ORIGINAL ARTICLE

Adsorption of Heavy Metals from Soil Contaminated with Sewage Sludge by Graphene Oxide

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ABSTRACT: Pollution caused by heavy metals, due to their toxicity and harmful effects on plant species, is considered one of the fundamental problems. The unique properties of graphene oxide (GO) in adsorbing heavy metals from contaminated soil systems have garnered attention in the past decade. This study evaluates the effects of different doses of graphene oxide (0.5 and 1.5 g kg^{-1}) on the adsorption and distribution of heavy metals, including Cadmium (Cd) and Copper (Cu) fractions, in soil contaminated with two rates (2% and 10%) of sewage sludge (SS) over a 92-day period. Subsamples were collected from the soils at intervals of 5, 15, 29, 57, and 92 days, air-dried, and subjected to metal fractionation. The aim was to evaluate the separation of Cd and Cu by employing a five-stage sequential extraction technique, where each stage involved 2 grams of air-dried soil as a sample. The overall metal quantity in the treatments was determined by utilizing aqua regia digestion. The recovery rate was determined by calculating the percentage of the total metal concentration obtained from acid digestion, involving the summation of the concentrations of the five metal fractions. The results showed that the F1 fraction of heavy metals in the soil with a 2% SS rate decreased with an increase in GO dosage. The calculated mobility factor of the metals at five incubation times (5, 15, 29, 57, and 92 days) was found to be in the range of 28.1-60.9 for Cd and 14.4-25.3 for Cu, indicating a higher mobility of Cd. The findings suggest that the presence of GO as an adsorbent and the incubation time were critical parameters in stabilizing heavy metals in the soil with different rates of SS. Additionally, the nature of the applied SS was found to influence the fractionation of heavy metals in the soil, besides providing elements.

INTRODUCTION

Presently, farmers are increasingly employing sewage sludge (SS) in agriculture, particularly in arid and semi-arid cropping systems, to sustain productivity and augment crop yields [1]. The utilization of SS enhances specific physical and chemical soil properties such as cation exchange capacity (CEC), water retention, aeration, and porosity [2]. However, detrimental components like heavy metals and pathogens cannot be disregarded [1]. Heavy metal contamination poses a significant environmental hazard due to its persistent toxicity, carcinogenic or mutagenic effects, and capacity for bioaccumulation, even at minute concentrations [3]. Extracting and reclaiming heavy metals is crucial from environmental and financial perspectives [4]. Several techniques are available for cleaning soils contaminated with metals, ranging from conventional methods like

landfilling and soil washing to more eco-friendly options like phytoremediation and immobilization [5]. Moreover, their harmful effects can be reduced by adding soil enhancements such as iron ions and limestone [6]. However, the most efficient environmental approach to reduce the presence of metals in soil revolves around immobilizing them with high-capacity materials. These stabilizers primarily target transforming soil metals into extremely stable chemical phases through processes such as surface adsorption, deposition, and complex formation [2]. The movement and harmful effects of metals in polluted soils typically depend on their distribution [7], which can be evaluated using sequential extraction methods.

The movement and harmful impact of metals in polluted soils typically depend on how they are spread [7], a factor that can be evaluated through consecutive extractions [8, 9]. Among the methods for removing pollutants, the adsorption technique has gained widespread usage due to its heightened efficacy, reduced cost, and ease of operation. Adsorption denotes a surface phenomenon in which pollutant molecules adhere to the adsorbent surface through intermolecular forces [10]. Surface adsorption occurs through two mechanisms: physical surface adsorption, a reversible process occurring at or below the boiling point of the adsorbed material, exhibiting a high adsorption rate without specificity to particular sites, enabling multi-layer adsorption on all adsorbent surfaces; and chemical surface adsorption, involving electron exchange between adsorbent surface sites and adsorbed molecules, leading to irreversible bonding at temperatures exceeding the boiling point of the adsorbed material [11].

An effective adsorbent should boast high adsorption capacity and rapid surface adsorption rate, coupled with a substantial specific surface area and plentiful surface adsorption sites [12]. Synthesized nanoparticles have been increasingly employed as adsorbents for pollutant removal in recent decades. Graphene, among these nanomaterials, has garnered considerable scientific attention. It is a novel two-dimensional nanoparticle comprising pure carbon atoms, constituting a two-dimensional carbon allotrope. Graphene's distinctive features encompass high density, chemical inertness, elevated thermal conductivity, optical transparency, and

ultra-hydrophobicity at the nanoscale, with a carbon-carbon bond length of approximately 0.142 nm [13]. Each carbon atom on a graphene plate is bonded to six other carbon atoms, forming a network of regular hexagons in a graphene plane. Additionally, each carbon atom possesses a free bond off the plate, suitable for various functional groups or hydrogen atoms [14]. Recent extensive research has focused on the utilization of new-generation adsorbents like graphene owing to its substantial cross-sectional area. Graphene, with a cross-section of 2630 m^2 g^{-1}, has been acknowledged as a high-performance adsorbent. However, a major limitation of high-level nanoparticles is their tendency to agglomerate, significantly reducing their efficiency. The presence of chemical groups on graphene nanoparticle surfaces impedes their aggregation, promoting better dispersion in the polymeric field. Graphene suspensions can comprise up to five layers, termed graphene with a low number of layers [15].

Currently, multiple research studies have explored how graphene oxide behaves in the environment and its impacts, either in controlled settings or on land. This focus persists despite widespread soil contamination found in different parts of the world, notably in China. Around 3.3 million hectares of agricultural land have faced significant pollution due to both organic and inorganic substances used in farming practices [16].

In a study observing the impact of graphene oxide on soil microbial populations, researchers noted a reduction in enzymatic activity upon introducing graphene oxide into the soil over a brief period. However, the microbial community's mass remained unaffected and gradually improved over 59 days [17]. Generally, after 90 days of graphene oxide interaction, bacterial performance and population diversity were selectively enhanced [18]. Additional studies have shown that graphene oxide is efficient in soaking up and removing heavy metals from the soil by using surface complexation and electrostatic reactions [19, 20]. Nonetheless, the interaction between heavy metals and graphene oxide may have a limited impact on bacterial populations in already metal-contaminated soils [21, 22]. In one study, researchers discovered that electrolysis-prepared graphene oxide displayed a high affinity for absorbing lead and cadmium [23]. Furthermore, graphene oxide exhibited effective

adsorption for removing arsenic from soil [24]. This study aims to explore the influence of graphene oxide nano-plates on the adsorption of heavy metals, such as cadmium and copper, from unsaturated porous soil media."

MATERIALS AND METHODS

Graphene oxide

The Hummers method was used to synthesize graphene oxide (GO). The process involved mixing 1 gram of graphite with 200 milliliters of sulfuric acid at 66°C until the graphite was soaked in the solution. The mixture was then cooled to 0°C, and 9 grams of potassium permanganate were added slowly, causing the solution to turn green. After stirring for 24 hours, approximately 200 milliliters of deionized water were added slowly, changing the color to brown. The oxidation process was stopped by adding 35 milliliters of H_2O_2, and the solution was stirred for an additional half-hour. The resulting compound was highly corrosive. To wash the solution, a 9:1 mixture of hydrochloric acid and water was used in a centrifuge at 4000 rpm. The plates of graphene oxide were then diluted in deionized water and stirred for two minutes to obtain graphene oxide monolayers [25]. All utilized chemicals and reagents were of analytical quality, and solutions were made with deionized water. Spectroscopic assessments were conducted to examine the physical properties of the GO sample. The X-ray diffraction (XRD) pattern was assessed using a STADIP diffractometer (X'Pert, Germany) to identify mineral phases in the SS sample. The scanning electron microscope (SEM, Tescan, Czech Republic) was employed to analyze the structure and dimensions of the solid phase surface of the GO sample. Prior to examination, the GO sample was evenly dispersed on adhesive tape, coated with a thin layer of gold, and exposed to an electron acceleration voltage of 30 kV. These examinations were conducted at the laboratories of Lorestan University in Khorramabad, Iran.

Preparation and analysis of soil and sewage sludge samples

A study was conducted in which surface soil samples (0-30 cm) of 20 points were collected in the summer season from the research farm at Agriculture College, Lorestan University located in Khorramabad, Iran. Standard methods [26]. were used to measure the physico-chemical properties of the soil after air-drying and sieving, as presented in Table 1.

Table 1. The physicochemical properties in the used soil.

Characteristics	Value
pH	6.9
Electrical conductivity (EC), ds m^{-1}	0.81
Organic matter, %	0.73
Total Nitrogen, %	0.19
Soil texture	clay loam
FC (%)	32.37
Permanent Wilting Point (PWP) (%)	10.95
CaCO$_3$ (%)	18.36
Cation exchange capacity (CEC), C$_{mol}$ kg^{-1}	26.48
P, ppm	3.26
K, ppm	401
Fe, ppm	3.6
Zn, ppm	1.5
Mn, ppm	13

The SS sample, originating from Boroujerd city's wastewater treatment plant, was gathered in February 2020 and air-dried through continuous mixing. After drying, the sample underwent filtration using a 2 mm sieve to eliminate stones, roots, plastic, and other debris. Table 2 provides a comprehensive breakdown of the SS

sample's chemical properties and concentrations of heavy metals. pH and EC values were determined using specific pH and EC meters. The total concentrations of heavy metals, particularly Cd and Cu, were evaluated using Atomic Absorption Spectrophotometry (AAS, Agilent, and 240FS AA). Total nitrogen concentration was assessed following the Kjeldahl digestion method established by a previous researcher [27]. The determination of total organic carbon concentration followed the Walkley and Black method [28]. Identification of amorphous Fe (Fe-Am), crystal Fe (Fe-Cr), amorphous Al (Al-Am), and crystal Al (Al-Cr) was carried out using the Mehra and Jackson method [29].

Table 2. Chemical properties and heavy metal concentration in the sewage sludge sample

Characteristics	value
pH	7.51
Electrical conductivity (EC), ds m^{-1}	7.46
C:N	4.16
Total Nitrogen, %	1.47
Fe, ppm	4381
Cd, ppm	17
Pb, ppm	105
Na, ppm	249
K, ppm	214
Mn, ppm	338
Cu, ppm	311
Zn, ppm	398

Experimental design

The soil samples collected were divided into 9 experimental pots, each containing 1000 g of soil. Duplicate samples were obtained for each pot. The soil was mixed with SS samples at rates of 2% (2 g SS in 100 g soil) and 10% (10 g SS in 100 g soil), respectively. Two different concentrations of graphene oxide as an adsorbent (0.5 and 1.5 g kg^{-1} dry weight soil) were added to the soil-SS mixtures and mixed thoroughly until completely homogeneous. The pH of the soil mixture was adjusted to 3, which is the optimum pH for adsorbing heavy metals, based on the results of previous research [30]. A control experiment was designed with soil only, without any SS samples or graphene oxide. In total, 51 treatments were placed in a greenhouse at a constant temperature of 25°C and a moisture level of 30%, with deionized water added to the soil every week, during the 92-day experiment. After 5, 15, 29, 57, and 92 days, subsamples were taken from all soils and air-dried before metal fractionation.

Fractionation of heavy metals

To evaluate how Cd and Cu were distributed in the treatments, a sequential extraction method consisting of five steps was employed using 2 grams of soil that had been air-dried as a subsample. The specific procedure for fractionation and the conditions for extraction are outlined in detail in Table 3. The metal levels in each fraction were measured using Atomic Absorption Spectrophotometry (AAS, Agilent, 240FS AA). The total metal content in the treatments was analyzed using aqua regia digestion. The recovery rate was computed as the percentage of the total metal concentration acquired from acid digestion, involving the summation of concentrations from the five metal fractions [31].

Table 3. Procedure for extracting heavy metals from soil [32].

| Fraction	Reagent	Condition
Easily soluble and exchangeable	20 mL 1 M MgCl$_2$ (pH 7.0)	0.5 h shake
Bound to carbonates	20 mL 1 M NaOAc adjusted to pH 5 with HOAc	0.5 h shake
Fe–Mn oxide bound	20 mL 0.04 M NH$_2$OH HCl in 25% CH$_3$COOH	6 h in shacking bath in 60°C
Bound to organic matter and sulphides	14 mL 8.8 M H$_2$O$_2$ in 0.02 M HNO$_3$ 6mL 3.2 M CH$_3$COONH$_4$ in 25% HNO$_3$	5 h in shacking bath at 85oC 0.5 h shake
Residual	Digestion with 10 mL of aqua regia	1 h shake, 22 h left followed by1 h shake

Statistical analysis

The data was subjected to analysis of variance (ANOVA), and for comparing means, the Duncan test (with a significance level of $P<0.05$) was utilized through the SPSS software (version 20.0). The absorption capacity and removal percentages were calculated using Microsoft Excel 2016. To draw diagrams and determine the degree of conformity with the isothermal equations of absorption, Sigma Plot software (version 12.0, USA) was used.

RESULTS AND DISCUSSION

Morphology and characterization of GO

To investigate the physical morphology of the GO surface SEM study was done in this research. The SEM image of GO is presented in Figure 1. Pores of different size and shape were observed in surface of samples.

The XRD graph in Figure 2 displays the pattern of graphene oxide. At 25.84°, distinct diffraction peaks of the GO samples were identified. These findings suggest that the introduction of oxygen-containing functional groups into the layers caused an expansion in the interlayer spacing, leading to the disruption of the natural graphite sample's original crystals [33].
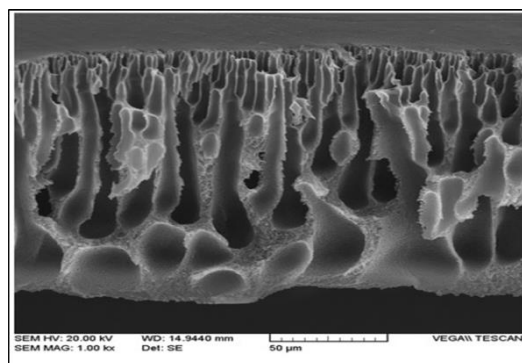
Figure 1. The scanning electron microscope (SEM) spectra of the graphene oxide Nano- plates.
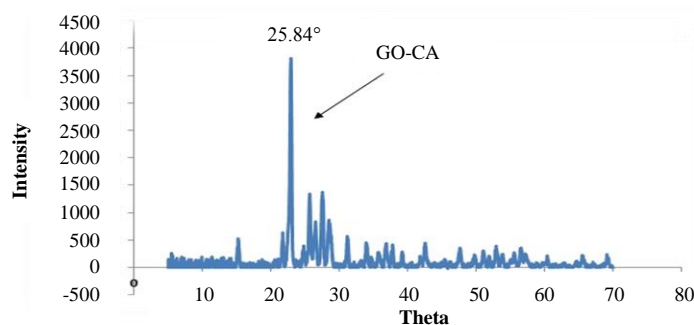

Figure 2. The X-ray diffraction (XRD) of the graphene oxide

Heavy metal fraction analysis at initial time

Figure 3 illustrates the fractions of Cd and Cu in soil with varying rates of SS and doses of GO after 5 days of incubation. In the soil featuring a 2% SS rate and 3% GO dose, Cd predominantly existed in F1 at a concentration of 0.6 mg kg^{-1}. Increasing the GO dose resulted in a reduction of F1 and an elevation of F5, signaling a decrease in Cd's availability and toxicity [34]. Notably, the maximum adsorption capacity of GO for Cd in an aqueous solution is approximately 106.3 mg g^{-1} [35], suggesting its potential to immobilize the free fraction of Cd through surface complexation and electrostatic interaction, subsequently reducing its solubility and bioavailability [36, 37]. However, factors like microorganisms, natural organic matter, and soil texture might also influence Cd sorption [38]. Cd can bind to various soil constituents, such as iron oxides, organic matter, clay minerals, or microorganisms, through surface adsorption, exchange reactions, and complexation. Chelation processes can transform highly soluble Cd forms into more stable ones [22]. Copper, on the other hand, was predominantly detected in F5, ranging from 6 to 7.8 mg kg^{-1} in soils with 2% and 10% SS rates. These findings highlight that the efficiency of acid digestion in recovering heavy metals from SS depends on the specific heavy metal and SS type. Consistently, other researchers have reported recoveries within the range of 116-309% for Cd and 83-97% for Cu [39, 40], indicating variability in the extractability of heavy metals in SS likely due to diverse chemical and physical properties [40].
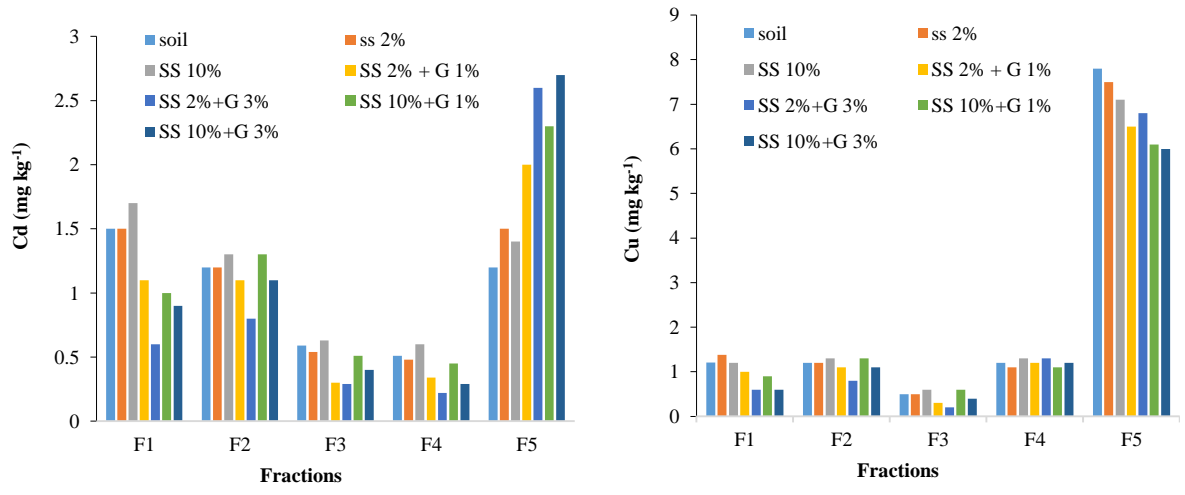
Figure 3. Separation of Cd and Cu in soil samples treated with varying sewage sludge rates and Graphene oxide amounts after a 5-day incubation period. 'SS' and 'G' represent the different sewage sludge and Graphene oxide, respectively, used in the soil.
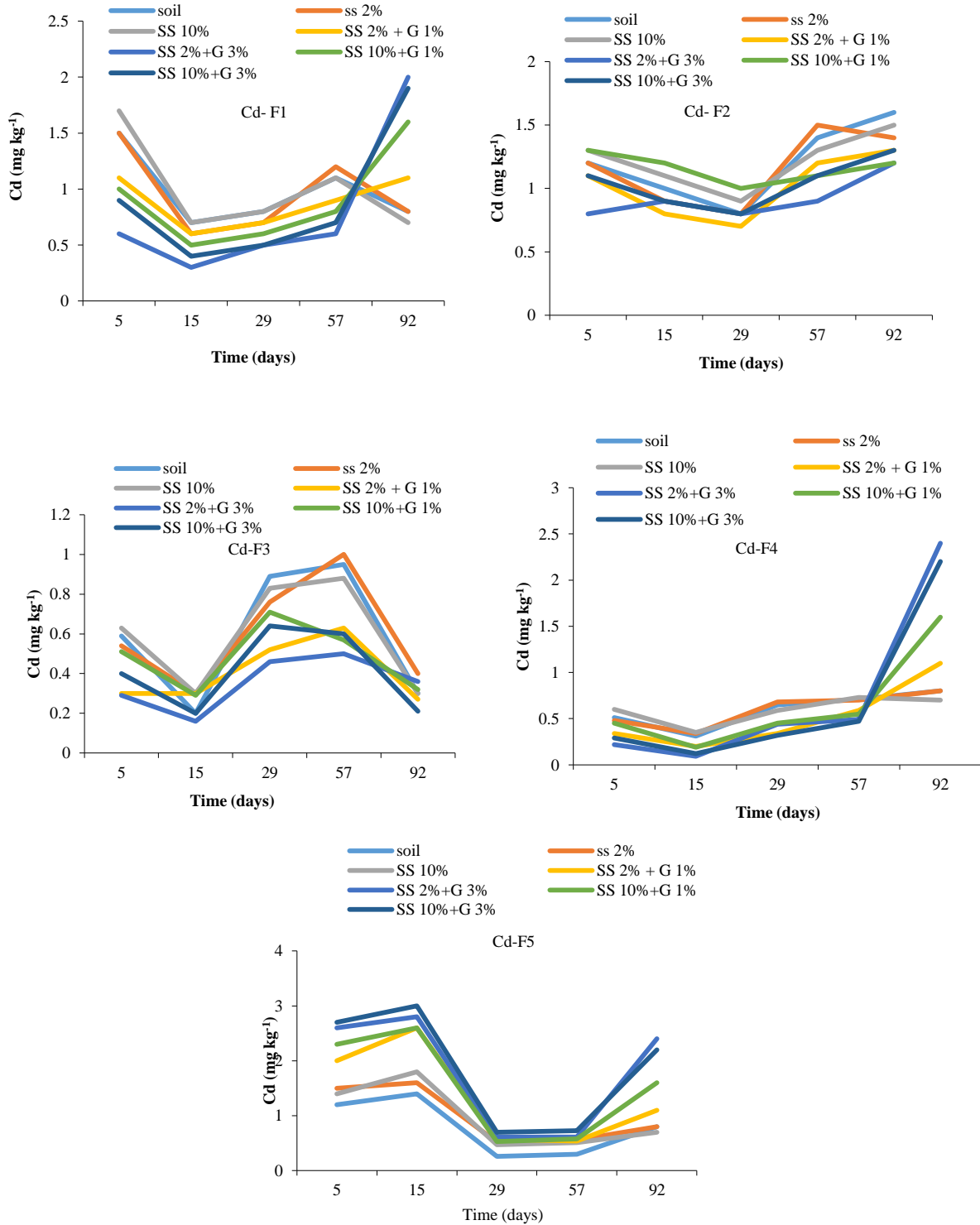
Heavy metal fraction throughout the incubation time

Figures 4 and 5 display the alterations in Cd and Cu distribution in the soil over time with varying levels of sewage sludge (SS) and doses of graphene oxide (GO). Figure 4 illustrates an increase in Cd concentration in fractions F2 and F4, along with a decrease over a span of 92 days in soil subjected to different SS rates and in the control soil. Notably, a significant rise in Cd concentration in F4 was observed during incubation. Regarding Cu fractionation (Figure 5), there was an increase in F3 and a simultaneous decrease in F2 between days 5 and 15 of incubation. Table 4 exhibits notable differences in the average distribution of metal fractions during different incubation times for soils treated with varying SS rates and GO values. At time 1 (5 days) and time 5 (92 days), the dominant fraction for both Cd and Cu was F5. The application of SS was found to enhance Cd availability, potentially displaying distinct behaviors in retaining or releasing specific elements in the soil. A boost in available metal fractions was evident, particularly in the short term following SS application, aligning with earlier research on SS-amended soils [41, 42]. However, the presence of adsorbents decreased

metal availability. Overall, incubation duration significantly influenced heavy metal fractions in soils treated with different SS rates and GO values. The initial 15 days notably impacted Cd and Cu fractionation. Cd availability declined within the first two weeks of incubation, followed by an increase. The environmental implications of such fluctuations warrant further investigation [9].

Figure 4. Changes in Cd fractions in the soil with different rates of sewage sludge samples and doses of Graphene oxide throughout the incubation time. Letter SS and G indicate the soil with different sewage sludge and Graphene oxide respectively.
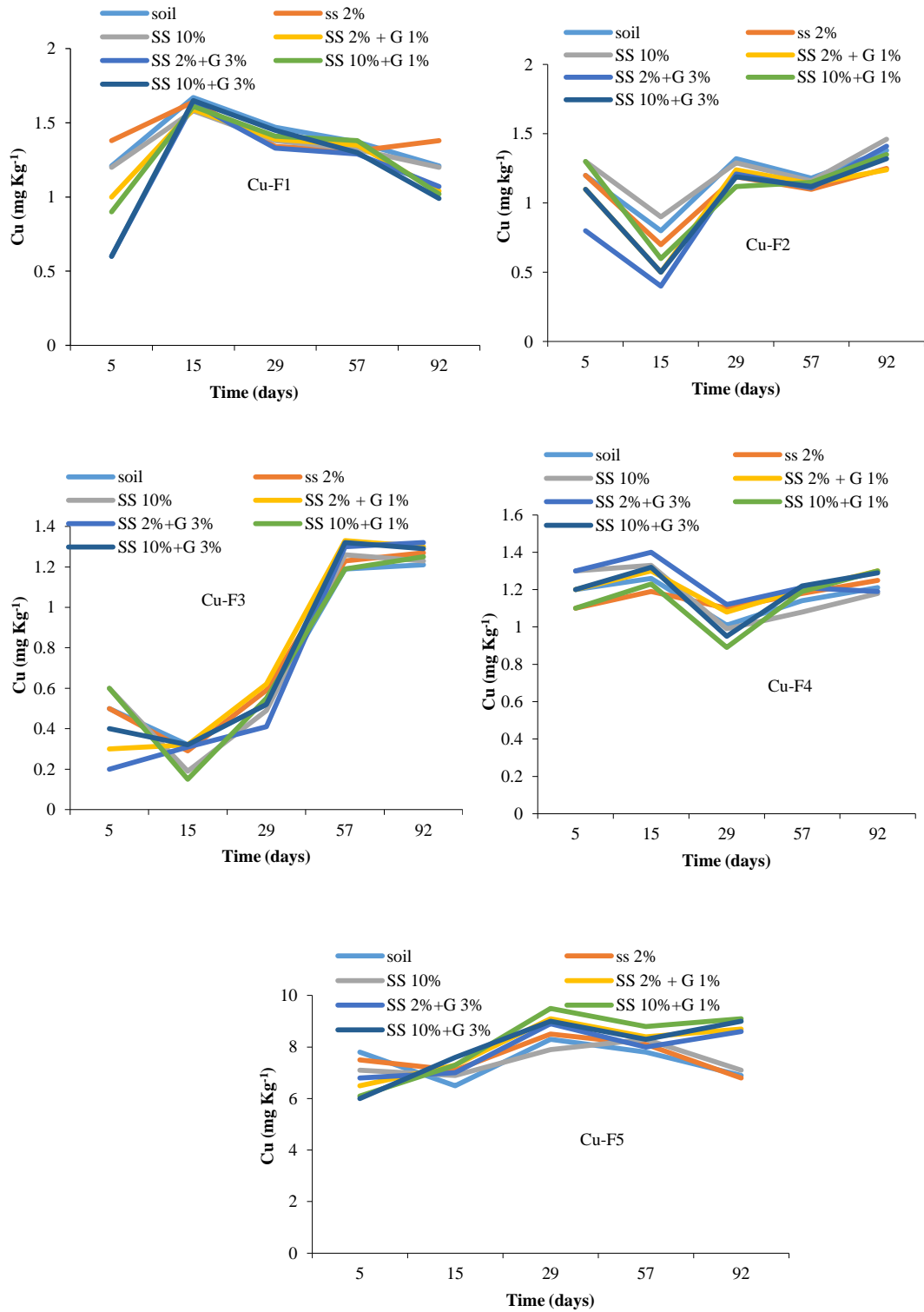
Figure 5. Changes in Cu fractions in the soil with different rates of sewage sludge samples and doses of Graphene oxide throughout the incubation time. Letter SS and G indicate the soil with different sewage sludge and Graphene oxide respectively.

The mobility factor (MF) of heavy metals was calculated according to the following equation:

$$MF = \frac{F1+F2}{F1+F2+F3+F4+F5} \times 100 \qquad (1)$$

The calculated average range of Mobility Factor (MF) for heavy metals across all treatments over the incubation period, based on data from Table 4, was 28.1-60.9 for Cd and 14.4-25.3 for Cu. These figures indicate a notably high mobility of Cd within soils treated with various SS

rates and GO doses. The observed increase in MF values for most elements over time may significantly influence the sorption and desorption of metals in soils amended with SS [43]. Despite the lower total concentration of Cd compared to Cu in soils treated with different SS rates and GO doses, its MF during incubation was higher than that of Cu. This highlights that the overall metal content might not be a suitable indicator to assess their availability in amended soil [44].

Table 4. Metal fractionation (mg kg^{-1}) in the soil with different rates of sewage sludge samples and doses of Graphene oxide during the incubation time. Metal fractions are: easily soluble and exchangeable (F1), bound to carbonates (F2), bound to Fe–Mn oxides (F3), bound to organic matter and sulfides (F4), and residual (F5).

Metal	Fractions	Incubation time (days)				
		5	15	29	57	92
	F1	1.18[a]	0.54[d]	0.65[e]	0.91[bc]	1.27[a]
	F2	1.14[b]	0.97[c]	0.82[d]	1.21[ab]	1.35[a]
Cd	F3	0.46[c]	0.25[d]	0.68[a]	0.73[a]	0.30[d]
	F4	0.41[e]	0.22[f]	0.49[de]	0.60[d]	1.37[a]
	F5	1.99[ab]	2.24[a]	0.52[d]	0.54[d]	1.37[c]
	F1	0.98[c]	1.62[a]	1.39[b]	1.33[b]	1.13[b]
	F2	1.14[b]	0.62[d]	1.22[a]	1.13[b]	1.34[a]
Cu	F3	0.44[d]	0.27[e]	0.52[d]	1.26[a]	1.26[a]
	F4	1.2[a]	1.29[a]	1.02[b]	1.17[a]	1.24[a]
	F5	6.82[b]	7.1[b]	8.74[a]	8.24[ab]	8.02[ab]

Different superscript on values in the same raw indicate significant difference between concentrations (P < 0.05) for each fraction

Effect of graphene oxide values on the heavy metal fraction

The impact of various doses of GO on the distribution of heavy metal fractions in soil amended with 2% and 10% SS, observed over five incubation periods, is detailed in Table 5. All doses of GO resulted in a decrease in the F1 fraction of Cd, with the most significant reduction seen in the treatment involving 3% GO and 2% SS. Concurrently, the F5 fraction of Cd increased with all GO doses, particularly evident in the 3% GO treatment. For Cu, the 1% and 3% GO doses decreased the F1 fraction in both 2% and 10% SS treatments. Moreover, the addition of GO augmented the F5 fraction of Cu in both soil-SS mixtures, notably prominent in the treatment with 1% GO and 2% SS. After a 92-day incubation period, the decrease observed in the F1 fraction of both Cd and Cu in soils with varying SS rates and GO doses underscored the significant influence of time on metal mobility in soil. These discoveries align with Renella et al.'s research

[45], emphasizing that upon sewage sludge (SS) application in soils, the mobility of Cd typically reached a saturation point shortly after SS incorporation, particularly in agricultural soils. In some cases, the introduced SS acted as a metal adsorbent, diminishing metal availability. For instance, in soil treated with a 2% SS rate, there was an increase in the percentage of Cd in the F5 fraction. Significantly, fractions F1 and F2 are crucial indicators linked to metal bioavailability and soil toxicity [46]. Jalali and Arfania's analysis [41] of heavy metal distribution in soil amended with municipal SS suggested that a major portion of Cd, Pb, and Ni resided in fractions F1 and F4, while Cu and Zn were predominantly present in fraction F4. Similarly, findings from Jamali et al. [47] indicated that the most easily mobilized forms (F1 and F2) were predominant for Cd, Ni, and Zn in the original SS.

Table 5. Cumulative effect of modified clays and nanoparticles on metal fractionation (mg kg^{-1}) the soil with different rates of sewage sludge samples and doses of Graphene oxide during the incubation time.

Metal	Fractions	Control treatments				Values GO		
		soil	SS 2%	SS 10%	SS 2% + G 1%	SS 2%+G 3%	SS 10%+G 1%	SS 10%+G 3%
Cd	F1	1.08 [a]	1.1 [a]	1.1 [a]	0.86 [b]	0.52 [d]	0.76 [b]	0.62 [c]
	F2	1.2 [a]	1.16 [a]	1.22 [a]	1.02 [ab]	0.92 [b]	1.16 [a]	1.04 [a]
	F3	0.58 [a]	0.6 [a]	0.58 [a]	0.40 [c]	0.35 [d]	0.48 [b]	0.41 [bc]
	F4	0.58 [a]	0.58 [a]	0.60 [a]	0.41 [c]	0.35 [c]	0.42 [c]	0.34 [c]
	F5	0.79 [de]	0.99 [d]	0.97 [d]	1.36 [c]	1.80 [a]	1.52 [b]	1.86 [a]
Cu	F1	1.38 [a]	1.41 [a]	1.33 [b]	1.27 [c]	1.18 [d]	1.26 [c]	1.19 [d]
	F2	1.17 [a]	1.08 [ab]	1.22 [a]	1.04 [b]	0.98 [b]	1.04 [b]	1.04 [b]
	F3	0.74 [c]	0.77 [a]	0.75 [b]	0.77 [a]	0.70 [e]	0.74 [c]	0.77 [a]
	F4	1.16 [d]	1.16 [d]	1.17 [d]	1.21 [b]	1.24 [a]	1.14 [e]	1.19 [c]
	F5	7.46 [d]	7.6 [c]	7.49 [d]	8.0 [ab]	7.86 [b]	8.16 [a]	7.98 [ab]

Distinct superscripts attached to values within the same row indicate a significant variance in concentrations ($P < 0.05$) for each fraction.

CONCLUSIONS

This research holds considerable significance, considering the potential use of nanoparticles (NPs) such as graphene oxide (GO) as an effective means to address environmental risks linked to soils contaminated with metals. However, the possible hazards to human health and ecosystems due to these NPs are still under assessment. Specifically, this study examined the effects of incorporating three distinct dosages of GO into contaminated soil, in combination with two different rates (2% and 10%) of sewage sludge (SS), on the adsorption and distribution of Cd and Cu fractions over a 92-day period. The results indicated that besides supplying essential elements, the type of applied SS significantly influenced the distribution of heavy metal fractions within the soil. Aging emerged as a crucial factor affecting metal distribution in the soil-SS mixture. Nevertheless, it's crucial to approach the long-term effects of SS on metal distribution cautiously, considering various factors such as soil characteristics, SS components, types of metals, and the study duration, all playing a significant role in determining the fate of metals in the soil. The study revealed that an increase in GO dosage resulted in a reduction in the F1 fraction of these heavy metals in soil treated with a 2% SS rate. Moreover, the calculated mobility factor of the heavy metals at five incubation periods (5, 15, 29, 57, and 92 days) ranged from 28.1 to 60.9 for Cd and 14.4 to 25.3 for Cu, signifying a higher mobility for Cd.

Conflict of interests

Authors declare that there are no financial or other relationships that might lead to a conflict of interest.

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