



Potential use of Thiol-Modified Biochar Nanoparticles for Hg Immobilization in Amazon Biome Soil

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Abstract Mercury (Hg) contamination associated with artisanal gold mining represents a major environmental and public health concern in South America, particularly in fish-dependent Amazonian communities. In this study, we investigated the potential of nanobiochar and thiol-modified nanobiochar to reduce Hg bioavailability in an Amazonian soil contaminated by illegal gold mining. Pristine nanobiochar (NB) and thiol-functionalized nanobiochar (NBS) were produced from açai (*Euterpe oleracea*) processing residues and applied to soil under controlled incubation conditions. Changes in Hg partitioning were assessed using sequential chemical extractions, allowing the evaluation of exchangeable, reducible, oxidizable, and residual Hg fractions. Both NB and NBS decreased the exchangeable Hg fraction, while increasing Hg associated with more stable soil compartments. The application of NBS reduced Hg availability of up to 17%, with enhanced Hg retention relative to NB. This behavior was attributed to differences in surface chemistry, including oxygen-containing functional groups (carboxyl, and phenolic) in NB and the presence of sulfur-containing groups in NBS, which promoted stronger Hg binding through covalent interactions. Although the low Fe content of

the soil limited Hg stabilization in the reducible fraction, thiol modification enhanced Hg immobilization overall. These results demonstrate that thiol-modified nanobiochar can effectively alter Hg partitioning toward less bioavailable forms, highlighting its potential as a soil amendment for mitigating Hg risks in contaminated tropical environments.

Keywords Soil contamination · Mercury · Particle size · Soil pollution · Sequential extraction

1 Introduction

Mercury (Hg) and its compounds are dangerous environmental pollutants related to strong carcinogenic effects that cause great harm to the human body and ecosystem (Soares et al., 2024). The Hg pollution exists worldwide to varying degrees, and a prolonged exposure to soils contaminated with Hg can lead to neurological and kidney damage and even death (Wu et al., 2024). Emissions from artisanal gold mining accounted for approximately 38% of total global Hg emissions in 2019 and are the main responsible source of Hg emissions in South America and sub-Saharan Africa (UN Environment Programme, 2019).

The World Health Organization (WHO) has proposed a tolerable weekly intake level for methyl mercury of 1.6 $\mu\text{g kg}^{-1}$ of body mass (WHO, 2016). However, some riverside populations in the Amazon

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consume quantities well above this limit (Crespo-Lopez et al., 2021). In this region, artisanal and small-scale mining is responsible for emitting more than 200 t of Hg per year, which represents approximately 27% of global emissions and 80% of total emissions in South America originating in this biome (Crespo-Lopez et al., 2021). This fact makes it necessary to develop environmentally friendly, financially economical, and effective strategies to reduce or alleviate the contamination load generated by Hg.

Many remediation technologies have been developed to reduce or control soil contamination by Hg in response to the growing need to address environmental contamination (Wang et al., 2021). Among the remediation methods, in situ stabilization is a non-destructive method that immobilize contaminants through chemical reactions between the stabilizing agent and the contaminant, which reduces their mobility and the potential to cause toxicity (Xiang et al., 2022).

Biochars have been one of the organic compounds used in remediation as a promising alternative for managing contaminated soils (Soares et al., 2021). Biochar acts in remediation through several mechanisms, such as i) electrostatic interactions with a surface charged with positive/negative charges, ii) surface complexation with functional groups, iii) (co) precipitation on the surface of the conditioner, and iv) physical adsorption on the internal surface of pores (Gao et al., 2022).

Feedstock is important in determining the function of biochar in the soil, but there is no consensus on the ideal feedstock for use in the soil and energy production, mainly because commercial pyrolysis plants are scarce and those that exist are associated with byproducts of specific materials that are abundant in each region (Borges et al., 2025). The main waste generated by the agroindustry in the Amazon region comes from the processing of açai (*Euterpe oleracea* Mart) and is composed by seeds and fibers often disposed inappropriately, causing blockages in sewers and waterways (Sato et al., 2020). Biochar from açai residues is a sustainable alternative by providing a noble destination for waste and can contribute to the viability of biochar production in the Amazon region.

Brazil produced 222,706 tons of açai in 2019, with 95% of the total produced in the state of Pará (IBGE, 2019). Açai is economically and culturally vital to this state. As a major producer of açai, Pará

plays a crucial role in the global supply chain of this superfood, which has gained international popularity for its health benefits. Economically, this production provides livelihoods for thousands of local families involved in harvesting, processing, and selling the berries (Freitas et al., 2021).

The use of pristine biochar has limited remediation capacity because of its low specific surface area and low number of charges present on the material's surface (Jadhav et al., 2025). The combination of biochar production with emerging technologies, such as biotechnology and nanotechnology, can create biochars with improved characteristics (Sani et al., 2023). With the use of nanotechnology, reducing the particle size of biochars to the nanometric range promotes improvements in their physicochemical properties due to the increase in specific surface area and reactivity (Raczkiewicz et al., 2024). Biochars with nanometric particles make it possible to accelerate sorption kinetics, which increases the possibilities for remediation of groundwater, surface water, and subsurface water (Chausali et al., 2021).

The main advantages of using nanoparticles as a remediation technique are the low energy demand and the possibility of in situ treatment (Ma et al., 2023). However, the potential effects of nanoparticles on the environment have not yet been fully investigated, and this method requires testing under real contamination conditions before it can be considered a viable remediation technique.

In addition to the use of nanoparticles, the technique of chemical modification of biochar through S-containing groups is a known alternative to increase Hg sorption (Bulutcu et al., 2025). S-containing groups, such as thiol functional groups, play an important role in the methylation of Hg^{+2} because they directly affect the activity of some methylating bacteria (Huang et al., 2023). Introducing thiol functional groups (-SH) on the surface of biochar improves its affinity to bind metal ions such as Hg, lead, and cadmium, due to the strong sulfur-metal interaction. This modification can significantly increase the capacity and efficiency of biochar in removing contaminants from aqueous solutions.

In this study, we hypothesize that modifying biochar nanoparticles with thiol reduces the bioavailability and mobility of Hg, increasing the residual Hg pool and consequently decreasing its absorption by plants and its leaching into groundwater. Thus, our

objective was to evaluate the efficiency of thiol-modified biochar nanoparticles in reducing Hg availability in contaminated soils.

The scientific contribution of this study lies in providing a mechanistic view of how thiol functionalization alters the distribution of Hg among geochemical fractions in soil, particularly under conditions representative of Amazonian environments. By combining nanobiochar surface modification with sequential extraction approaches, this study deepens the understanding of Hg stabilization mechanisms in real contaminated soils and supports the potential application of biochar-based amendments to mitigate Hg bioavailability in humid tropical regions impacted by artisanal gold mining.

2 Material and Methods

2.1 Production and Chemical Modification of Nanobiochar

Nanobiochar was produced from agro-industrial waste açai, and the feedstock was placed in a sealed reactor to prevent the entry of O₂. The reactor furnace was heated at a rate of 5 °C min⁻¹, and the final pyrolysis temperature of 600 °C was maintained for 1 h. The choice of this pyrolysis temperature was based on previous studies in which the authors showed the efficiency of biochars pyrolyzed at temperatures close to 600 °C in removing Hg⁰ and Hg²⁺ species (Li et al., 2015; Park et al., 2019; Shen et al., 2015; Yang et al., 2016).

A ball mill was used to transform the biochar into nanobiochar (<30 nm). Ball mill has a low cost, is efficient and environmentally friendly since it does not use chemical reagents (Pratap et al., 2021). To avoid nanoparticle interaggregation, the nanobiochar was dispersed in ethanol (1:20) and subsequently sonicated (50 kHz for 30 min) using a probe-type ultrasonic vibrator (Dong et al., 2018).

The thiol-modified nanobiochar (NBS) was prepared by mixing water, ethanol, and (3-Mercaptopropyl)trimethoxysilane (3-MPTS) (3.6 mL of water, 114 mL of ethanol, and 2.4 mL of 3-MPTS). For this purpose, 3 g of nanobiochar were added to a 500 mL-agate crucible, and then 120 mL of the mixed 3-MPTS solution were added dropwise using a Pasteur pipette. Then, the samples were

homogenized using an agate ball mill. After grinding, the NBS was washed with ethanol and deionized water three times and lyophilized for 48 h (Lyu et al., 2020).

2.2 Physicochemical Characterization of Nanobiochar

The total carbon (C) and nitrogen (N) contents of the biochar were determined via combustion using a CHN elemental analyzer (Carmo & Silva, 2012), and then the C/N ratio was calculated. The specific surface area (SSA) was obtained by the N₂-BET method through N₂ sorption and BET isotherm fitting (Song & Guo, 2012). The morphology and particle size of the nanobiochar surface were obtained using a transmission electron microscope (TEM; FEI TECNAI G² F20 HRTEM, Fei Company). For this, nanobiochars were diluted in deionized water (final concentration of 0.1 g L⁻¹) and pipetted onto a copper grid with carbon tape, air-dried, and subsequently imaged at 200 kV at high temperature.

In TEM imaging, the samples were photographed with a 200 nm magnification and 200 kV transmission level. In addition to morphology and size, the surface charge of nanobiochars was estimated by zeta (ζ) potential. The samples were diluted in 0.01 M NaNO₃ with a final pH of 6.8 ± 0.1 and a concentration of 0.1 g L⁻¹. For analysis, the samples were loaded into capillary cells and later examined using a Malvern Zetasizer Nano Z (Malvern PANalytical). The quality of the analysis was ensured by using an external Malvern standard with a potential of -42 ± 4.2 mV.

The solid composition of the biochar was estimated by proximal analysis, in which the contents of fixed carbon (C-fixed), volatile carbon (C-volatile) and ash were estimated through the mass loss after heating in a muffle furnace (ASTM, 2007). The organic functional groups of the nanobiochars were determined by diffuse reflectance infrared Fourier transform spectroscopy (DRIFT). The spectral readings (n = 4) were obtained with reflectance spectrum from 4000 to 500 cm⁻¹ with a resolution of 2 cm⁻¹. The nanobiochars will be diluted in KBr in the proportion 1:300 because the color of the nanobiochar saturates the absorbance in the DRIFT equipment.

2.3 Soil Sampling and Characterization

Soil samples were collected from an area in Tucumã, state of Pará, Brazil (6°44'52" S, 51°09'39" W), contaminated with Hg due to artisanal gold mining. This region is part of the Amazon biome and is near the "Estação Ecológica Terra do Meio," a conservation unit managed by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) under the Brazilian Ministry of Environment supervision (ICMBio, 2005). The "Terra do Meio" reserve has 28 million hectares, making it Brazil's second-largest biodiversity corridor.

Topsoil samples (0–0.2 m) were taken using a Dutch auger, sealed, and stored at around 4 °C to prevent chemical changes. For Hg content analysis, 0.1 g of soil was digested in a water bath at 95 °C with a mixture of concentrated HCl and HNO₃ (3:1, v/v) (Man et al., 2021), and Hg contents were measured using cold vapor atomic absorption spectrometry (CVAAS). Quality control and validation were conducted using a standard sample (SRM 2710a, Montana Soil II) certified by the National Institute of Standards and Technology (NIST), and a Hg recovery rate of 91% was achieved. The soil organic carbon content was 0.7% and was determined by a LECO-Shimadzu elemental analyzer, while the proportion of sand (70%), silt (26%) and clay (4%) were estimated

by the Pipette method (Gee & Or, 2002). The soil pH (5.6) was determined using a pH meter by extraction with ultrapure water.

The mineralogical composition of sand and clay fractions was obtained in samples sieved and dispersed with 0.1 mol L⁻¹ NaOH (Soukup et al., 2008) to isolate the clay fraction (< 2 µm). The clay was separated by siphonation, and X-ray diffraction (XRD) analysis was conducted using a computer-controlled diffractometer with Cu-Kα radiation (0.1542 nm) at 40 kV and 40 mA, along with a graphite monochromator. The diffractograms were processed to correct peak positions, and the crystalline phases were identified using the Match!® software. The mineralogy evaluated by XRD showed specific phases, and peaks in the diffractograms of the sand and clay fraction indicated the presence of kaolinite, gibbsite, chamosite, anatase, illite, hematite, quartz, hausmannite, plagioclase and vermiculite (Fig. 1).

2.4 Soil Incubation

The soil contaminated with Hg was incubated with NB and NBS (n=5) for 60 days, and geochemical changes were evaluated at the end of the incubation period. A subsample of 200 g of soil was placed in glass jars with lids containing small holes to allow gas exchange, preventing pressure buildup while

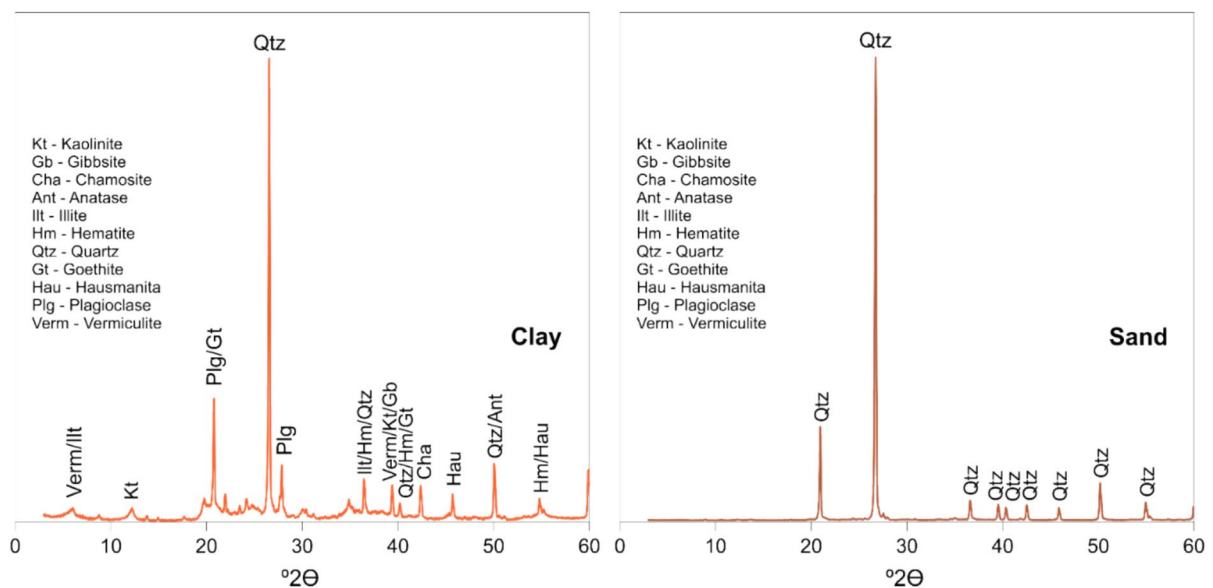


Fig. 1 X-ray diffraction pattern ($\lambda = 1.5405 \text{ \AA}$) of clay and sand fraction **B** of the contaminated soil

maintaining aerobic conditions. The nanobiochars were applied at a rate of 1% (w/w), and the soil moisture was adjusted to 70% of its water-holding capacity. All containers were kept in a temperature-controlled room (20 ± 2 °C) throughout the experiment. Under the same conditions, soil contaminated with Hg without the application of nanobiochar ($n=5$) was used as a control. After the end of the experiment, soil samples were collected and preserved at 4 °C until analysis.

The soil water-holding capacity was obtained using a gravimetric procedure. First, the soil was fully saturated with deionized water and kept in containers with the upper surface covered to prevent evaporative losses. After saturation, the soil was allowed to drain freely under gravity. The drainage period lasted up to 96 h, at which point a constant mass was reached. The wet soil was then weighed, oven-dried at 105 °C to constant mass, and weighed again. The maximum water retention capacity was calculated as the difference between the wet and dry masses, expressed on a mass basis (Embrapa, 2017).

After incubation, soil sampling was conducted to ensure that each replicate was representative of the entire incubation volume. Each glass jar contained 200 g of soil, and after opening the containers, the full soil mass was carefully loosened and homogenized using a pre-cleaned stainless-steel spatula. From this thoroughly mixed material, a 25 g subsample was collected for analysis. All utensils were rinsed with ultrapure water and dried with lint-free wipes between samples to prevent cross-contamination.

For DOC extraction, 5 g of soil were transferred to 100 mL of ultrapure water (1:20 w/v). The suspensions were shaken at 60 rpm for 16 h and subsequently filtered through a 0.45 µm membrane filter (Wei et al., 2019). This filtrate was used to determine dissolved or suspended nitrogen (DN) and the C:N ratio using a TOC elemental analyzer (TOC-L, Shimadzu) (Yoon et al., 2018). The same extract was also analyzed for pH and for calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), and sulfur (S) concentrations via inductively coupled plasma optical emission spectrometry (ICP-OES).

2.5 Sequential Chemical Extraction of Mercury

Changes in mercury (Hg) geochemical fractions were assessed by sequential chemical extractions

using pre-cleaned 50 mL polytetrafluoroethylene (PTFE) centrifuge tubes. The method followed the European Union's Bureau Communautaire de Référence (BCR) protocol: 0.11 M acetic acid (solution A) for extracting the exchangeable fraction, 0.1 M hydroxylamine hydrochloride (solution B) for the reducible fraction, and 8.8 M hydrogen peroxide at pH 2–3 (solution C) along with 1 M ammonium acetate at pH 2 (solution D) for the oxidizable fraction (Sahuquillo et al., 1999).

The sequential extraction process had four steps:

- Step 1: 1 g of soil was mixed with 40 mL of solution A in a 100 mL centrifuge tube and stirred for 16 h at 25 °C. The mixture was centrifuged at $1500 \times g$, and an aliquot of the extract was filtered (0.22 µm) and either analyzed immediately or stored at 4 °C. The residue was washed with 20 mL of ultrapure water, stirred for 15 min, and centrifuged again. The supernatant was decanted without losing the solid residue.
- Step 2: The residue from Step 1 was treated with 40 mL of solution B, and the extraction and cleaning were repeated as in Step 1.
- Step 3: 10 mL of solution C were added to the solid residue slowly to avoid a violent reaction. The tube was covered, left at room temperature for 1 h with occasional stirring, and then heated at 85 °C for 1 h in a water bath. After cooling, the volume was reduced to a few milliliters and then heated again at 85 °C for 1 h. Finally, 50 mL of solution D were added to the residue and extracted as described previously.
- Blank extractions (without soil) were performed for each analysis set using the same reagents to ensure accuracy. For dry mass correction, a separate 1 g sediment sample was dried at 105 ± 2 °C until a constant mass was achieved.

The residual fraction was determined by digesting with HCl: HNO₃ (3:1) following EPA method 3051A (USEPA, 2007), similar to the method for extracting semitotal Hg content in the soil. For quality control, the procedures were also applied to the NIST SRM 2710A Montana Soil II certified sample, and the recovery rate for the certified sample was 84%. Extracts were acidified and stored at 4 °C until analysis.

2.6 Statistical Analysis

All the results (soil, solution and nanobiochar attributes) were evaluated using the Shapiro–Wilk normality test. The non-normality of the data was checked and compared using the non-parametric Bootstrap mean comparison test ($p < 0.05$).

3 Results and Discussion

3.1 Changes in the Physicochemical Properties of Nanobiochar

The formation of biochar's nanoparticles was successful, as demonstrated by the results in the TEM images (Fig. 2) that revealed nanoparticles consistently uniform in size and shape, with a precise distribution and well-defined structure. The high resolution and clarity of the TEM images allowed for in-depth analysis, confirming that the milling process produced nanoparticles with an average size of 27 nm. The TEM images also showed interaggregation between the biochar nanoparticles. This aggregation was expected and is due to their high surface energy. In general, aggregation can be a problem because it drastically affects the reactivity, behavior, fate, function, and hazard of biochar nanoparticles in the natural environment and/or in engineered systems (Xing et al., 2023).

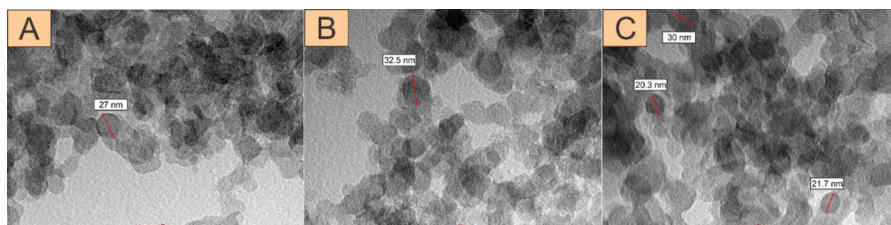
The modification with thiol groups promoted significant changes in the physicochemical properties of nanobiochar (Figs. 3 and 4). In general, a reduction in total C, N, fixed carbon (C-fix), and ash content was observed after the functionalization process (Figs. 3A, B, C and D). This trend can be largely attributed to the rinsing step subsequent to the anchoring of the thiol groups, necessary to remove excess reagent not chemically bound to the material's surface. During this process, fractions of carbon

and nitrogen weakly associated with the carbonaceous matrix, especially those present in more labile domains or in less condensed surface structures, may have been removed. Furthermore, the decrease in ash content suggests the solubilization or partial leaching of inorganic components, such as carbonates, oxides, and soluble salts, originally present in the biochar, in agreement with previous observations for materials subjected to intensive washing steps (Wei et al., 2022).

These compositional modifications reflect not only quantitative losses of certain constituents, but also a structural reorganization of the nanobiochar after functionalization. The reduction in fixed carbon and ash indicates that the modification process may have promoted a cleaner and more chemically active surface, dominated by covalently bonded sulfur functional groups, at the expense of mineral phases or less stable organic fractions. From a functional point of view, these changes are relevant because they suggest that the efficiency of the modified nanobiochar is not associated with maintaining the original composition, but rather with the introduction of specific sites of high chemical affinity, capable of governing the interaction mechanisms with contaminants such as Hg. Thus, despite the reduction in some classic characterization parameters, the modification with thiol represents a qualitative optimization of the material's surface, potentially favoring strong and selective complexation processes.

The changes in the physicochemical properties of the nanobiochar after thiol sorption were verified by FTIR spectroscopy (Fig. 4). The chemical modification affected not only the intensity but also specific spectral features, including peak positions and absorbance patterns. Prior to modification, the FTIR spectra of NB indicated the presence of oxygen-containing functional groups, such as hydroxyl (–OH), carboxylic (–COOH), and phenolic groups, which are commonly formed during low- to

Fig. 2 Transmission Electron Microscopy images of different regions of the nanobiochar **A**, **B**, and **C**. Images obtained with resolution of 30 nm and accelerating voltage of 200 kV



intermediate-temperature pyrolysis and are known to play an important role in metal(loid) sorption. In particular, phenolic and carboxylic hydroxyl groups promote strong and, in some cases, irreversible Hg^{2+} complexation through inner-sphere interactions, as previously demonstrated for low-temperature biochars (Dong et al., 2013).

After thiol modification, an increase in the absorbance intensity of bands related to aliphatic C–H and C–O groups was observed, indicating changes in the surface chemistry of the nanobiochar (Peltre et al., 2014). These changes suggest that the original oxygen-containing functional groups, including phenolic moieties, may act as anchoring sites for thiol functionalization or be partially altered during the modification process.

The modification with thiol also generated Si–OH and Si–O–Si functional groups, evidenced by absorptions in the regions of 3500–3300 and 1100–900 cm^{-1} , respectively. The presence of Si-containing groups was expected, as the thiol source used [$\text{HS}(\text{CH}_2)_3\text{Si}(\text{OCH}_2\text{CH}_3)_3$] contains silicon as the central atom. No –SH peaks were observed in NBS, which may be due to the low concentration of thiol groups, their chemical bonding to the biochar surface, or the overlap of –SH bands with Si–X vibrational peaks, making them difficult to detect by FTIR.

The increase in C-volatile in NBS may be related to the loss of compounds containing SH groups, not necessarily associated with carbon-based organic compounds. The thiol groups added to the nanobiochar structure were likely still present but may have been lost during the proximate analysis or remained below the method's detection limit, which explains the higher C-volatile values observed for NBS. Yang et al. (2022) investigated the contribution of volatile thiols to rapeseed (*Brassica napus*) oil and found that temperatures close to 50 °C were efficient for the formation of volatile compounds containing sulfur, but this temperature is much lower than the temperature used to determine volatile compounds in biochar. This supports the interpretation that the increase in C-volatile in NBS is plausibly linked to the volatilization or thermal degradation of SH groups during the analysis.

The loss of compounds after modification with thiol not only resulted in the reduction of phases of the solid structure of the biochar, but also altered phases extractable with water, as in the case of the

DOC content and consequently the pH of the biochar (Fig. 3). The modification with thiol reduced the pH of the nanobiochar by 16%, and this might be related to the reduction of ash and DOC contents in the NBS. The ash of the biochar has oxides and hydroxides capable of increasing the pH of the suspension (Escuer et al., 2021), while the DOC can be formed by several organic acids of low molar mass capable of reducing the pH due to the interaction with OH in solution and deprotonation (Wang et al., 2022a).

The combination of these factors increased negative charges on the surface of the NBS in relation to the NB (Fig. 5A). The ζ potential of the NBS increased by 30% after sorption and chemical modification with thiol and may be directly linked to the insertion of reduced sulfur groups, which can generate extra negative charges on the surface of the nanobiochar (Lyu et al., 2020). The SH groups generate a negative charge due to their chemical composition and through bonds with other functional compounds present on the surface of the nanobiochar.

The chemical modification process changed the SSA of the nanobiochar (Fig. 5B). The desorption of excess SH on the surface of the nanobiochar removed part of the ash and dissolved carbon that possibly obstructed some pores of the nanobiochar, and this resulted in a 13% increase in the SSA of NBS compared to NB. During pyrolysis, the pores can be blocked and filled with ash after transformation and migration into the biochar structure, particularly to micropores, which results in a reduction in SSA (Wang et al., 2022b).

3.2 Changes in Soil Solution

The addition of NB and NBS resulted in chemical changes in the soil solution (Fig. 6). In general, an increase in the values of the chemical attributes of the soil solution was observed after incubation with biochar nanoparticles. The pH of the solution increased from 5.4 to 5.9 and 6.8 after the addition of NB and NBS, respectively, and is related to the ash content of NB and NBS (Fig. 3). The nanobiochar with higher ash content resulted in a higher pH of the soil solution.

The increase in the pH of the solution affects the release of nutrients in the soil and is crucial for Hg retention. The mobility and bioavailability of Hg are strongly influenced by soil pH levels (Hussain et al.,

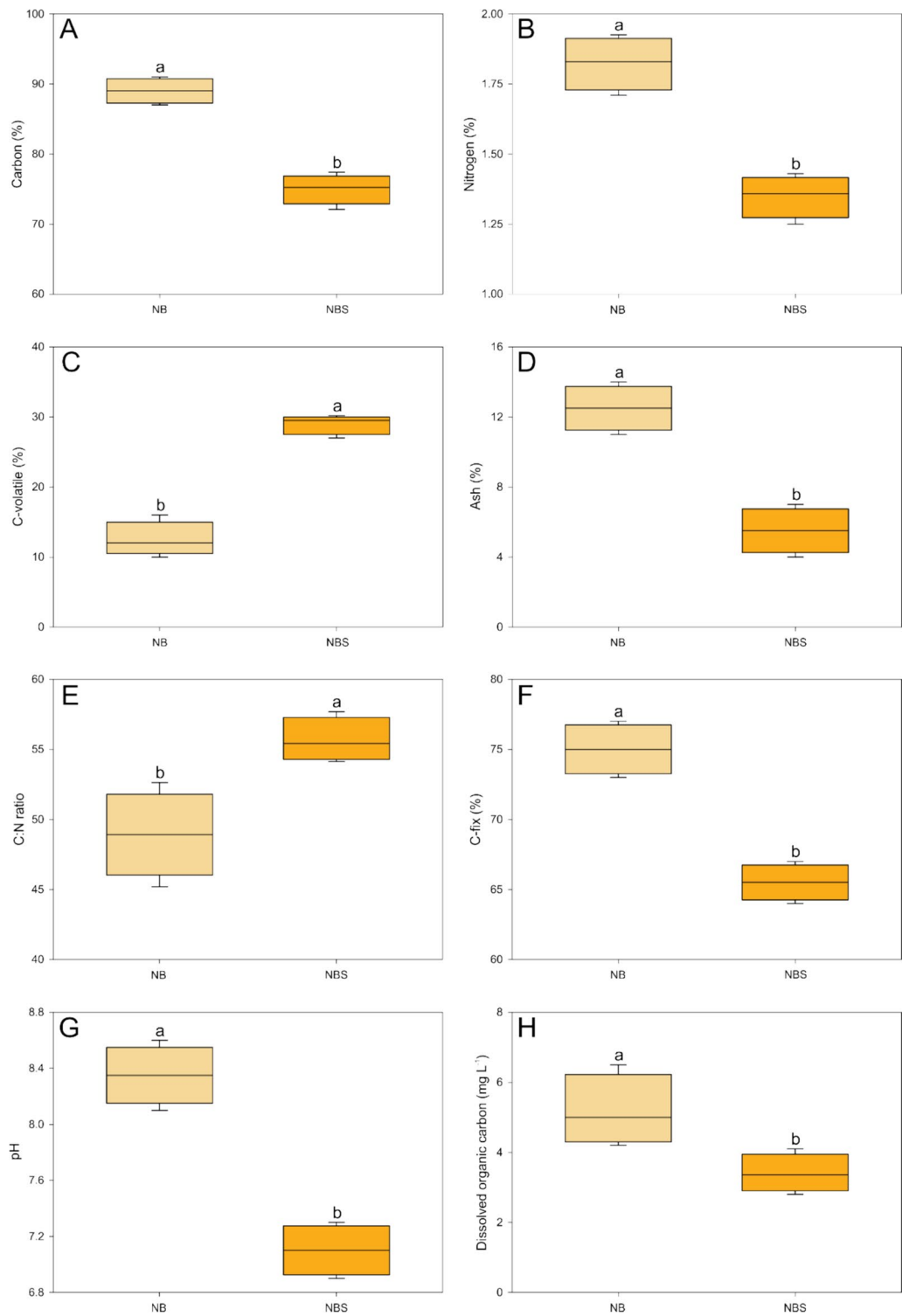


Fig. 3 Physicochemical attributes of pristine (NB) and thiol-modified (NBS) nanobiochars. Panel A is total carbon content, B total nitrogen content, C volatile carbon, D ash content, E C:N ratio, F fixed carbon, G pH, and H dissolved organic carbon. The boxplots display the median, interquartile range (IQR), and minimum–maximum values for each treatment. Error bars represent the standard deviation of four replicates. Different lowercase letters indicate differences between treatments (Bootstrap test, $p < 0.05$)

2022): under acidic conditions (low pH), Hg tends to form soluble and more toxic methylmercury compounds that can easily leach into groundwater and be absorbed by plants, posing serious risks to ecosystems and human health. As the soil pH increases, Hg can undergo pH-induced precipitation (e.g., as $\text{Hg}(\text{OH})_2$ or mixed hydroxy complexes) or bind more strongly to functional groups present in soil organic matter and mineral surfaces, reducing its solubility and mobility (O'Connor et al., 2019). Higher pH conditions enhance deprotonation of organic ligands and mineral surface sites, increasing their affinity for Hg and thereby promoting immobilization.

The pH interferes with Hg dynamics and affects the dynamics of anions and other cations in solution (Fig. 6). The increase in DOC in solution after the addition of NB and NBS may be related either to the nanobiochar being a source of DOC or to the increase in solution pH favoring the desorption of natural soil organic matter (Grybos et al., 2009). The increase in DOC content was greater in NB than NBS because of the ability of NB to supply DOC or increase pH (Fig. 3). This increase in solution was observed for nitrogen content, C:N ratio, calcium and magnesium, however, the values of these attributes did not differ between NB and NBS, but only between control soil and soil with nanobiochar.

The differences between these attributes in the solution as a function of the addition of nanobiochar may be related to their release into the solution through the dissolution of soluble phases present in the nanobiochar structure, while the lack of difference between NB and NBS is related to how these elements may be bound in the nanobiochar in a way that was not impacted by the chemical modification with thiol.

Only potassium and sulfur contents differed between NB, NBS, and control (Fig. 6), and the difference between them was due to the washing process and the addition of thiol. The addition of thiol in NBS

may have removed potassium from the nanobiochar during the cleaning process and increased the sulfur content due to the insertion of SH groups. It is likely that during the experiment, part of the SH present in the nanobiochar was released into the soil solution.

The SH is important for Hg retention, and its release into solution may be favorable for the formation of the HgS ionic pair in which the compound is insoluble, but it may not be retained in the soil and be carried by water, which in the medium and long term may not be efficient in reducing Hg toxicity in the environment (Zhang et al., 2012).

3.3 Sequential Chemical Extraction and Changes in Soil Hg Pools

The addition of nanobiochar reduced the exchangeable Hg contents by up to 18%, while increasing the reducible, oxidizable, and residual Hg fractions (Fig. 7). Regarding the modification with thiol, NBS was more efficient than NB in reducing the exchangeable and most available fractions of Hg in the soil. The NBS was 10% more efficient in reducing exchangeable Hg compared to NB by promoting a 9% increase in the Hg content associated with the reducible, oxidizable and residual fractions. This improved performance of NBS is consistent with previous studies reporting that thiol-functionalized sorbents, including thiolated biochars, MOF-based materials, and biopolymer composites, exhibit markedly higher affinity for Hg due to the formation of strong Hg-S complexes (Bulutcu et al., 2025; Louca et al., 2016).

The main differences in exchangeable Hg content between soils amended and not amended with biochar nanoparticles were attributed to the functional organic groups present in the biochars, which retained Hg through ion-exchange processes or covalent bonding (Rizwan et al., 2024). Another indirect mechanism of interaction is promoted by sulfur cycling that favors soil-biochar-Hg interaction (Yang et al., 2021). In this context, the superior reduction of exchangeable Hg by NBS aligns with observations from aqueous systems, where thiol-modified biochars and sulfur-functionalized MOFs achieved substantially higher Hg removal efficiencies than non-functionalized materials due to enhanced surface density of reactive sulfur sites (Huang et al., 2023; Li et al., 2022). Although the absolute efficiencies observed in soil systems are lower than those reported for engineered sorbents

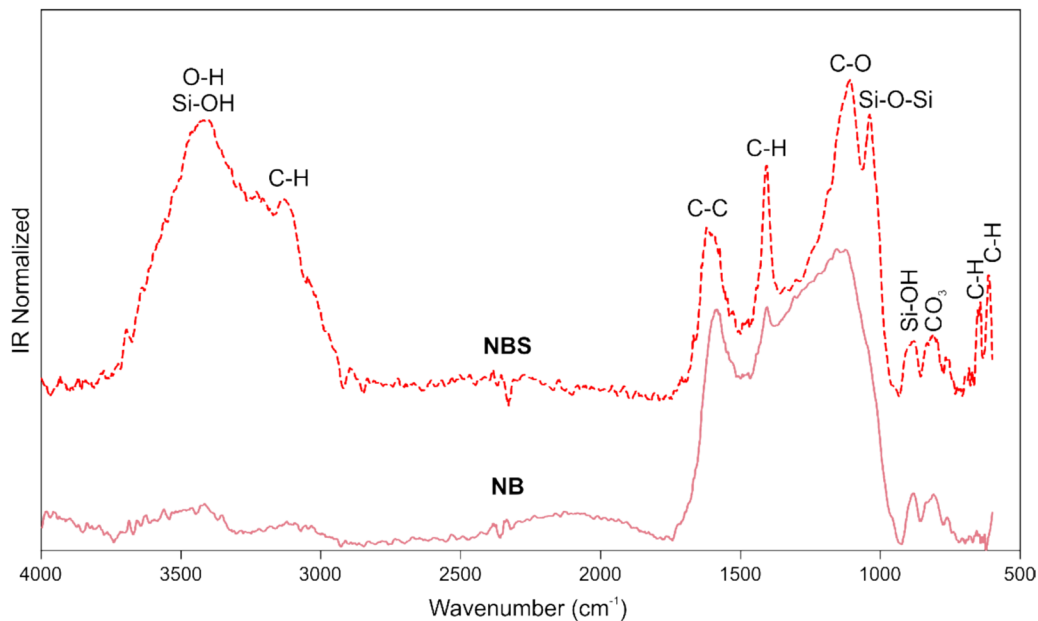


Fig. 4 Diffuse reflectance Fourier transform infrared (DRIFT) spectroscopy spectra of pristine (NB) and modified with thiol (NBS) nanobiochars. DRIFT peaks for regions corresponding to O–H and/or Si–OH ($3480\text{--}3300\text{ cm}^{-1}$),

C–H ($3300\text{--}3000\text{ cm}^{-1}$), C–C ($1660\text{--}1580\text{ cm}^{-1}$), C–H ($1440\text{--}1395\text{ cm}^{-1}$), C–O ($1160\text{--}1000\text{ cm}^{-1}$), Si–O–Si ($1000\text{--}900\text{ cm}^{-1}$), Si–OH ($900\text{--}800\text{ cm}^{-1}$), CO_3 (850 cm^{-1}), and C–H ($700\text{--}500\text{ cm}^{-1}$)

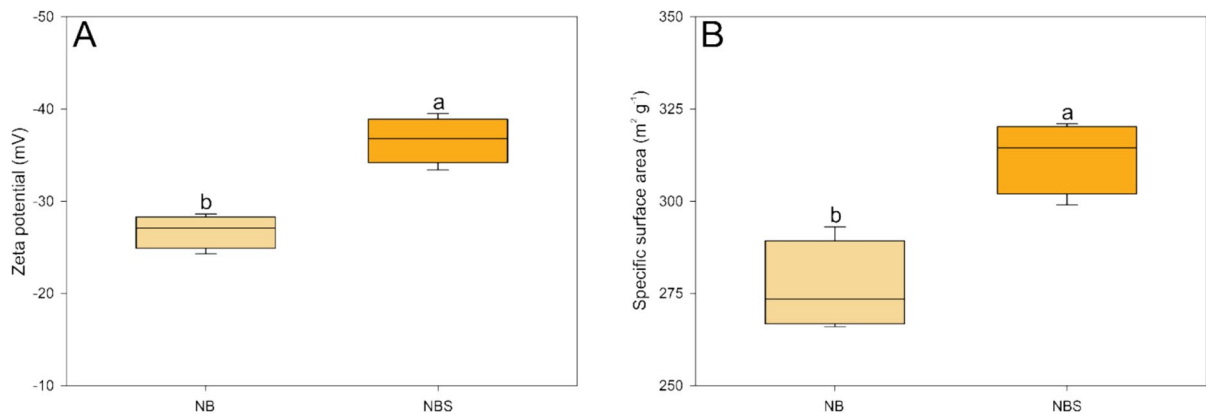


Fig. 5 Zeta potential **A** and specific surface area **B** of pristine (NB) and thiol-modified (NBS) nanobiochars. The boxplots show the median, interquartile range (IQR), and minimum–maximum values for each treatment. Error bars represent the

standard deviation of four replicates. Different lowercase letters indicate differences between treatments (Bootstrap test, $p < 0.05$)

in water, the relative improvement induced by thiol functionalization remains consistent across matrices.

The differences between NB and NBS in the oxidizable fraction of Hg can be attributed to the quality of organic functional groups present on the surface of biochar nanoparticles, but can also be related

to differences in the content of sulfides (Richardson, 2022), capable of retaining Hg^{2+} by the reaction $\text{R-SH} + \text{Hg}^{2+} \rightarrow \text{R-S-Hg} + \text{H}^+$. The functional groups on the surface of biochars are critical for Hg complexation (Amalina et al., 2022). Xu et al. (2016) found that Hg^{2+} sorption by bagasse-derived

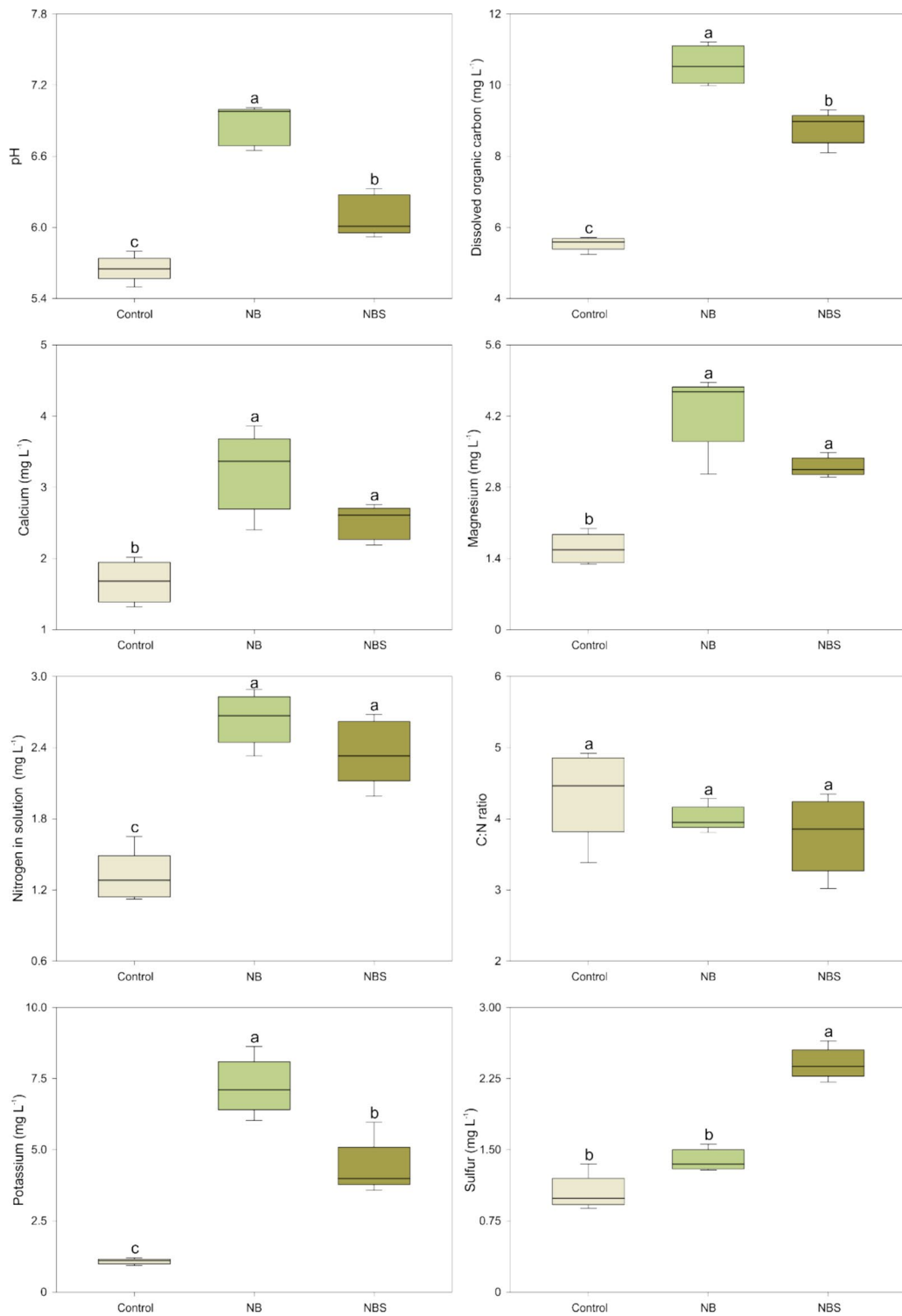


Fig. 6 Values of pH and levels of dissolved organic carbon, calcium, magnesium, nitrogen, potassium, and sulfur in solution of a soil amended of pristine (NB) and modified with thiol (NBS) nanobiochars. The boxplots show the median,

interquartile range (IQR), and minimum–maximum values for each treatment. Error bars represent the standard deviation of four replicates. Different lowercase letters indicate differences between treatments (Bootstrap test, $p < 0.05$)

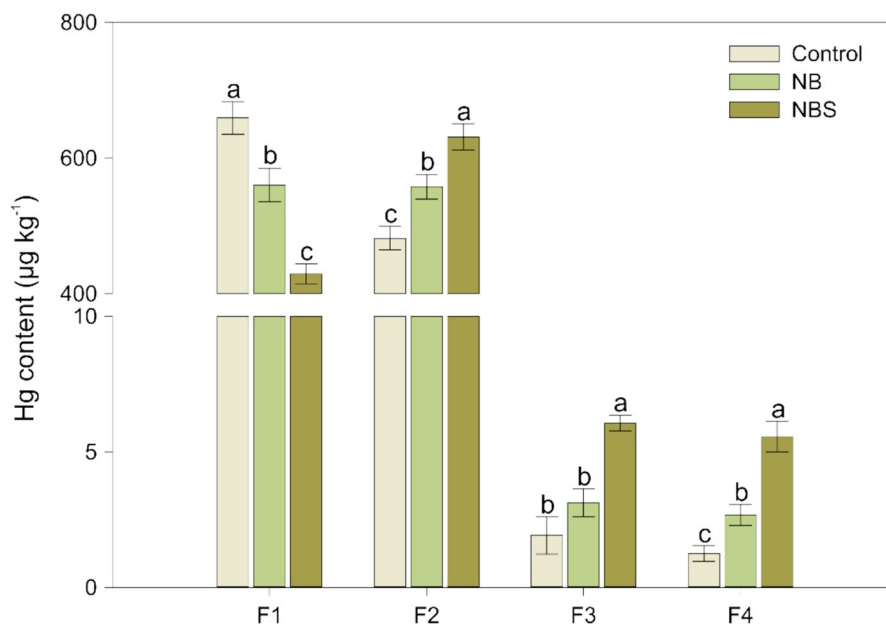


Fig. 7 Mercury (Hg) fractions (F1–F4) in the soil from the mining area amended with pristine (NB) and thiol-modified (NBS) nanobiochars. F1 corresponds to the exchangeable Hg fraction extracted with 0.11 M acetic acid; F2 represents the reducible Hg fraction extracted with 0.1 M hydroxylamine hydrochloride; F3 refers to the oxidizable Hg fraction extracted

using 8.8 M hydrogen peroxide at pH 2–3, followed by 1 M ammonium acetate at pH 2; and F4 corresponds to the residual Hg fraction determined by acid digestion according to EPA Method 3051A. Box plots followed by the same letter do not differ among treatments (Bootstrap test, $p < 0.05$)

biochar was mainly attributed to the complexation of Hg^{2+} with phenolic hydroxyl (COH) and carboxylic (COOH) groups and the formation of $(-\text{O})_2\text{Hg}$ and $(-\text{COO})_2\text{Hg}$. These organic groups were identified in NB and NBS in this study by FTIR spectroscopy (Fig. 4). Compared to polymer-based hydrogels incorporating thiolated minerals (Bulutcu et al., 2025) or MOF composites with engineered porosity and high sulfur loading (Li et al., 2022), NBS presents a lower but environmentally realistic density of reactive sites, which may explain the moderate, yet significant shifts observed in oxidizable Hg fractions under soil conditions.

Although the soil was maintained under aerated conditions, the reducible Hg fraction represents Hg associated with Fe (oxyhydr)oxides formed under oxic environments but extractable under reducing conditions. The application of NB and NBS increased the amount of Hg associated with this fraction. The low Fe content of the soil, however, constrained the overall pool of Fe (oxyhydr)oxides available for Hg stabilization. As a result, despite the observed increase, the magnitude of Hg accumulation in the

reducible fraction remained limited when compared to the potential retention capacity of the amendments. This contrasts with Fe-enriched sorbents such as $\text{Fe}_3\text{O}_4@\text{DTIM-MOF@SH}$ (Li et al., 2022), where iron phases directly contribute to Hg binding and recyclability, highlighting that the effectiveness of nanobiochar in soils is partially governed by intrinsic soil mineralogy.

The increase in the residual Hg fraction after NB and NBS addition may be related to interactions of Hg^{2+} with $\text{C}=\text{C}$ and $\text{C}=\text{O}$ organic groups to form $\text{Hg}-\pi$ bonds. Such results were verified after Hg^{2+} sorption on walnut (*Juglans regia*) chips or wood-derived biochar (Park et al., 2019; Xu et al., 2016). In addition to functional groups, Dong et al. (2013) suggested that Hg^{2+} can be irreversibly sorbed through complexation with phenolic and carboxylic hydroxyl groups in low-temperature (400 and 500 °C) pyrolyzed Brazilian pepper (*Schinus terebinthifolia*) biochars and by graphite-like structures in high-temperature (600 °C) biochar. Taken together, these findings indicate that, while engineered materials such as thiolated MOFs and biopolymer hydrogels can achieve

higher absolute Hg sorption capacities in aqueous systems (Bulutcu et al., 2025; Li et al., 2022), thiol-functionalized nanobiochar offers a balanced and scalable approach for soil remediation by promoting a consistent redistribution of Hg from labile to more stable fractions under environmentally realistic conditions.

3.4 Main Mechanisms of Mercury Retention

Mercury retention by nanobiochar is governed by four main mechanisms: *i*) ion exchange, *ii*) covalent bonding via inner-sphere complexation, *iii*) surface complexation, and *iv*) physical entrapment through pore filling (Fig. 8). These mechanisms are controlled by the combined influence of surface chemistry and structural properties, which together determine the efficiency and stability of Hg immobilization in soil systems (Al-Wabel et al., 2013).

Ion exchange represents a relatively labile interaction, in which Hg species are reversibly associated with charged functional groups on the nanobiochar surface, contributing primarily to the initial decrease in exchangeable Hg (Chaubey et al., 2023).

In contrast, covalent bonding via inner-sphere complexation involves the formation of strong and specific bonds between Hg and functional groups, particularly thiol (-SH) groups in NBS, leading to the formation of highly stable Hg–S complexes. This process results in chemisorbed Hg species with high thermodynamic stability and resistance to remobilization (Serrano et al., 2016). These mechanisms explain the redistribution of Hg from labile pools toward more stable fractions following nanobiochar amendment (Fig. 7).

Surface complexation, distinct from covalent bonding, involves coordination interactions between Hg species and oxygen-containing functional groups (e.g., carboxyl and hydroxyl groups) on the nanobiochar surface. These interactions may include both inner- and outer-sphere complexes and contribute to Hg stabilization with intermediate binding strength, depending on environmental conditions such as pH and ionic strength (Fazi et al., 2026).

In addition to chemical stabilization pathways, physical immobilization through pore filling plays an important complementary role. Mercury species, including Hg associated with dissolved organic matter, may become physically entrapped within the

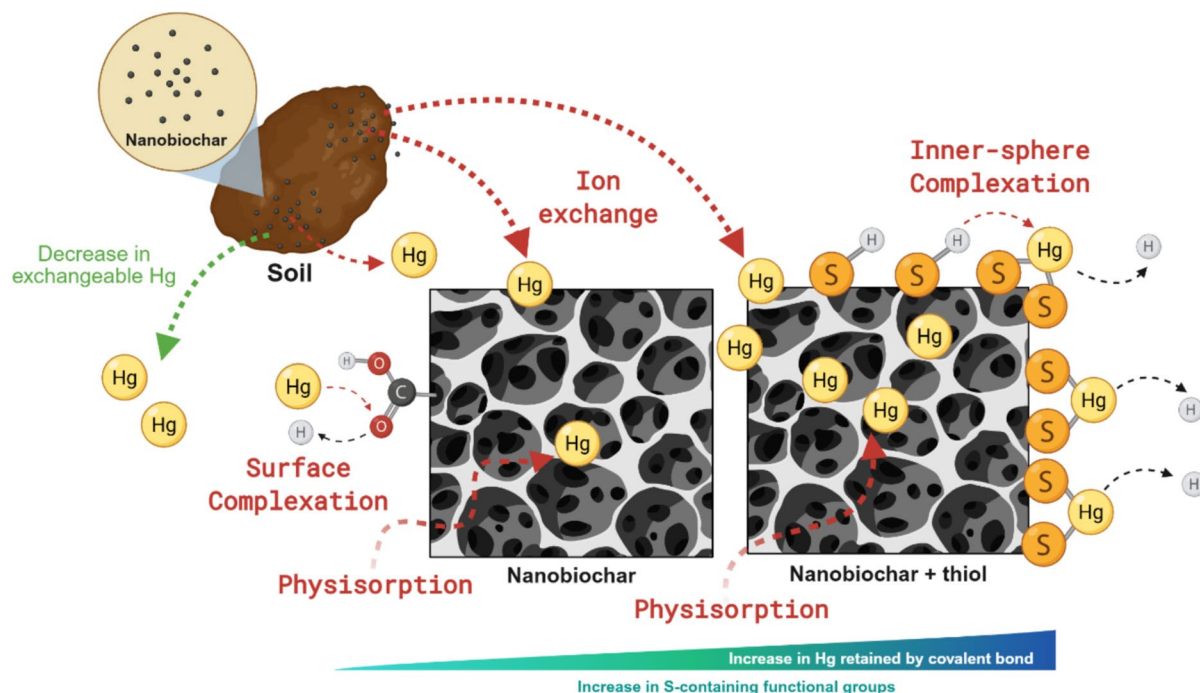


Fig. 8 Main mechanisms of mercury retention in pristine (NB) and thiol-modified (NBS) nanobiochars. The four main mechanisms (ion exchange, surface complexation, physisorption, and inner-sphere complexation) are highlighted in red

micro- and mesoporous structure of nanobiochar, limiting their accessibility and mobility without necessarily altering their chemical speciation (Rizwan et al., 2024). Together, these mechanisms contribute to Hg immobilization in nanobiochar-amended soils, with their relative importance governed by nanobiochar properties and soil geochemical conditions (Li et al., 2019).

4 Final Remarks

The use of biochar nanoparticles (nanobiochar) represents a promising strategy for the remediation of Hg-contaminated soils in the Amazon. Our results demonstrate that the application of nanobiochar effectively reduces the exchangeable fraction of Hg, thereby decreasing Hg bioavailability and associated environmental and human health risks. These findings highlight the potential of nanobiochar-based amendments as a viable approach for mitigating Hg mobility in tropical soils impacted by artisanal gold mining.

Complexation, ion exchange, physisorption, and electrostatic interactions were identified as the main mechanisms governing Hg retention by nanobiochar. Functional groups such as carboxyl, hydroxyl, and phenolic moieties play a key role in stabilizing Hg through surface complexation processes. In thiol-modified nanobiochar, the enhanced Hg retention is likely associated with the formation of stronger covalent interactions between Hg and sulfur-containing functional groups, further reinforcing Hg immobilization.

Overall, this study provides mechanistic insight into how surface functionalization of nanobiochar influences Hg partitioning among geochemical fractions, particularly under conditions representative of Amazonian soils. From a practical perspective, the results support the use of nanobiochar, and especially thiol-modified nanobiochar, as a potentially scalable amendment that may offer a cost-effective option for reducing Hg bioavailability and ecological risk in contaminated humid tropical environments.

The addition of nanobiochar in Hg-contaminated Amazonian soils is therefore an effective strategy to mitigate Hg-related ecological risks. However, continued research, including long-term field trials, is needed to optimize application rates, assess

amendment stability, and evaluate the persistence of Hg immobilization under variable soil and climatic conditions. Future studies could further enhance this efficiency by optimizing sulfur loading and functional group accessibility, tailoring pore size distribution to favor Hg retention, and combining thiol-functionalized nanobiochar with complementary amendments (e.g., Fe-based phases) to promote multi-mechanistic stabilization under variable redox and aging conditions.

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Authors' Contributions MBS: Conceptualization, Methodology, Investigation, Formal analysis, Writing—Original Draft, Review & Editing and Data processing. RAGS: Formal analysis; Writing—Original Draft, Review & Editing. LRFA: Conceptualization, Methodology, Supervision, Funding acquisition, and Writing—Review & Editing.

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Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent for Publication Not applicable.

Competing interests The authors declare no competing interests.

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