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Nano zerovalent Fe did not reduce metal(loid) leaching and ecotoxicity further than conventional Fe grit in contrasting smelter impacted soils: A 1-year field study

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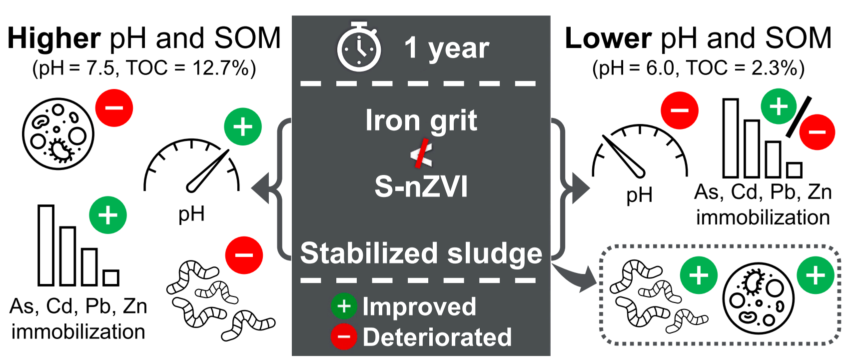
HIGHLIGHTS

- Nano and micro Fe materials were applied to two metal(loid) contaminated soils.
- The evaluation was performed for 1-year after amendment application.
- Nanoiron and iron grit performed similarly at metal(loid) immobilization.
- Amendment effectivity was largely dependent on soil pH.
- The combination of sewage sludge with nanoiron was most beneficial for soil health.

GRAPHICAL ABSTRACT



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ABSTRACT

The majority of the studies on nanoscale zero-valent iron (nZVI) are conducted at a laboratory-scale, while field-scale evidence is scarce. The objective of this study was to compare the metal(loid) immobilization efficiency of selected Fe-based materials under field conditions for a period of one year. Two contrasting metal(loid) (As, Cd, Pb, Zn) enriched soils from a smelter-contaminated area were amended with sulfidized nZVI (S-nZVI) solely or combined with thermally stabilized sewage sludge and compared to amendment with microscale iron grit. In the soil with higher pH (7.5) and organic matter content (TOC = 12.7 %), the application of amendments resulted in a moderate increase in pH and reduced As, Cd, Pb, and Zn leaching after 1-year, with S-nZVI and sludge combined being the most efficient, followed by iron grit and S-nZVI alone. However, the amendments had adverse impacts on microbial biomass quantity, S-nZVI being the least damaging. In the soil with a lower pH (6.0) and

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organic matter content (TOC = 2.3 %), the results were mixed; 0.01 M $CaCl_2$ extraction data showed only S-nZVI with sludge as remaining effective in reducing extractable concentrations of metals; on the other hand, Cd and Zn concentrations were increased in the extracted soil pore water solutions, in contrast to the two conventional amendments. Despite that, S-nZVI with sludge enhanced the quantity of microbial biomass in this soil. Additional earthworm avoidance data indicated that they generally avoided soil treated with all Fe-based materials, but the presence of sludge impacted their preferences somewhat. In summary, no significant differences between S-nZVI and iron grit were observed for metal(loid) immobilization, though sludge significantly improved the performance of S-nZVI in terms of soil health indicators. Therefore, this study indicates that S-nZVI amendment of soils alone should be avoided, though further field evidence from a broader range of soils is now required.

1. Introduction

Nanoscale zero-valent iron (nZVI) materials are gaining recognition in the field of pollutant remediation for a wide range of organic and inorganic compounds (Ken and Sinha, 2020). Their high reactivity, large specific surface area, and abundant sorption sites (Liang et al., 2022; Reddy et al., 2016) are potentially suited to their application to contaminated soils and waters. The unique core-shell structure of nZVI is composed of metallic iron (Fe^0) and acts as an electron donor source to reduce pollutants. The shell consists of a layer of iron oxides/hydroxides formed from the oxidation of Fe^0, which occurs when the nZVI is exposed to oxidative environment (e.g., oxygen dissolved in water) and is naturally generated during the nZVI synthesis. The shell acts as an electron acceptor providing sites for adsorption and surface complexation reaction. In particular, these qualities are ideal for metal(loid) immobilization (Cai et al., 2019; Jiang et al., 2015).

Notwithstanding the advantageous qualities of nZVI materials, their bare application within the environment has limitations related to rapid oxidation and agglomeration of the particles due to magnetic and electrostatic attraction, van der Waals forces, and high surface energy, resulting in the decline of activity, longevity, and efficiency (Zhou et al., 2022). Due to its oxidation in the presence of oxygen and water, nZVI is able to generate reactive oxygen species, which have strong oxidizing ability against various organic compounds. However, it can cause oxidative stress for microbial cells. Cells under severe oxidative stress demonstrate diverse dysfunctions of membrane lipids, proteins, and DNA (Semerád et al., 2019). In addition, the nZVI particles adsorbed on cell membranes can obstruct cellular ducts, induce structural alterations to the membranes, or restrain mobility and nutrient intake, which often lead to disturbance in the functioning of the cell and may be lethal to bacteria (Stefaniuk et al., 2016).

The organisms inhabiting the soil play a vital role in maintaining a healthy soil ecosystem (Custódio et al., 2022). Soil life is dominated by microorganisms, which perform a number of essential soil functions by decomposing organic matter, defining soil structure, adjusting nutrient cycling, endorsing plant productivity, and restraining soil-borne plant diseases. As such, the structure and diversity of microbial community can be used as indicator of soil health (Coban et al., 2022). Elevated concentrations of metal(loid)s in soils can change the taxonomic structure, increase the overall community tolerance, or decrease enzymatic activity, microbial biomass and the diversity of metabolic processes performed by a microbial community (Stefanowicz et al., 2020). Likewise, the exposure to nZVI can immensely change the structure and composition of soil bacterial communities (Liu et al., 2022). Therefore, it is crucial to conduct ecotoxicological assessments and investigate changes in the microbial community structure to obtain insights into how S-nZVI and iron grit influences soil microorganisms and consequences for general soil health.

Earthworms represent an important component of the soil macrofauna due to their participation in nutrient cycling, decomposition of organic matter, and formation of soil structure – they have been regarded as soil ecosystem engineers because of their burrowing, feeding, and casting activities (Liu et al., 2020). Earthworms stay in close contact with soil particles via their permeable skin and digestive system. The presence of sensory tubercles and chemoreceptors on their body make earthworms highly susceptible to contaminants in their surroundings, allowing them to avoid hostile environments (Yadav et al., 2023). As such, earthworms can be reliable indicators of soil quality and observation of their behavior can serve as first screening tool for evaluating the ecotoxic potential of metal-polluted or amended soils (Udovic and Lestan, 2010; Xiao et al., 2022).

Many surface modification techniques have been employed to enhance the stability and reactivity and reduce the aggregation and toxicity of bare nZVI, such as emulsification, metal doping, or surface coating (Ken and Sinha, 2020). Recently, sulfide-modified nZVI, synthesized from surface modification of nZVI with reducing sulfur compounds such as sulfate, dithionite, thiosulfate, and sulfide, has been widely investigated (Cheng et al., 2019; Nunez Garcia et al., 2020). Sulfidation improves the stability and reactive lifetime of nZVI by two orders of magnitude owing to the formation of a FeS shell on the surface of nZVI (Xu et al., 2021). The shell selectively facilitates the electron transfer from the Fe^0 core to the target pollutants while protecting the core from the swift oxidation and aggregation of nZVI particles (Mangayayam et al., 2022; Tang et al., 2021). Although sulfidized nZVI (S-nZVI) gained significant attention as a promising remediation material, knowledge of the fate and potential risk of S-nZVI during remediation in the field is limited (Cheng et al., 2019). Most of the experiments are conducted solely in laboratory settings (e.g., Guan et al., 2019; Guo et al., 2021; Liang et al., 2021; Liu et al., 2023), whereas studies describing how this amendment performs under field conditions remains scarce. However, long-term field studies are critical for better understanding the behavior of the amendments under natural climatic conditions (Kumpiene et al., 2019). Consequently, a thorough investigation is needed to connect the dots and understand the environmental impacts of this remediation practice, long-term immobilization of the pollutants, and performance of amendments under field conditions, which is what this research aims to.

Comparing S-nZVI and iron grit for soil remediation, S-nZVI should offer enhanced reactivity and improved remediation efficiency compared to (micro) iron grit (Dong et al., 2022) though the application of S-nZVI is expensive in comparison to iron grit, hindering its widespread usage (Crane and Scott, 2012). Additionally, the impacts of S-nZVI on soil health and its potential toxicity is vastly understudied compared to iron grit (Cheng et al., 2019). As with all soil amendments, their effectiveness is greatly influenced not only by their individual properties but by the characteristics of the soils they are amending (Fangueiro et al., 2018). For example, the reactivity of S-nZVI and iron grit in soils will be materially affected by soil pH, temperature, soil organic matter content, dissolved oxygen (redox), soil moisture status etc., though very few studies have investigated these interactions (Jiang et al., 2018; Xu et al., 2020), and none at field scale across seasons to the best of our knowledge.

In this study, the pilot test of metal(loid)s in situ immobilization in contaminated soils was carried out via small-scale field experiments situated on two sampling localities, both affected by former smelting activities. The used soil amendments included S-nZVI, S-nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS), and iron grit as conventional, microscale, and commercially available form of zero-valent iron. Sewage sludge, a residual product of wastewater treatment, which is sufficiently treated to a certain quality that reduces or eliminates health and environmental risks, can be used as a soil conditioner (Marchuk et al., 2023) and thus improve the stabilization process and mitigate the possible toxicological effects of nZVI after its application to soils.

The principal aim of this research is to test S-nZVI under field conditions for one year and investigate its behavior in comparison to iron grit. The specific objectives of the present study were to (i) measure the mobility of As, Cd, Pb and Zn after S-nZVI and iron grit field application to contrasting soils, (ii) evaluate the the tested amendments in a 1-year field trial; (iii) discuss the findings in the context of potential impacts to soil health via microbial and earthworm indicators after nZVI application to soils, including its comparison with conventional iron grit.

2. Materials and methods

2.1. Soils and their amendments in the field

Two sampling sites were selected for this study of differing soil pH and organic matter content but having received enriched metal(loid) deposition from the same local source; their basic characteristics are presented in Table 1. Soil denoted as "Fluvisol" originates from an alluvium of the Litavka River which flows through Příbram (Czech Republic). The town and its surroundings have an increased load of pollution owing to past mining and smelting activities, which generated fly ash, slag and other waste material that was deposited in the area. The alluvium is contaminated as a result of historical atmospheric deposition as well as occasional floods, i.e., as the contaminants are washed out from deposited waste materials. This soil displays, due to its fluvic nature, reasonable homogeneity of basic soil characteristics between field plots (see Table S1). The area belongs to the Příbram ore district and is characterized by vein-type Ag, Pb, and Zn ore deposits (Ettler et al., 2006). Accordingly, local soils are contaminated with As, Cd, and especially Pb and Zn. The soils and sediments in this area have been thoroughly investigated several times and the studies showed that Cd and Zn are mainly present in the mobile fractions (e.g., Ettler et al., 2006; Kotková et al., 2019; Teodoro et al., 2020; Vaňková et al., 2021). The weather conditions during the duration of the experiment were typical for Central Europe.

Soil denoted as "Technosol" is naturally more alkaline ($pH_{H_2O} = 7.5$) than the Fluvisol ($pH_{H_2O} = 6.0$), which is common in technogenic substrates due to the presence of human artifacts, such as slags, glasses, coal combustion products, or crushed building materials containing calcium carbonate ($CaCO_3$) (Bilibio et al., 2022; Riddle et al., 2022). Thus, the electrical conductivity is higher in the Technosol (30.4 mS/m) than the Fluvisol (6.4 mS/m). Comparatively higher content of total organic carbon is observed in the Technosol (average value 12.7 %), though with greater heterogeneity in the tested field plots utilized for this study (see Table S1). Both soils exhibit similar particle size distribution, the Technosol can be categorized as loamy sand and the Fluvisol as sandy loam. Both soils are significantly contaminated with As, Cd, Pb and Zn (Table 1).

Table 1
Basic soil characteristics of the soils from the study localities.

Soil properties	Technosol	Fluvisol
pH_{KCl}	6.7	5.1
pH_{H_2O}	7.5	6
Electrical conductivity (mS/m)	30.4	6.4
TOC (%) (n = 4)	12.7 ± 2.7	2.27 ± 0.51
Particle size distribution		
Sand (%)	81	75
Slit (%)	11	20
Clay (%)	8	5
Aqua regia extracted (mg/kg) (n = 4)		
As	205 ± 17	400 ± 29
Cd	39.5 ± 6.6	45.0 ± 3.9
Pb	9357 ± 802	5730 ± 211
Zn	2771 ± 370	4443 ± 502

Based on the preliminary results of the incubation experiment (details are available in Supplementary Material and Fig. S1), three amendment materials were selected for this trial. Sulfidized nano zero-valent iron nanopowder material (Nanofer 25DS) was purchased from NANO IRON Ltd., Czech Republic. According to the manufacturer specification this nanopowder material is composed of 80 % zerovalent iron nanoparticles of the size 50 nm and average surface area 20–25 m^2/g, which are then dispersed in a slurry comprised of 20 % nanopowder to 80 % water to enhance the stability of the finished product (Nanoiron Ltd., 2024). Therefore, the zero-valent iron content of the material applied to soil in the present study is ~16 %. Sewage sludge was obtained from a small wastewater treatment plant in the Czech Republic and is meeting all the limits for use in agriculture set by the Ministry of the Environment of the Czech Republic no. 273/2021 (Table S1). The collected sludge was subjected to thermal drying at 90 °C to ensure sanitation of the material. Fine waste cast iron particles (particle size <2 mm), referred to as iron grit, were acquired from Dekonta, Czech Republic.

Two replicates of four different treatments were used at both localities (i) control (soil without any amendments); (ii) iron grit, applied at 1 % (*w*/w); (iii) sulfidized nano zero-valent iron (S-nZVI), applied at 1 % (w/w); (iv) combination of 1 % (w/w) S-nZVI and 3 % (w/w) thermally stabilized sewage sludge. The purpose of the sludge was to mitigate potential toxicity issues associated with the application of S-nZVI alone. The sludge dose was calculated primarily to comply with the local regulations. The necessary amount of S-nZVI slurry was recalculated according to the mass of pure Fe. A total of 16 experimental plots (4 treatments, 2 replicates, 2 soils, each with an area of 1 m^2) were monitored. The treatments were applied by removing the topsoil layer (ca. 20 cm), mixing it thoroughly with the amendments in a plastic container, and then returning it back to its original place. The detailed chemical composition of each study plot after the application of amendments along with material characterization of iron grit and thermally stabilized sewage sludge is expressed in Table S1 in the Supplementary Material.

2.2. Soil sampling and characterization

The soil samples have been periodically collected from the study area (after 1 week, 6 weeks, 3 months, 6 months, 9 months, 1 year). In order to obtain a representative sample, the soil was taken from 5 points and depths (0–20 cm) within each sampling plot and a mixed sample was used. The soil samples were sieved through a 4-mm stainless sieve to remove any unwanted stones and debris from the soil. After that, part of the fresh soil was frozen (−18 °C) and within one week lyophilized prior to microbial analyses. Another part of the fresh soil was oven-dried (40 °C) overnight in order to determine its current water content. Such samples were later homogenized, sieved through a 2-mm stainless sieve, and kept for further laboratory analyses.

The pseudo-total content of metals was determined according to USEPA 3051A using digestion with 9 mL HNO_3 + 3 mL HCl under microwave conditions (Multiwave PRO, Anton Paar, Austria) (US EPA, 2007). After the digestion, 1 mL of H_2O_2 was added, then the mixture of chemicals was evaporated from the sample (120 °C), and the sample was dissolved in 2 % HNO_3 (*v*/v). The SRM NIST 2711a Montana II Soil was used to validate the results.

The particle size distribution was determined using the hydrometer method (Gee and Bauder, 1986). Soil pH was measured in a soil suspension of 1:5 ratio (*w*/*v*) prepared with deionized water (pH_{H_2O}) and 1 M KCl solution (pH_{KCl}). The $CaCl_2$ extraction was performed according to Quevauviller (1998) to characterize easily extractable metal(loid) concentrations in soils. Bioavailability indicates the amount of dissolved metal(loid) fraction in the pore water which can be taken up by plant roots or other soil organisms and can cause an adverse physiological or toxicological response (Kim et al., 2015; National Research Council, 2003). A metal(loid) is bioavailable if it is present as a free-ion species or can be readily transformed as such. Plenty of single extraction methods have been extensively used to predict bioavailable, furthermore phytoavailable, fractions of elements in soil (Caporale and Violante, 2016). Yet no extraction procedure can accurately predict the bioavailability of metal(loid)s due to its operationally defined nature. Nonetheless, $CaCl_2$ extraction is considered as a good proxy for bioavailability of metals, particularly in relation to plant quality and toxicity as the ionic strength of 0.01 M $CaCl_2$ is similar to that of pore water (Kim et al., 2015). For this extraction, 2 g of soil were shaken horizontally with 20 mL of 0.01 M $CaCl_2$ for 3 h on a reciprocating shaker at 200 RPM. After shaking, the soil slurry was centrifuged at 5500 rpm for 10 min, and the supernatant was filtered through a 0.45 μm membrane filter. The BCR extraction method was performed in accordance with Rauret et al. (2000). All extractions were carried out in duplicates or triplicates. All chemicals used in this study were of analytical grade.

2.3. Determination of pore water properties & DGT

The extraction of soil pore-waters using Rhizon samplers has been proposed as a practical non-destructive method to assess metal(loid) solubility and mobility especially after remedial amendment of contaminated soils (Moreno-Jiménez et al., 2011). In the present study the remaining fresh soil described previously was kept in a closed plastic bag to preserve its current moisture content. Once the moisture content was determined, a fresh soil aliquot equivalent to 150 g of dry soil was placed into a plastic container, moistened to the maximum water holding capacity with demineralized water, and left to equilibrate for 24 h at room temperature. The soil was equally separated into three plastic cups, each containing about 50 g of incubated soil (dry weight equivalent). During the pre-testing it was found that the pore water collection in the field is not always successful due to fluctuating soil moisture, which can result in inconsistent sampling of pore water volume. Therefore, the samples of soil solution were incubated in the laboratory using fresh soil and Rhizon samplers (Rhizosphere Research Products, Wageningen, The Netherlands).

Given that the two studied field sites displayed sporadic vegetation cover, from where plant material was not consistently available for sampling, the DGT sampler was thought to be a suitable additional proxy for bioavailable metal(loid)s further to the $CaCl_2$ extraction, as described by Hooda and Zhang (2008). Two remaining cups of soil were used for the diffusive gradients in thin films (DGT) analysis, one for a DGT sampler (DGT Research Ltd., Lancaster, UK) suitable for cationic metals in soils using a Chelex binding layer, and one appropriate for oxyanionic metals in soil using a ferrihydrite binding layer. The DGT sampler was deployed onto the soil surface and incubated at room temperature for 24 h. Then the DGT sampler was disassembled to remove the resin and obtain the DGT extracts following instructions provided by the manufacturer.

The Eh and electrical conductivity (EC) of the soil solution samples were measured with a multimeter Multi 3420 (WTW, Germany) immediately after sampling. The pH was measured with a pH-meter 3310 (WTW, Germany). The content of organic and inorganic carbon (TOC/TIC) was determined using a carbon analyzer (TOC-L CPH, Shimadzu, Japan). The concentrations of anions in the soil solution samples and water extracts were determined by ionic chromatography (Dionex ICS-5000+, Thermo Fischer Scientific). The concentrations of major and trace elements in all tested solutions were determined using inductively coupled plasma optical emission spectrometry (ICP OES; iCAP 7000 series, Thermo Scientific, Germany).

2.4. Microbial analyses

Soil microorganisms play an essential role in the nutrient cycling, decomposition and mineralization of organic matter, and pollutant degradation (Gong et al., 2023). In this study, microbial populations were monitored using two different approaches – Phospholipid fatty acid (PLFA) analysis and 16S rRNA sequencing during the pilot test. The PLFA analysis is used to quantify microbial biomass and determine community composition, particularly in soils and sediments. This analysis serves as a very good indicator of soil microbial communities because some PLFAs – main structural components of biological membranes, are specific to a single microbial taxon and hence can be used as taxonomic biomarkers allowing identification and quantification of microbial groups (Lewe et al., 2021). In addition, since PLFAs are swiftly degraded and metabolized upon cell death, total PLFA biomarkers indicate the biomass of living microorganisms (Agnihotri et al., 2023). The PLFA analysis was performed using freeze-dried soil samples. The samples were extracted in triplicate using a mixture of chloroform, methanol, and phosphate buffer (1:2:0.8; *v*/v/v), transmethylated and analyzed using tandem gas chromatography–mass spectrometry (GC–MS; 450-GC, 240-MS Varian, 72 Walnut Creek, CA, USA); and processed as described by Covino et al. (2016). The bacterial biomass was quantified as a sum of the specific methyl ester markers: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 16:1ω5, 16:1ω7, cy17:0, 18:1ω7, cy19:0, 10Me-16:0, 10Me-17:0, 10Me-18:0 (for actinobacteria); 15:0, 17:0, and 16:1ω9 (Šnajdr et al., 2008; Stella et al., 2015).

For Illumina MiSeq sequencing, total DNA for microbial community analysis was extracted in triplicate from all soil samples using a NucleoSpin soil kit (Macherey-Nagel) and purified using a GENECLEAN Turbo kit (MP Biomedicals) according to the manufacturer's instructions. The V4 region of the bacterial 16S rRNA was amplified using the barcoded primers 515F (5′ GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2012). The 16S polymerase reaction was carried out for 4 min at 94 °C, 25 cycles (45 s at 94 °C, 60 s at 50 °C and 75 s at 72 °C) with a final extension at 72 °C for 10 min (Bosch et al., 2023). For the purified pool of products from tree PCR reactions, the MinElute PCR Purifcation Kit (Qiagen) was used, and the concentration of amplicons was measured using the Qubit 2.0 Fluorometer (Thermo Fisher Scientifc). Sequencing libraries were prepared using the TruSeq PCR-Free Kit (Illumina) according to the manufacturer's instructions and sequencing was performed in-house on Illumina MiSeq (2 × 250 bases).

Amplicon sequencing data were processed with the SEED 2.1.2 pipeline (Větrovský et al., 2018). Paired-end reads were merged using fastq-join (Aronesty, 2013). Two external algorithms implemented in Vsearch (Rognes et al., 2016) and Usearch (Edgar, 2010) were used to cluster similar sequences into OTUs (Operational Taxonomic Units) and remove chimaeras. Sequences were clustered at an identity level of 97 %. The most abundant sequences were considered representative of each OTU. The closest bacterial hits at the species level were identified using the BLAST alignment software (Altschul et al., 1997). BLAST searches were performed remotely using the NCBI API. The number of sequences was rarefied to the smallest number of sequences per sample (6781 sequences) and the final data consisted of 20,730 bacterial OTUs.

2.5. Earthworm avoidance test

Earthworms represent an important part of the soil fauna contributing to many beneficial ecological services. Owing to their permeable skin and continual contact with soil via their digestive tract, they are extremely sensitive to soil contamination (Yadav et al., 2023). Thus, they can be regarded as useful indicator of soil health and quality. The standardized earthworm avoidance test (ISO 17512-1, 2008) provides a fast screening method for evaluating the habitat function of soils and the impact of contaminants and chemicals on earthworm behavior. In this study, this test was slightly modified as the control soil was not without contaminants but instead a contaminated soil sample from the field without any amendments added. Details on the test are available in Supplementary Material.

2.6. Statistical analysis

Upon the organization of data, appropriate statistical analyses were carried out. Analysis of variance ($p < 0.05$) of the data was performed using one-way ANOVA (Tukey-HSD test). The statistical analyses were performed using the software R 4.3 (https://www.R-project.org).

3. Results and discussion

3.1. Amendment effects on soil pH and metal(loid) mobility

One of the central principles of applying soil amendments to contaminated soils is to induce changes in 'master variables' which influence metal(loid) mobility, such as pH (Fangueiro et al., 2018). Soil pH of the Technosol (Fig. 1a) increased in all treatments compared to the control and remained higher even after one year after application. While the soil pH of control samples remained stable in the Technosol, it was affected by seasonal fluctuations in the Fluvisol. As can be seen in Fig. 1b, the pH was higher than 6.0 in samples from 1 week, 9 months and 1 year, which corresponds to observed changes in metal(loid) availability in control soil during the year. Regarding the effects of amendments in the Fluvisol, there was a significant increase of pH (Fig. 1b) after the application of all amendments in comparison to the control samples. This effect lasted for three months after the application. After one year, all amended samples exhibited lower pH than that the control samples. Measured properties of soil solution including statistical significance are given in Table S2.

The pH increase can be attributed to the consumption of H^+ cations during the oxidation of zero-valent iron (Fe^0) and precipitation of amorphous iron oxyhydroxides causing decrease in H^+ activity (increase of pH) (Qiao et al., 2018). The zero-valent iron corrosion is enhanced by acidic pH values (Rezaei and Vione, 2018). As can be seen in Fig. 1a, b, the initial increase in pH of treated soils was more pronounced in the Fluvisol, which is naturally more acidic ($pH_{H_2O} = 6.0$), than in the Technosol ($pH_{H_2O} = 7.5$).

After one year, S-nZVI with thermally stabilized sludge showed marginally better metal(loid) stabilization than both iron grit and S-nZVI on Technosol in some cases (Fig. 2c, g, k). Yet pristine S-nZVI was not superior to iron grit in terms of metal(loid) stabilization. Though the differences have not been found to be statistically significant. In comparison, the results for Fluvisol are not as decisive as the amendments performed differently for each metal(loid).

Seasonal variations may have impacted mobility and bioavailability of some elements, with this effect being more prominent in the Fluvisol – in spring and summer (i.e., sampling time of 3 months and 6 months), Cd and Zn concentrations increased (Fig. 2d, l) and As concentrations decreased (Fig. 2b) in all monitored plots including control. Regarding As, all the amendments reduced its concentration in the soil solution on both localities (Fig. 2a, b), yet this decrease was not found to be statistically significant. The prevailing forms of As in soils are inorganic species, occurring as arsenite (As(III); predominantly $H_3AsO_3^0$) and arsenate (As(V); predominantly $H_2AsO_4^-$ or $HAsO_4^{2-}$). Under oxidizing conditions (in aerobic environments), oxidation of arsenite to arsenate can decrease As mobility in soil, because arsenates are strongly sorbed onto organic matter, Al, Fe and Mn (hydr)oxides, and clays compared to arsenite (Mandal and Suzuki, 2002; Pigna et al., 2015). Since the redox conditions of the experimental soils were consistently oxidized since week 1 with an Eh values of the Rhizon extracts greater than +300 mV on both localities regardless of the treatment (Fig. S2a, b), As mobility was mainly driven by pH. Further information on observed soil Eh and electrical conductivity (EC) values can be found in Supplementary Material and Fig. S2.

Cadmium and Zn followed the same pattern over time in the Fluvisol as a result of their similar geochemical behavior (Biswas et al., 2022). Apart from that, the available concentrations of these elements were much lower in the Technosol though the total concentrations present on both localities are alike. For example, the average Zn concentration of control samples over time was 0.87 mg/L in Technosol and 12.5 mg/L in the Fluvisol. This could be attributed to differences in pH and organic matter (OM), which are key factors controlling bioavailability and mobility of metal(loid)s (Liu et al., 2016). The debris occurring on the Technosol site might contain materials effective for immobilization of metal(loid)s, such as lime (CaO) (Tak et al., 2023). Indeed, the Technosol has comparably higher content of total Ca present in soil (≤ 19.6 g/kg) than Fluvisol (≤ 1.7 g/kg) (Table S1) as well as Ca available in control Rhizons samples (average concentration ≤ 51.7 mg/L in the Technosol vs. ≤18.5 mg/L in the Fluvisol throughout time) (Table S3). Additionally, Fig. S3 shows the percentage of metals extracted using the BCR method from untreated samples in each locality. As can be seen, the elements in the Fluvisol have generally a higher proportion in the first, easily soluble, fraction than in the Technosol. In contrast, the elements in the Technosol have two to three times higher portion of oxidizable fraction (bound to organic matter) in comparison to the Fluvisol. Correspondingly, the amount of dissolved organic carbon occurring in control Rhizon samples in the Technosol is twofold compared to the Fluvisol, on average 146 mg/L and 73.1 mg/L in time, respectively (Table S3).

In soils, both Cd and Zn belong to the most readily available metals, as they both typically occur as free divalent cations, i.e., Cd^{2+} at pH values lower than 6.5 and Zn^{2+} at a pH values below 7.7 (Balafrej et al., 2020; Kubier et al., 2019). Thus, their mobility highly depends on pH conditions. Regarding the treatments, concentrations of both metals were lower than control even after one year in the Technosol (Fig. 2c, k), S-nZVI with treated sewage sludge having the best outcome. In contrast,

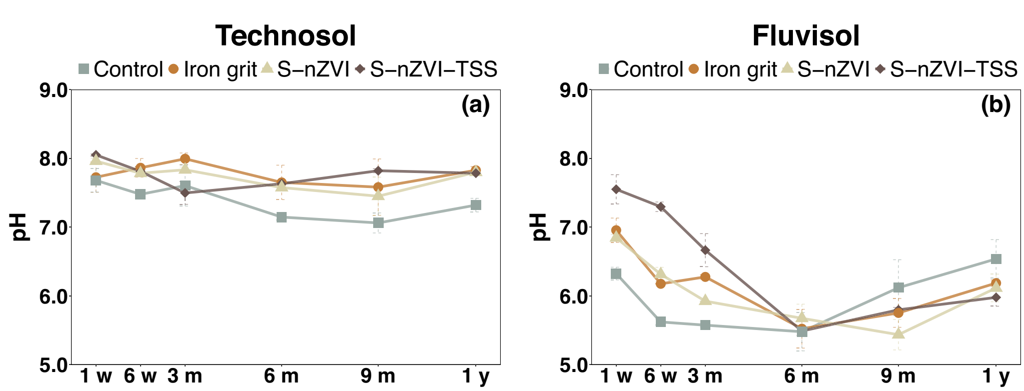

Fig. 1. Porewater pH following the application of amendments with time (1 week, 6 weeks, 3 months, 6 months, 9 months, 1 year) for the non-amended soil (control) and the soils amended with iron grit, S-nZVI and S-nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS) ($n = 2$).

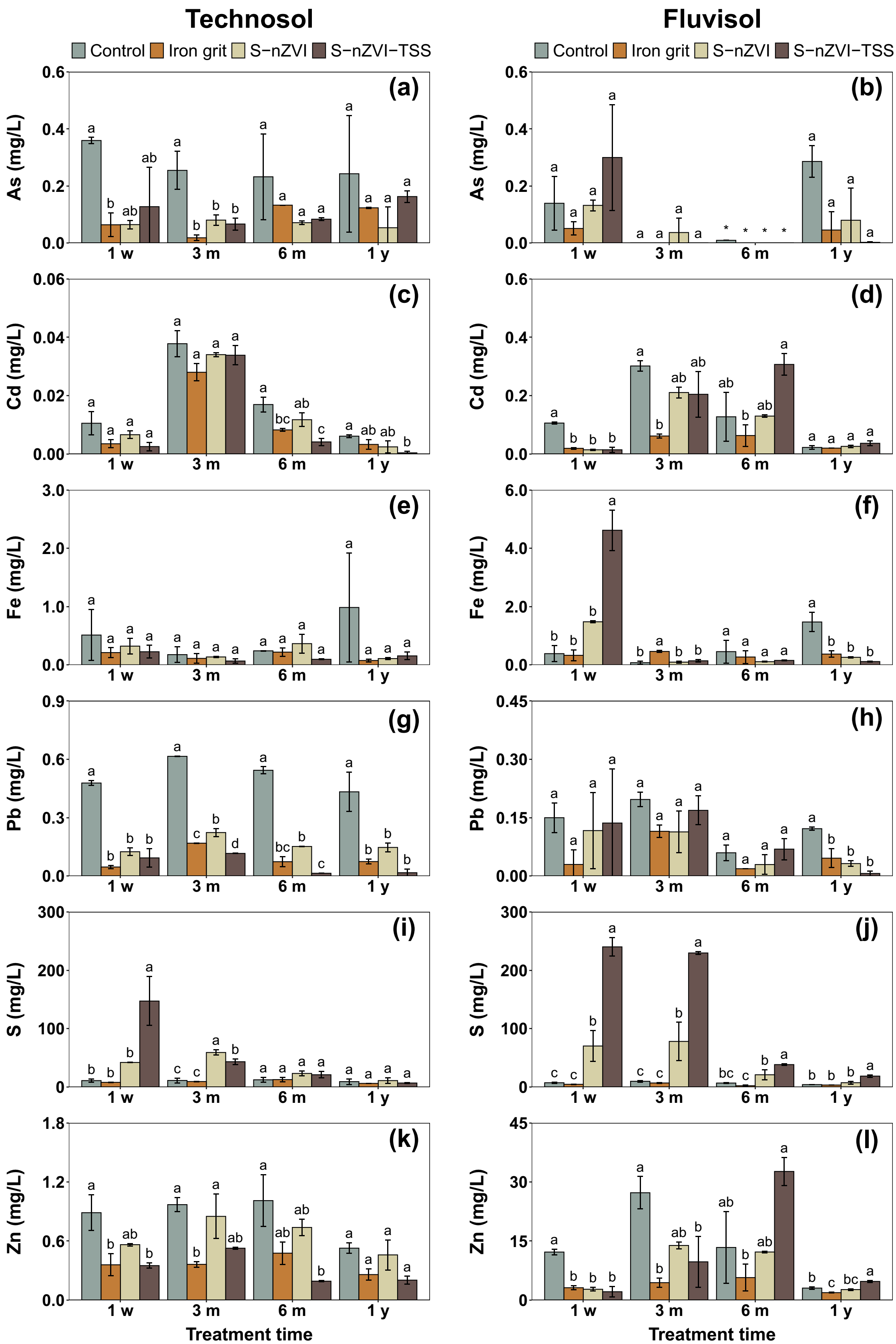

Fig. 2. Concentrations of As, Cd, Fe, Pb, S and Zn in porewater extracted by Rhizon samplers measured for the non-amended soil (control) and the soil amended with iron grit, S-nZVI and S-nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS) (n = 2) after 1 week, 3 months, 6 months, 1 year. The letters compared the different treatments at each sampling time. Different letters correspond to values statistically different ($p < 0.05$) within each treatment time and soil. The asterisks (*) represent values below the detection limit.

the Fluvisol plots amended with S-nZVI supported with thermally treated sewage sludge had raised concentrations of both metals and only iron grit retained the immobilizing effect (Fig. 2d, l).

Although Pb is present in studied soils in excessive concentrations (≤ 10,000 mg/kg), <0.7 mg/L of Pb was found in the soil solution extracted by Rhizon samplers from soils in Technosol and <0.2 mg/L in Fluvisol, demonstrating its relative immobility. Lead is generally a very immobile element in soils due to its strong tendency to be adsorbed onto organic matter, clay minerals and oxides (Sipos et al., 2005). It may even form stable insoluble precipitates such as chloropyomorphite ($Pb_5(PO_4)_3Cl$), which is considered as one of the most stable forms of Pb in the soil environment (Pontoni et al., 2020). All the treatments decreased the Pb concentrations in the soil solution after one year, S-nZVI with thermally treated sludge being the most effective on both localities (Fig. 2g, h).

In this study, much of the impact of soil amendments were site specific due to the differences in pH and organic matter between sites. The Technosol exhibits a higher pH value ($pH_{H_2O} = 7.5$) and soil organic matter content (TOC = 12.7 %) content than the Fluvisol ($pH_{H_2O} = 6.0$, TOC = 2.27 %), leading to naturally lower mobility of elements (specifically Cd, Zn) on this site. Cations (such as Cd, Pb, Zn) have usually raised tendency to be released towards low pH values, whereas increased release of anions (including As) is usually observed towards high pH values (Król et al., 2020). The changes in pH throughout time were more pronounced in Fluvisol, causing distinct differences in mobility. While Cd and Zn were more soluble at lower pH (Fig. 2d, l; treatment time 3 m, 6 m), As mobility was reduced below the limit of detection (Fig. 2b; treatment time 3 m, 6 m), but appeared remobilized with increasing pH after 12 months (Fig. 2b; treatment time 1 y).

Since the applied materials were based on Fe and some of them contain S (S-nZVI and S-nZVI-TSS), the change of concentrations of these two elements in soil solution was investigated as well. Concerning Fe, 1 week after application of treatments were its concentrations higher in S-nZVI and S-nZVI with thermally treated sewage sludge samples compared to iron grit in Fluvisol (Fig. 2f). Nonetheless, 1 year after the application, the concentrations of Fe were not higher than those observed in control samples on both localities (Fig. 2e, f). The concentrations of S were notably increased on both localities for the first 3 months after the application of amendments (Fig. 2i, j). Although the concentrations remarkably declined throughout the time, the S-nZVI-TSS treated plots in Fluvisol still demonstrated raised S concentrations even after 1 year (Fig. 2j). The raised concentrations correspond to increased values of electrical conductivity (Fig. S2c, d). Both S-nZVI and especially S-nZVI in combination with thermally stabilized sewage sludge increased soil electrical conductivity (EC) in the first three months after the application of the amendments on both localities. Additionally, the data obtained by ion chromatography (Table S3) demonstrate that S in the soil solution of plots amended with S-nZVI and S-nZVI with thermally stabilized sludge was mainly present as sulfate (SO_4^{2-}) indicating that the sulfide layer of S-nZVI particles oxidized to sulfates.

Of other elements detected by ICP OES in the soil solution (Table S3), notable leaching of Na was observed in the first 3 months of the experiment on both localities up to 221 ± 37 mg/L (while control sample had <10 mg/L) on the plots treated with S-nZVI and especially S-nZVI with thermally stabilized sludge, which results into increased ionic strengths of the soil solution. Gil-Díaz et al. (2020) also observed increased leaching of Na and it was attributed to the presence of a stabilizing agent (Na salt of polyacrylic acid) in the nanoparticles used in their study. Although different types of nZVI particles were used in our study, Na compounds (e.g., $Na_2S_2O_4$ and $NaBH_4$) can be used to synthesize S-nZVI (Zhang et al., 2022). Therefore, increased Na concentrations following S-nZVI application might be remnants of the S-nZVI synthesis, but the effect is transient as concentrations decreased over time close to those of the control concentrations. Regarding the S-nZVI with thermally treated sludge variant, another source of Na is apparently the sludge itself as the total concentration of Na present in sludge is 1.3 g/kg (Table S1).

Since the results for 6 weeks and 9 months obtained for soil solution were similar to 1 week and 1 year respectively, they were omitted. Table S3 provides complete results for soil solution, including the content of dissolved organic and inorganic carbon, the concentrations of anions, and other measured elements in all monitored time intervals.

3.2. Metal bioavailability in the treated soils

The bioavailable levels of Cd, Pb, and Zn in the control soil (without amendments) and the amended samples were assessed by 0.01 M $CaCl_2$ extraction (Fig. 3) as well as the DGT technique (Figs. S4-S7). Both DGT and $CaCl_2$ methods are supposed to mimic plant bioavailable metals (Hooda and Zhang, 2008). However, the DGT extractable Cd and Zn were correlated with $CaCl_2$ concentrations (S5 & S7), while As and Pb (S4 & S6) were not, which demonstrates a limitation of the usage of this expensive and time-consuming diffusion method on multi-element contaminated soils. It thus appears that DGT offers no advantages over the traditional $CaCl_2$ extraction towards understanding metal(loid) bioavailability in the studies soils. Therefore, only $CaCl_2$ data will be discussed further. The mobility of selected metals was generally higher in the Fluvisol than in the Technosol; the lowest extractability was observed for Pb, for which <0.02 % of the pseudo-total soil metal concentrations (≤ 10,000 mg/kg) were extracted from the Technosol samples (Fig. 3c) and <0.1 % from the Fluvisol samples (Fig. 3d). Whereas Cd (Fig. 3a, b) demonstrated the highest extractability, < 2 % and <25 % of the pseudo-total soil metal concentrations (≤ 45 mg/kg) in the Technosol and the Fluvisol, respectively.

Concerning the immobilizing efficiency, all selected elements exhibited similar trends over time. In the Technosol (Fig. 3a, c, e), all amendments were effective in decreasing the mobility of aforementioned metals, even after one year after application. In contrast, in Fluvisol (Fig. 3b, d, f), only the combination of S-nZVI with thermally stabilized sewage sludge (S-nZVI-TSS) remained effective for decreasing the mobility of metals. Yet the effect was more prominent in first 3 months and therefore it might diminish eventually. Otherwise, Fluvisol samples treated with iron grit and S-nZVI exhibited either the same or higher concentrations of metals extracted in the solution than the control soils.

The main mechanisms involved in treatment of metal(loid) pollution with ZVI include adsorption onto the iron (oxyhydr)oxide shell, (co-)precipitation, and redox reactions (O'Carroll et al., 2013). Depending on the particle size, ZVI can be typically transformed into the less stable iron oxides, ferrihydrite or lepidocrocite with high surface area and sorption capacity (Ma et al., 2016; Tiberg et al., 2016; Yu et al., 2016). Such oxides can sorb metal(loid)s and thus reduce their available fraction (Oustriere et al., 2016). With time, these (oxyhydr)oxides can gradually transform into more crystalline iron oxides (e.g., goethite or hematite) which have a lower specific surface area. Consequently, previously sorbed metal(loid)s may be released, increasing their leaching over time (Danila et al., 2020). Nonetheless, it had been reported that nZVI oxidation is not directly proportional to time and the coexistence of poorly crystalline (i.e., ferrihydrite) and more crystalline (i.e., magnetite, lepidocrocite, goethite) Fe (oxyhydr)oxides is possible (Mitzia et al., 2023). The additional reaction mechanism between S-nZVI and metal(loid)s involves sulfide formation, i.e., the substitution of Fe in FeS to form metal(loid) sulfides, which are very stable implying that the immobilized metal(loid)s are not likely to be released into solution again. Apart from possible sulfide formation and/or surface complexation, sulfur can be also oxidized to sulfate (SO_4^{2-}) during the reaction (Liang et al., 2021; Su et al., 2015). As indicated by the data obtained from the ion chromatography (Table S3), this mechanism seems to be prevailing in our study due to oxic conditions of the experiment.

It seems from the observed results that the immobilizing effects of the amendments tend to fade away over time in the Fluvisol, which might be attributed to the occurring desorption. In contrast, the metal(loid)s in

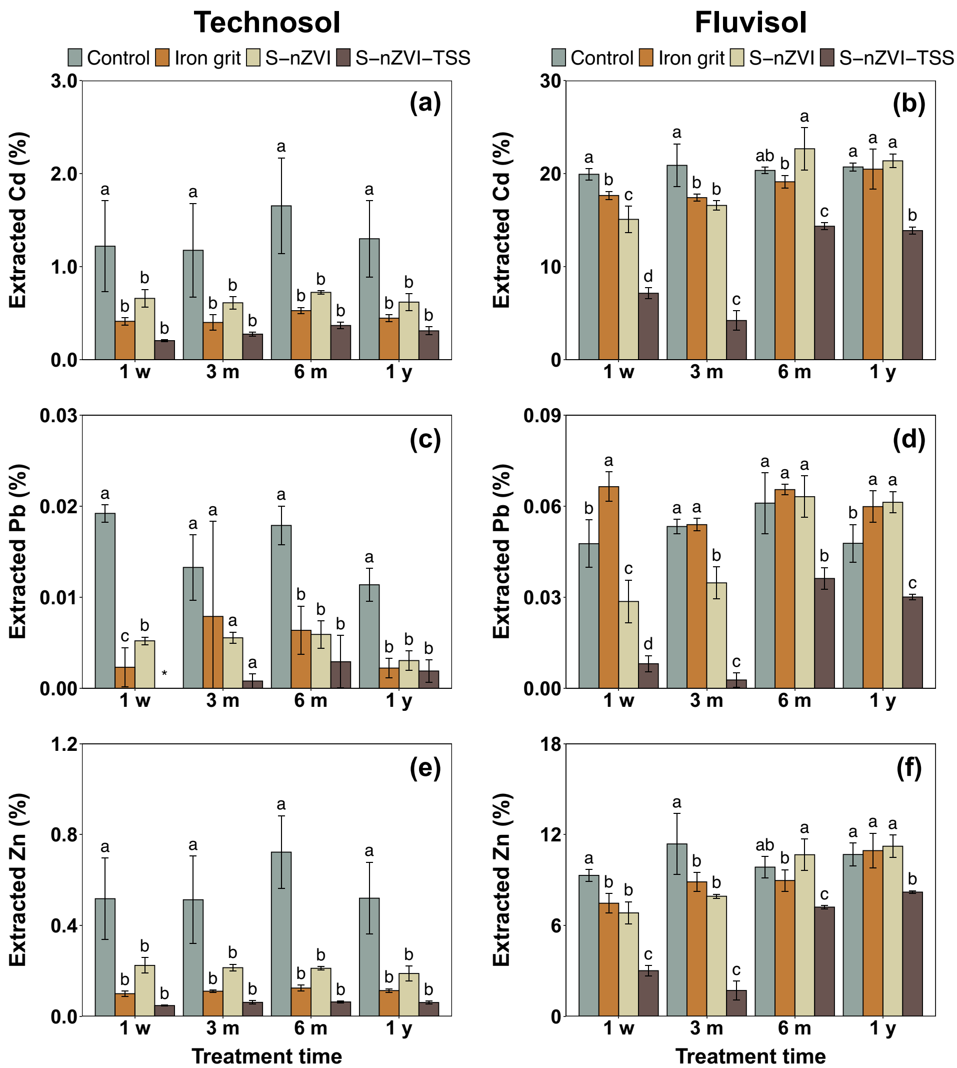

Fig. 3. 0.01 M $CaCl_2$-extractable metal(loid) concentrations in soils (expressed as the percentage of the pseudo-total soil metal concentrations) in the non-amended soil (control) and the soil amended with iron grit, S-nZVI and S-nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS) ($n = 4$) after 1 week, 3 months, 6 months, 1 year. The letters compared the different treatments at each sampling time. Different letters correspond to values statistically different ($p < 0.05$) within each treatment time and soil. The asterisk (*) represent value below the detection limit. Note the different scales of the y-axes.

the Technosol appear to be immobilized longer, indicating that the less stable and thus the most reactive Fe (oxyhydr)oxides might still be present one year after the application of the amendments. It has been reported that ferrihydrite can be present in micro-ZVI amended soils in considerable quantities even after 15 years indicating the possibility of the long-term immobilization efficiency using micro-ZVI under certain conditions (Kumpiene et al., 2021). Nonetheless, the sulfidation had no beneficial impact on immobilizing efficiency of nZVI compared to bare micro-ZVI (iron grit).

The natural pH of the soils seems to be an important factor influencing the longevity of metal(loid) immobilization with selected materials. While the metal(loid)s remained immobilized in the Technosol ($pH_{H_2O} = 7.5$) even after one year, they were mobilized in the Fluvisol ($pH_{H_2O} = 6.0$). The acidic pH enhances Fe corrosion, causing a decrease of the sites available for contaminant adsorption (Rezaei and Vione, 2018). Consequently, the occurring reactions could be accelerated, and the material efficiency seems to be depleted faster.

Direct comparison between our study and other studies is difficult due to the relative lack of knowledge about the performance of nZVI-based amendments under field conditions. Of those limited field studies on this topic, efficiency of nZVI was observed for the immobilization of Cr (Singh et al., 2012), As and Hg (Gil-Díaz et al., 2019) in metal(loid) enriched postindustrial soils. In general, there is an inclination towards a viewpoint that the differences between effectiveness of micro-ZVI and nZVI are negligible in relation to plant growth, foliar elemental concentrations, and exchangeable metal concentrations in the short term (Dovletyarova et al., 2022). Yet the retention of metal(loid)s over a long period of time could be more efficient in soils treated with micro-ZVI, due to the higher amount of poorly crystalline Fe in the ZVI-treated soil (Danila et al., 2020).

3.3. *Influence of the amendments on soil biota and implications for soil health*

Since the experimental plots were left unplanted and naturally occurring plants in the area did not grow well enough to make any conclusions about soil health by that indicator (i.e., phytotoxicity), the avoidance response of earthworms, microbial biomass and community structure were employed to investigate soil health.

3.3.1. *Soil microbial composition*

Soil microorganisms contribute to important soil processes. The results of the PLFA analysis (Fig. 4) demonstrated that the Technosol has generally more microbial biomass (control around 33 mg/kg of bacterial

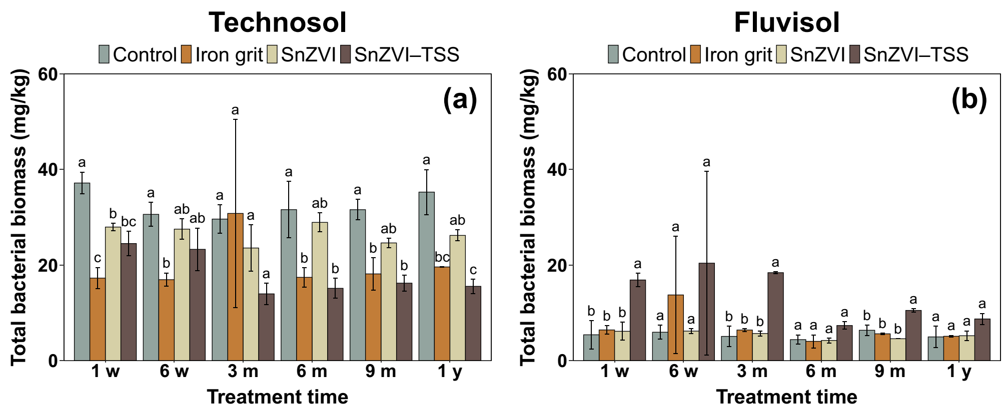

Fig. 4. Total bacterial biomass as measured by phospholipid fatty acid (PLFA) analysis in the Technosol (a) and Fluvisol (b) for the non-amended soil (control) and the soil amended with iron grit, S-nZVI and S-nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS) 1 week, 6 weeks, 3 months, 6 months, 9 months and 1 year after the application. Error bars represent standard deviation (n = 4) around the mean. Bars with the same letter show no significant differences in terms of total bacterial biomass at $p < 0.05$ within one treatment time and soil.

PLFA throughout time) than the Fluvisol (control around 5 mg/kg of bacterial PLFA throughout time). One week after the application of amendments to the Technosol, bacterial biomass decreased in all treated plots compared to control soil, iron grit having the biggest impact on reduction. This effect persisted even after 1 year. Anza et al. (2019) reported that no adverse effects of nZVI on microbial parameters were observed in uncontaminated soil. Whereas in contaminated soil, the microbial quality was diminished by nZVI treatments. Authors suggest that it might demonstrate a "stress-on-stress" effect, meaning that microbial populations previously exposed to stress (contaminated soil) are more susceptible to the application of nZVI (secondary stress) than those in uncontaminated soil.

In contrast, none of the amendments decreased the total bacterial biomass one week after the application of amendments in Fluvisol. Moreover, the addition of S-nZVI in combination with thermally stabilized sewage sludge significantly increased the total bacterial biomass. Although this increase was apparent even after 1 year, it was not found statistically significant. The increased soil microbial biomass along with activity after application of sewage sludge has been already reported several times and it is probably due to the supply of additional organic matter and nutrients (Urra et al., 2019).

Both fungi and bacteria (Fig. S8) were significantly affected by the addition of thermally stabilized sewage sludge on Technosol, followed by iron grit and plain S-nZVI was the least damaging. On contrary, both groups were unaffected by iron grit and S-nZVI on Fluvisol and S-nZVI with thermally stabilized sludge improved their biomass. The ratio of gram-positive to gram-negative bacterial PLFAs (Fig. S9a, b) was higher in the Fluvisol than in the Technosol and although the value obtained from S-nZVI treated with thermally stabilized sludge was different at the beginning in both localities, no significant differences among treatments were observed after 1 year. Minor differences were also observed in the ratio of fungal to bacterial PLFAs (Fig. S9c, d) on both localities.

The compositions of the microbial communities were characterized using amplicon sequencing analysis. Since the greatest changes in amendments' behavior were observed after 1 week, 6 months and 1 year in other analyses (i.e., Rhizon and 0.01 M $CaCl_2$ extraction, PLFA analysis), samples from these time stamps were selected for further investigation using amplicon sequencing analysis. After quality filtering, denoising, and removal of short and chimeric sequences, the sequences were clustered into 20,730 bacterial operational taxonomic units (OTUs). Taxonomic profiles of the bacterial communities in all treatments are displayed in Fig. 5. The analysis was performed at the genus level and at first glance the effect of the iron grit addition did not cause substantial changes in any of the soils as well as the addition of S-nZVI into the Technosol (Fig. 5a). In the case of the S-nZVI treatment of the Fluvisol (Fig. 5b), the system did tend to return to the original bacterial community within 6 months. Only the sludge addition brought about greater changes in the communities of both soils. Nevertheless, the Technosol bacterial community also restored quickly and only the Fluvisol bacteria remained altered during the whole experiment. Cluster analysis dendrograms documenting the similarities of the bacterial communities in the samples are shown in Fig. S10.

Regardless of the time point or the soil type, the bacterial communities in all the samples except the sludge treatments were dominated by the genera of *Sphingomonas*, *Rhodoplanes*, *Bradyrhizobium* belonging among the class of soil and rather widespread *Alphaproteobacteria*. The genus *Sphingomonas* was found to positively correlate with the presence of Zn, Sb, As, and Cd (Ma et al., 2020). *Rhodoplanes* together with *Bradyrhizobium* belong to the family of Nitrobacteraceae. *Bradyrhizobium* was found to be dominant in a mining soil considering its resistance to As (López-Pérez et al., 2021). The soils in this study differed in a few more dominant genera represented by *Piscinibacter* and *Thermoactinomyces* in the Technosol, and *Arthrobacter* and *Actinoallomurus* in the Fluvisol. The sludge addition had enabled development of *Flavobacterium*, *Pseudomonas*, *Arthrobacter* populations; however, their abundances decreased during the duration of the experiment. *Flavobacterium* as well as *Arthrobacter* were also found in soils contaminated with As, Cd and Zn (Ma et al., 2020) and *Pseudomonas* spp. were found to possess Zn and Cd resistance genes (Puthusseri et al., 2021).

Considering the class level, the soils were dominated by *Alphaproteobacteria* and *Actinomycetes*, that were diversified with *Gammaproteobacteria*, *Betaproteobacteria*, and *Flavobacteriia* after the sludge addition. Many bacterial species can reduce the accumulation and toxicity of toxic metals due to lowering their availability (Kumar et al., 2023). However, based on our microbial analyses, how the communities contributed to this phenomenon is not clear. On the other hand, the present communities exhibited high resilience and resistance towards the added reactive agents as well as the bacterial inoculum coming from the sludge (Technosol). The latter one could be also attributed to the specific conditions in the disturbed soils and certain adaptation of the restoring microflora.

3.3.2. Avoidance response of earthworms

Earthworms are vital part of the soil fauna portraying a valuable indicator of soil health and quality (Sizmur and Hodson, 2009). Thus, the avoidance response of earthworms to tested soils provides simple indicator of soil quality (Xiao et al., 2022). Results of the earthworm avoidance test (Fig. 6) illustrate that there are no significant differences between earthworms' behavior in the Technosol. Avoidance in soil containing the amendment is denoted by a positive value and attraction to the treated soil by a negative value. Although portion of earthworms was attracted to S-nZVI with treated sewage sludge variant to some

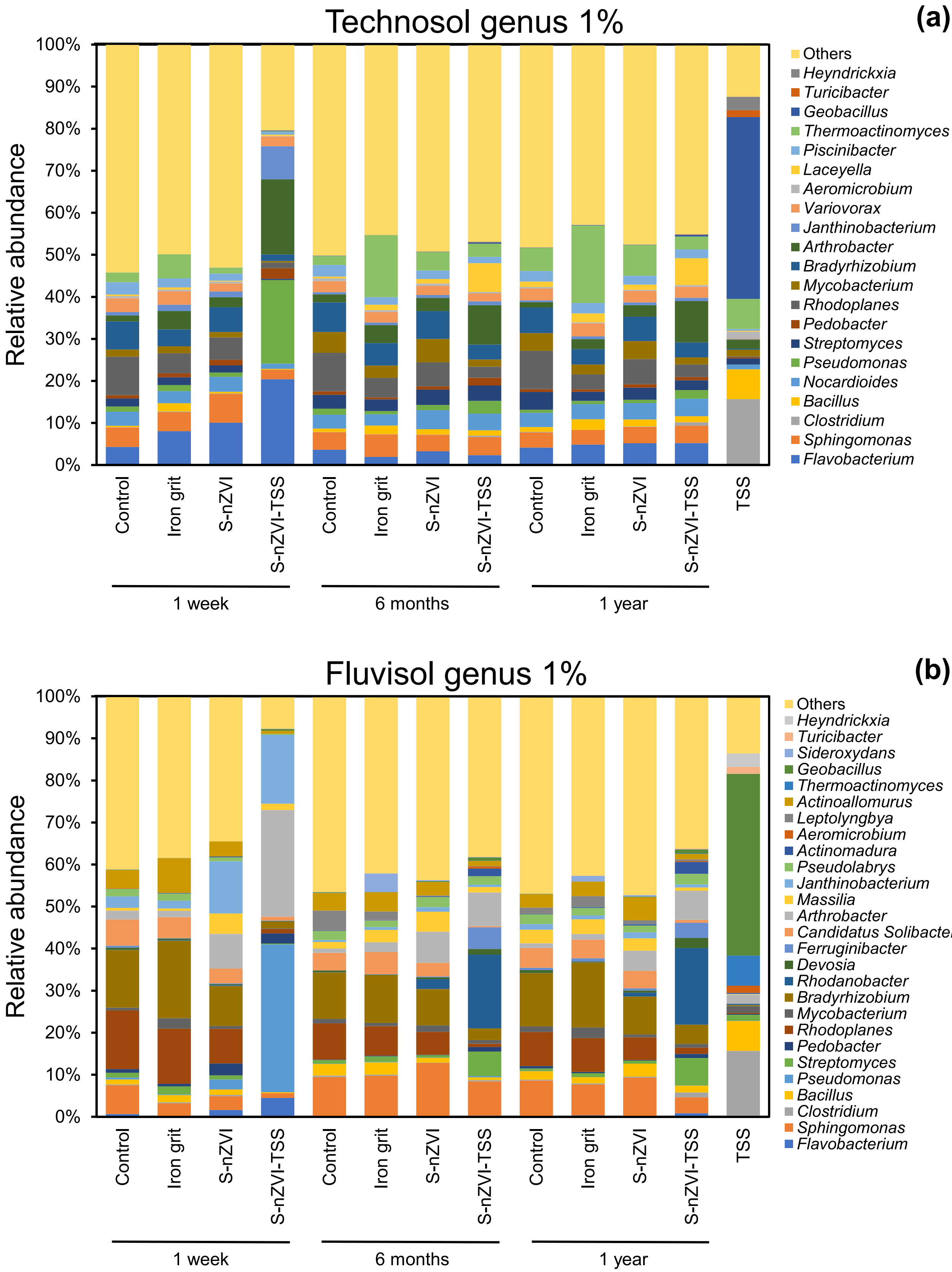

Fig. 5. Taxonomic profiles of the bacterial communities in all treatments of the Technosol (a) and Fluvisol (b). The analysis was performed at the genus level for the non-amended soil (control) and the soil amended with iron grit, S-nZVI and S nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS) 1 week, 6 months, and 1 year after the application. The profile of the applied thermally stabilized sewage sludge (TSS) is presented as well. Others represent genera with abundancy below 1 %.

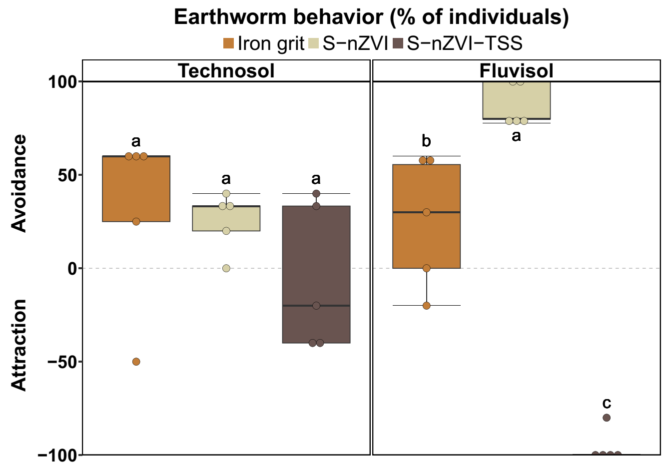

Fig. 6. Attraction/avoidance ($n = 5$) of earthworms to amendments (iron grit, S-nZVI, S-nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS)) in comparison to control (non-amended) from the Technosol and the Fluvisol soils. Letters represent statistical differences ($p < 0.05$) between treatments on each of the localities.

extent, the repulsion towards the amended soils prevails. As such, the earthworms generally preferred to stay in the non-amended soil.

On the contrary, the earthworms' behavior in the Fluvisol was significantly different for various amendments. A strong attraction to the S-nZVI with treated sewage sludge variant was observed. Whereas in the soil treated with S-nZVI the earthworms distinctly preferred the control (non-amended) soil and avoided the S-nZVI-amended soil. As for the iron grit, the earthworms tended to avoid it.

It had been previously observed that the avoidance of earthworms to freshly added nZVI increases with increasing dosage of nZVI, particularly above 500 mg/kg, in response to the oxidative stress caused by exposure to nZVI (El-Temsah and Joner, 2012; Liang et al., 2017). Yet the earthworms in this study were exposed to a soil amended one year beforehand implying that the negative impacts might be longstanding. The avoidance response may be affected by several factors, including pH, electrical conductivity (EC), texture, and organic matter content (Delgadillo et al., 2017). That could explain the different behavior on the two sites. As the Technosol had comparatively higher SOM content, the earthworms appeared to prefer to settle in a control (contaminated) soil away from additional stressor – added Fe, regardless of its form. In contrast, in Fluvisol the earthworms seem to be foraging for organic matter (nourishment), added in the form of thermally stabilized sludge.

In general, the earthworms preferred to remain in the non-amended soil on both localities. The aversion was observed for both iron grit and S-nZVI. Nonetheless, the thermally stabilized sludge considerably improved the environment for the earthworms suggesting thus its positive effect on soil biota.

3.4. Implications for soil amendment with nano vs. micro Fe materials

In the present study, S-nZVI with treated sewage sludge demonstrated generally the best performance as it remained the only effective amendment for stabilization of metal(loid)s in the Fluvisol after one year, as observed from the $CaCl_2$ extraction, where it also improved the environment for soil biota. Interestingly, compared to the other two amendments, increased leaching of Cd and Zn into soil solution was observed (through Rhizons) on plots amended with S-nZVI in combination with thermally treated sewage sludge. On the other hand, no considerable distinctions between performance of S-nZVI and iron grit were observed in Fluvisol. Apart from S-nZVI having the lowest impact on reduction of microbial biomass, no major differences among treatments were observed also in the Technosol, which implies that all amendments on this locality successfully decreased metal(loid) mobility even after one year. Table 2 summarizes our results, showing the influence on different parameters one year after the introduction of amendments into the soil.

Regarding soil properties, all amendments increased the pH of Technosol. This results into increased sorption of cationic metals and/or subsequent (co-)precipitation of metal-bearing phases. On the contrary, all amended plots exhibited lower soil pH compared to control (untreated) plots on Fluvisol. Besides, it should be noted that unlike iron grit, both S-nZVI and S-nZVI with treated sewage sludge remarkably increased electrical conductivity at first. Although after one year, these elevated values practically returned to the base values.

The behavior and potential toxicity of iron-based nanoparticles in soils is influenced by various geochemical and biological factors. For instance, the pH and ionic strengths change the extent of nanomaterial aggregation in the environment, and organic matter, clays, or microorganisms further impact their colloid stability (Lei et al., 2018). The alterations in the reactivity due to environmental specifics were also observed in this study, demonstrating the importance of specific soil and site properties during the remediation process, though firm conclusions should only be drawn after trials on a greater range of soils.

Overall, iron grit showed slightly better performance than S-nZVI. Considering iron grit is a by-product from industrial activities, such as iron melting process, it is a cost-efficient and widely available material. The utilization of iron grit is likely to be considerably less expensive than S-nZVI, especially if widely applied at a whole site scale. However, S-nZVI in combination with thermally treated sludge has proven to be the best amendment in this study. In view of cost-effectiveness and availability, similar to iron grit, sewage sludge is generated in vast quantities and ubiquitously used, after treatment, for land application (Capodaglio, 2023). There are potential negative aspects of sewage sludge application to consider, such as the entrainment and release of metal(loid)s into soils and proximal waters, disruption to microorganism assemblages and function, and introduction of emerging micropollutants associated with sewage sludge. Thus, this material must be used with caution and a comprehensive evaluation of the characteristics of soil and sewage sludge is required prior to land application of sewage sludge in general (Singh and Agrawal, 2008).

Table 2
Overview of effects of applied amendments (iron grit, S-nZVI, and S-nZVI in combination with thermally stabilized sewage sludge (S-nZVI-TSS)) on selected parameters one year after their application to two contrasting soils (Technosol and Fluvisol) compared to control samples (without amendments). The double plus symbol (++) denotes improved and statistically significant ($p < 0.05$) parameter, single plus symbol (+) denotes improved parameter, the double minus sign (– –) stands for deteriorated and statistically significant ($p < 0.05$) parameter, single minus symbol (–) means deteriorated parameter, and zero (0) stands for no effect.

		Technosol			Fluvisol		
		Iron grit	S-nZVI	S-nZVI-TSS	Iron grit	S-nZVI	S-nZVI-TSS
pH		++	++	++	–	–	–
Immobilization (Rhizon-extractability)	As	+	+	+	+	+	+
	Cd	+	+	++	0	0	–
	Pb	++	++	++	++	++	++
	Zn	+	0	+	++	0	– –
Immobilization (0.01 M $CaCl_2$-extractability)	As	+	+	+	–	0	0
	Cd	++	++	++	0	0	++
	Pb	++	++	++	– –	– –	++
	Zn	++	++	++	0	0	++
Earthworm avoidance		–	–	–	–	– –	++
Microbial biomass		– –	–	– –	0	0	+

4. Conclusions

The feasibility of Fe-based materials for the in situ stabilization of metal(loid)s in two contrasting soils severely polluted by past smelting activities was investigated. The application of sulfidized nanoscale zero-valent iron (S-nZVI) did not reduce metal mobility further than micro-scale zero-valent iron (iron grit). In fact, only the addition of thermally treated sewage sludge resulted in any other remedially advantageous effects (e.g., improved environment for earthworms, microbial biomass etc.) in the Fluvisol. Considering our results, and that S-nZVI with thermally treated sewage sludge (S-nZVI-TSS) also decreased the concentration of studied elements in 0.01 M $CaCl_2$ extracts furthest in both soils, it was the only effective treatment for the holistic remediation of the tested soils.

Notwithstanding the impacts of the individual amendments to the individual soils, much of their effect can be attributed to base soil characteristics (e.g., pH, SOM). Therefore, extrapolating the results of this study, it can be said that the choice of an appropriate Fe-based material used for soil remediation should primarily depend on soil properties and site-specific conditions, with the general principle being to neutralize extreme pH, add organic matter and maintain, or enhance, microbial biomass. Further work should now commence to chart the onward trajectory of the effects seen here for the following two years, and also expand to cover a broader range of soils.

CRediT authorship contribution statement

Šárka Lewandowská: Writing – original draft, Visualization, Investigation, Data curation. **Zuzana Vaňková:** Writing – review & editing, Validation, Methodology, Investigation. **Luke Beesley:** Writing – review & editing. **Tomáš Cajthaml:** Writing – review & editing, Methodology, Investigation, Data curation. **Niluka Wickramasinghe:** Investigation. **Jiří Vojar:** Resources, Methodology. **Martina Vítková:** Writing – review & editing, Conceptualization. **Daniel C.W. Tsang:** Writing – review & editing, Methodology. **Kuria Ndungu:** Writing – review & editing, Validation, Methodology. **Michael Komárek:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171892.

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