pratense* accumulated 10 times higher Pb concentration (127 mg kg⁻¹) while *M. sativa* and *F. arundinacea* plants – 2 times higher concentrations (27 mg kg⁻¹ and 26 mg kg⁻¹) compared to the control (13 mg kg⁻¹, Fig. 5). *M. sativa* and *F. arundinacea* accumulated significantly more Ni in the aboveground part, about a quarter more than plants grown in the uncontaminated soil (p < 0.05). *M. sativa* accumulated significantly higher amounts of Sb in the soil of the shooting range compared to the control – 3 times higher (p < 0.05, Fig. 5).

The roots of all treated plants accumulated significantly higher concentrations of Pb compared to the control (Fig. 5). The highest concentrations of Pb were determined in *F. arundinacea* (147 mg kg⁻¹) and *T. pratense* (111 mg kg⁻¹), almost 11 and 7 times higher compared to the control. Roots of *M. sativa* accumulated 43% higher concentration of Pb than control. Only the roots of *M. sativa* and *F. arundinacea*, accumulated higher concentrations of Ni – 8 and 11 mg kg⁻¹, respectively. Similar results were obtained with Sb – in the roots of *M. sativa* and *F. arundinacea*, Sb concentrations were found to be 2 and 3 times higher,



Fig. 3. Shoot height and root length (cm) of *M. sativa, T. pratense* and *F. arundinacea* after 3 months of growth in the control and shooting range soils (mean \pm SE, n_{control} = 9, n_{shooting range} = 27). Different letters above columns indicate significant different between means (U test, p < 0.05).



Fig. 4. Chlorophyll content in *M. sativa, F. arundinacea* and *T. pratense* grown in control and shooting range soils (mean \pm SE, n_{control} = 9, n_{shooting range} = 27). Different letters above the columns indicate significant difference (p < 0.05) among the treatments (U test).

respectively, compared to controls. Among all studied plants, *F. arundinacea* was the plant capable of accumulating significantly higher amounts of Pb, Ni and Sb in roots than in shoots (p < 0.05).

The accumulation of trace elements in plants, expressed as a BCF, did not show a significant bioconcentration of lead in the plants studied (Fig. 6). The significantly higher BCF_{Pb} was determined in *F. arundinacea* but it still was lower than 1.0. According to the plant ability to accumulate Pb in roots the subsequent ability of different plant species was as follows: *F. arundinacea* > *T. pratense* > *M. sativa*. BCF_{Ni} values in roots of *F. arundinacea* was 1.9 while of other treated plant species were not significantly different in the ability to absorb elements from the soil and BCF_{Ni} values were near 1.0 (Fig. 6). BCF_{Sb} values were very low for all treated plants with mean value 0.0025–0.0027.

T. pratense had the highest TF values, being able to translocate a high proportion of Pb and Sb from roots to shoots, while *F. arundinacea* had the lowest efficiency. The translocation from roots to shoots of *M. sativa* was similar for all elements, with TF ranging from 0.96 (Ni) to 1.09 (Pb) (Fig. 7).

4. Discussion

Shooting activities at firing ranges lead to the accumulation of trace elements in range soils, which is one of the largest sources of contamination in the world, for example in the United States, it is the second largest source of Pb contamination (Ahmad et al., 2012a). Ammunition used for firing contains a wide range of metals including arsenic, zinc, nickel, etc. but lead is one of the most prevalent contaminants in the shooting ranges. The contamination of the soils investigated confirmed that shooting range is heavily contaminated with trace elements, especially Pb and Sb. The high level of contamination is due to accumulation of lead and antimony fired at the shooting ranges (Dinake et al., 2019; Sanderson et al., 2018). High levels of these elements, above the permitted levels, have the greatest potential to harm human health and the environment (Bai and Zhao, 2020; Christou et al., 2022; Dinake et al., 2021; Fayiga and Saha, 2016).

The high levels of contamination found at the firing ranges is an indication of a need for remediation. On the one hand, this is local pollution, which is easier to control and manage. On the other hand, there is an accumulation of a various potentially toxic elements in the environment of shooting ranges resulting from shooting activities. Phytoremediation is one of the most cost-effective technologies for removing pollutants and has been tested in the case of firing range pollution (Bandara and Vithanage, 2016).

The success of phytoremediation depends on the plant's tolerance to trace elements and its ability to accumulate them of under extreme



Fig. 5. Concentration of Pb, Ni, Sb in the shoot and root biomass of *M. sativa, F. arundinacea, T. pratense* after 3-month phytoremediation (mean \pm SE, n_{control} = 9, n_{shooting range} = 27). Different letters above the columns indicate significant difference (p < 0.05) among the treatments (U test).



Fig. 6. The bioconcentration factor of trace elements (Pb, Ni) in roots of *M. sativa, F. arundinacea* and *T. pratense* after 3-month of phytoremediation (mean \pm SE, $n_{control} = 9$, $n_{shooting range} = 27$). Different letters above the columns indicate significant difference (p < 0.05) among the treatments (U test).

pollution conditions, such as growing in the contaminated soil of the shooting range. Our results showed that exposure to contaminated soil did not adversely affect the germination of the selected plant species. This is important for achieving phytoremediation objectives. Cao et al. (2004) showed that plant seeds sown in a soil contaminated with trace elements with a total Pb concentration of 21,540 mg kg⁻¹ germinated in only one of the three selected plant species - Helichrysum italicum L., Juncus compressus L., and F. arundinacea - only the latter was able to germinate and grow. Our results are in agreement with the results of phytotoxicity studies carried out by Lago-Vila et al. (2019), where it was found that Sinapis alba L., Lactuca sativa L. and Festuca ovina L. were able to germinate and grow in the shooting range soil in spite of the high Pb content. The absence of germination inhibition can be considered as a sign of tolerance of these plants, which may partly explain why high level of lead contamination did not have a significant effect on germination. Other studies have shown that Pb toxicity resulted in significantly lower germination of lettuce in the shooting range soil, with germination rates as low as 15% (Ahmad et al., 2012b) or even 5% (Wolf et al., 2020) and also caused the low germination of Solanum lycopersicum (Yoo et al., 2016).

The growth of the plants confirmed the tolerance of these species to

pollutants – plants exposed to the shooting range soil produced the same biomass as plants growing in uncontaminated soil. Despite the fact that the contaminated soil from the firing range did not adversely affect the biomass of any of the plant species, plant growth was impaired. The growth of *M. sativa* and *T. pratense* was significantly reduced leading to shorter in height plants. This harmful effect is suggested to be caused by the damaged roots which length was significantly lower than of control plants. Inhibition of root elongation is the primary effect of Pb toxicity, which can be explained by impaired root cell division (Eun et al., 2000). Root growth inhibition was also observed in three different species (*Sinapis alba* L., *Lactuca sativa* L. and *Festuca ovina* L.), exposed to a Pb polluted trap shooting range and small-arms firing range soils through phytotoxicity assays (Lago-Vila et al., 2019). *F. arundinacea* was the most tolerant species because the shoot height was reduced to a minimum and root length was not adversely affected by contaminants.

Chlorophyll content is an indicator of photosynthetic mechanism and of plant metabolism. The photosynthetic pigments were affected by stress from metal contaminants - the decrease in chlorophyll content in plants was determined. It is argued that chlorophyll levels generally decrease with increasing stress levels — nutrient deficiencies and chlorophyllase activity under stress conditions can provoke a decrease in



Fig. 7. The translocation factor (TF) of heavy metals (Pb, Ni, Sb) of *M. sativa, F. arundinacea* and *T. pratense* after 3-month of phytoremediation (mean \pm SE, n_{control} = 9, n_{shooting range} = 27). Different lowercase letters above the columns indicate significant difference (p < 0.05) among the treatments (U test).

chlorophyll biosynthesis (Piotrowska-Niczyporuk et al., 2015). Reduction of photosynthetic pigments by the heavy metals also indirectly influences the photosynthesis (Aggarwal et al., 2012) and this reduction in photosynthesis efficiency is partly responsible for the overall reduction in plant growth and biomass production (Chandra and Kang, 2016).

Plants grown on the contaminated soils accumulated high amounts of trace elements. All species showed the ability to accumulate Pb, and the highest Pb concentration was found in shoots of *T. pratense*, which was up to 10 times higher than the control. The studies confirmed that *T. pratense* can accumulate high levels of Pb and the accumulation was linearly related to the Pb concentration in the soil (Malizia et al., 2012). Translocation factor of Pb and Sb also indicated ability of this species to translocate these elements from roots to shoots and this is responsible for phytoextraction (Nirola et al., 2015) especially for *T. pratense* in this study.

Among the plants studied, *F. arundinacea* was able to accumulate about twice the concentration of elements such as Pb, Ni and Sb in the stems as in the control, but this efficiency was much higher in the roots. Several authors also indicated that the roots of *F. arundinacea* had higher accumulation ability compared to the shoots (Lou et al., 2017). Results of other studies also indicate that most of Pb from the contaminated soil was translocated to aerial parts of plants. Root Pb concentration of St. Augustine grass was significantly higher than in shoot biomass – 74-93% of total plant Pb in the roots (Fayiga and Saha, 2016). Much higher Pb accumulation in roots of *Agrostis capillaris* L. grown in soil of an old trap shooting range indicated the phytostabilization properties of this species (Rodríguez-Seijo et al., 2016). Pb phytostabilization in root tissues leads in a delay of Pb translocation to aboveground plant parts (Lou et al., 2017).

Higher accumulation in the roots was reflected in the highest value of the bioconcentration factor (BCF), which indicates the plant's ability to absorb elements from the environment, among the plants studied. *F. arundinacea* was considered to be able to absorb more Pb and other elements than other herbaceous plants due to its tolerance and strong root system (Khashij et al., 2018). The high concentration of Pb in roots was shown by the BCF of Pb in roots, which was higher than the TF and can be explained by its adhesion properties (Albornoz et al., 2016). The roots acted as a barrier preventing from Pb transfer to the shoots, and Pb could be precipitated in the form of Pb-phosphate at the root surface (Lou et al., 2013).

In contrast to *F. arundinacea*, *M. sativa* showed a similar accumulation of Pb in shoots as in the roots. Other studies have shown that the concentration of metals, including Pb, were significantly higher in *M. sativa* roots than in the shoots (Midhat et al., 2018). Baker (1981) suggested that this is a typical tolerance mechanism of accumulator species. Many heavy metal-tolerant plants exhibit a characteristic property of reducing root-to-shoot transport of trace elements at higher concentrations, for example above 2000 mg kg⁻¹ Pb (Steliga and Kluk, 2020).

5. Conclusions

In conclusion, the three studied plant species were able to germinate and to form vegetative cover in the contaminated soil and accumulate trace elements in their roots and shoots, indicating their usefulness for phytoremediation of contaminated soil. The soil of the examined shooting range contained elevated Pb concentration, resulting in Pb accumulation in roots and shoots of all plants. The highest bioaccumulation was observed in T. pratense, with most of it translocating from roots to shoots. F. arundinacea showed the highest accumulation ability of trace elements in the roots. In summary, the much higher accumulation of Pb in the roots of the plants grown in the soil of the shooting range was indicative of their phytostabilising properties, which delayed the translocation of Pb to the aerial parts of the plants. Field studies are useful for finding the most effective ways to control and manage pollution from local sources such as shooting ranges. Our results also point to the need to test plants on a wide range of outdoor soils, as there is a lack of field studies conducted under natural conditions and using a variety of plant species. This suggests the need for clear guidelines on how to implement phytoremediation cost-effectively and easily.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2023.107134.

Credit author statement

Jūratė Mankė: Study design; Investigation; Statistical Analysis, Writing - Original Draft Preparation, Reviewing and Editing. Gintarė Sujetovienė: Supervision, Conceptualization, Methodology, Writing-Reviewing and Editing. Marius Praspaliauskas and Nerijus Pedišius: Sample analysis, Visualization, Investigation, Data Curation. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

Data availability

Data will be made available on request.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- Aggarwal, A., Sharma, I., Tripathi, B.N., Munjal, A.K., Baunthiyal, M., Sharma, V., 2012. Metal toxicity and photosynthesis. In: Itoh, S., Mohanty, P., Guruprasad, K.N. (Eds.), Photosynthesis: Overviews on Recent Progress & Future Perspectives. I.K. International Publishing House Pvt. Limited, pp. 229–236.
- Ahmad, M., Lee, S.S., Moon, D.H., Yang, J.E., Ok, Y.S., 2012a. A review of environmental contamination and remediation strategies for heavy metals at shooting range soils. Environ. Protect. Strateg. Sustain. Dev. 437–451 https://doi.org/10.1007/978-94-007-1591-2_14/COVER.
- Ahmad, M., Lee, S.S., Yang, J.E., Ro, H.M., Han Lee, Y., Sik Ok, Y., 2012b. Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. Ecotoxicol. Environ. Saf. 79, 225–231. https://doi.org/10.1016/J.ECOENV.2012.01.003.
- Albornoz, C.B., Larsen, K., Landa, R., Quiroga, M.A., Najle, R., Marcovecchio, J., 2016. Lead and zinc determinations in *Festuca arundinacea* and *Cynodon dactylon* collected from contaminated soils in Tandil (Buenos Aires Province, Argentina). Environ. Earth Sci. 75, 1–8. https://doi.org/10.1007/S12665-016-5513-9/FIGURES/7.
- Bai, J., Zhao, X., 2020. Ecological and Human Health risks of Heavy Metals in Shooting Range Soils: a Meta Assessment from China. Toxics 8, 32. https://doi.org/10.3390/ toxics8020032.
- Baker, A.J.M., 1981. Accumulators and excluders -strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654. https://doi.org/10.1080/ 01904168109362867.
- Bandara, T., Vithanage, M., 2016. Phytoremediation of Shooting Range Soils, in: Phytoremediation. Springer International Publishing, Cham, pp. 469–488. https:// doi.org/10.1007/978-3-319-40148-5 17.
- Barker, A.J., Mayhew, L.E., Douglas, T.A., Ilgen, A.G., Trainor, T.P., 2020. Lead and antimony speciation associated with the weathering of bullets in a historic shooting range in Alaska. Chem. Geol. 553, 119797 https://doi.org/10.1016/j. chemee.2020.119797.
- Barker, A.J., Clausen, J.L., Douglas, T.A., Bednar, A.J., Griggs, C.S., Martin, W.A., 2021. Environmental impact of metals resulting from military training activities: a review. Chemosphere 265, 129110. https://doi.org/10.1016/J. CHEMOSPHERE.2020.129110.
- Begonia, M.T., Begonia, G.B., Ighoavodha, M., Gilliard, D., 2005. Lead Accumulation by Tall Fescue (*Festuca arundinacea* Schreb.) Grown on a Lead-Contaminated Soil. Int. J. Environ. Res. Public Health 2, 228–233.
- Cao, A., Cappai, G., Carucci, A., Muntoni, A., 2004. Selection of Plants for Zinc and Lead Phytoremediation. J. Environ. Sci. Health A 39, 1011–1024. https://doi.org/ 10.1081/ESE-120028410.
- Česynaitė, J., Praspaliauskas, M., Pedišius, N., Sujetovienė, G., 2021. Biological assessment of contaminated shooting range soil using earthworm biomarkers. Ecotoxicology 30, 2024–2035. https://doi.org/10.1007/S10646-021-02463-W/ TABLES/1.
- Chandra, R., Kang, H., 2016. Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. For. Sci. Technol. 12, 55–61. https://doi.org/10.1080/21580103.2015.1044024.
- Christou, A., Hadjisterkotis, E., Dalias, P., Demetriou, E., Christofidou, M., Kozakou, S., Michael, N., Charalambous, C., Hatzigeorgiou, M., Christou, E., Stefani, D., Christoforou, E., Neocleous, D., 2022. Lead contamination of soils, sediments, and vegetation in a shooting range and adjacent terrestrial and aquatic ecosystems: a holistic approach for evaluating potential risks. Chemosphere 292, 133424. https:// doi.org/10.1016/J.CHEMOSPHERE.2021.133424.
- Clarke, C.E., Mohammed, F.K., Hamid, A., Bent, G.-A., 2022. Quantification and health risk assessment of heavy metals in residual floor dust at an indoor firing range: a case study in Trinidad, WI. Int. J. Environ. Health Res. 32, 652–664. https://doi.org/ 10.1080/09603123.2020.1793917.
- Delorme, T.A., Gagliardi, J.V., Angle, J.S., Chaney, R.L., 2001. Influence of the zinc hyperaccumulator Thlaspi caerulescens J. & C. Presl. And the nonmetal accumulator *Trifolium pratense* L. on soil microbial populations. Can. J. Microbiol. 47, 773–776. https://doi.org/10.1139/w01-067.
- Dinake, P., Kelebemang, R., Sehube, N., Kamwi, O., Laetsang, M., 2018. Quantitative assessment of environmental risk from lead pollution of shooting range soils. Chem. Speciat. Bioavailab. 30, 76–85. https://doi.org/10.1080/09542299.2018.1507689.
- Dinake, P., Kelebemang, R., Sehube, N., 2019. A Comprehensive Approach to Speciation of Lead and its Contamination of Firing Range Soils: a Review. Soil Sediment Contam. Int. J. 28, 431–459. https://doi.org/10.1080/15320383.2019.1597831.
- Dinake, P., Mercy Mokgosi, S., Kelebemang, R., Trinity Kereeditse, T., Motswetla, O., 2021. Pollution risk from Pb towards vegetation growing in and around shooting ranges-a review. Environ. Pollut. Bioavailabil. 33, 88–103. https://doi.org/10.1080/ 26395940.2021.1920467.

- Eun, S.O., Shik Youn, H., Lee, Y., 2000. Lead disturbs microtubule organization in the
- root meristem of Zea mays. Physiol. Plant. 110, 357–365. https://doi.org/10.1111/ J.1399-3054.2000.1100310.X.
 Evangelou, M.W.H., Hockmann, K., Pokharel, R., Jakob, A., Schulin, R., 2012.
- Accumulation of Sb, Pb, Cu, Zn and Cd by various plants species on two different relocated military shooting range soils. J. Environ. Manag. 108, 102–107. https:// doi.org/10.1016/J.JENVMAN.2012.04.044.
- Fayiga, A.O., Saha, U.K., 2016. Soil pollution at outdoor shooting ranges: Health effects, bioavailability and best management practices. Environ. Pollut. 216, 135–145. https://doi.org/10.1016/j.envpol.2016.05.062.

Grandahl, K., Suadicani, P., Jacobsen, P., 2012. Individual and environmental risk factors for high blood lead concentrations in Danish indoor shooters. Dan. Med. J. 59, 1–5.

- Hashimoto, Y., Taki, T., Sato, T., 2009. Sorption of dissolved lead from shooting range soils using hydroxyapatite amendments synthesized from industrial byproducts as affected by varying pH conditions. J. Environ. Manag. 90, 1782–1789. https://doi. org/10.1016/J.JENVMAN.2008.11.004.
- HN 60-2004, 2004. Lithuanian hygienic norm HN 60:2004. Maximum permitted concentrations of hazardous substances in soil (in Lithuanian). Valstybės žinios no, 41–1357.
- Hu, Z., Xie, Y., Jin, G., Fu, J., Li, H., 2015. Growth responses of two tall fescue cultivars to Pb stress and their metal accumulation characteristics. Ecotoxicology 24, 563–572. https://doi.org/10.1007/S10646-014-1404-6/FIGURES/6.
- Hui, C.A., 2002. Lead distribution throughout soil, flora, and an invertebrate at a wetland skeet range. J. Toxicol. Environ. Health A 65, 1093–1107. https://doi.org/10.1080/ 152873902760125246.
- Islam, M.N., Nguyen, X.P., Jung, H.-Y., Park, J.-H., 2016. Chemical speciation and quantitative evaluation of heavy metal pollution hazards in two army shooting range backstop soils. Bull. Environ. Contam. Toxicol. 96, 179–185. https://doi.org/ 10.1007/s00128-015-1689-z.
- Khan, A.Z., Khan, S., Muhammad, S., Baig, S.A., Khan, A., Nasir, M.J., Azhar, M., Naz, A., 2021. Lead contamination in shooting range soils and its phytoremediation in Pakistan: a greenhouse experiment. Arab. J. Geosci. 14, 1–7. https://doi.org/ 10.1007/S12517-020-06301-X/TABLES/5.
- Khashij, S., Karimi, B., Makhdoumi, P., 2018. Phytoremediation with *Festuca arundinacea*: a mini review. Int. J. Health Life Sci. 4, 86625. https://doi.org/ 10.5812/JJHLS.86625.
- Lach, K., Steer, B., Gorbunov, B., Mička, V., Muir, R.B., 2015. Evaluation of exposure to airborne heavy metals at gun shooting ranges. Ann. Occup. Hyg. 59, 307–323. https://doi.org/10.1093/annhyg/meu097.
- Lachapelle, A., Yavari, S., Pitre, F.E., Courchesne, F., Brisson, J., 2020. Co-planting of Salix interior and Trifolium pratense for phytoremediation of trace elements from wood preservative contaminated soil. Int. J. Phytoremed. 1–9 https://doi.org/ 10.1080/15226514.2020.1847034.
- Lago-Vila, M., Rodríguez-Seijo, A., Vega, F.A., Arenas-Lago, D., 2019. Phytotoxicity assays with hydroxyapatite nanoparticles lead the way to recover firing range soils. Sci. Total Environ. 690, 1151–1161. https://doi.org/10.1016/J. SCITOTENV.2019.06.496.
- Lee, I., Baek, K., Kim, H., Kim, S., Kim, J., Kwon, Y., Chang, Y., Bae, B., 2007. Phytoremediation of soil co-contaminated with heavy metals and TNT using four plant species. J. Environ. Sci. Health, Part A: Tox. Hazard. Subst. Environ. Eng. 42, 2039–2045. https://doi.org/10.1080/10934520701629781.
- Liu, C., Lin, H., Dong, Y., Li, B., Liu, Y., 2018. Investigation on microbial community in remediation of lead-contaminated soil by Trifolium repensL. Ecotoxicol. Environ. Saf. 165, 52–60. https://doi.org/10.1016/J.ECOENV.2018.08.054.
- Lou, Y., Luo, H., Hu, T., Li, H., Fu, J., 2013. Toxic effects, uptake, and translocation of Cd and Pb in perennial ryegrass. Ecotoxicology 22, 207–214. https://doi.org/10.1007/ S10646-012-1017-X/TABLES/2.
- Lou, Y., Zhao, P., Wang, D., Amombo, E., Sun, X., Wang, H., Zhuge, Y., 2017. Germination, physiological responses and gene expression of tall fescue (*Festuca arundinacea* Schreb.) growing under Pb and Cd. PLoS One 12, e0169495. https://doi.org/10.1371/journal.pone.0169495.
- Malizia, D., Giuliano, A., Ortaggi, G., Masotti, A., 2012. Common plants as alternative analytical tools to monitor heavy metals in soil. Chem. Cent. J. 6, S6. https://doi. org/10.1186/1752-153X-6-S2-S6.
- Mench, Michel, Schwitzguébel, Jean-Paul, Schroeder, Peter, Bert, Valérie, Gawronski, Stanislaw, Gupta, S., Schröder, P., Mench, M., Schwitzguébel, J.-P., Schroeder, P., Gupta Agroscope, S., Reckenholz, F., Gawronski, S., Bert, V., Sci, E., Res, P., 2009. Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. Environ. Sci. Pollut. Res. 16, 876–900. https://doi.org/ 10.1007/S11356-009-0252-Z.
- Midhat, L., Ouazzani, N., Hejjaj, A., Bayo, J., Mandi, L., 2018. Phytostabilization of polymetallic contaminated soil using *Medicago sativa* L. in combination with powdered marble. Sustain. Rehabil. 20, 764–772. https://doi.org/10.1080/ 15226514.2018.1425665.
- Moon, I., Kim, H., Jeong, S., Choi, H., Park, J., Lee, I., 2021. Chemical properties of heavy metal-contaminated soils from a Korean military shooting range: evaluation of Pb sources using Pb isotope ratios. Appl. Sci. 11, 7099. https://doi.org/10.3390/ APP11157099.
- Motuzova, G.V., Minkina, T.M., Karpova, E.A., Barsova, N.U., Mandzhieva, S.S., 2014. Soil contamination with heavy metals as a potential and real risk to the environment. J. Geochem. Explor. 144, 241–246. https://doi.org/10.1016/J. GEXPLO.2014.01.026.
- Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Remediation technologies for metalcontaminated soils and groundwater: an evaluation. Eng. Geol. 60, 193–207. https://doi.org/10.1016/S0013-7952(00)00101-0.

Nirola, R., Megharaj, M., Palanisami, T., Aryal, R., Venkateswarlu, K., Naidu, Ravi, 2015. Evaluation of metal uptake factors of native trees colonizing an abandoned copper mine – a quest for phytostabilization. J. Sustain. Min. 14, 115–123. https://doi.org/ 10.1016/J.JSM.2015.11.001.

- Orru, H., Pindus, M., Harro, H., Maasikmets, M., Herodes, K., 2018. Metallic fumes at indoor military shooting ranges: Lead, Copper, Nickel, and Zinc in different fractions of airborne particulate matter. Propell. Explos. Pyrotech. 43, 228–233. https://doi. org/10.1002/prep.201700225.
- Peddicord, R.K., LaKind, J.S., 2000. Ecological and human health risks at an outdoor firing range. Environ. Toxicol. Chem. 19, 2602–2613. https://doi.org/10.1002/ ETC.5620191029.
- Pedersen, K.B., Jensen, P.E., Ottosen, L.M., Barlindhaug, J., 2018. The relative influence of electrokinetic remediation design on the removal of as, Cu, Pb and Sb from shooting range soils. Eng. Geol. 238, 52–61. https://doi.org/10.1016/J. ENGGEO.2018.03.005.
- Piotrowska-Niczyporuk, A., Bajguz, A., Talarek, M., Bralska, M., Zambrzycka, E., 2015. The effect of lead on the growth, content of primary metabolites, and antioxidant response of green alga Acutodesmus obliquus (Chlorophyceae). Environ. Sci. Pollut. Res. 22, 19112–19123. https://doi.org/10.1007/s11356-015-5118-y.
- Porfido, C., Gattullo, C.E., Allegretta, I., Fiorentino, N., Terzano, R., Fagnano, M., Spagnuolo, M., 2022. Investigating lead bioavailability in a former shooting range by soil microanalyses and earthworms tests. Soil Syst. 6, 25. https://doi.org/10.3390/ SOILSYSTEMS6010025.
- Reigosa-Alonso, A., Lorenzo Dacunha, R., Arenas-Lago, D., Vega, F.A., Rodríguez-Seijo, A., 2021. Soils from abandoned shooting range facilities as contamination source of potentially toxic elements: distribution among soil geochemical fractions. Environ. Geochem. Health 43, 4283–4297. https://doi.org/10.1007/s10653-021-00900-7.
- Rodríguez-Seijo, A., Lago-Vila, M., Andrade, M.L., Vega, F.A., 2016. Pb pollution in soils from a trap shooting range and the phytoremediation ability of *Agrostis capillaris* L. Environ. Sci. Pollut. Res. 23, 1312–1323. https://doi.org/10.1007/s11356-015-5340-7.
- Rodríguez-Seijo, A., Cachada, A., Gavina, A., Duarte, A.C., Vega, F.A., Andrade, M.L., Pereira, R., 2017. Lead and PAHs contamination of an old shooting range: a case study with a holistic approach. Sci. Total Environ. 575, 367–377. https://doi.org/ 10.1016/j.scitotenv.2016.10.018.
- Rodríguez-Seijo, A., Vega, F.A., Arenas-Lago, D., 2020. Assessment of iron-based and calcium-phosphate nanomaterials for immobilisation of potentially toxic elements in soils from a shooting range berm. J. Environ. Manag. 267, 110640 https://doi.org/ 10.1016/J.JENVMAN.2020.110640.

- Sanderson, P., Naidu, R., Bolan, N., 2016. The effect of environmental conditions and soil physicochemistry on phosphate stabilisation of Pb in shooting range soils. J. Environ. Manag. 170, 123–130. https://doi.org/10.1016/J. JENVMAN.2016.01.017.
- Sanderson, P., Qi, F., Seshadri, B., Wijayawardena, A., Naidu, R., 2018. Contamination, fate and management of metals in shooting range soils—a review. Curr. Pollut. Rep. 4, 175–187. https://doi.org/10.1007/s40726-018-0089-5.
- Scheinost, A.C., Rossberg, A., Vantelon, D., Xifra, I., Kretzschmar, R., Leuz, A.K., Funke, H., Johnson, C.A., 2006. Quantitative antimony speciation in shooting-range soils by EXAFS spectroscopy. Geochim. Cosmochim. Acta 70, 3299–3312. https:// doi.org/10.1016/J.GCA.2006.03.020.
- Selonen, S., Liiri, M., Setälä, H., 2014. Can the soil fauna of boreal forests recover from lead-derived stress in a shooting range area? Ecotoxicology 23, 437–448. https:// doi.org/10.1007/s10646-014-1210-1.
- Skalny, A.V., Aschner, M., Bobrovnitsky, I.P., Chen, P., Tsatsakis, A., Paoliello, M.M.B., Buha Djordevic, A., Tinkov, A.A., 2021. Environmental and health hazards of military metal pollution. Environ. Res. 201, 111568 https://doi.org/10.1016/J. ENVRES.2021.111568.
- Sorvari, J., 2007. Environmental risks at Finnish Shooting Ranges—a Case Study. Hum. Ecol. Risk Assess. Int. J. 13, 1111–1146. https://doi.org/10.1080/ 10807030701506124
- Steliga, T., Kluk, D., 2020. Application of *Festuca arundinacea* in phytoremediation of soils contaminated with Pb, Ni, Cd and petroleum hydrocarbons. Ecotoxicol. Environ. Saf. 194, 110409 https://doi.org/10.1016/j.ecoenv.2020.110409.
- Sujetovienė, G., Česynaitė, J., 2021. Assessment of air pollution at the indoor environment of a shooting range using lichens as biomonitors. J. Toxicol. Environ. Health A 84, 273–278. https://doi.org/10.1080/15287394.2020.1862006.
- Tariq, S.R., Ashraf, A., 2016. Comparative evaluation of phytoremediation of metal contaminated soil of firing range by four different plant species. Arab. J. Chem. 9, 806–814. https://doi.org/10.1016/J.ARABJC.2013.09.024.
- Urrutia-Goyes, R., Mahlknecht, J., Argyraki, A., Ornelas-Soto, N., 2017. Trace element soil contamination at a former shooting range in Athens, Greece. Geoderma Reg. 10, 191–199. https://doi.org/10.1016/j.geodrs.2017.08.002.
- Wolf, D.C., Cryder, Z., Khoury, R., Carlan, C., Gan, J., 2020. Bioremediation of PAHcontaminated shooting range soil using integrated approaches. Sci. Total Environ. 726, 138440 https://doi.org/10.1016/j.scitotenv.2020.138440.
- Yoo, J.C., Shin, Y.J., Kim, E.J., Yang, J.S., Baek, K., 2016. Extraction mechanism of lead from shooting range soil by ferric salts. Process. Saf. Environ. Prot. 103, 174–182. https://doi.org/10.1016/J.PSEP.2016.07.002.