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Research Article

Fractionation of water-soluble organic matter (WSOM) with polyvinylpyrrolidone: A study on antimony associated with WSOM in contaminated soils

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ABSTRACT

Water-soluble organic matter (WSOM) significantly influences the transport of metals and organic contaminants in soils, yet the interaction specifics with antimony (Sb) remain largely unexplored. Antimony is of particular environmental concern due to its toxic properties and harmful effects on ecosystems and human health. Employing a three-step fractionation method with polyvinylpyrrolidone (PVP), this study aimed to isolate and analyze humic acids (HA), PVP-non adsorbed fulvic acids (FAA), and PVP-adsorbed fulvic acids (FAB) from WSOM in soil spiked with Sb and incubated for 18 months. These fractions underwent chemical analysis for carbon (C), nitrogen (N), total organic carbon (TOC), and Sb, complemented by FTIR and 1H NMR spectroscopic characterization. The study revealed that HA was more aliphatic, with Sb predominantly associating with the fulvic acid (FA) fraction, accounting for 97 % of Sb in extracts. Specifically, the FAA subfraction held substantial portions of total carbon (TC), total nitrogen (TN), total organic carbon (TOC), and Sb. Correlations between Sb concentrations and TN, TC, and TOC were significant. Extraction methods showed NaOH and $Na_4P_2O_7$ outperformed HCl and deionised water in extracting TC, TN, and TOC, with higher Sb concentrations found in $Na_4P_2O_7$ and NaOH extracts. This underscores the role of Fe/Al-SOM complexes in Sb soil availability. The results revealed that FAA subfraction accounted for 76 %, 64 % and 94 % of TN, TOC and Sb, respectively. Therefore, this research highlights the FAA fraction's central role, predominantly comprising non-humic substances

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like amines, in the availability of C, N, and Sb in Sb-impacted soils. The findings offer insights for environmental management and remediation strategies.

Introduction

Antimony (Sb), a metalloid classified within group 15 of the periodic table, has found extensive utilization in diverse industrial sectors including batteries, flame-retardants, paints, automobile brakes, ammunition, and the plastic industry (e.g., polyethylene terephthalate) (Bagherifam et al., 2019; Bagherifam et al., 2022). The primary environmental forms of Sb, namely Sb(V) and Sb(III), predominantly exist based on the oxidation/reduction state prevailing in environmental systems (Bagherifam et al., 2019). Antimony (Sb) is often released into the environmental systems through mining, industrial emissions, and waste disposal. Due to its toxicity, Sb exposure can have severe health impacts on humans, including detrimental effects on heart, lung, eye and skin, and it is also recognized as a potential carcinogen. In soils, the bioavailability of Sb is influenced by organic matter, pH, redox conditions, source of contaminants, ageing and speciation, Fe and Mn oxides, soil types and chemical composition (Bagherifam et al., 2019). The WHO has set the 20 µg Sb/L regulatory guidelines for Sb in drinking water (WHO, 2003). Owing to its detrimental effects on both human health and ecological receptors, Sb has been recognized as a priority contaminant by entities such as the European Union (EU) and the U.S. Environmental Protection Agency (USEPA) (Fan et al., 2020).

Soil organic matter (SOM) is an intricate amalgamation of colloidal substances predominantly comprised of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulfur (S), and trace quantities of other nutrients. It plays a fundamental role in the carbon dynamics and biogeochemical cycles within the soil. SOM consists of a fusion of aromatic and aliphatic hydrocarbons that possess diverse functional groups, including amid, carboxyl, hydroxyl, phenoxyl, and ketone (Nkhili et al., 2012; Strawn et al., 2019). While water-soluble organic matter (WSOM) and dissolved organic matter (DOM) make up only a small proportion of SOM, they are regarded as the most active and unstable fraction of SOM (Chantigny, 2003; Scaglia and Adani, 2009; Nkhili et al., 2012). DOM and WSOM primarily comprise organic compounds that mirror the chemical composition of SOM, and they can influence soil's biological functions, the transportation of organic pollutants and metal(loid)s, as well as the processes of mineral weathering in soils (Chantigny, 2003). Dissolved organic matter (DOM) can have a significant impact on the bioavailability of metals in soils. DOM acts as a complexing agent, forming soluble metal-organic complexes that can affect the mobility, transport, and availability of metals to organisms in the soil ecosystem through complexation, competition, metal speciation, colloidal transport and biological interactions. The solubility of SOM is influenced by factors such as the pH and ionic strength of the soil solution, while clay minerals, as well as iron (Fe) and aluminum (Al) oxides, impact the equilibrium between DOM and solid SOM (Chantigny, 2003). Nevertheless, due to its high polarity, low concentrations, and intricate nature, water extractable organic matter has long been regarded as an analytical challenge (Nkhili et al., 2012). Understanding these complex dynamics is essential for assessing the environmental fate and potential risks associated with metal contamination in soils. Environmental chemistry of Sb and its stabilization in soils is affected by different types of natural organic matter (Zheng et al., 2025). Fan et al. (2019) elucidated the substantial influence exerted by DOM on the mass transfer rate and adsorption of Sb in soil environments. Their findings highlighted the formation of Sb(V)-DOM complexes through functional groups such as amide, phenol, polysaccharides, and aromatic rings, which in turn affected Sb adsorption in a pH-dependent manner. Furthermore, Grob et al. (2018) examined the release of Sb in soils of shooting ranges subjected to flooding and noted that the escalation of DOM, along with the dissolution of Fe and manganese (Mn) oxyhydroxides, could contribute to the release of Sb in the soil. The characteristics of soil and the content of DOM within the soil exert a significant influence on the environmental availability of Sb in soil systems. Despite thorough and the elimination of humic and fulvic acids using 0.5 mol/L NaOH and 0.1 mol/L $Na_4P_2O_7$, a considerable portion of Sb persists within recalcitrant components of SOM (glycerol extractable) and humin fraction (Bagherifam et al., 2023a). Due to varying complexing capacities, biological interactions and effects of pH on various WSOM functional groups, understanding the speciation of WSOM is needed to elucidate the mechanisms by which metal(loid)s interact with WSOM. This can provide insights into the fate, transport, and potential impacts of metal(loid)s on ecosystems. Nonetheless, there is a considerable knowledge gap concerning the effects of WSOM speciation on the chemistry of Sb and other metals in soil systems.

The insoluble variant of polyvinyl pyrrolidine has found diverse applications in research endeavors, such as the segregation of plant phenols and elimination of tannins. This is attributed to the inherent ability of polyvinyl pyrrolidine to establish robust hydrogen bonds with phenolic, hydroxyl, and carboxyl groups present in DOM (Harrison, 1971; Lowe, 1975). It has also been proven instrumental in the isolation of plant hormones (Glenn et al., 1972). Moreover, polyvinyl pyrrolidone (PVP) has been employed advantageously as a selective adsorbent for the extraction of phenolic and polyphenolic compounds (Lowe, 1975). Scientists have effectively utilized PVP under acidic conditions to partition WSOM into fractions enriched in humic substances (HS) and non-humic substances (NHS) (Aoyama, 2002).

Lowe (1975) conducted a partitioning procedure on the acid-soluble constituents of WSOM by utilizing insoluble PVP, successfully segregating polyphenolic components from polysaccharide compounds. Their findings revealed notable variations in the levels of fulvic carbon within the polyphenolic subfraction, contingent upon the source of SOM and specific soil horizons. Aoyama (2006) observed that the PVP-non-adsorbed fraction constituted the largest portion of organic matter in the soil extracts. Furthermore, the humic substances found within the HA and PVP non-adsorbed FA fractions exhibited greater bioavailability to microorganisms in comparison to the PVP-adsorbed FA fraction. Watanabe and Kuwatsuka (1992) investigated the chemical attributes of fulvic acid fractions obtained through insoluble PVP and documented that the PVP-non-adsorbed fraction of soil FAs primarily comprised NHS, including carbohydrates and peptides. WSOM plays a vital role in influencing the mobility and bioavailability of Sb in soils. This study investigates how different WSOM fractions interact with Sb in contaminated soils, offering insights into the mechanisms governing Sb distribution and potential environmental implications. By fractionating WSOM into HA and FA, including non-adsorbed (FAA) and PVP-adsorbed (FAB) subfractions, this study aims to clarify how each fraction impacts Sb binding and transport in soil matrices.

To date, no comprehensive investigation has been conducted to systematically explore the interactions between Sb and other heavy metal(loids) with WSOM and the association of Sb with HA and FA fractions. Therefore, the specific objectives of this study encompass the following: (a) the separation of WSOM into three distinct fractions, namely HA, PVP-non adsorbed FA, and PVP-adsorbed FA; (b) the examination of the speciation of organic carbon (C) and nitrogen (N) forms within the WSOM fractions; (c) the analysis of the relative distribution of Sb within the HA and FA fractions of WSOM; and (d) the comparison of extraction efficiency using alkali, acidic, and deionized water for TC, TN, total organic carbon (TOC), and Sb in the experimental soils. The results of this research are likely to augment our comprehension of the interplay between labile and recalcitrant forms of Sb present within WSOM, thus furnishing a valuable resource for future researchers to explore the correlations and bioavailability of other metallic and metalloid elements within WSOM.

1. Materials and methods

1.1. *Experimental setup*

Composite soil samples were collected from a depth of 0-30 cm of a hill rich in soil organic matter (SOM). Air-dried and sieved to 2 mm, soil samples were mixed with antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2{\cdot}xH_2O$) to prepare Sb-contaminated soils with 1000 mg/kg Sb. The dry salt application provided a consistent Sb concentration in the soil, simulating long-term contamination scenarios. Soils were maintained at 70 % water-holding capacity for 18 months by regular additions of double-distilled water. Following extraction, aqueous samples were stored at 4°C and analyzed within two weeks to preserve sample integrity. The Sb contaminated soils were used for the extraction and fractionation of WSOM using Polyvinylpyrrolidone (PVP), CAS: 25249-54-1, cross linked, Thermo Scientific Chemicals, and single step extractions with alkali and acidic solutions. The concentrations of Sb in organic extracts were determined by ICP-MS. The experimental soil had a clay texture and with abundant amounts of nutrients, with the total nitrogen and estimated OM of 0.58 %, and 12 %, respectively, while organic carbon (OC%) content (Walkley & Black method) was estimated to be 7.4 % (Rayment and Lyons, 2011). Nevertheless, the DOC, Fe and Mn (aqua regia extractable) concentrations were 708, 22571 and 748 mg/kg, respectively. The DTPA extractable content of Fe and Mn were 130 and 50 mg/kg, respectively, and the soil acidity (1:5 water, S/L) was 7.1. Soil characteristics were determined based on (Rayment and Lyons, 2011) soil chemical methods. All single and sequential extractions in this research were performed in triplicate.

1.2. *Fractionation of water-soluble organic matter (WSOM) in soils*

1.2.1. *Extraction of soil water extract*

The WSOM fractionation procedure used in this study was based on the method previously used (Aoyama, 2002, 2006; Lowe, 1975) with modifications. For each extraction, 30 g of soil samples was shaken with 120 mL of distilled water for 2 h on the horizontal shaker and the resulting slurry was centrifuged at 8000 $\times g$ for 15 min. The supernatant was collected and the extraction of residues was repeated 5 times. The resulting soil extract were combined and passed through 0.45 µm syringe filter. The organic C and total N of extracts were determined by colorimetric method after dichromate addition and persulphate method.

1.2.2. *HA, PVP adsorbed FA and PVP-non-adsorbed FA fractions*

The 200 mL of water extract was placed in a small beaker and the pH of the extracts were adjusted to 1 using H_2SO_4 (1:5, V/V) and centrifuged at 12,000 r/min for 15 min. The residual fraction (dark precipitate) in each centrifuge tube was washed with 10 ml of distilled water and supernatant was collected using centrifugation. The supernatant and the washings were combined (PVP adsorbed FA + PVP-non-adsorbed FA fractions) and stored for further extractions.

1.2.3. *HA fraction*

The residual fractions were dissolved in 10 mL of 0.1 mol/L NaOH and combined. The pH of the extracts was adjusted to 8 with 0.5 mol/L H_2SO_4 and stored for further analysis.

1.2.4. *PVP non-adsorbed FA*

The final resulting solution was mixed with PVP (1 g/100 mL) and shaken for 1 h on a rotatory shaker (Aoyama, 2002, 2006; Lowe, 1975). The PVP was separated by passing the SOM suspension through an Whatman ashless filter paper and washed with 10 mL of 0.5 mol/L H_2SO_4. The filtrate and the washing were combined and adjusted to pH 8.0 with 0.5 mol/L NaOH and made up to a total volume of 300 mL with distilled deionized water (PVP non-adsorbed FA fraction). PVP was employed to separate fulvic acids based on adsorption affinity, without column chromatography. Rather, PVP was added directly to the aqueous solution, desorbed, and then separated through filtration for subsequent analysis.

1.2.5. *PVP adsorbed FA*

The soil organic fractions adsorbed to PVP were desorbed with 30 mL of 0.1 mol/L NaOH and adjusted to pH 8 with 0.5 mol/L HCl and made up to a total volume of 100 mL with distilled water.

1.3. *Single extractions*

1.3.1. *HCl*

5 g of soil was shaken with 25 mL of 0.5 mol/L HCl in 50 mL centrifuge tubes for 1 h and centrifuged at 25,000 ×*g* for 30 min and passed through 0.45 µm syringe filter (Fox et al., 2017).

1.3.2. *Sodium pyrophosphate (PP)*

25 mL of 0.1 mol/L $Na_4P_2O_7$ was shaken with 5 g of air-dried soil in 50 mL centrifuge tubes for 20 h, centrifuged at 25,000 ×*g* for 30 min and passed through 0.45 µm syringe filter (Fox et al., 2017).

1.3.3. *NaOH*

The 25 mL of 0.5 mol/L NaOH was shaken with 5 g of air-dried soil in 50 mL centrifuge tubes for 20 h, centrifuged at 25,000 ×*g* for 30 min and passed through 0.45 µm syringe filter (Fox et al., 2017).

1.4. *^{1}H-NMR and FTIR characterisation of WSOM fractions*

Spectroscopic Analysis FTIR and 1H NMR characterizations were performed to analyze the chemical properties of WSOM fractions. FTIR spectra were obtained using a Perkin Elmer FTIR spectrometer within the range of 650-4000 cm^{-1}, while 1H NMR spectra were recorded using a Bruker 500 MHz NMR spectrometer (USA) at UNE, Australia. For sample preparation, 20 mg of freeze-dried humic acids were dissolved in 0.6 mL of 0.1 mol/L NaOD/D_2O solution. Samples were filtered through glass wool, transferred to NMR tubes, and scanned. The spectra were analyzed by integrating areas under three chemical shift regions: 0.5-3 ppm (aliphatic H), 3-4.2 ppm (carbohydrate H), and 6-8 ppm (aromatic H), following standard protocols (Baglieri et al., 2007; Francioso et al., 2003). Additional methodological details, including regression and statistical analyses, are provided in the Results Section for coherence with spectroscopic and Sb fractionation data. Regression and statistical analysis were performed using Microsoft Excel and GenStat 12 software and differences between soil organic extracts were calculated by ANOVA using Duncan's multiple range test for means comparison ($P < 0.05$). The figures presented in this article were meticulously crafted using Microsoft Excel, and Python programming language and the powerful visualization library, Matplotlib.

2. Results and discussion

2.1. *Isolated WSOM characteristics*

2.1.1. *FTIR spectra of water extractable organic matter*

The FTIR spectra of WSOM is depicted in Fig. 1. The small band at about 1081 cm^{-1} could be assigned to C-O stretching vibrations of C-O-C groups in cellulose, while the intense peak at 1740 cm^{-1} could be ascribed to the stretching vibrations of the C=O group in the structure of cyclic and acyclic aldehydes and ketones as well as carboxylic acids. Furthermore, the adsorption bands observed at 1000-1100 cm^{-1} might be related to the Si-O-Si groups of silicates due to the presence of negligible ash content in isolated water-soluble organic matter. The intense signal at 1217 cm^{-1} could be ascribed to -C-O ester linkages and phenolic C-OH (Suman et al., 2020). The intense signal at 1372 cm^{-1} could be attributed to C-N stretching (Hestrin et al., 2019). The region below 900 cm^{-1} (peaks at 800, 780 and 695 cm^{-1}) might also be linked to the silicates and quartz (Nuzzo et al., 2020).

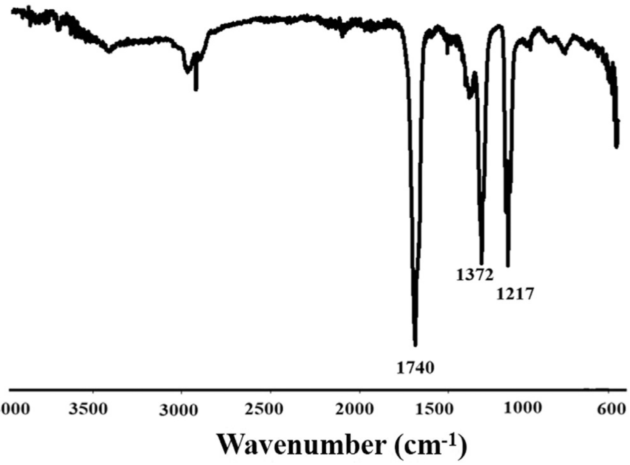

Fig. 1 – FTIR spectra of humic acid (HA) fraction of purified water-soluble organic matter (WSOM).

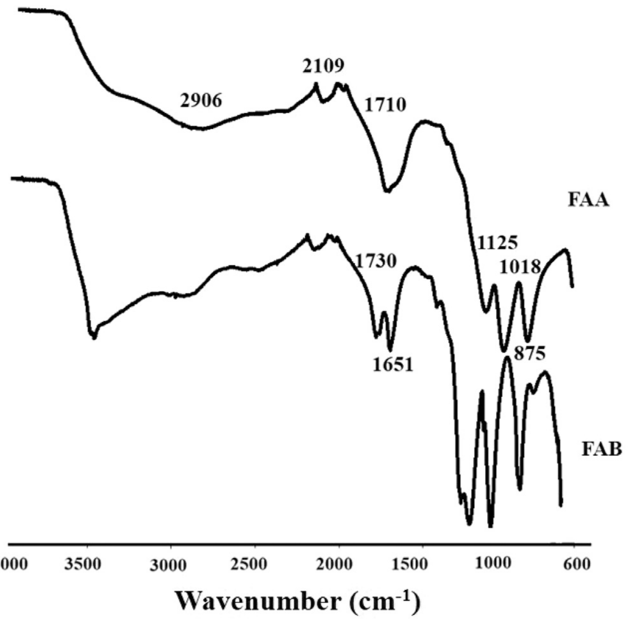

Fig. 2 – FTIR spectra of PVP-non adsorbed fulvic acids (FAA), and PVP-adsorbed fulvic acids (FAB) subfractions separated using polyvinylpyrrolidone (PVP).

The FTIR spectra of the PVP-non adsorbed FA and PVP adsorbed FA are illustrated in Fig. 2. The broad peak observed at 2906 cm^{-1} could be attributed to the C-H stretching of aliphatic groups of FA (Suman et al., 2020). The minor peak appeared at 2091 cm^{-1}, however, could be attributed to the C-H stretching of methyl and methylene groups of aliphatic chains (Yusuf and Audu, 2017). The sharp and intense band at 1710 cm^{-1} might be assigned to the C=O stretching band of carboxyl functions in soluble organic matter (Ellerbrock and Gerke, 2004), while dissociation of COOH functional group may bring about unmasking of other absorptions at about 1730-1720 cm^{-1} due to functions such as carbonyl groups in conjugated or heterocyclic moieties, whose absorption frequency range is slightly higher than functional carboxyl groups (Nuzzo et al., 2020). The signal at 1651 cm^{-1} in the FTIR peak of PVP- adsorbed FA might be related to either C=O stretch of conjugated quinones, ketones or to the C=O stretch of amide I groups, or could be due to the O-H bond of interlaminar water of clays minerals, depending on the ash content of the isolated FA fractions (Baglieri et al., 2007; Fischer, 1977). The sharp peak appeared at the 1125 cm^{-1} could be ascribed to -C-C, C-OH, C-O-C typical of glucosidic linkages, polymeric compounds and Si-O bonds in isolated organic matter fractions (Suman et al., 2020). The band at 1018 cm^{-1} could be assigned to C-O vibrations of polysaccharides (Parikh et al., 2014), while the band at 875 cm^{-1} might be due to carbonates remain in isolated FA fractions (Tatzber et al., 2007). Appearance of the peaks at the region below 900 cm^{-1} might also be linked to the silicates and quartz (Nuzzo et al., 2020).

2.1.2. 1H NMR spectra

The ^1H NMR spectra of water extractable, and alkali extractable organic fractions are illustrated in Fig. 3. It has been reported that ^1H NMR is more feasible than ^{13}C NMR for samples with high ash continent, it may underestimate the aromaticity of organics specially for some aromatic compounds (Baglieri et al., 2007; Stevenson, 1982a). The relative distribution of the corresponding region was calculated as % of the total area under the curve and is presented in Table 1. The spectra of organic silicon compounds (e.g., Si-R and Si-O-R) are appeared in the region of less than 0.5 ppm of the ^1H NMR spectra (Baglieri et al., 2007; Francioso et al., 2003; Kovac et al., 2002). The results showed that water extracts composed mostly of aliphatic (46 %) and aromatic compounds, while NaOH extracted organics mostly made up of aliphatic and carbohydrate H. Nonetheless, the highest content of aliphatic compounds was found to be associated with the $Na_4P_2O_7$ extractable organics, with 57 % of the total surface area under spectrum.

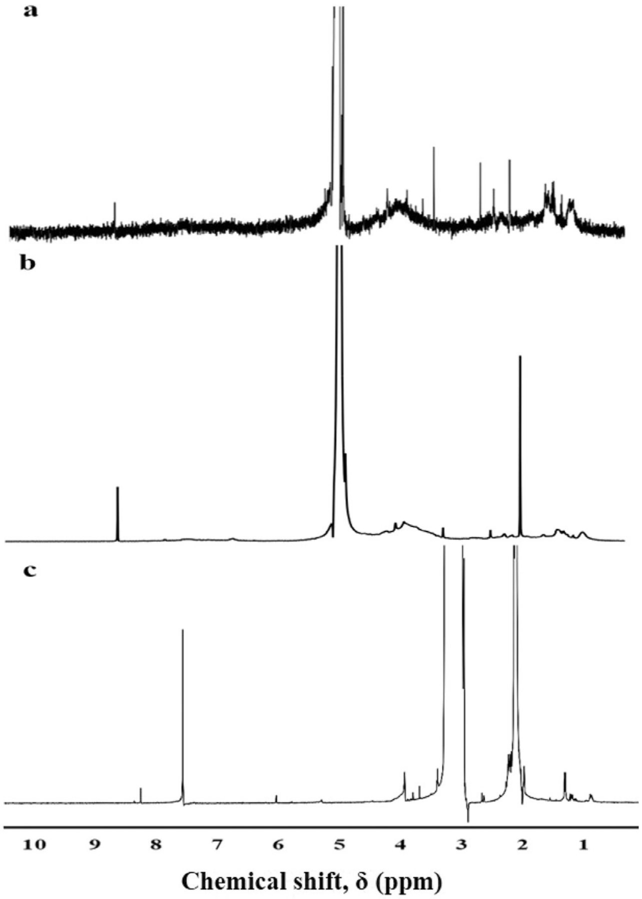

Fig. 3 – ^1H NMR spectra of (a) water-soluble organic matter (WSOM), (b) NaOH, and (c) $Na_4P_2O_7$ extractable soil organic matter (SOM).

Table 1 – Relative distribution of aliphatic, carbohydrates and aromatic H in SOM extracts.

Sample	Aliphatic H% 0.5 – 3 (ppm)	Carbohydrate H% 3 – 4.2 (ppm)	Aromatic H% 6 – 8 (ppm)
WE	46	23	31
NaOH	46	45	9
$Na_4P_2O_7$	57	35	8

WE: water extract.

2.2. PVP fractionation of humic acid and fulvic acids of WSOM

The results of chemical analysis of HA and FA fractions of isolated WSOM are presented in Appendix A Table S1. The results revealed that the concentrations of inorganic nitrogen forms (NO_3^-, NO_2^-, NH_4^+), phosphate (PO_4^{3-}) and total nitrogen content of FA extract were greater than HA extracts, whereas the total carbon, total inorganic carbon (TIC) were found to be greater in HA fraction of WSOM. Even though the concentrations of Al and Fe in HA were remarkably higher than FA, the amount of Sb associated with the FA fraction was greater than isolated HA, with 77 and 3 µg Sb/g dry soil for FA and HA, respectively. This is in harmony with the significantly higher TOC concentrations of FA compared with that of HA, demonstrating the central role of organic carbon in adsorption mechanisms and controlling the chemistry of Sb in soils. The strong correlations between Sb and TOC have been further discussed in Section 2.4.

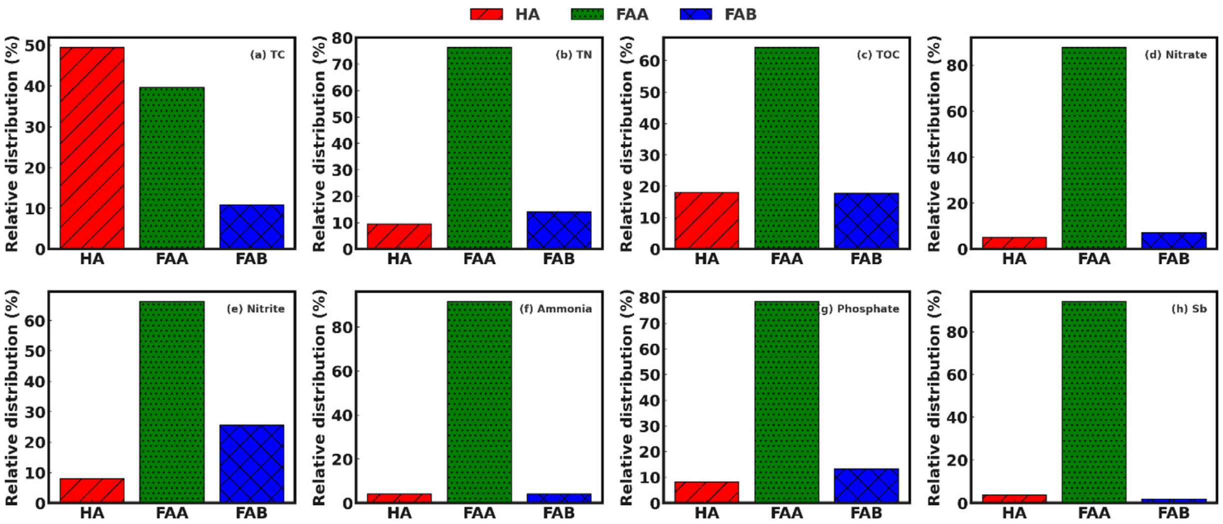

Fig. 4 – Relative distribution of (a) total carbon, (b) total nitrogen, (c) total organic carbon, (d) nitrate, (e) nitrite, (f) ammonia, (g) phosphate, and (h) Sb in humic acid and polyvinylpyrrolidone PVP fractions.

2.3. *The relative distribution of C, N and P forms in HA and PVP extracted FA fractions*

Distribution of TC, TOC, TIC (total inorganic carbon), TN, phosphate and inorganic nitrogen forms in humic and fulvic acid fractions are depicted in Fig. 4. The results revealed that more than 49 % of the total carbon in soil water extracts was associated with the HA fraction, followed by the FAA and FAB fractions, whose C content accounted for 40 % and 11 % of the total carbon respectively. However, FAA fraction accounted for 76 % of the total N of the soil water extracts, which was the largest N bearing constituents of WSOM subfractions. Furthermore, the relative distribution of inorganic N forms in WSOM extracts showed that the FAA fractions are responsible for 92 %, 88 % and 66 % (Fig. 4d, e, and f) of the concentrations of ammonium, nitrate and nitrite in soil extracts. In the nitrogen mineralization process, microorganisms break down complex large proteins into amino acids, amides, and amines through the aminization, and the amino group consequently are converted to ammonium. Through the activities of Nitrosomonas and Nitrobacter, ammonium is transformed to nitrate and nitrate. While nitrite is considered toxic to plants, ammonium (NH_4^+) and nitrate (NO_3^-) are the only phytoavailable inorganic forms of nitrogen in soils. Even though ammonium is more readily available in some soils, NO_3^- are preferentially taken up by plant roots due to its high diffusion coefficient (Cruz et al., 2021; Datta et al., 2019). Furthermore, FAA fraction was responsible for 79 % of the total amounts of dissolved phosphate in WSOM fractions, which is the most readily bioavailable form of phosphorus (Reynolds and Davies, 2001). The results of this article are in agreement with those of (Aoyama, 2006) who studied the properties of bioavailable organic matter in soil extracts during the incubation period and reported that the HA fraction disappeared and organic C and N in the PVP-non-adsorbed FA fraction (FAA fraction in the present study) declined considerably, showing that HA and PVP-non-adsorbed FA (FAA) are the more bioavailable fractions. Hence, the predominance of inorganic and organic forms of nitrogen and phosphorus in FAA fraction of WSOM might be attributed to the higher phytoavailabilty and bioavailability of this subfraction to plants and microorganisms. FAA accounted for the largest fraction of TOC pool with 64 % of total organic carbon reservoir of WSOM. The findings of this research are in agreement with (Aoyama, 2002) who showed that the PVP-non-adsorbed fulvic acid fraction contained the largest concentrations of the total water-soluble organic carbon pool, depending on the soil type. Moreover, Sb was mostly associated with FAA fraction and 94 % of the total Sb pool in WSOM was found to be bound with PVP non adsorbed fraction of FA, whereas only negligible amount of Sb was associated with HA and FAB fractions, with 4 % and 2 % of the total Sb in WSOM, respectively. Water soluble fraction of heavy metals in soils has been used for assessing their environmental availability and risk assessments (Wang et al., 2025). Fan et al. (2019) demonstrated that specific functional moieties, such as amide I and hydroxyl groups from phenols, display significant binding affinity for antimonate (Sb(V)) under natural pH conditions. Dissolved organic matter (DOM) plays a crucial role in modulating the bioavailability of heavy metals within terrestrial ecosystems. Additionally, it has been identified that water-extractable organic matter of higher molecular weight substantially enhances the formation and stability of soil aggregates (Sonsri and Watanabe, 2023). Variations in dissolved organic matter types critically affect the bioavailability of heavy metals and microbial activity in the rhizosphere of a plant-wetland soil system (Li et al., 2021). Li et al. (2019) observed that nitrogen supplementation expedites the transition of heavy metal speciation from bioavailable to organically bound forms by prolonging the organic matter decomposition period. Zhong et al. (2023) investigated the efficacy of soluble straw-derived humic substances in remediating soils contaminated with various heavy metals, utilizing spectroscopic and mass spectrometric techniques to elucidate the contribution of phenolic, carboxylic, and aromatic components in hu-

Table 2 – Correlation coefficients of soil parameters in WSOM.

	Total N (µg/g)	TC (µg/g)	TIC (µg/g)	TOC (µg/g)	Sb (µg/g)	C/N ratio	CO/N ratio	E2/E3	E4/E6
TN(µg/g)	1.00								
(TC) (µg/g)	0.97***	1.00							
(TIC)(µg/g)	ns	ns	1.00						
(TOC)(µg/g)	0.97***	1.00	ns	1.00					
Sb (µg/g)	0.88***	0.94***	ns	0.95***	1.00				
C/N ratio	ns	ns	ns	ns	ns	1.00			
CO/N ratio	ns	ns	ns	ns	ns	ns	1.00		
E2/E3	-0.88***	-0.83***	ns	-0.83***	-0.68**	ns	ns	1.00	
E4/E6	-0.72**	-0.80***	ns	-0.80***	-0.77**	ns	ns	0.42**	1.00

ns: not significant, ***significant at $p < 0.01$, **significant at $p < 0.05$.

mic substances to the formation of complexes that immobilize heavy metals. They found that the processed aerobic humic substances effectively removed metals from soils. DOM is also implicated in the detoxification mechanisms for metalloids in soils. The bioavailability of SOM within WSOM can significantly impact the toxicity of Sb in soils. These organic matter forms can bind with Sb, affecting its mobility and availability for uptake by plants and microorganisms. Enhanced bioavailability of organic matter can lead to the formation of complexes with Sb, potentially affecting its toxicity by alteration of its solubility and mobility. Ultimately, the interplay between organic matter forms and heavy metals in soils determines the extent of its toxicity, affecting plant growth, microbial activity, and overall soil health. Hence, it is essential to consider the complex interactions between Sb and organic matter when assessing soil contamination and implementing remediation strategies (Bagherifam et al, 2023b). It has been shown that PVP-none adsorbed fraction of fulvic (FAA fraction in our study) is largely composed of non-humic substances such as carbohydrates and peptides (Aoyama, 2002). Amino acids in the soil solution are formed as a result of the microbial conversion of N in proteins to NH_3 (Proteins → peptides → amino acids → NH_3) (Stevenson, 1982b).

Due to the dominance of TOC, N and Sb in FAA subfraction, it could be partly explained by the high affinity between the amin functional groups or reactions of soluble organic N compounds and antimony species in soil solution. Furthermore, this finding highlights the importance of organic carbon in environmental chemistry of Sb as FAA subfraction was found to be containing the largest reservoir of organic carbon of WSOM.

2.4. *Correlation between experimental parameters and Sb*

The correlations between WSOM and Sb are presented in Table 2 and Appendix A Fig. S1. The results revealed that there are significant and high correlations between experimental parameters, but the highest correlations were found between Sb, TOC, TC and TN. Furthermore, the concentrations of Sb were negatively correlated with the E2/E3 and E4/E6 values in the studied soils. SOM can greatly affect the bioavailability and adsorption behavior of Sb oxyanions in soils (Dousova et al., 2015). Tella and Pokrovski (2009) elucidated the significant interaction between antimonite and dissolved organic carbon (DOC) through carboxylic groups. They highlighted the capacity of humic substances to facilitate the rapid photooxidation of antimonite. Buschmann et al. (2005) documented a swift photooxidation process from Sb(III) to Sb(V) mediated by humic acids. Inam et al. (2019) conducted research on the complex formation between humic acid (HA), salicylic acid (SA), and L-cysteine (L-cys) with antimony in its trivalent Sb(III) and pentavalent Sb(V) forms, finding that hydrophobic HA had a higher binding affinity for Sb compared to more hydrophilic forms of natural organic matter, with the resulting complexes showing greater stability for Sb(V) over Sb(III). Additionally, it was observed that natural organic matter significantly hampers the removal of Sb during the coagulation process using ferric chloride, as reported by Guo et al. (2009).

Total organic carbon (TOC) is pivotal in facilitating the mobilization of antimony (Sb) in aqueous environments, with studies indicating that hydrophobic humic substances enhance the mobility of Sb in environments affected by alkalinity (Guo et al., 2009). Furthermore, the interaction between HA and Fe complexes is known to decrease Sb adsorption onto iron oxides (Gu et al., 1994), thereby augmenting the bioavailability of Sb by inducing a preferential adsorption of Fe onto organic substrates (Verbeeck et al., 2019). WSOM is crucial for the mobility and complexation of Sb in soils, promoting the formation of Sb-organic matter complexes and the oxidation of antimonite. The soil organic nitrogen fraction, primarily consisting of amino acids, amino sugars, nucleic acids, and other nitrogenous organic compounds, plays a significant role in soil chemistry (Stevenson, 1982b). The protonation of amine groups linked to carboxylic acid functionalities within soil organic matter can enhance the attraction of anions from the soil solution to the positively charged amine sites, owing to their electron pairs, despite the prevalence of negative charges from carboxylic and phenolic groups over positive charges (Strawn et al., 2019). Sb was predominantly associated with the low molecular weight FA fractions (96 %) of WSOM and only small amounts of Sb was bound to humic acids. Hence, the high correlations observed between TOC, TN and Sb could be attributed to the formation of WSOM-Sb complexes and N- containing organic matter such as amin functional groups.

The E2/E3 and E4/E6 values in WSOM after each sequential extraction and mixture of all 5 extraction steps (AVG) are presented in Appendix A Table S2. Humic acids with E4/E6 values of less than 5 are considered more aromatic (Steely et al., 2007; Stevenson, 1982a). Likewise, the lower the E2/E3 ratio, the

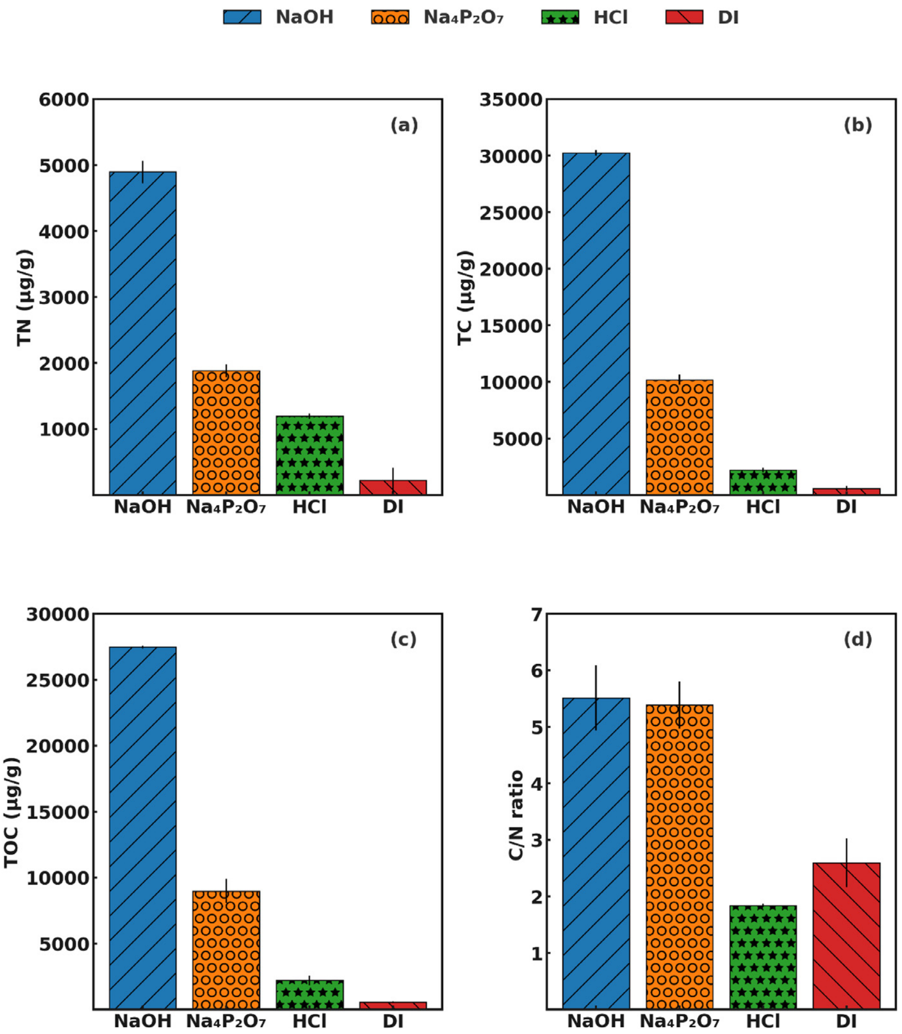

Fig. 5 – Extraction efficiency of (a) total nitrogen (TN), (b) total carbon (TC), (c) toral organic carbon (TOC) and (d) C/N ratio in soil extracts.

more aromatic the organic compound is (Steely et al., 2007). Based on the E4/E6 values, the degree of aliphaticity of WSEOM increased after the first two extraction stage in soil organic solutions. E4/E6 after the second extraction ranged between 5.13 and 5.71, showing the more aliphatic nature of the water extract with increasing the extraction steps. The mixture of all WSOM extracts (WSOM-AVG) showed the E4/E6 value of 0.12 ± 0.11, suggesting the WSEOM was in a high degree of aromaticity, which was somewhat in agreement with the trend observed for E2/3. However, these trends might be different in various soils, depending on the soil chemical properties and organic matter composition.

2.5. *Efficacy of single extraction in the removal of TN, TC, TOC and Sb from soils*

Formation of complexes and bridges between Fe and Al oxyhydroxides and SOM plays an important role in chemical behaviour, leaching processes and bioavailability of metal(loids) in soils. Numerous efforts have been made to target Fe and Al fractions associated with SOM. Alkaline extracts such as NaOH have long been used for extraction of SOM from soils (Stevenson, 1982a; Strawn et al., 2019). Sodium pyrophosphate alkali extract has been used to specifically solubilize SOM associated with mineral surfaces, ligands, cation bridges and organic matter occluded in metal-SOM complexes due to their metal-chelating properties (Ellerbrock and Kaiser, 2005; Fox et al., 2017). However, HCl extraction is mostly used for dissolution of amorphous or easily reducible Fe-oxides and SOM associated with them, and therefore it releases acid-soluble organic matter (Chao and Zhou, 1983; Fox et al., 2017). As can be seen in Fig. 5, the amounts of extractable TN and TC in extracting solution varied between 223-4895 μg/g soil and 581-30241 μg/g soil, respectively. The extracting efficiency for TN and TC followed the order: NaOH> $Na_4P_2O_7$>HCl>DI water. A similar decreasing trend was observed in the case of TOC in soil extracts, and the highest concentration of TOC was found to be 27428 μg/g soil in NaOH extract. The result was in contrast with those of (Fox et al., 2017) who studied the extraction efficiency of OC in low carbon sediments and reported a sequence $Na_4P_2O_7$> NaOH >HCl>DI. The observed differences could be attributed to the variations of physiochemical and

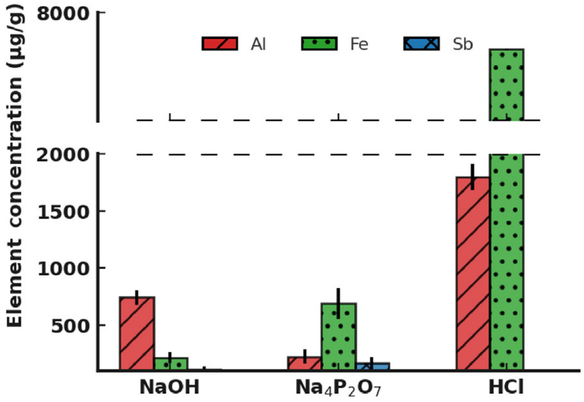

Fig. 6 – Concentrations of extractable Fe, Al and Sb using acidic and alkaline extractants.

mineralogical properties of authors' studied sediments from our experimental soils. It has been reported that a considerable amount of N in soils are recovered as acid insoluble, which could be because N exists in the molecular structure of humic substances (Stevenson, 1982b). The published literature on the recovery of acid-soluble forms of N are conflicting. While Cheng et al. (1975) reported that HF pre-treatment increased the acid-soluble N recovery due to the release of amino acids, other studies showed that acid insoluble N fraction reduced dramatically by using dilute base (Griffith et al., 1976; Stevenson, 1982b). In the present research, the amounts of TN, TC and TOC removal by HCl were moderately higher than DI water, and the C/N ratio in DI water extract was higher than HCl. It could be assumed that at lower C:N ratio, N would be more readily available to be released into the soil, and that decreasing C:N ratio could lead to increasing nitrate leaching rate (Brust, 2019; Dise et al., 1998).

2.6. *Extractable Fe, Al and Sb concentrations in soils*

The amounts of extractable Fe, Al and Sb using acidic and alkaline extractants are illustrated in Fig. 6. The results revealed that HCl extracted the maximum amounts of Al and Fe from soils with extraction yields of 1795 and 5968 μg/g soil, respectively. Even though NaOH extracted more Al from soils than $Na_2P_4O_7$, the amounts of Fe release from soils were higher in $Na_4P_2O_7$ extracts. In case of Sb, the highest concentrations of Sb were extracted with $Na_4P_2O_7$ solutions, with 165 μg/g soil. The Sb extractability sequence followed the order of $Na_4P_2O_7 >$ NaOH> HCl. Kaiser and Guggenberger (2000) reported the correlations between Al and Fe oxyhydroxides and the concentrations of organic carbon in soils. Soil physiochemical characteristics such as organic matter content, dissolved organic matter and Fe content of the soils can greatly influence the Sb bioavailability in soils. The interaction between Sb species and Fe and Al hydroxides has been a significant focus of research in recent years. He et al. (2015) explored the adsorption dynamics of Sb(III) and Sb(V) on freshly synthesized ferric hydroxide, noting that adsorption equilibrium was reached within 2 h. They identified the maximum adsorption capacities as 12.77 and 10.21 mmol/g for Sb(III) and Sb(V), respectively, proposing the establishment of inner-sphere complexes with ferric hydroxide. Research indicates that both Sb(III) and Sb(V) exhibit stronger affinities for Fe, Mn, and Al hydroxides compared to their interaction with clay minerals (Leuz and Johnson, 2005). Besold et al. (2019) discovered that the immobilization of Sb in peatlands was predominantly influenced by its binding to thiol and oxygen-rich functional groups within organic matter at greater depths, and its associations with Fe-containing phases closer to the surface. Phyllosilicates tend to from close associations with SOM via cation bridges facilitated by Al^{3+} and Fe^{3+}, which could promote soil aggregation (Strawn et al., 2019). It is evident that although the concentrations of Fe and Al extracted with HCl were dramatically higher than those of NaOH and $Na_4P_2O_7$, the amounts of extractable Sb associated with this fraction was considerably lower than NaOH and $Na_4P_2O_7$. Furthermore, the highest concentrations of Al (743 μg/g soil) were extracted with NaOH alkali solution in which Sb concentration was moderately lower than $Na_4P_2O_7$. Moreover, $Na_4P_2O_7$ extracted the maximum concentrations of Fe and Sb with 692 and 165 μg/g soil, respectively. The results revealed that releasing Fe and Al with alkali extracts brought about the dramatic increase in Sb removal from soils. Hence, the findings of this research indicate the central role of Fe-SOM as well as Al-SOM bridges in controlling the bioavailability and mobility of Sb in soils.

3. Conclusions

In order to elucidate the characteristics and Sb binding behaviour of WSOM, a fractionation method was used to isolate three dissimilar fractions based on their acid solubility and affinity to PVP. It was found that 94 % of Sb in WSOM was associated with FAA fraction, while in WSOM almost all Sb was bound to FA fraction (97 %). Single extraction analyses demonstrated that the concentrations of Fe and Al were higher in hydrochloric acid (HCl) extractions compared to other solvents, while Sb was more effectively mobilized by alkaline extracts that interacted with Fe/Al-soil organic matter (SOM) linkages. This suggested a pivotal role of these metal-organic frameworks in the immobilization of Sb within soil matrices. The FA fraction exhibited the highest levels of TOC, TN, and Sb, implicating those non-humic entities within FA, such as amines, could significantly influence the bioavailability and translocation of Sb in soil environments. Nonetheless, comprehensive investigations across diverse soil types with varied physicochemical attributes and SOM compositions employing this fractionation method are essential to elucidate the intricate interactions between WSOM and Sb.

Declaration of competing interest

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

CRediT authorship contribution statement

Saeed Bagherifam: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Trevor C. Brown:** Writing – review & editing, Supervision, Funding acquisition. **Andrea Baglieri:** Writing – review & editing. **Binoy Sarkar:** Writing – review & editing. **Jörg Rinklebe:** Writing – review & editing.

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Appendix A Supplementary data

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jes.2025.03.001.

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