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Structural characterization of humic acids from chromium and nickel polluted and unpolluted Alfisols Inceptisols and Vertisols of India

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Abstract

Heavy metal contamination significantly alters soil organic matter dynamics, particularly the structural and functional characteristics of humic acids (HA), with implications for metal mobility and soil quality. This study characterized HA extracted from metal-polluted and adjoining non-polluted soils across three major Indian soil orders: Alfisols (Sukinda Valley, Odisha; Cr-polluted), Inceptisols (Sonipat, Haryana; Ni-polluted), and Vertisols (Dewas, Madhya Pradesh; Cr/Ni-polluted). Soil physicochemical properties and HA characteristics, including E4/E6 ratio, total acidity, carboxylic and phenolic OH content, and FTIR spectral features, were evaluated. Soil order was the dominant determinant of pH, texture, electrical conductivity, organic carbon, and cation exchange capacity, with Vertisols showing the highest clay content and CEC due to smectitic mineralogy, while Alfisols were acidic with lower CEC. Metal pollution reduced the E4/E6 ratio across all soil orders, most markedly in Vertisols, indicating enhanced humification and aromatic condensation under metal stress. Total and carboxylic acidity declined under pollution, reflecting preferential complexation of metals with carboxyl groups, whereas phenolic OH content showed a slight increase, suggesting oxidative structural rearrangement. FTIR spectra corroborated these findings, revealing intensified carbonyl and aromatic absorption bands alongside diminished aliphatic and polysaccharide signals in polluted soils, particularly in Alfisols and Inceptisols, while Vertisols exhibited greater structural resilience owing to strong clay–humus interactions. These results demonstrate that inherent soil mineralogy governs the magnitude of pollution-induced HA

transformation, with heavy metal stress consistently driving humic structures toward more condensed, aromatic, and recalcitrant forms, offering insights relevant to soil carbon stabilization and remediation strategies.
Keywords: Humic acid; Heavy metal pollution; FTIR spectroscopy; E4/E6 ratio; Soil organic matter

INTRODUCTION

Heavy metal contamination has emerged as a global environmental concern owing to its adverse impacts on ecosystems, agricultural productivity, and human health (Li *et al*., 2006). Heavy metals are introduced into soil, water, and air through both natural and anthropogenic sources. Natural processes such as rock weathering, geological transformations, and soil erosion contribute to the release of metals into the environment (Singh *et al*., 2023). However, rapid industrialization, mining activities, indiscriminate use of agrochemicals, urbanization, and industrial effluent discharge have significantly intensified metal accumulation in environmental compartments (Raj and Maiti, 2020). Human exposure to toxic metals, particularly cadmium (Cd), lead (Pb), and copper (Cu), through contaminated food and water can result in severe health disorders, including neurological impairment, haemolysis, metabolic dysfunction, renal and hepatic damage, reproductive abnormalities, and diseases such as Wilson's disease and itai-itai disease (Islam *et al*., 2021; Golui *et al*., 2019; Mishra *et al*., 2018). Due to their non-biodegradable nature and long-term persistence in the environment, heavy metals pose a significant threat to food safety, environmental quality, and public health, necessitating effective monitoring and remediation strategies.

Among the various remediation approaches, in situ technologies employing naturally occurring attenuating agents have gained considerable attention because of their cost-effectiveness and environmental sustainability. Humic substances (HS), particularly humic acids, play an important role in regulating the mobility, bioavailability, and fate of heavy metals in soil and aquatic environments. Humic acids are dark-colored, amorphous organic fractions formed through the chemical and biological decomposition of plant and animal residues. These substances possess complex macromolecular structures characterized by varying degrees of aromaticity and a high abundance of reactive functional groups, notably hydroxyl (-OH) and carboxyl (-COOH) groups (Khan and Schnitzer, 1972; Stevenson, 1994). Such functional groups provide strong metal-binding capacity, enabling humic acids to adsorb, chelate, and complex with metal ions, thereby influencing their chemical speciation and environmental

behaviour. Through these mechanisms, HS not only reduce the bioavailability of toxic metals but also contribute to soil structure, nutrient cycling, and overall ecosystem resilience, highlighting their significant potential in sustainable remediation practices.

MATERIAL AND METHOD

Soil sampling

Soil samples were collected from selected metal-polluted sites and adjoining non-polluted areas across different soil orders of India. Polluted sites were chosen based on proximity to industrial zones, mining areas, and wastewater-irrigated agricultural lands, while non-polluted sites served as controls.

1. Soil 1: Sukinda valley, Jajpur, Odisha (Alfisols)-Cr polluted (chromite mining)
2. Soil 2: Sonipat, Haryana (Inceptisols)-Ni polluted (Atlas cycle factory effluent irrigated)
3. Soil 3: Dewas Industrial area, Madhya Pradesh (Vertisols)-Cr, Ni polluted (industrial effluent irrigated)

From each location, composite samples were taken from the topsoil (0–15 cm depth) and subsoil (15-30 cm depth) and were air-dried, ground, and passed through a 2 mm sieve for further analysis.

General characteristics of the soils

Soil pH was measured in a 1:2.5 (soil: water) ratio using a combined electrode. At 25°C, the electrical conductivity (EC) of the supernatant liquid was determined with Conductivity Bridge and expressed in dS m^{-1} (Jackson, 1973). The hydrometer technique was used to determine the mechanical composition of the experimental soil, i.e., the proportion of sand, silt, and clay size particles (Bouyoucos, 1962). The USDA proposed textural triangle was used to determine the texture of the soil (Brady and Weil, 1999). The soil's cation exchange capacity (CEC) was determined using Jackson's (1973) ammonium acetate method. The soil sample was sieved to pass through a 0.2 mm sieve for the measurement of oxidizable organic carbon. The organic carbon content of soil was determined using the Walkley and Black (1934) wet oxidation technique with $K_2Cr_2O_7$.

Extraction of humic acid from soil samples

Humic acid was extracted from soil samples following the IHSS method (Swift, 1991). Soil samples were acidified (pH 1-2) with HCl to remove non-humic components and then extracted with 0.1 N NaOH under a nitrogen atmosphere. The alkaline extract was acidified to pH 1 using 6 M HCl, causing humic acid to precipitate while fulvic acid remained in solution. The humic acid fraction was separated by centrifugation, purified through repeated dissolution and precipitation steps using KOH and HCl, and further treated with HCl-HF to remove mineral impurities. The purification process was continued until the ash content was below 1%. Finally, the humic acid was dialyzed against distilled water until free of chloride ions.

Characterization of Humic Acid

The E4/E6 ratio was determined by measuring absorbance at 465 and 665 nm after dissolving humic acid in 0.05 N $NaHCO_3$ solution (Chen *et al*., 1977). This ratio provides an indication of the degree of humification and molecular complexity of humic substances.

Total acidity and carboxylic acidity were determined following Schnitzer and Gupta (1965). Phenolic hydroxyl content was calculated as the difference between total acidity and carboxylic acidity.

Purified humic acid samples were vacuum-dried, finely ground, and analyzed using a Bruker Alpha FTIR spectrometer. Spectra were recorded in the range of 4000-600 cm^{-1} to identify the major functional groups and characterize the chemical structure of the humic acid.

RESULT

The study (**Table 1**) revealed significant variations in soil properties across soil orders. Alfisols (Sukinda Valley, Odisha) were loamy sand, Inceptisols (Sonipat, Haryana) sandy loam, and Vertisols (Dewas Industrial area) clay with 50.3% clay content. Soil pH varied significantly with soil order, while pollution level and depth showed no statistical effect. Alfisols were acidic (≈5.8), increasing slightly under pollution (≈6.12), Inceptisols remained near-neutral to slightly alkaline (7.57–7.85), and Vertisols were alkaline (7.99–8.52). Electrical Conductivity (EC) also differed significantly by soil order (CD = 0.048, P = 0.05), but not by pollution or depth. Polluted Alfisols and Inceptisols exhibited higher EC than non-polluted samples, whereas Vertisols showed a mixed trend—an increase at 0–15 cm (0.57 dS m^{-1}) and a decrease at 15–30 cm (0.18 dS m^{-1}).

The data on organic carbon (OC) and cation exchange capacity (CEC) under different

soil orders and pollution levels are presented in **Table 2**. In Alfisols (Sukinda Valley, Odisha), OC ranged from 1.20 to 1.59 g/100 g, being slightly higher in polluted soils (1.59 and 1.24 g/100 g at 0–15 cm and 15–30 cm) than in non-polluted soils (1.53 and 1.20 g/100 g). However, CEC was lower in polluted soils (10.6 and 6.2 cmol(p^+)/kg) compared to non-polluted (12.3 and 8.1 cmol(p^+)/kg). In Inceptisols (Sonipat, Haryana), OC was relatively low (0.65–0.89 g/100 g), with polluted soils showing higher values (0.89 and 0.77 g/100 g) than non-polluted (0.70 and 0.65 g/100 g). CEC showed little variation between polluted (16.3 and 11.0 cmol(p^+)/kg) and non-polluted soils (16.7 and 11.2 cmol(p^+)/kg). Vertisols (Dewas Industrial Area) had OC between 1.08 and 1.17 g/100 g, slightly lower in polluted soils (1.15 and 1.08 g/100 g) than non-polluted (1.17 and 1.09 g/100 g). CEC was markedly higher in Vertisols, with non-polluted soils recording 50.9 and 47.0 cmol(p^+)/kg, and polluted soils slightly reduced (48.6 and 43.9 cmol(p^+)/kg).

Table 1 Texture, pH and EC of polluted and non-polluted soils under major soil orders of India

Soil orders		**pH**		**EC (dS m^{-1})**		**Clay (%)**	**Texture**
		0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**		
Alfisol (Sukinda valley, Odisha)	NP	5.86	5.82	1.49	1.47	6.46	Loamy sand
	P	5.64	6.12	1.58	1.57		
Inceptisol (Sonipat, Haryana)	NP	7.66	7.85	0.76	0.88	14.1	Sandy loam
	P	7.57	7.62	0.98	0.93		
Vertisol (Dewas Industrial area)	NP	8.03	8.52	0.32	0.47	50.3	Clay
	P	7.99	8.02	0.57	0.18		
CD (*P=0.05*)		A-0.215, B-NS, C-0.175		A-0.048, B-NS, C-NS			
A-Soil order, B-Pollution level, C-Depth							

Table 2 Organic carbon and CEC of soil collected from polluted and non-polluted soils under major soil orders of India

Soil orders		**OC (g/100g)**		**CEC (Cmol(p^+)/kg)**	
		0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**
Alfisol (Sukinda valley, Odisha)	NP	1.53	1.20	12.3	8.1
	P	1.59	1.24	10.6	6.2
Inceptisol (Sonipat, Haryana)	NP	0.70	0.65	16.7	11.2
	P	0.89	0.77	16.3	11.0
Vertisol (Dewas industrial area)	NP	1.17	1.09	50.9	47.0

	P	1.15	1.08	48.6	43.9
CD (*P=0.05*)		A-0.039, B-0.032, C-0.032		A-1.68, B-1.37, C-1.37	
A-Soil order, B-Pollution level, C-Depth					

Table 3 The E4/E6 ratio of humic acid extracted from different metal polluted and non polluted soils under major soil orders of India

E4/E6 ratio			
	NP	**P**	**Mean A**
Alfisol (Sukinda valley, Odisha)	5.11	5.03	5.07
Inceptisol (Sonipat, Haryana)	4.31	4.27	4.29
Vertisol (Dewas industrial area)	3.63	3.35	3.49
Mean B	4.35	4.22	
Factors	Factor(A)	Factor(B)	Factor (A X B)
CD (*P=0.05*)	0.123	0.1	N/A

The E4/E6 ratio of humic acid extracted from soils under different soil orders showed significant variation between metal polluted (P) and non-polluted (NP) conditions (Table 3). Among the soil orders, the highest mean E4/E6 ratio was observed in Alfisol (5.07), followed by Inceptisol (4.29), whereas the lowest value was recorded in Vertisol (3.49).

Between pollution conditions, non-polluted soils (4.35) exhibited slightly higher mean E4/E6 ratios compared to polluted soils (4.22). In Alfisol, the E4/E6 ratio decreased marginally from 5.11 (NP) to 5.03 (P), while in Inceptisol it decreased from 4.31 (NP) to 4.27 (P). A more pronounced reduction was observed in Vertisol, where the ratio declined from 3.63 (NP) to 3.35 (P).

Table 4 Total acidity of humic acid extracted from different metal polluted and non-polluted soils under major soil orders of India

Total acidity (meq/g)			
	NP	**P**	**Mean A**
Alfisol (Sukinda valley, Odisha)	6.20	6.23	6.28
Inceptisol (Sonipat, Haryana)	5.98	5.91	5.95

Vertisol (Dewas industrial area)	3.84	3.68	3.76
Mean B	5.34	5.27	
Factors	Factor(A)	Factor(B)	Factor (A X B)
CD (*P=0.05*)	0.097	N/A	N/A

The total acidity of humic acid extracted from soils under different soil orders exhibited significant variation between metal polluted (P) and non-polluted (NP) conditions (Table 4). Among the soil orders, the highest mean total acidity was recorded in Alfisol (6.28 meq g^{-1}), followed by Inceptisol (5.95 meq g^{-1}), whereas the lowest value was observed in Vertisol (3.76 meq g^{-1}).

Across pollution conditions, non-polluted soils showed a slightly higher mean total acidity (5.34 meq g^{-1}) compared to polluted soils (5.27 meq g^{-1}). In Alfisol, the total acidity increased marginally from 6.20 (NP) to 6.23 (P). In contrast, a slight decline was observed in Inceptisol (from 5.98 NP to 5.91 P) and Vertisol (from 3.84 NP to 3.68 P).

The critical difference (CD) at 5% probability for soil orders (Factor A) was 0.097, indicating statistically significant variation among soil types. However, Factor B (pollution status) and the interaction effect (A × B) were found to be non-significant.

Table 5 Carboxylic and Phenolic OH group of humic acid extracted from different metal polluted and non-polluted soils under major soil orders of India

	Carboxylic OH			**Phenolic OH**		
	NP	**P**	**Mean A**	**NP**	**P**	**Mean A**
Alfisol (Sukinda valley, Odisha)	3.67	3.63	3.65	2.53	2.60	2.56
Inceptisol (Sonipat, Haryana)	3.46	3.33	3.40	2.52	2.58	2.55
Vertisol (Dewas industrial area)	2.44	2.28	2.36	1.40	1.40	1.40
Mean B	3.19	3.08		2.15	2.19	
Factors	Factor(A)	Factor(B)	Factor (A X B)	Factor(A)	Factor(B)	Factor (A X B)

CD (*P=0.05*)	0.094	0.077	N/A	0.075	N/A	N/A

The content of carboxylic and phenolic OH groups of humic acid varied across soil orders and pollution conditions **(Table 5).** For the carboxylic OH group, the highest mean value was recorded in Alfisol (3.65 meq g^{-1}), followed by Inceptisol (3.40 meq g^{-1}), whereas the lowest value was observed in Vertisol (2.36 meq g^{-1}). Across pollution conditions, non-polluted soils exhibited a higher mean value (3.19 meq g^{-1}) compared to polluted soils (3.08 meq g^{-1}). In Alfisol and Inceptisol, a slight decrease in carboxylic OH was observed under polluted conditions (from 3.67 to 3.63 and from 3.46 to 3.33, respectively). A more pronounced reduction occurred in Vertisol (from 2.44 NP to 2.28 P). For the phenolic OH group, Alfisol (2.56 meq g^{-1}) and Inceptisol (2.55 meq g^{-1}) recorded higher mean values, while Vertisol showed the lowest (1.40 meq g^{-1}). Interestingly, polluted soils exhibited slightly higher phenolic OH content (2.19 meq g^{-1}) compared to non-polluted soils (2.15 meq g^{-1}). In Alfisol and Inceptisol, the phenolic OH increased marginally under polluted conditions (from 2.53 to 2.60 and from 2.52 to 2.58, respectively), whereas Vertisol showed no change (1.40 under both conditions).

Characterization of HA extracted from polluted and non-polluted soils of Alfisol by FT-IR

The FT-IR spectra of humic acid (HA) extracted from non-polluted (NP) and polluted (P) Alfisol soils of Sukinda Valley revealed the typical functional groups associated with humic substances; however, distinct differences were observed between the two soils, indicating the influence of pollution on HA composition **(Figure 1).** Both spectra exhibited major absorption bands around 3400 cm^{-1} (O–H and N–H stretching), 2920–2850 cm^{-1} (aliphatic C–H), 1720–1650 cm^{-1} (C=O of carboxylic and carbonyl groups), 1620–1600 cm^{-1} (aromatic C=C and COO^{-}), 1420–1380 cm^{-1} (COO^{-} symmetric stretch), and 1250–1100 cm^{-1} (C–O stretching of polysaccharides and alcohols). Compared to the non-polluted soil, the polluted Alfisol showed stronger peaks in the 1720–1650 cm^{-1} region, indicating an increased abundance of carbonyl and carboxyl groups, possibly due to oxidation or metal–organic complexation. The aromatic region around 1600 cm^{-1} was also more pronounced in the polluted soil, suggesting a higher degree of aromatic condensation and humification. In contrast, the aliphatic C–H peaks (2920–2850 cm^{-1}) and polysaccharide-related bands (1100–1000 cm^{-1}) were relatively weaker, reflecting the loss of labile organic fractions under pollution stress. Additionally, the O–H/N–H stretching band near 3400 cm^{-1} appeared broader in the polluted sample, indicating enhanced

hydrogen bonding and increased acidic functional groups. Overall, the FT-IR results demonstrate that pollution has altered the chemical structure of HA in Alfisols by increasing aromaticity and carbonyl content, while reducing aliphatic and polysaccharide components, thereby affecting the quality and stability of soil organic matter.

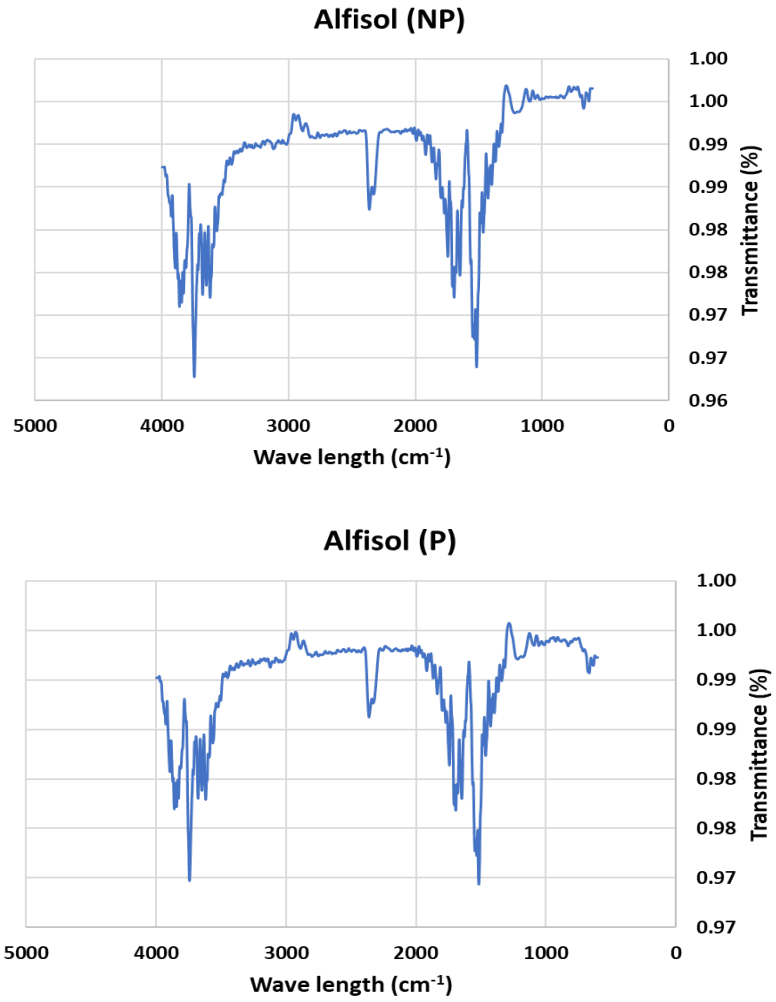

Fig 1 FT-IR characterization of HA extracted from polluted and non-polluted soils of Alfisol (Sukinda valley, Odisha)

Characterization of HA extracted from polluted and non-polluted soils of Inceptisol (Sonipat, Haryana) by FT-IR

The FT-IR spectra of humic acids (HA) extracted from non-polluted (NP) and polluted (P) Inceptisol soils of Sonipat, Haryana, displayed characteristic functional groups typically associated with humic substances, but clear modifications were observed due to pollution effects **(Figure 2).** Both samples exhibited major absorption peaks around 3400 cm^{-1} corresponding to O–H and N–H stretching, 2920–2850 cm^{-1} for aliphatic C–H stretching, a

strong band at 1720–1650 cm^{-1} associated with C=O stretching of carboxylic and carbonyl groups, and prominent peaks at 1620–1600 cm^{-1} representing aromatic C=C bonding and asymmetric COO^- stretching. Peaks at 1420–1380 cm^{-1} (symmetric COO^- stretch and phenolic OH) and 1250–1100 cm^{-1} (C–O stretching of polysaccharides, alcohols, and ether linkages) were also evident in both spectra. However, HA extracted from the polluted soil exhibited noticeably sharper and deeper peaks in the carbonyl (1720–1650 cm^{-1}) and aromatic (1620–1600 cm^{-1}) regions, suggesting an increased degree of oxidation, higher aromatic condensation, and enhanced metal–organic interactions. Conversely, the aliphatic C–H peaks (2920–2850 cm^{-1}) and polysaccharide-related bands (1100–1000 cm^{-1}) were slightly weaker in the polluted Inceptisol, indicating degradation of labile organic matter under pollution stress. The broadening of the O–H/N–H band near 3400 cm^{-1} in the polluted sample further suggests an increase in acidic functional groups and strengthened hydrogen bonding due to chemical transformations induced by pollutants. Overall, the FT-IR results reveal that pollution has substantially altered the structural chemistry of humic acids in Inceptisols, increasing aromatic and carbonyl functionalities while reducing aliphatic and carbohydrate-like components, thereby affecting the stability and composition of soil organic matter.

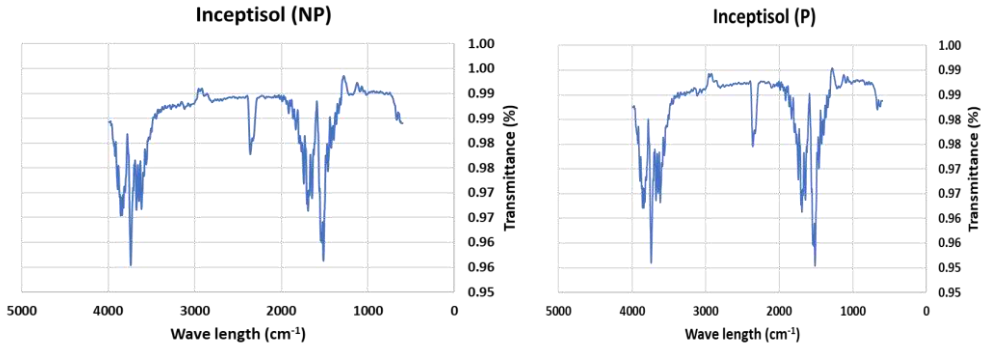

Fig 2 FT-IR characterization of HA extracted from polluted and non-polluted soils of Inceptisol (Sonipat, Haryana)

Characterization of HA extracted from polluted and non polluted soils of Vertisol (Dewas industrial area, Madhya Pradesh) by FT-IR

The FT-IR spectra of humic acids (HA) extracted from non-polluted (NP) and polluted (P) Vertisol soils of the Dewas industrial area, Madhya Pradesh, exhibited the characteristic absorption bands of humic substances; however, notable spectral differences indicate the influence of industrial pollution on HA structure and composition **(Figure 3)**. Both spectra showed a broad absorption band around 3400 cm^{-1} corresponding to O–H and N–H stretching vibrations, followed by aliphatic C–H stretching bands at 2920–2850 cm^{-1}. The strong peak in

the 1720–1650 cm^{-1} region, associated with C=O stretching of carboxylic and carbonyl groups, was more intense in the polluted soil, suggesting enhanced oxidation and possible metal–organic complex formation due to industrial contaminants. A prominent band around 1620–1600 cm^{-1}, representing aromatic C=C stretching and asymmetric COO^- stretching, was deeper in the polluted Vertisol, indicating an increased degree of aromaticity and humification. In contrast, the aliphatic C–H peaks and polysaccharide-associated peaks in the 1100–1000 cm^{-1} region were slightly weaker in the polluted sample, reflecting the degradation of labile organic components under pollution stress. The polluted Vertisol also exhibited a broader and more pronounced O–H/N–H stretching region, indicating greater hydrogen bonding and the formation of acidic functional groups. Overall, the FT-IR analysis demonstrates that industrial pollution has altered the chemical composition of humic acids in Vertisols by increasing carbonyl and aromatic functionalities while reducing aliphatic and carbohydrate-like structures, thereby affecting the stability, reactivity, and quality of soil organic matter.

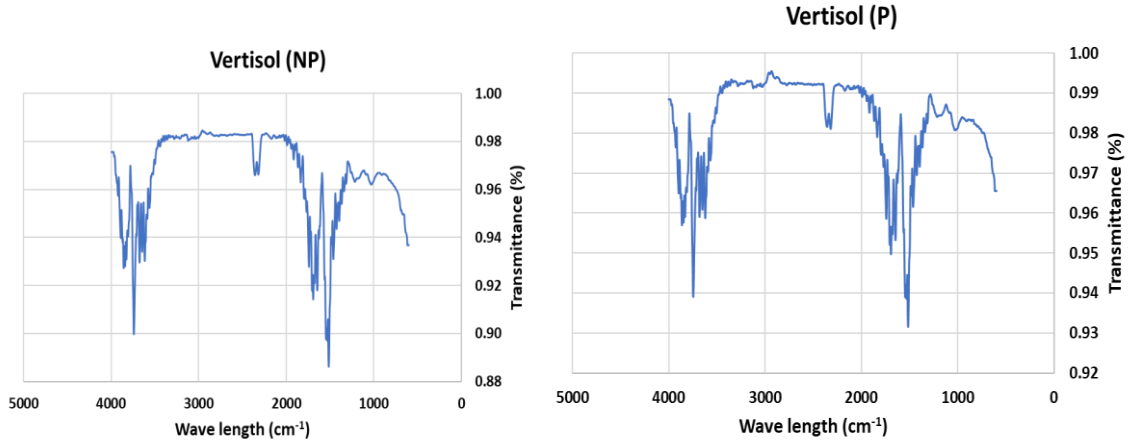

Fig 3 FT-IR characterization of HA extracted from polluted and non polluted soils of Vertisol (Dewas industrial area, Madhya Pradesh)

DISCUSSION

Effect of polluted and non-polluted soils on soil pH, EC texture

The present study revealed **(Table 1)** distinct variations in soil physicochemical properties across the three evaluated soil orders- Alfisols, Inceptisols, and Vertisols—indicating that inherent pedogenic characteristics strongly influence soil pH, texture, and electrical conductivity (EC). Textural differences were substantial, with Alfisols exhibiting a loamy sand texture, Inceptisols a sandy loam texture, and Vertisols a clayey texture. The high clay content

of Vertisols (50.3%) is consistent with their well-known smectitic mineralogy and advanced weathering stage, which impart pronounced shrink–swell behaviour (Brady & Weil, 2013).
Soil pH varied significantly across soil orders (factor A), whereas pollution level (B) and soil depth (C) did not show significant effects. Acidic conditions in Alfisols of the Sukinda Valley (pH ~5.8) correspond with their highly leached nature and low base saturation in humid tropical climates (Nair *et al*., 2019). Under polluted conditions, Alfisols exhibited a slight rise in pH at deeper depths, possibly due to deposition of alkaline industrial particulates. Inceptisols maintained near-neutral to slightly alkaline pH across depths, reflecting their moderate weathering status and presence of carbonates, which provide buffering capacity (Paton *et al*., 1995). Vertisols consistently showed alkaline pH values (7.99–8.52), a typical trait linked to base-rich parent materials and high cation retention associated with 2:1 clay mineral (Datta *et al*., 2020)
Electrical conductivity (EC) also differed significantly by soil order, while pollution and depth effects remained statistically non-significant. Polluted Alfisols and Inceptisols showed marginally higher EC values compared to non-polluted soils, likely due to the influx of soluble ions from industrial emissions and surface deposition. Vertisols displayed an increase in EC at the surface layer (0–15 cm) under polluted conditions, followed by a decrease at 15–30 cm. This pattern may be attributed to the strong adsorption and limited vertical mobility of salts in clay-rich horizons (Guo *et al*., 2021). The high clay fraction in Vertisols restricts percolation and promotes stratification of soluble salts within the profile. Overall, the results indicate that soil order is the dominant factor governing soil pH and EC, confirming that inherent pedogenic controls outweigh short-term anthropogenic pollution effects. The resilience and response of each soil order—acidic Alfisols, buffered Inceptisols, and alkaline, clay-rich Vertisols—reinforce the understanding that soil mineralogy and development stage strongly regulate chemical behaviour, even under external pollution stress.

Effect of polluted and non-polluted soils under major soil orders of India on Organic carbon, CEC

Organic carbon (OC) and cation exchange capacity (CEC) showed clear differences among the three soil orders, reflecting their inherent mineralogical and organic matter characteristics as well as the influence of metal pollution **(Table 2).** Alfisols from Sukinda Valley, being highly weathered and low in clay activity, exhibited moderate OC but relatively low CEC, consistent with their dominance of low-activity kaolinitic clays and limited humus stabilization. Although polluted Alfisols showed slightly elevated OC, possibly due to deposition of carbon-rich

industrial particulates or reduced microbial decomposition under metal stress, their CEC declined under pollution. This reduction may be attributed to the displacement or blockage of exchange sites by heavy metals, which often bind strongly to clay–humus surfaces and reduce effective exchange capacity. Inceptisols of Sonipat, with their intermediate weathering stage and mixed mineralogy, maintained low OC but moderate CEC across depths. The slight increase in OC under polluted conditions may again be related to inhibited decomposition or external carbon deposition, whereas the relatively stable CEC indicates that these soils possess a more resilient exchange complex, likely due to a mixture of 2:1 and 1:1 clay that provide balanced buffering against metal interference (**Juo, & Franzluebbers, (2003**). Vertisols, characterized by highly expansive smectitic clays, unsurprisingly recorded the highest CEC values among the soil orders, reaffirming the strong charge characteristics of 2:1 clay mineral. The marginal decline in CEC under polluted conditions suggests partial occupation or saturation of exchange sites by metal ions, which can reduce the overall effective exchange capacity despite the inherent buffering strength of smectites. Across all soils, depth-wise declines in OC and CEC reflect typical pedogenic gradients where organic matter inputs and biotic activity decrease with depth. Notably, both OC and CEC were significantly influenced by soil order, pollution level, and depth, indicating that metal contamination alters chemical functional groups and exchange reactions differently depending on the underlying soil mineralogy and organic matter dynamics (Li *et al*., 2023).

Characterization of HA extracted from polluted and non-polluted soils

E4/E6 ratio

The E4/E6 ratio of humic acid is widely recognized as an indicator of humic substance maturity, molecular condensation, and aromaticity (Stevenson, 1994; Chen *et al*., 1977). In the present study, significant variations in the E4/E6 ratio were observed across soil orders as well as between metal-polluted and non-polluted conditions **(Table 3)**. Alfisols exhibited the highest mean E4/E6 ratio, followed by Inceptisols, whereas Vertisols showed the lowest values, suggesting a comparatively higher degree of aromatic condensation and humification in Vertisols due to their clay-rich nature and slower organic matter turnover (Schnitzer, 1999). Across soil orders, metal-polluted soils consistently displayed lower E4/E6 ratios than non-polluted soils, indicating that heavy metal stress may enhance structural condensation or promote the formation of more polymerized humic substances (Kalbitz *et al*., 2000). The decline in the ratio was marginal in Alfisol and Inceptisol but more pronounced in Vertisol, implying that clay–metal interactions in Vertisols could further stabilize humic molecules and reduce their optical absorbance ratio. Overall, the results reaffirm that both inherent soil

properties and metal contamination significantly influence humic acid characteristics, consistent with global observations on SOM–metal interactions (Michalzik & Matzner, 1999; Guo & Chorover, 2003).

The total acidity

The total acidity and functional group composition of humic acids are key indicators of their reactivity, structural complexity, and capacity to interact with metal ions and soil minerals (Stevenson, 1994; Swift, 1996). In the present study **(Table 4),** total acidity exhibited significant variation among soil orders, with Alfisol showing the highest values, followed by Inceptisol, while Vertisol recorded notably lower acidity. This trend reflects inherent differences in organic matter turnover, clay mineralogy, and degree of humification, as soils with higher weathering intensity and organic matter decomposition tend to accumulate more acidic functional groups (Schnitzer & Khan, 1975). Although the mean total acidity was marginally higher in non-polluted soils than in metal-polluted soils, the differences were small, and the pollution effect remained statistically non-significant, suggesting that metal contamination exerted only a limited influence on the overall acidic character of humic substances. However, the slight reduction in acidity in Inceptisol and Vertisol under polluted conditions may indicate partial neutralization or complexation of acidic groups by heavy metals, a phenomenon earlier reported by Senesi *et al*. (1990).

The carboxylic and phenolic OH group

The carboxylic and phenolic OH group distribution further supports this interpretation. Carboxylic acidity, which plays a dominant role in metal binding, was highest in Alfisol and lowest in Vertisol, and it consistently decreased under polluted conditions across soil orders **(Table 5)**. This reduction, particularly pronounced in Vertisol, aligns with previous findings that metals preferentially form stable complexes with carboxyl groups, effectively lowering measurable acidity (Guo & Chorover, 2003). Conversely, phenolic OH groups displayed an opposite trend, with polluted soils showing slightly higher mean values than non-polluted soils. This increase in Alfisol and Inceptisol may be attributed to oxidative transformations or partial depolymerization of humic macromolecules under metal stress, which can enhance the exposure of phenolic moieties (Kalbitz *et al*., 2000). Vertisol, however, showed no change in phenolic OH content, likely due to its high clay content and strong organo–mineral associations that buffer functional group expression (Piccolo, 2001). Overall, the results highlight that soil order exerts a stronger influence on humic acid functionality than pollution status, but metal

contamination can differentially affect specific functional groups, particularly carboxyl and phenolic OH groups, consistent with earlier global observations on SOM–metal interactions.

FT-IR Characterization of Humic Acids

The FT-IR characterization of humic acids extracted from polluted and non-polluted soils across Alfisol, Inceptisol, and Vertisol clearly revealed that pollution induces substantial chemical modifications in humic substances, particularly in their functional group composition and structural organization **(Figure 1, 2, 3).** The spectra from all soil orders exhibited typical humic substance absorption bands, including broad O–H/N–H stretching around 3400 cm^{-1}, aliphatic C–H stretching at 2920–2850 cm^{-1}, strong carbonyl (C=O) peaks at 1720–1650 cm^{-1}, aromatic C=C and COO^- stretching at 1620–1600 cm^{-1}, and C–O stretching of polysaccharides and alcohols between 1250–1100 cm^{-1}, consistent with classical humic fingerprints (Stevenson, 1994; Swift, 1996).

Across all soil orders, polluted samples consistently showed stronger and sharper peaks in the carbonyl and aromatic regions (1720–1600 cm^{-1}), indicating enhanced oxidation, increased aromatic condensation, and greater metal–organic complexation. This trend suggests that pollution accelerates the transformation of humic substances toward more recalcitrant and aromatic structures, a phenomenon also reported by Senesi *et al*. (1991) and Kalbitz *et al*. (2000). Conversely, the aliphatic C–H bands and polysaccharide-associated peaks were weaker in polluted soils, demonstrating the loss of labile, aliphatic, and carbohydrate-like components, likely due to pollutant-induced decomposition or selective preservation of more stable fractions (Piccolo, 2002). A pronounced broadening of the 3400 cm^{-1} band in polluted soils across all soil orders further suggests increased hydrogen bonding and the proliferation of acidic functional groups, which may arise from oxidation or metal-induced structural rearrangements (Guo & Chorover, 2003). While the general trend was similar across soil orders, the magnitude of spectral changes varied. Alfisol and Inceptisol exhibited stronger increases in aromatic and carbonyl peaks, reflecting greater sensitivity to pollution-induced oxidative modification. Vertisol showed comparatively moderate spectral shifts, likely due to its high smectitic clay content, which stabilizes humic–clay complexes and buffers against structural transformation (Schnitzer & Khan, 1975). Overall, the FT-IR results conclusively demonstrate that pollution alters the chemical composition of humic acids by increasing aromaticity and carbonyl functionality while reducing aliphatic and polysaccharide components. These changes indicate a shift toward more condensed and stable organic structures, with significant implications for soil organic matter reactivity, nutrient dynamics, and metal-binding behavior.

CONCLUSION

Soil order significantly governed physicochemical and humic acid (HA) properties, while pollution acted as a modifier of variable significance. Texture, pH, and EC varied significantly with soil order (CD = 0.215, 0.048) but not pollution. Alfisols were acidic (pH ~5.8–6.1, CEC 6.2–12.3 cmol(p^+)/kg), Inceptisols near-neutral (pH 7.6-7.9, CEC 11.0-16.7), and Vertisols alkaline (pH 8.0–8.5, clay 50.3%, CEC 43.9-50.9) due to smectitic mineralogy. OC and CEC varied significantly with both soil order and pollution (CD = 0.032-1.68): OC rose slightly under pollution (e.g., 1.53-1.59 g/100g, Alfisol), while CEC declined (e.g., 12.3-10.6 cmol(p^+)/kg), reflecting metal occupation of exchange sites.

Among HA parameters, E4/E6 ratio (5.07 to 3.49, Alfisol–Vertisol) and carboxylic OH varied significantly with soil order and pollution, with polluted soils showing lower E4/E6 (4.35 to 4.22) and carboxylic OH (3.19 to 3.08 meq/g), confirming preferential metal–carboxyl complexation. Total acidity and phenolic OH showed no significant pollution effect. FTIR spectra supported these trends, showing enhanced carbonyl/aromatic bands and reduced aliphatic/polysaccharide peaks in polluted soils-most pronounced in Alfisols and Inceptisols-while Vertisols remained comparatively resilient.

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Authors' contributions

Chinmayee Behera - CB: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Nayan Ahmed -NA: Data curation, Investigation, Methodology, Supervision, Writing – review & editing. Kanchikeri Math Manjaiah- KMM: Writing – review & editing. Debasis Golui -DG: Data curation, Writing – review & editing. Bhupinder Singh - BS: Methodology, Writing – review & editing. Siyaram Meena -SM: review & editing

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Declarations

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