EFFECT OF INCUBATION TIME AND SOIL MOISTURE CONTENTS ON LEAD SPECIATION IN SEWAGE SLUDGE AMENDED SOIL

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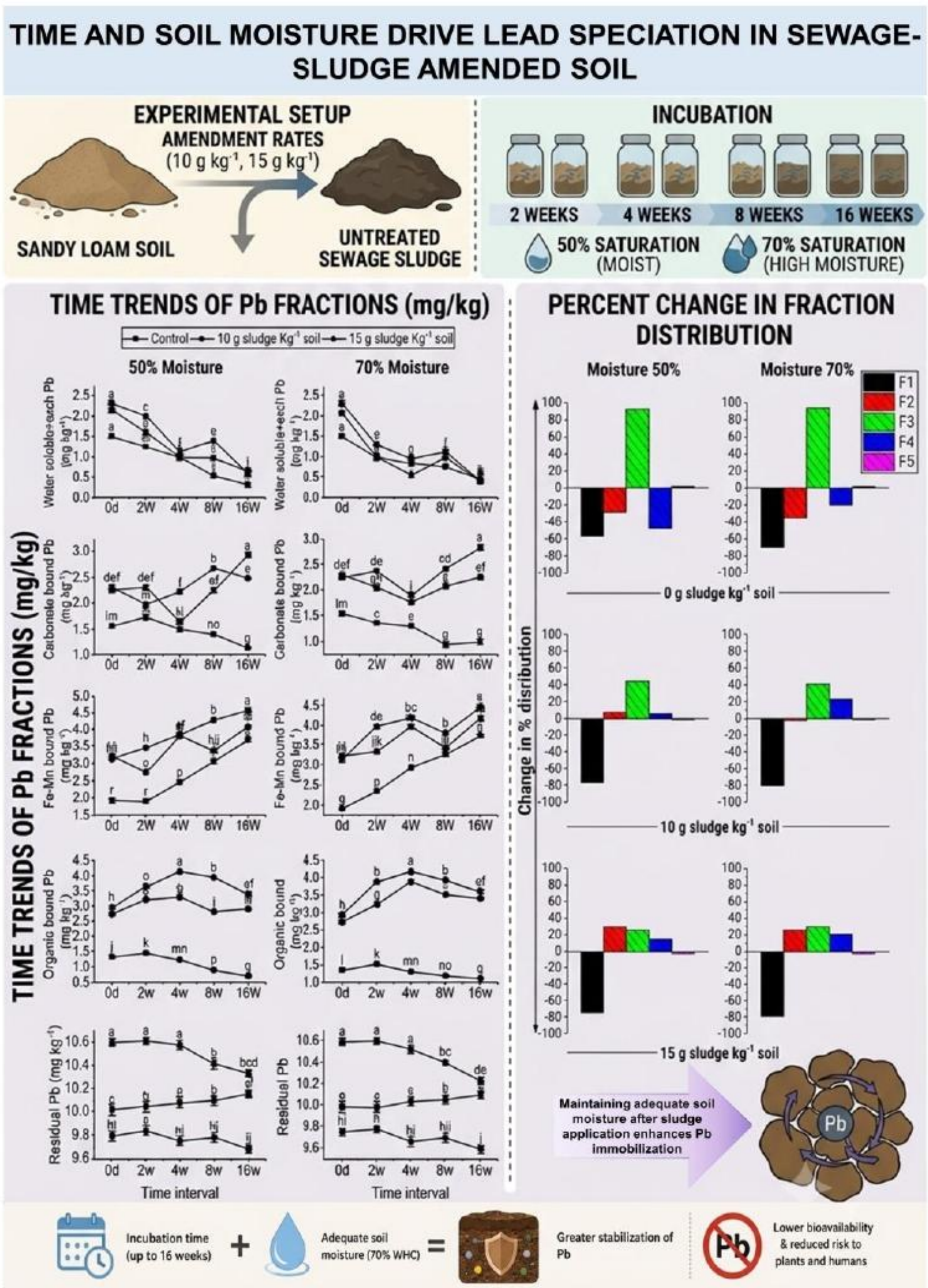
Graphical Abstract



Abstract

The application of sewage sludge to agricultural soils is increasingly considered a sustainable option for waste disposal, nutrient recycling, and soil fertility improvement. However, sewage sludge may also introduce potentially toxic elements (PTEs), particularly lead (Pb), into soil systems, where their environmental risk depends largely on chemical speciation rather than total concentration alone. Lead in sewage sludge-amended soils poses a potential threat to food sustainability through crop uptake and subsequent entry into the human food chain. This study investigated the effects of incubation time and soil moisture on Pb fractionation in sewage sludge–amended soil under controlled experimental conditions. The sandy loam soil, amended with untreated sewage sludge at rates of 10 and 15 g kg^{-1} soil, was incubated for 16 weeks under two moisture conditions (50% and 70% saturation percentage). Soil samples were collected after 2, 4, 8, and 16 weeks and subjected to sequential extraction to determine exchangeable (F1), carbonate-bound (F2), Fe–Mn oxide-bound (F3), organic-bound (F4), and residual (F5) Pb fractions. The application of sewage sludge significantly increased total Pb concentration compared with the unamended control, with higher application rates showing higher levels of Pb accumulation. Across all treatments, Pb was predominantly associated with the F5, followed by F3 and F4. With increasing incubation time, Pb was progressively redistributed from more labile forms, particularly the F1, into less bioavailable fractions such as F3 and F4 pools. Higher soil moisture (70%) accelerated the decline in exchangeable Pb (F1) and enhanced Pb stabilization compared with 50% moisture. In contrast, the F5 remained relatively constant throughout the incubation period. The results indicate that both ageing time and soil moisture conditions regulate Pb transformation in sludge-amended soils. Maintaining high soil moisture levels may promote the transformation of Pb into stable geochemical forms, thereby reducing its mobility and bioavailability, and further mitigating potential environmental risks

Keywords: metals accumulation; soil contamination; Pb speciation; organic amendments; bioavailability; sewage sludge; sequential extraction.

INTRODUCTION

Sewage sludge is a by-product of municipal and industrial wastewater treatment processes. It is widely recognized as a valuable source of organic matter and essential nutrients for soil amendment. As a pre-treatment step for land application, sewage sludge is typically stabilized through processes such as anaerobic digestion, composting, lime stabilization, or thermal drying to reduce pathogen content and improve its operational characteristics [1, 2]. the application of sewage sludge in agriculture has received widespread attention due to its ability to recycle nutrients, improve soil fertility, and serve as an environmentally sustainable disposal method [3]. However, in order to prevent soil pollution and potential environmental risks, the land use of sludge is strictly regulated, especially with clear restrictions on the concentration and cumulative application of heavy metals [4].

Despite its agronomic benefits, sewage sludge often contains a range of potentially toxic elements (PTEs), including cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) [5-7]. The concentration of Pb in sewage sludge vary widely depending on the source, typically ranging from 50 to 300 mg kg^{-1} in treated biosolids [8]. Compared with other PTEs, Pb has attracted much attention due to its persistence in the environment, strong adsorption capacity for soil components, and easy long-term accumulation characteristics [9, 10]. Unlike micronutrients such as Zn and Cu, Pb has no known biological function and is highly toxic even at relatively low concentrations, causing neurotoxicity and damage to important organs in the human body [11, 12].

Long term use of sewage sludge containing high concentrations of Pb may lead to sustained accumulation of Pb in the soil, which can have adverse effects on soil quality, crop safety, and human health. Pb can be absorbed by plants and enriched in edible tissues, thereby entering the food chain. This not only reduces the quality of agricultural products, but may also pose a serious threat to human health and further affect food security and the sustainable development of agriculture. The environmental risks posed by Pb not only depend on its total content, but are also closely related to its chemical or existing forms, as these factors determine the mobility, bioavailability, and reactivity of Pb [13, 14]. Ageing processes play a crucial role in determinin Pb behavior in soil systems. In the initial stage of sludge application, Pb usually combines with easily decomposable organic matter and exists in a more available form; However, over time, Pb will gradually undergo transformation through processes such as adsorption, complexation, precipitation, and diffusion [15].

To better understand the transformation processes of Pb in soil, researchers typically classify it into different defined forms, including exchangeable, carbonate-bound, Fe–Mn oxide-bound, organic matter-bound, and residual forms [16]. Among them, exchangeable Pb has high mobility and bioavailability, making it easier for plants to absorb; However, residual Pb is relatively stable and is usually difficult to release or utilize under natural conditions [16]. The distribution of Pb among its various forms is influenced by different soil physicochemical properties, such as soil pH, ion composition, and cation exchange capacity. At the same time, environmental factors such as soil moisture status and redox conditions also play an important role in it [17-20].

Due to the strong adsorption capacity of oxide minerals, the iron manganese oxide bound state can usually fix a large amount of Pb, while the organic matter bound state indicates that Pb can form relatively stable complexes with organic ligands [14, 21, 22]. However, these associations are dynamic; for example, Pb bound to organic matter may be released under oxidizing conditions due to decomposition processes [22]. Similarly, changes in soil moisture can also affect redox conditions and thereby affect Pb redistribution among fractions [23]. In contrast, residual Pb is mainly fixed within the mineral lattice and is usually more stable in the short to medium term, with relatively low activity [24].

Given the increasing use of sewage sludge in agriculture and the environmental significance of Pb, it is essential to understand its transformation and redistribution in amended soils under varying environmental conditions. Therefore, the present study was designed to (i) evaluate total Pb concentration and its fractionation in soil amended with sewage sludge at different application rates over time, and (ii) assess the influence of moisture levels on Pb transformation and redistribution among different geochemical fractions.

MATERIALS AND METHODS

An incubation pot experiment was conducted in growth room, of Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF). The experiment was designed to study the effects of incubation time and moisture contents on chemical speciation of Pb in sewage sludge amended soil.

Collection and preparation of soil and sewage sludge

Soil in bulk was collected from the top fertile layer (20 cm) of farm area of UAF, air-dried and passed through a 2-mm sieve. After drying, 300 g soil capacity pots covered with parafilm and having 2-3 hole in them for proper gaseous exchange were filled with 200 g of soil per pot. Dried and 2 mm sieved sewage sludge was collected from I-9 Sector, Islamabad Wastewater Treatment Plant, Pakistan. The detailed characteristics of sewage sludge is presented in table 1.

Table 1: Properties of sewage sludge used in experiment.

Parameter	**Unit**	**Value**
pH	-	7.6
Organic matter	%	32
$EC_{1:10\ (\text{sewage sludge : water})}$	dS m^{-1}	3.7
Nitrogen	%	0.95
Phosphorus	%	0.24
Potassium	%	0.27
Total Pb	mg kg^{-1}	88.71
Exchangeable Pb	mg kg^{-1}	4.65
Carbonate bound Pb	mg kg^{-1}	7.46
Fe and Mn oxide bound Pb	mg kg^{-1}	10.67
Organic bound Pb	mg kg^{-1}	22.76
Residual Pb	mg kg^{-1}	40.56

Treatments layout

The incubation experiment was laid-out in completely randomized design with three replications of each treatment and conducted under controlled laboratory conditions. Soil samples were incubated at a constant temperature of 25 ± 2 °C. Soil moisture levels were maintained at the designated treatments (50% and 70% of saturation percentage) throughout the incubation period by periodic weighing and replenishing water losses with deionized water. The samples were incubated in a well-ventilated environment to avoid excessive humidity buildup. Treatments were divided into 2 sets i.e., soil saturation percentage (SP) of 50 and 70%. The controls were un-amended soil with 50 and 70% SP, while the second and third treatments of the set-1 and set-2 were with 50 and 70% SP with 10 and 15 g kg^{-1} application of sewage sludge. Soil moisture treatments were maintained at 50% and 70% SP. Moisture content was controlled gravimetrically by weighing pots at regular intervals and replenishing water losses with deionized water throughout the incubation period. To determine the initial distribution of Pb fractions in various soil components and provide a comparative baseline for subsequent changes, homogenized soil samples (10 grams) were collected from each treatment at the beginning of the experiment (Week 0). Additional samples were collected after 2, 4, 8 and 16 weeks of incubation for measuring Pb concentration in different soil fractions using sequential extraction.

Soil analysis

Soil pH was measured using the portable pH meter (model HM-12P) after calibrating it with buffer solutions of pH 4 and 9. Soil and sewage sludge EC was measured through Lovibond Model SensoDirect Con200 conductivity meter after taking the saturated paste extract through applying positive pressure via filter press. Soluble cation and anions were determined using the methods described by US Salinity Lab. Staff protocols (Table 2).

Table 2. Properties of soil used for pot experiment.

Parameter	Value
Textural class	Sandy loam
Sand %	74.48
Silt %	12.28
Clay %	13.24
Saturation percentage %	29.13
pH_s	7.7

EC_e (dS m^{-1})	2.01
TSS ($mmol_c$ L^{-1})	14.3
Ca^{2+} + Mg^{2+} ($mmol_c$ L^{-1})	13.10
CO_3^{2-} ($mmol_c$ L^{-1})	-
HCO_3^- ($mmol_c$ L^{-1})	2.20
Cl^- ($mmol_c$ L^{-1})	4.5
SO_4^{2-} ($mmol_c$ L^{-1})	13.4
Na^+ ($mmol_c$ L^{-1})	4.12
K^+ ($mmol_c$ L^{-1})	0.6
SAR (mmol L^{-1})$^{1/2}$	1.60
Calcium carbonate %	1.72
Nitrogen %	0.06
Phosphorus (mg kg^{-1})	5.3
CEC ($cmol_c$ kg^{-1})	5.42
OM %	0.68
Exchangeable Ca^{2+} + Mg^{2+} ($cmol_c$ kg^{-1})	3.85
Exchangeable Na^+ ($cmol_c$ kg^{-1})	0.20
Exchangeable K^+($cmol_c$ kg^{-1})	0.25
ESP %	4.65
Total Pb (mg kg^{-1})	17.62
Exchangeable Pb (mg kg^{-1})	1.53
Carbonate bound Pb (mg kg^{-1})	1.57
Fe and Mn oxide bound Pb (mg kg^{-1})	1.95
Organic bound Pb (mg kg^{-1})	1.37
Residual Pb (mg kg^{-1})	10.02

Note: EC: electrical conductivity, TSS: total soluble salts, SAR: sodium adsorption ratio, CEC: cation exchange capacity, OM: organic matter, ESP: exchangeable sodium percentage.

Metals analysis

Lead concentrations were determined using atomic absorption spectrophotometer (Thermo Electron AA, Solar-Series, Waltham, USA) following acid digestion with HNO_3 and HCl (USEPA Method 3050B). Quality assurance and quality control procedures included calibration using standard solutions, analysis of reagent blanks, triplicate sample analysis, and periodic verification of instrument performance during Pb determination. These procedures were used to ensure analytical accuracy and precision.

Sequential Extraction for Pb Fractionation

Sequential fractionation was performed according to the Tessier protocol, yielding exchangeable, carbonate-bound, Fe–Mn oxide-bound, organic-bound, and residual fractions. Pb fractionation in soil was performed according to [25] with modifications. The procedure operationally separates Pb into five fractions (Table 3).

Extraction Recovery Assessment of Pb

The completeness of the sequential extraction procedure was evaluated by comparing the sum of Pb recovered in fractions F1–F5 with the independently measured total Pb concentration. Recovery (%) was calculated as:

$$Recovery\ (\%) = \frac{\mathrm{F1 + F2 + F3 + F4 + F5}}{Total\ Pb} \times 100$$

Recovery values were used to assess the completeness of the sequential extraction procedure.

Table 3. Sequential extraction procedure for Pb fractionation in soils.

Fraction	Notation	Extractant & Conditions
Exchangeable	F1	1 g soil + 8 mL 1 M $MgCl_2$ (pH 7), shaken 1 h at RT, centrifuge (10,000 rpm, 30 min)
Carbonate-bound	F2	Residue + 8 mL 1 M NaOAc (pH 5, HOAc), shaken 6 h at RT centrifuged
Fe–Mn oxide-bound	F3	Residue + 20 mL 0.04 M $NH_2OH{\cdot}HCl$ in 25% HOAc, heate 96 °C for 6 h
Organic-bound	F4	Residue + 3 mL 0.02 M HNO_3 + 5 mL 30% H_2O_2 (pH 2), heate 85 °C (2 h), repeated, then 3 mL 3.2 M NH_4OAc in 20% HNO added, shaken 30 min

Fraction	Notation	Extractant & Conditions
Residual	F5	Residue + 8 mL HNO_3 + 2 mL HCl, near boiling for 45 min

Statistical analyses

The experimental data were analyzed using a three-way analysis of variance (ANOVA) to evaluate the effects of incubation time, sewage sludge application rate, moisture level, and their interactions. Treatment means were compared using the least significant difference (LSD) test at a significance level of $P \leq 0.05$. All statistical analyses were performed using Statistix version 8.1 software. Graphical illustrations were prepared using OriginLab software.

RESULTS

At the start of experiment, exchangeable fraction (F1) remained the same 1.53, 2.17 and 2.34 mg kg^{-1} at both moisture levels i.e., 50 and 70% in un-amended soil (control), 10 g sludge kg^{-1}, and 15 g sludge kg^{-1} respectively. After 2 weeks of incubation, Pb concentration decreased by about 16, 24, and 13% in T_1, T_2 and T_3, respectively with respect to that of day 0 at 50% moisture level. In case of 70 % moisture level, 33, 53 and 43% less Pb concentrations was recorded in T_1, T_2 and T_3, respectively. After 16 weeks of incubation, concentration decreased by 56.20% in un-contaminated soil (T_1) at 50% moisture, and with 70% moisture 69.29% decrease was observed, compared to that of initial concentration. With 10 g sludge kg^{-1} soil, F1 decreased by 77.88% at 50% moisture and by 80.73% at 70% moisture compared to that of initial Pb concentration. At higher sewage sludge level (15 g kg^{-1} soil), 73.50% and 79.05% decrease in Pb concentration was observed at 50% and 70% SP respectively.

The carbonate bound fraction of Pb (F_2) in response to application of sewage sludge at different rates (10 and 15 g sludge kg^{-1} soil) under varying moisture regimes (50 % and 70 % SP) is shown in (Fig. 1). At the start of incubation, the F_2 concentration (mg kg^{-1}) in T1, T2 and T3 was 1.57, 2.33 and 2.27 respectively. In control treatment, at 50 and 70% moisture the concentration of F2 fraction decrease (35. 95 and 38.18 %) after 16 weeks incubation. In treatment T2 (10 g sludge kg^{-1} soil), F2 first decrease and then increase with respect to initial (0 day) concentration. After 16 week, ~7.72 and 1.78% increase was observed at moisture level 50 and 70% respectively. Similarly, with treatment T3 (15 g sludge kg^{-1} soil) ~29.95 and 25.99 % increase was observed at moisture level 50 and 70% respectively after 16-week incubation. As shown in Fig. 1, the F_3 concentration (mg kg^{-1}) was ranged between 1.95 and 3.26 mg kg^{-1}. In control treatment, F3 gradually increase with time at both moisture levels whereas in sludge amended treatments, variable trend was observed. The Pb concentration for F3 at moisture level 50% was 3.72, 4.61 and 4.12 with T1, T2 and T3 respectively after 16 weeks of incubation. At moisture level 70% was 3.76, 4.51 and 4.22 mg kg^{-1} respectively.

The organic bound Pb (F_4) was 1.37 mg kg^{-1} in T_1 and T_4. In soil applied with sewage sludge at 10 g sludge kg^{-1} soil at 50 % SP (T_2) and 70 % SP (T_5), F4 was 2.76 mg kg^{-1}. At level 15 g sludge kg^{-1} soil T_3 and T_6, the Pb concentration was 2.98 mg kg^{-1}. It was observed that the F_4 Pb concentration was increased at both moisture levels in all the treatments after 2 weeks of incubation. The maximum concentration for F_4 was recorded after 4 weeks incubation time. The organic bound fraction (F_4) Pb was 1.26 and 1.31 mg kg^{-1} in T_1 and T_4 after 4 weeks of incubation. With T_2 and T_5, F_4 Pb was 3.32 and 3.92 mg kg^{-1} respectively. The concentration of Pb for F_4 was 4.18 and 4.22 (mg kg^{-1}) with T_3 and T_6, respectively. After 16-week incubation, in control treatments, with moisture 50 and 70%, F4 concentration decreased about 47.44 and 18.97% respectively, with respect to initial (0 day) concentration. On the other hand, in sludge amended treatments, Pb concentration was increased up to 6.15, 14.76, 24.27 and 21.47% with T_2, T_3, T_5 and T_6, respectively. Residual Pb fraction (F5) was little affected with moisture level and time. After 16-week incubation, the Pb concentration for Residual fraction (F_5) with T_1, T_2, T_3, T_4, T_5 and T_6 was 10.16, 9.69, 10.34, 10.1, 9.62 and 10.24 mg kg^{-1} respectively.

Total concentration of Pb in unamended soil (T_1 and T_4) was ranged between 18.32 - 18.59 mg kg^{-1}. The Pb concentration ranged 22.31- 22.57 (mg kg^{-1}) with treatments T_2 and T_5 (soil amended with 10 g sludge per kg soil). Treatments with 15 g sewage sludge per kg soil shows high total Pb contents that was 23.81 to 24.09 (mg kg^{-1}). In sludge-amended soil, Pb concentration was higher than in the control soil, and increased with increasing sludge application rate (Table 4).

Table 4: Effect of moisture, sewage sludge rates and incubation time on total concentration of Pb concentration (mg kg^{-1}).

Moisture level	Sludge rate (g/kg)	Symbol	0-week	2-week	4-week	8-week	16-week
50	**0**	**T1**	18.43±0.05 lm	18.33±0.05 mn	18.55±0.03 k	18.42±0.04 lmn	18.39±0.02 lmn
50	**10**	**T2**	22.40±0.02 ij	22.31±0.03 j	22.45±0.05 hi	22.51±0.03 gh	22.57±0.02 g
50	**15**	**T3**	23.97±0.04 cd	23.81±0.03 f	24.05±0.03 abc	24.07±0.02 ab	23.92±0.03 de
70	**0**	**T4**	18.35±0.05 lmn	18.43±0.05 l	18.59±0.05 k	18.41±0.03 lmn	18.32±0.03 n
70	**10**	**T5**	22.31±0.03 j	22.38±0.05 ij	22.55±0.04 g	22.43±0.04 hi	22.57±0.02 g
70	**15**	**T6**	23.84±0.03 ef	23.97±0.02 cd	23.98±0.03 bcd	24.09±0.03 a	23.93±0.05 de

Values are presented as mean ± standard error (SE, n = 3). Different letters indicate significant differences among treatment combinations (moisture × sludge rate × time) at $p \leq 0.05$.

Changes in Percent Distribution of Pb Fractions

The effect of sewage sludge application on the percent distribution of Pb among different chemical fractions in soil at two moisture regimes (50% and 70% of water holding capacity) after incubation is presented in Fig. 2. Distinct shifts in Pb partitioning were observed depending on both sludge amendment rate and soil moisture level.

The exchangeable fraction (F1) of Pb decreased across all treatments, with a more pronounced reduction under 70% moisture compared to 50%. Among the sludge-amended soils, the decline was most substantial in the treatment receiving 10 g sludge kg^{-1} soil, suggesting greater immobilization of Pb in less labile forms.

The Carbonate bound fraction (F2) decrease in control treatment whereas increased in sludge amended treatments. The decrease in this fraction was more at 70% moisture level than 50%. The magnitude of increase in this fraction was more at 50% moisture with 15 g sludge per kg soil in comparison to 70% moisture level.

The Fe-Mn oxide bound fraction (F3) increased in all the treatments and the magnitude of increase was more in control as compared to other sludge amended treatments. This suggests that in the absence of organic inputs, Pb tends to stabilize preferentially in Fe–Mn oxide forms.

The organically bound Pb fraction (F4) decreased in the case of control and the magnitude of decrease was more under 50% moisture than with 70% moisture. In the case of sludge amended treatments, this fraction increased, and the magnitude of increase was higher at 70% moisture than 50%.

The residual fraction (F5) showed only minor changes overall. In control soils, a slight increase was observed, especially at 50% moisture. Conversely, in sludge-amended treatments, a modest decline was recorded, particularly under 70% moisture.

Overall, after 16 weeks of incubation, Pb in sludge-amended soils was progressively redistributed from the F1 into the less labile carbonate-bound F2, Fe–Mn oxide-bound F3, and organic-bound F4 fractions. The residual fraction F5 remained relatively stable throughout the incubation period, indicating that it was minimally influenced by soil moisture.

Extraction Recovery of Pb

The recovery of Pb based on the sum of sequential extraction fractions (F1–F5) relative to total Pb concentration ranged from 88.14 to 90.72%, with an average recovery of approximately 89.5%. This result indicates that the sequential extraction process has acceptable completeness and good analytical reliability of the sequential extraction procedure.

DISCUSSION

Redistribution of Pb among geochemical fractions

The sequential extraction results showed that after the application of sewage sludge, Pb in the soil gradually transformed from a more available form to a more stable form over time, and this trend was basically consistent under two moisture conditions of 50% and 70% SP. However, under higher moisture content conditions, the process of Pb transformation and redistribution is more rapid, indicating that although soil moisture does not change the overall trend of Pb aging, it will significantly affect its transformation rate [26, 27].

The exchangeable fraction (F1) Pb significantly decreased with time under all treatments, reaching a decrease of 56-80% after 16 weeks, and the decrease was greater under 70% SP than 50% SP. This indicates that higher soil moisture content may be more conducive to the transformation of Pb from a labile form to a stable form, promoting its fixation in the soil. The reason may be that higher water content enhances the wetting effect on the surface of soil particles, making it easier for Pb ions to come into contact with adsorption sites in the soil and be fixed. Although this study did not monitor the soil pH during the incubation period, previous studies have suggested that a wetter environment may alter soil pH and increase the negative charge on the colloid surface by affecting soil buffering capacity and related biogeochemical processes, thereby further promoting Pb adsorption [28, 29]. However, this mechanism remains theoretical without direct measurements. The study [30] found that amendments that enhance surface reactivity can usually reduce exchangeable Pb across diverse systems, supporting the general pattern observed here.

The carbonate bound Pb (F2) showed different trends of change: in the control soil, its content decreased, while in the treatment group treated with sewage sludge, it showed an increasing trend, especially under the treatment of 15 g kg $^{-1}$, with an increase of 26-30% after 16 weeks. This difference may be related to the organic matter and dissolved inorganic carbon in sewage sludge promoting the formation of Pb carbonate complexes. F2 exhibits a non monotonic pattern of first decreasing and then increasing over time, which is inconsistent with the irregular fluctuations observed in Pb added soil [26]. The reason for this difference may be that microbial respiration in the soil treated with sewage sludge continuously produces carbonates, while this process does not exist in the nitrate addition system. Similar degrees of F2 changes have also been reported by [31] , and it is believed to be related to the dynamic changes in soil chemical properties under different moisture conditions and the limitations of carbonate precipitation kinetics.

The Fe–Mn oxide-bound fraction (F3) increased progressively in all treatments, which is consistent with characteristics of Pb that is affinity for hydrous Fe and Mn oxides [32]. Compared to the treatment with sewage sludge, the increase of F3 in the control soil was more significant, indicating that the occupation of oxide surfaces by organic ligands competes with Pb adsorption. The competitive adsorption phenomenon involving organic matter has been confirmed in multiple studies on soil organic-amendments [33, 34].

The organic-bound Pb fraction (F4) showed a decreasing trend (18-47%) in some control soils, which may be related to the mineralization and decomposition of the original organic matter in the soil; In some soils treated with sewage sludge, F4 increased by 6-24% and reached its highest value after 4 weeks of incubation, then stabilized. This indicates that the initial complexation between Pb and active organic components occurs rapidly, while the further transformation towards more stable forms is relatively slow. Under 70% SP conditions, the F4 content is relatively high, indicating that the wetting effect of water may be the main factor promoting the formation of metal organic complexes, which may be related to the enhanced microbial activity under higher moisture conditions [35]. Since the oxidation-reduction potential was not measured in this study, the inference about the reduction conditions is mainly based on existing literature.

The residual Pb fraction (F5) showed little change throughout the entire incubation period, which is consistent with its stable form fixed in mineral structures on geological timescales [31, 36].

Role of soil moisture in Pb transformation kinetics

Soil moisture has a significant impact on Pb transformation rates, with Pb redistribution significantly faster under 70% SP than under 50% SP. This difference may be related to the following three aspects. Firstly, higher moisture content can more fully wet soil micropores, thereby increasing the contact opportunity between Pb ions and soil reaction surfaces, and enhancing their interaction with active sites [37].

Secondly, under 70% SP conditions, higher soil moisture content can sustain more active microbial activity and promote organic matter decomposition, thereby enhancing Pb fixation through organic ligands produced by microorganisms. In this study, the increase in F4 content under 70% SP treatment further indicates that the organic matter-mediated Pb fixation process may dominate under this condition. However, it is not absolutely stable. During the late stage of crop growth or seasonal drying, if the soil is transformed back into an aerobic environment, Pb, which was originally bound to organic matter, may still be released again. Previous studies have shown that under oxidative conditions, mineralization of organic matter can lead to the re-entry of bound Pb into the soil environment [33]. This study also revealed that F4 tended to stabilize after the 4^{th} week, indicating that some Pb may have been transformed into relatively stable organic complex states. However, since the ratio of labile and stable organic carbon has not been analyzed in this study, further research is still needed on this process.

Third, moisture influences Fe and Mn oxide stability. Under high moisture conditions, a reducing environment may gradually develop in the soil. Research in paddy soil has shown that changes in redox conditions can affect the solubility of Cd through the dissolution and reprecipitation processes of Fe oxides. Although this study mainly focuses on Cd, the redox mechanisms involved can also provide reference for understanding the behavioral changes of Pb in soil. Results showed increasing trend of F3 under 70% SP conditions, indicating the immobilization effect of Pb may outweigh the impact of Fe-Mn oxide instability. However, this inference still needs to be verified by directly measuring the redox potential.

Although pH, Eh, and dissolved organic carbon were not directly measured during the incubation period, the results still provide important references for understanding the transformation behavior of Pb in soil improved by sewage sludge. However, if future research can combine continuous monitoring of these parameters and further analyze microbial activity and organic carbon components, it will help to reveal more deeply the underlying mechanisms of Pb redistribution and its transformation process.

Conclusion

This study demonstrated that Pb in sewage sludge-treated soil, gradually transforms from a labile form to a more stable geochemical form during short-term incubation, and soil moisture has a significant impact on this transformation process and its rate. At higher water content (70% SP), Pb transforms more rapidly into relatively stable forms such as carbonate bound fraction, Fe-Mn oxide bound fraction, and organic matter bound fraction, but its overall transformation trend is consistent with the results observed at lower water content (50% SP). This change may be related to enhanced soil surface interactions, active microbial activity, and the impact of changes in redox conditions on oxide stability. However, in order to more accurately elucidate these mechanisms, direct measurements of pH, redox potential (Eh), and dissolved organic carbon are still needed.

From a practical application perspective, in the early stage of sewage sludge application, reasonable irrigation or short-term maintenance of high soil moisture content may help reduce the bioavailability of Pb, thereby reducing its accumulation risk in plants. This is particularly important for ensuring food security, as the decrease in Pb availability in soil can limit crop uptake of Pb, thereby reducing its likelihood of entering the food chain and reducing potential harm to human health. However, as this study is only based on short-term incubation results of 16 weeks, further research is needed on the long-term stability of Pb under different environmental conditions and the possibility of reactivation. In the future, in order to improve the accuracy of environmental risk assessment and promote sustainable management of sewage sludge, it is recommended to strengthen the analysis and monitoring of its different chemical forms (species) while monitoring the total amount of metals.

Author Contributions

Conceptualization, methodology, software, formal analysis, investigation, resources, data curation, visualization, and writing—original draft preparation: A.A.Q.; Supervision and writing—review and editing: G.M.; Data analysis and writing—review and editing: Z.U.R.F.; Writing—review and editing: U.R.; Writing—review and editing: P.I.

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All data supporting the findings of this study are included within the manuscript. Additional supporting data are available from the corresponding author upon reasonable request.

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AI Declaration:

During the preparation of this work the authors used (ChatGPT, https://chatgpt.com) in order to improve the English language of this manuscript as well as taking ideas for the figures drawn. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

List of Abbreviations

AI	Artificial Intelligence
ANOVA	Analysis of Variance
CEC	Cation Exchange Capacity
CRMs	Certified Reference Materials
DOC	Dissolved Organic Carbon
EC	Electrical Conductivity
Eh	Redox Potential
ESP	Exchangeable Sodium Percentage
F1	Exchangeable Fraction
F2	Carbonate-bound Fraction
F3	Fe–Mn Oxide-bound Fraction
F4	Organic Matter-bound Fraction
F5	Residual Fraction
LSD	Least Significant Difference
OM	Organic Matter
Pb	Lead
PTEs	Potentially Toxic Elements
RT	Room Temperature
SAR	Sodium Adsorption Ratio
SE	Standard Error
SP	Saturation Percentage
T1–T6	Treatment Codes
TSS	Total Soluble Salts
U.S. EPA	United States Environmental Protection Agency

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