



Research article

Complexation, retention and release pattern of arsenic from humic/fulvic acid extracted from zinc and iron enriched vermicompost

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ABSTRACT

Arsenic (As) is a highly poisonous heavy metal with major environmental ramifications. Inorganic components such as zinc (Zn) and iron (Fe), as well as organic vermicompost, have been used as management solutions, with limited attempts of using them together. The current study involved preparing non-enriched vermicompost as well as six distinct Zn and Fe enriched vermicomposts and analyzing their chemical composition using the standard procedures. Organic fractions from these seven vermicompost and arsenic polluted soils of West Bengal, India were recovered and separated into humic (HA) and fulvic acid (FA) fractions. Potentiometric titrations, viscometric assays, and visible spectrophotometry were used to characterize the HA and FA samples. In aqueous phase the stability constant (log K) of the complexes formed with As indicates that stability of FA extracted from enriched vermicompost V₄ (Zn and Fe sulphate @ 10% w/w dry weight basis of composting substrates before application of vermiforms) was maximum as 10.20 with a mole ratio (x) value of 1.36. Fourier-transform infrared (FT-IR) spectroscopy and Scanning Electron Microscopy (SEM) studies confirmed the complexation of As with HA/FA. The release isotherm of As from the HA/FA complexes in the presence of competitive oxy-anions was found to follow the order of sulphate > nitrate > phosphate.

1. Introduction

Arsenic (As) poisoning which equally threatens the terrestrial and aquatic systems, is a persistent problem, particularly for South and Southeast Asian and Latin American countries (Mandal et al., 2021; Hussain et al., 2021). Around 200 million people are thought to be at the risk of As poisoning, either directly by drinking As-contaminated water or by consuming As-laden food stuffs. Rice (*Oryza sativa* L.) being a high water requiring crop is severely influenced by the use of As-contaminated irrigation water (Shakoor et al., 2017). Bioavailability of As in soil has been found to be controlled by a number of parameters, including soil type, pH, texture, redox potential (Eh), organic carbon content, and existence of other competing ions in soil solution (Kumari et al., 2021; Mandal et al., 2019a). Immobilisation of As via adsorption by chemical amendments such as iron (Fe) (Liu et al., 2015; Yu et al., 2017), zero valent Fe (Qiao et al., 2018), and nitrate (Wang et al., 2019) has piqued attention in recent years due to its capability in reducing As mobility in contaminated soil. Arsenic ions bind to the adsorption sites

in the soil organic matter (SOM), which reduces their kinesis in the soil (Sinha et al., 2011; Mandal et al., 2019a). Amino acids, aliphatic acids, sugar acids, and phenols form the organic matter by the decomposition of biomass. According to Stevenson (1994), SOM may be defined as water-soluble fractions of different molecular weight and chemical configuration of humic acids (HA) and fulvic acids (FA) containing functional groups with a variety of characteristics.

Use of organic amendments (sugarcane bagasse, farm yard manure and vermicompost) to reduce the As uptake in wheat and maize crop was reported by Mandal et al. (2019b) and Kumar et al. (2021). The efficacy of vermicompost in reducing the As accumulation in vegetable edibles and rice grain has been reported by Bhattacharyya et al. (2021) and Sengupta et al. (2021). Three steps are involved in the binding of As to the active sites of organic molecules. Buschmann et al. (2006) recommended a covalent link between As and the active sites, but Saada et al. (2003) favoured ionic association while Lin et al. (2004) advocated cationic bridging complexes.

Case studies show that organic matter has a lot of potential to

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Table 1
Different combinations of vermicompost samples prepared.

Treatment	Treatment Details
V ₁	Non-enriched vermicompost
V ₂	Zn and Fe sulphate @ 5% w/w dry weight basis of composting substrates applied before application of vermiworms
V ₃	Zn and Fe sulphate @ 7.5% w/w dry weight basis of composting substrates applied before application of vermiworms
V ₄	Zn and Fe sulphate @ 10% w/w dry weight basis of composting substrates applied before application of vermiworms
V ₅	Zn and Fe sulphate @ 5% w/w dry weight basis of composting substrates applied at peak stage of vermiworm multiplication
V ₆	Zn and Fe sulphate @ 7.5% w/w dry weight basis of composting substrates applied at peak stage of vermiworm multiplication
V ₇	Zn and Fe sulphate @ 10% w/w dry weight basis of composting substrates applied at peak stage of vermiworm multiplication

influence As adsorption behavior through interaction, directly with the mineral surfaces or with As itself, and that it could play a significant part in release of As (Sinha and Bhattacharyya, 2011; Ghosh et al., 2012; Mandal et al., 2019a). SOM can increase As release by competing for accessible adsorption sites, creating aqueous compounds, and altering the redox chemistry of site surfaces and As species (Hussain et al., 2021; Mandal et al., 2021). Organic acids, on the other hand, can limit As mobility by acting as a complexing agent especially in presence of other metal cations (Pandey et al., 2000). These results are significant in fostering our perception about the fate of As and its transport cycle in the biosphere. It also has implications for regulating mobility of As and developing remediation techniques to prevent and surpass additional pollution (Hussain et al., 2021). However, the amount of knowledge available is restricted, and there is still a lot that is unknown regarding the nature of complexes and how far stable they are to reduce As phyto-availability (Costa et al., 2022).

A number of inorganic amendments have also been attempted over the decades of which application of zinc (Zn) (Craw and Chappell, 2000; Karmakar et al., 2021) and Fe (Bandyopadhyay et al., 2004; Matsumoto et al., 2015) were observed to be the most efficient regarding reduced availability and uptake of arsenic in soil and by crop. This led to our assumption that if we combine the most efficient inorganic amendment (Zn and Fe) with the most efficient organic (vermicompost), then there might be a better possibility of As retention from stable complex formation.

With this background, an attempt has been made (i) to study the characteristics of HA/FAs extracted from Zn and Fe enriched vermicompost (ii) to investigate the equilibria of organo (HA and FA)-As complexes and also the impact of competing ions, and (iii) to understand the binding of As with HA/FA by Fourier-transform infrared (FT-IR) spectroscopy and Scanning Electron Microscopy (SEM) studies.

2. Materials and methods

2.1. Production of Zn and Fe enriched vermicompost

Fe and Zn enriched vermicompost was made by adding ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 19 percent Fe) and zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 34 percent Zn) to pre-digested composting substrates following Hashemi-majd and Jamaati-e-Somarin (2011). Cow dung and local farm wastes at 6:4 ratio was used as composting substrate. The greater amount of dry dung was applied as it ensures easiest source of food for rapid growth and development of earthworms and further ensures more microbial activity (Manyuchi and Whingiri, 2014). In three sets of enriched vermicompost, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ were added to the composting substrate mixture at 5, 7.5, and 10% w/w on a dry weight basis and pre-decomposed for 15 days. Fe and Zn rich pre decomposed materials were fed to surface dwelling vermiworm (*Eisinea foetida*) inoculated at a rate of 10 worms per kg substrate (dry weight basis). In the other three sets of enriched vermicomposts, vermiworms were allowed to grow in

composting substrate mixture without Fe and Zn, and when the maximum vermiworm multiplication stage was reached (about 25–30 days after application), Fe and Zn sulphate was applied (5, 7.5, and 10% w/w dry weight basis). The entire activity was carried out for 3 months in the first cycle and 35/45 days for the third cycle and onwards, in a cemented trench of 0.305 m depth and appropriate length, shaded with thatched roof. Watering, vermiworm prevention, harvesting, and post-harvest processing were all done according to the requirement following Roy et al. (2022). By varying the time of application of Zn and Fe sulphate and release of vermiworms, seven different types of vermicompost have been prepared as can be observed from Table 1.

2.2. Vermiworm count, microbial biomass carbon, C:N ratio and microbial respiration of the enriched vermicompost

During vermicomposting, vermiworm counts were obtained at intervals of 0, 30, 60, and 90 days. Feedstocks were obtained from pits, spread out in aluminium trays, and vermiworms were sieved through 3–4 mm iron mesh for this purpose. After calculating their numbers, the vermiworms and feedstocks were moved back to the specified pits using the previously employed techniques (Goswami et al., 2016; Roy et al., 2022), ensuring normal and uninterrupted vermiworm reproduction and growth. The vermiworms were removed from the vermi-processed feedstocks by sieving at the end of vermicomposting period (i.e. 90 days) and their population was counted. The vermiworms were then returned to the stock vermiculture unit for future cycles.

The microbial biomass carbon (mg/kg) was determined by fumigation extraction method (Alef, 1995). Further throughout this time period, the microbial respiration and C: N (based on total C and N) ratio was computed. The CO_2 evolved during incubation of the samples (with or without glucose) in a closed environment was estimated to determine microbial respiration. The CO_2 was eventually trapped in a NaOH solution that was then titrated with HCl (Alef, 1995).

2.3. Estimation of physicochemical and biological characters of prepared vermicompost samples

After collection of the vermicompost samples they were separately stored in pre-marked polythene packets in a cool environment (4°C) to ensure better activity. The samples were then subjected to physicochemical characterization following standard analytical procedures. The moisture content (%) was measured by drying vermicompost samples at 105°C for about 24 h or at constant weight (ASAE, 1998; Khater, 2015); the water holding capacity (%) by determining the amount of water retained by the dry sample (g/g) after water soaking as outlined by Ahn et al. (2008); bulk density (kg m^{-3}) was determined by placing the compost material in a container to a definite level (known volume) and weighing it as outlined by Schaub-Szabo and Leonard (1999) and the particle size distribution was determined by sieving air-dried vermicompost samples and particle fractions retained on 4 mm sieve was weighed and expressed as percentage of the total weight (Bachman and Metzger, 2007). The pH and EC of the vermicompost samples were analyzed in 1:5 compost:water suspension as outlined by Jackson (1967). Total organic carbon (wet oxidation and titration method) and total organic matter (loss on ignition using muffle furnace) was analyzed as per the methods proposed by Tiessen and Moir (1993) and Abad et al. (2002) respectively. The macronutrient status of the vermicompost samples mainly nitrogen (digestion, distillation and Micro-Kjeldahl method), phosphorus (tri-acid digestion and spectrophotometry), potassium (tri-acid digestion and flame photometry) was determined as per the methods outlined by Jackson (1973) and the micronutrients like Fe, manganese (Mn), Zn, copper (Cu) was determined by acid digestion and atomic absorption spectrophotometry (Jackson, 1967). The enzymatic activity namely dehydrogenase (DHA), FDA hydrolysable enzyme and alkaline phosphatase were determined by reacting with their corresponding substrates and determining the intensity of colour by

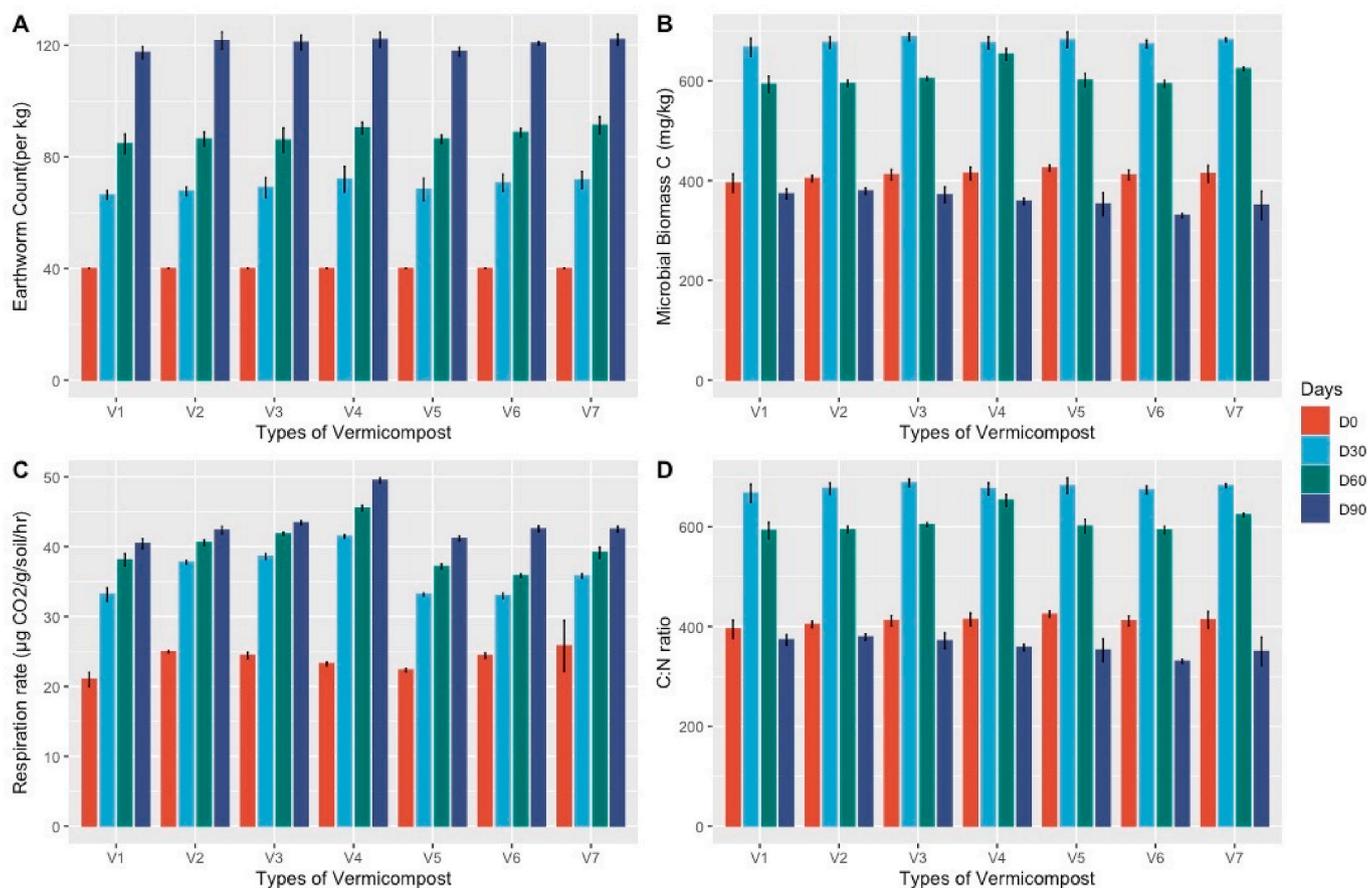


Fig. 1. (A) Earthworm count, (B) Microbial biomass C, (C) Microbial respiration, and (D) C: N ratio in case of vermicompost samples prepared over a period of 0, 30, 60, and 90 days. Results represented as the mean of three observations \pm standard deviations.

spectrophotometer, following Casida et al. (1964), Schnürer and Ross-wall (1982) and Tabatabai and Bremner (1969) respectively.

2.4. Organo - arsenic complexation study

Prior to studying the humic/fulvic-arsenic complexation equilibria, the organic fraction from the experimental soil and vermicompost was extracted, and separated into HA and FA fractions (Mukhopadhyay and Sanyal, 2004).

2.4.1. Extraction of HA and FA from soil and vermicompost

The soil and vermicompost materials were dried in the open air, crushed, and sieved at 2 mm. In a Winchester bottle, the samples were mixed with 0.5 M Na_2CO_3 and kept overnight. The humic acid (HA) was precipitated by siphoning out the supernatant and acidifying it with HCl to a pH of 2.0–3.4. The apparatus was left overnight to ensure that all the HA was precipitated. The supernatant was removed, and the precipitate was dialyzed until chloride was removed (Mukhopadhyay and Sanyal, 2004). The fulvic acid (FA) was precipitated as Ba-fulvate after the supernatant was corrected to pH 8.0 and reacted with BaCl_2 . To get FA, the Ba-fulvate was dialyzed until it was chloride-free, and then passed through an H^+ ion resin column (Kononova and Newman, 1969). The same procedure was followed to extract HA/FA from the experimental soils. The HA extracted from the vermicomposts were categorized as HA-V₁ to HA-V₇ (V₁ to V₇ refers to the seven types of vermicompost, described earlier). The FA fractions were named accordingly.

2.4.2. Determination of contents of carboxylic and phenolic groups of HA and FA by potentiometric titration

Different amounts of standard NaOH (0.01 N) solution, viz. 0 (i.e. no

alkali), 0.5 ml, 1.0 ml, 1.5 ml, etc., were added to equal volumes (10 ml) of HA/FA suspension in a series of beakers. The suspension was then stirred well with a glass rod, and the pH measurements were taken using a pH meter. The pH was measured at the first and last inflection points of the differential titration curves (Mandal et al., 2019a), and the ΔpH value was calculated. In addition, the amount of fresh alkali required to achieve a stable pH was recorded, as well as the time taken to achieve that pH.

Schnitzer and Gupta (1964) described $\text{Ba}(\text{OH})_2$ method was used to determine the total acidity of HA and FA. Determination of carboxylic groups was based on the liberation of acetic acid when acids were treated with calcium acetate and titration with standard 0.1 N NaOH (Schnitzer and Khan, 1972). The difference between total acidity and COOH acidity was used to compute the phenolic OH groups.

2.4.3. Viscometric measurement

In complexation study there is a special significance of determination of viscosity of HAs/FAs as it indicates the complex nature and molecular weight of the molecules which can directly influence the retention pattern of heavy metals (Ghosh et al., 2012). With the addition of appropriate amount of alkali (NaOH) to the first inflection point of the potentiometric titration curves all the carboxylic acid groups in each HA/FA sample were converted to carboxylate form. An Ostwald/Ubbelohde viscometer was used to measure the viscosities of the HA/FA suspensions (Sinha and Bhattacharyya, 2011). At four different concentrations the viscosities of each sample were tested. The mean flow time at each concentration, as well as the laboratory's ambient temperature and suspension densities, were all recorded using a specific gravity bottle. The ratio of the mean flow time is used to calculate the relative viscosity (η_{rel}) of varying concentrations of each HA/FA sample

(η) regarding the viscosity of distilled water (η_0), because viscosity is directly proportional to mean flow time (Chen and Schnitzer, 1976). The specific viscosity ($\eta_{sp} = \eta_{rel}-1$) of each HA/FA sample was then determined at various concentrations, and a corresponding plot of η_{sp}/\sqrt{C} vs. \sqrt{C} was created. The Jones-Dole equation's A and B coefficients (Chen and Schnitzer, 1976) were determined (Sinha and Bhattacharyya, 2011), and by deducting the reference value of B_{Na+} (Stokes and Mills, 1965) from the B_{expt} value of sodium humate/fulvate, the B_{expt} values of the humate/fulvate anions were determined (Sanyal and Mandal, 1983). The viscosity-average molecular weight (M) of the humic/fulvic substance was calculated using the B_{expt} values as approximated to the respective intrinsic viscosities, $[\eta]$ (Chen and Schnitzer, 1976), by setting $K = 7.3 \times 10^{-4}$ and $\alpha = 0.65$ (Hansen and Schnitzer, 1969; Relan et al., 1984; Mukhopadhyay and Sanyal, 2004) in the modified Staudinger's equation $[\eta] = K (M)^\alpha$.

2.4.4. Visible spectrophotometric measurement

At pH 7.0 and 9.0, the optical densities of dilute solutions of HA/FA (Na-form) were measured using a spectrophotometer at 465 nm (E_4) and 665 nm (E_6) (Sinha and Bhattacharyya, 2011; Mandal et al., 2019a). After that, the difference between the E_4 and E_6 ratios at pH 7.0 and 9.0 was calculated. This estimation indicates the measure of degree of aromaticity or degree of coiling of HAs/FAs which directly influence its complexation and retention of As in aqueous phase.

2.4.5. Determination of stability constant of organo-arsenic complexes

The stability constants of the arsenic-humic/fulvic complexes generated were determined using the standard ion-exchange method described by Schnitzer and Skinner (1966), and the complexation equilibria between arsenic and the humic/fulvic compounds were studied as per the methods outlined by Sinha and Bhattacharyya (2011) and Mandal et al. (2019a).

2.5. Exchangeability of phosphate, sulphate, and nitrates (replacing anions) with humic/fulvic-arsenate complexes

By putting equal volumes of HA or FA suspensions in stoppered bottles, the exchangeability of phosphate, sulphate, and nitrate with arsenate-humic/fulvic complexes was determined by oxyanion exchange phenomenon as per the methods outlined by Sinha and Bhattacharyya (2011) and Mandal et al. (2019a).

Table 2

Physical, chemical and biological (enzymatic) characterization of prepared vermicompost samples.

	V ₁	V ₂	V ₃	V ₄	V ₅	V ₆	V ₇
Moisture (%)	19.12d	21.00 ab	21.60a	21.52a	20.31b	19.82c	20.11b
WHC (%)	78.77d	86.52b	88.99a	88.58a	83.64c	81.57cd	82.81cd
BD (kg/m ³)	0.71c	0.72b	0.73b	0.74a	0.69d	0.72b	0.75a
Particle Size (%)	91.20d	92.10bc	92.50b	93.20a	91.80c	92.40b	93.00a
Dehydrogenase ($\mu\text{g TPF g}^{-1} 24 \text{ h}^{-1}$)	78.82e	91.23 ab	89.98b	92.34a	84.36d	89.64b	87.23c
Alkaline Phosphatase ($\mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$)	165.92e	192.04a	189.41 ab	194.38a	177.58d	188.69b	183.62c
FDA enzyme assay ($\mu\text{g fluorescein g}^{-1} \text{ h}^{-1}$)	87.65d	101.45a	100.06 ab	102.68a	93.81c	99.68 ab	97.00b
pH	7.12a	7.01b	6.94c	6.85d	7.04b	7.00b	6.91c
EC (dSm ⁻¹)	3.64e	4.12c	4.86b	5.45a	3.96d	4.32c	4.88b
Total OM (%)	25.00e	29.65d	33.14 ab	34.30a	29.07d	30.23c	31.19b
Total OC (%)	21.20f	25.79d	27.77b	28.64a	24.37e	26.03cd	26.98c
Total N (%)	1.04e	1.23d	1.39c	1.58a	1.21d	1.42bc	1.44b
Total P (%)	0.96d	0.98c	1.00c	1.14a	0.98c	1.08b	1.12a
Total K (%)	0.68d	0.78c	0.94b	1.05a	0.79c	0.94b	0.96b
Total Zn (mg kg ⁻¹)	44.9e	175.55d	216.5c	235.95b	177d	226.3bc	252.36a
Total Cu (mg kg ⁻¹)	27.4f	43.55d	61.85b	68.95a	34.45e	58.8c	64.25 ab
Total Fe (mg kg ⁻¹)	820.12f	1335.22e	1463.49d	1628.12b	1372.59e	1529.14c	1782.49a
Total Mn (mg kg ⁻¹)	73.76e	78.74cd	80.67b	82.70a	77.16d	79.04c	80.87b
Total As (mg kg ⁻¹)	0.0016a	0.0015 ab	0.0009c	0.0009a	0.0014b	0.0010c	0.0011c

V₁ as non-enriched vermicompost; V₂, V₃ and V₄ (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied before application of vermicorms) and V₅, V₆ and V₇ (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied at peak stage of vermicorm multiplication, 25–30 days after vermicorm application). Values with different alphabets (to be read horizontally) are significantly different from each other according to the DMRT test ($P < 0.05$). Each value is a mean of three replicates.

2.6. FT-IR and SEM characterization for As sorption on vermicompost

In order to decipher, illustrate and characterize the possible As sorption on the vermicompost samples, they were analyzed by Fourier-transform infrared (FT-IR) spectroscopy using a PerkinElmer Spectrum 100 spectrophotometer and Scanning Electron Microscopy (SEM) using a ZEISS, EVO40 microscope.

3. Results and discussion

3.1. Vermiworm count, microbial biomass carbon, C: N ratio and microbial respiration during vermicomposting

The vermicorm count per kg of vermicomposted material (Fig. 1A) showed a gradual increase in all the treatments with the advancement of time presumably due to rapid rate of growth and multiplication of vermicorms over time. Zn and Fe enrichment of prepared vermicompost at higher doses was associated with larger vermicorm count per kg, with the maximum population being associated with the treatment V₄ which was at par with the treatment combinations of V₂ and V₇. In all cases, the minimum vermicorm count was associated with the non-enriched vermicompost (V₁) and was statistically dissimilar. This phenomenon is not uncommon and such high tolerance of *E. foetida* to metal rich condition has been vividly discussed by Paul et al. (2018) and Mondal et al. (2020). However, if the metal content in substrate is exceedingly high then over long time it can be a serious concern towards growth of vermicorm as well as its fecundity (Pączka et al., 2020). In a study involving *E. foetida* it was observed that exposure to As at 10.8 mg/kg for 21 days, Zn at 3605 mg/kg for 14 days can severely affect the reproduction (cocoon production and hatchling) of vermicorms (Sivakumar, 2015).

The microbial biomass carbon (MBC) of vermicompost was evaluated over the entire 90 days of vermicomposting (Fig. 1B) in the same way as the vermicorm count was estimated and it showed same pattern. Such findings have concordance with the findings of Roy et al. (2022) who found that the MBC increases over time. Statistical difference analysis suggests among the treatment, the highest MBC was recorded with the treatment V₄, while the minimum MBC was associated with V₁. The increase in metal concentration in vermicomposting material eventually induces microbial toxicity and disrupts interspecific association within microbial communities (Zhu et al., 2017), which could

Table 3
Observations from pH-based potentiometric titration of vermicompost samples.

	COOH group (cmol/kg)	Phenolic OH group (cmol/kg)	Total Acidity (cmol/kg)	pH at first inflexion point (pH ₁)	pH at final inflexion point (pH ₂)	$\Delta\text{pH} = \text{pH}_{3/4} - \text{pH}_{1/4}$ (pH = pH ₂)	Amount of fresh alkali required to attain stable higher pH (cmol/kg)	Time required to attain a stable higher pH (hr)
HA-soil	35.0	26.0	61.0	7.38	7.46	3.73	0.630	87
HA-V ₁	28.0	21.0	49.0	7.24	7.82	3.91	0.525	90
HA-V ₂	22.0	20.0	42.0	7.48	7.98	3.99	0.750	78
HA-V ₃	52.0	32.0	84.0	6.56	7.02	3.51	0.460	90
HA-V ₄	70.0	48.0	118.0	6.49	6.56	3.28	0.425	112
HA-V ₅	65.0	43.0	108.0	6.38	6.46	3.23	0.468	110
HA-V ₆	55.0	35.0	90.0	6.45	6.48	3.24	0.550	94
HA-V ₇	36.0	26.0	62.0	7.02	7.52	3.76	0.650	85
FA-soil	135.0	45.0	180.0	6.80	7.80	3.90	2.100	75
FA-V ₁	141.0	47.0	188.0	6.95	7.82	3.91	4.150	78
FA-V ₂	98.0	68.0	166.0	6.38	6.98	3.49	4.450	65
FA-V ₃	195.0	62.0	257.0	6.98	7.54	3.77	2.250	88
FA-V ₄	232.0	85.0	317.0	7.00	8.90	4.45	3.190	87
FA-V ₅	210.0	70.0	280.0	6.18	6.23	3.12	1.730	89
FA-V ₆	196.0	66.0	262.0	6.27	6.37	3.19	2.260	86
FA-V ₇	142.0	51.0	193.0	7.80	9.20	4.60	3.260	79

HA and FA designate humic and fulvic acid extracts of soil and vermicompost samples. V₁ as non-enriched vermicompost; V₂, V₃ and V₄ (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied before application of vermiworms) and V₅, V₆ and V₇ (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied at peak stage of vermiworm multiplication, 25–30 days after vermiworm application). Each value is a mean of three replicates.

explain the feedstocks' low initial microbial biomass load. Vermiworms, on the other hand, are known to aid microbial activity by contributing gut-associated bacteria (Sahariah et al., 2015). As a result, the performance of vermicomposting systems can largely be explained by the interplay of microbial abundance and metabolic efficiency (Domínguez et al., 2019). As a result, it appears that an increase in vermiworm population, aided microbial biomass during vermicomposting in an indirect manner.

The microbial respiration rate of vermicompost was evaluated over the entire 90 days of vermicomposting (Fig. 1C). The augment of microbial activity resulted in the increment in the microbial respiration rate over time (Goswami et al., 2016). Among the treatment combinations, the highest microbial respiration rate with statistical difference was recorded with the treatment V₄, while the minimum rate was associated with V₁. Respiration of metabolically inactive microorganisms, reflected as basal microbial respiration rate, is based on the activity of a slow autochthonous microbial community (Pal et al., 2007; Tripathy et al., 2014) rather of the fast developing zymogenous ones. As a result of these findings, the vermiworms and their intestinal microbes may have boosted the growth of autochthonous microorganisms throughout vermicomposting, resulting in an increase in microbial respiration rate over time (Roy et al., 2022).

The carbon: nitrogen (C:N) ratio of vermicompost was evaluated over the entire 90 days of vermicomposting (Fig. 1D) by estimating the total C and N content. The C:N ratio of vermicompost samples followed the trend of 25.8 ± 0.46 (0th day) > 21.1 ± 1.33 (30th day) > 17.1 ± 2.12 (60th day) > 16.6 ± 2.06 (90th day). The lowest C:N ratio was recorded with the treatment V₄, while the highest C:N ratio was associated with V₁, both having statistical variation. According to Domínguez and Edwards (2011) and Ramnarain et al. (2019), the C:N ratio, which is one of the most generally recognised indices of organic waste maturity, declined during the vermicomposting process, which was acceptable. The drop in the C:N ratio, according to Kaushik and Garg (2003), might be related to the rapid decomposition of organic waste, as well as mineralization and stabilisation throughout the vermicomposting process.

3.2. Physical, chemical and biological (enzymatic) characterization of prepared vermicompost samples

The final product of seven differently prepared vermicompost samples were characterized for different physical properties like moisture content (%), water holding capacity (%), bulk density (kg/m³) and particle size distribution (%). The results as evident from Table 2 suggested that the moisture content of the vermicompost samples ranged from 19.12 to 21.60%, while for the other parameters, the ranges were, 78.77–88.99% (for water holding capacity), 0.69–0.74 kg/m³ (for bulk density), and 91.20–93.20% (for particle size distribution). All the samples of vermicompost had the characteristics within the limits as set by Fertilizer (Control) Order, 1985 which is administered by Dept of Agriculture Cooperation, Govt. of India.

The results revealed that most of these properties had their best treatment as V₄, while the bulk density of V₄ and V₇ were at par. In all cases the poorer estimates were associated with V₁. As mentioned earlier there exists a high tolerance of *E. foetida* to metal rich condition (Goswami et al., 2016; Paul et al., 2018; Mondal et al., 2020) and thus there was better activity of the vermiworms as the time of vermicomposting progresses and the final product thus becomes well prepared and granulated.

The vermicompost samples were also analyzed for their enzymatic characters like dehydrogenase activity, alkaline phosphatase activity, and FDA enzyme assay. The results as evident from Table 2 suggested that the dehydrogenase activity of the vermicompost samples ranged from 78.82 to 92.34 $\mu\text{g TPF g}^{-1} 24 \text{ h}^{-1}$, while the values of alkaline phosphatase and FDA enzyme assay ranged from 165.92 to 194.38 $\mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$ and 87.65–102.68 $\mu\text{g fluorescein g}^{-1} \text{ h}^{-1}$

respectively. Further all these properties exhibited best value for V₄, while the lowest values were associated with V₁.

Greater activity of vermiworms with metal enriched feeding materials led to greater enzymatic activity as evident from the study of Roy et al. (2022). The overall oxidative activities of the soil microflora can be determined by examining a group of enzymes called as Dehydrogenase (Nikaeen et al., 2015). FDA hydrolysis by different microbial enzymes (proteases, lipases, and esterases), on the other hand, aids in the fast assessment of total bacteria and fungus in biomass and soil (Tian et al., 2015). The abundant intrinsic microbiological activity in cow dung enhanced the activity of DHG and FDA as reported by Sadet-Bourgeteau et al. (2018). The inhibitory effect of metal enrichment in the vermicomposting feedstock, on the other hand, which was predicted to disrupt microbial growth and cellular proliferation (Roy et al., 2022), was not observed in our investigation. This is due to the fact that vermiworms, along with their gut microbiota, may have effectively compensated for the metal enhancement of the food, reducing stress (Elbl et al., 2019).

Chemical characterization of the prepared vermicompost samples (Table 2) suggested that the ranges for the parameters were 6.85–7.12 (pH), 3.64–5.45 dSm⁻¹ (EC), 25.0–34.3% (Total OM), 21.2–28.64% (Total OC), 1.04–1.58% (Total N), 0.96–1.14% (Total P), 0.68–1.05% (Total K), 44.9–252.36 mg/kg (Total Zn), 27.4–68.95 mg/kg (Total Cu), 820.12–1782.49 mg/kg (Total Fe), 73.76–82.70 mg/kg (Total Mn), and 0.0009–0.0016 mg/kg (Total As). The characteristics of the samples of vermicompost were within the limits set by Fertilizer (Control) Order, 1985 administered by Dept of Agriculture Cooperation, Govt. of India. Further, most of these properties had their best treatment as V₄. Although the maximum Zn and Fe content were associated with V₇ yet the content was at par with V₄. In all cases, the poorest performer was V₁ treatment.

Enrichment of vermicompost with Zn and Fe increased the total content of these metals (Hashemajd and Jamaati-e-Somarin, 2011). pH reduction in all treatments of enriched vermicompost were observed in comparison to non-enriched vermicompost possibly due to the formation of sulfuric acid during oxidation and vermiworm activity (Atiyeh et al., 2000). Increase in the electrical conductivity of enriched vermicompost can be originated from solubility of Zn and Fe mineral salts (Geiklooi and Shirmohammadi, 2013). Although both the total organic carbon and total nitrogen increased in case of enriched vermicompost, the rate of increase in N was much higher than C resulting in lower estimates of C:N ratio that indicate advanced decomposition. Lower C:N may be due to the decomposition of organic matter and emission of carbon as CO₂ (Marzi et al., 2020). The enriched vermicompost showed higher content of other elements than non-enriched vermicompost similar to the findings of Hashemimajd et al. (2004) and Geiklooi and Shirmohammadi (2013).

3.3. Characterization of HA and FA extracted from the enriched vermicomposts

3.3.1. Determination of contents of carboxyl and phenolic (OH) groups of HA and FA by the potentiometric titration methods

The potentiometric information about the various HA/FA samples extracted from either soils or different combinations of vermicomposts (non-enriched vermicompost as well as the six different combinations of enriched vermicompost) have been recorded in Table 3. The carboxylic acidity was, in general, more than the phenolic (OH) group acidity which is in good agreement with the earlier observations (Sinha and Bhattacharyya, 2011; Ghosh et al., 2012; Mandal et al., 2019a).

Out of the given HA/FA samples extracted from selected soils and organic manures, FA-V₄ registered the strongest acidic character, as evident from the order: FA-V₄ > FA-V₅ > FA-V₆ > FA-V₃ > FA-V₇ > FA-V₁ > FA-soil > FA-V₂ > HA-V₄ > HA-V₅ > HA-V₆ > HA-V₃ > HA-V₇ > HA-V₄ > HA-V₂ > HA-soil. It has been found that total acidity was higher in the fulvic acid samples compared to the corresponding HA samples. This may be due to the fact that the later were higher polymers,

Table 4
Viscometric, spectrophotometric study, stability constant and mole ratio of HA/FA-Arsenic complexes.

	B of Na- humate/fulvate anion (percent ⁻¹)	B _{expt.} of Na- humate/fulvate (percent ⁻¹) [B _{expt.} = B - B _{Na} ⁺]	Intrinsic viscosity(η) (100 ml/g)	Molecular weight (M) of humic/fulvic acid samples	(E ₄ /E ₆) at pH 7.0	(E ₄ /E ₆) at pH 9.0	Δ (E ₄ /E ₆) = (E ₄ /E ₆) at pH 7.0 - (E ₄ /E ₆) at pH 9.0	Mole ratio (x)	Stability constant (log K)
HA-soil	0.3245	0.3241	0.3241	11770.88	7.86	8.12	-0.26	0.36	2.70
HA-V ₁	0.3754	0.3750	0.3750	14759.43	5.75	7.21	-1.46	0.52	3.90
HA-V ₂	0.3261	0.3257	0.3257	11882.92	5.56	7.12	-1.56	0.72	5.40
HA-V ₃	0.2567	0.2563	0.2563	8219.87	6.01	7.53	-1.52	0.6	4.50
HA-V ₄	0.4629	0.4625	0.4625	20377.52	5.84	7.42	-1.58	0.92	6.90
HA-V ₅	0.4560	0.4556	0.4556	19911.83	5.49	6.82	-1.33	0.56	4.20
HA-V ₆	0.4114	0.4110	0.4110	16994.10	5.58	7.01	-1.43	0.59	4.43
HA-V ₇	0.3470	0.3466	0.3466	13075.72	5.87	7.38	-1.51	0.84	6.30
FA-soil	0.2636	0.2632	0.2632	8562.67	8.84	10.01	-1.17	0.54	4.05
FA-V ₁	0.2742	0.2738	0.2738	9098.76	6.47	8.21	-1.74	0.74	5.55
FA-V ₂	0.2871	0.2867	0.2867	9766.37	6.26	7.86	-1.61	0.91	6.83
FA-V ₃	0.2135	0.2131	0.2131	6188.29	6.76	8.25	-1.49	0.85	6.38
FA-V ₄	0.2638	0.2634	0.2634	8572.68	7.83	9.65	-1.82	1.36	10.20
FA-V ₅	0.2646	0.2642	0.2642	8612.76	7.36	8.69	-1.33	0.87	6.53
FA-V ₆	0.2814	0.2810	0.2810	9469.34	6.86	8.12	-1.26	0.89	6.68
FA-V ₇	0.2650	0.2646	0.2646	8632.82	7.05	8.92	-1.87	1.19	8.93

HA and FA designate humic and fulvic acid extracts of soil and vermicompost samples. V₁ as non-enriched vermicompost; V₂, V₃ and V₄ (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied before application of vermiworms) and V₅, V₆ and V₇ (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied at peak stage of vermiworm multiplication, 25–30 days after vermiworm application). Each value is a mean of three replicates.

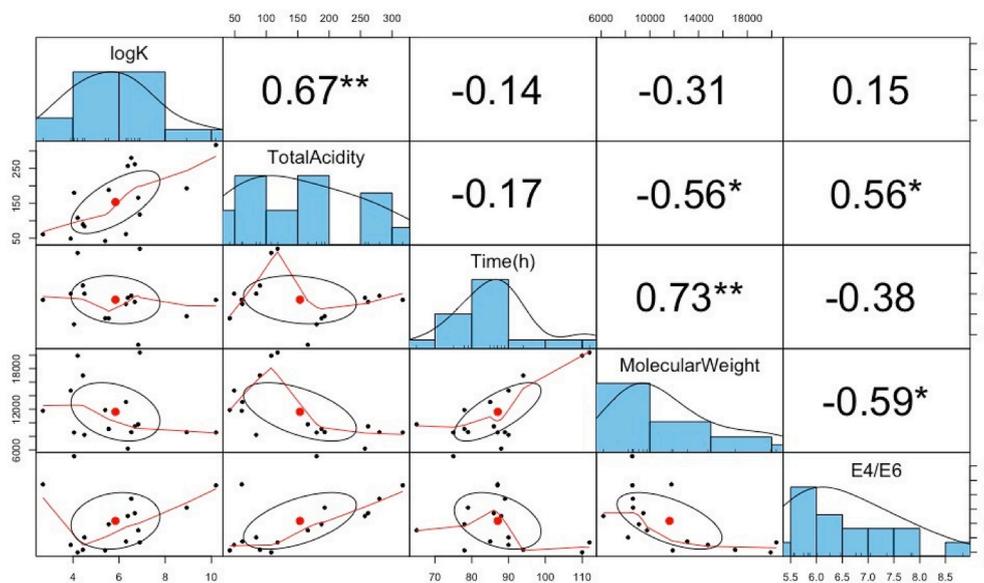


Fig. 2. Spearman correlation of stability constant of As-HA/FA with total acidity, time to attain stable pH, E4/E6 ratio and molecular weight of HA/FA.

formed through poly-condensation processes involving the functional groups.

The pH values pertaining to the first and final inflection points of the potentiometric titration curves for the various HA/FA samples were shown in Table 3. The pH variations in this table, namely $\Delta \text{pH} = \text{pH}_{3/4} - \text{pH}_{1/4}$, refer to the pH values at final inflection points that correspond to three-fourths and one-fourth of the pH. It was discovered that the ΔpH value of reported HA/FA samples was larger than 0.954, indicating that the samples were polyprotic (Sturrock, 1968), which is consistent with the result of Mandal et al. (2019a).

The amount of fresh excess alkali (ranging from 0.425 to 4.45 cmol/kg) required by HA/FA samples to reach a steady higher pH when HA/FA molecules were highly unfolded under thermodynamic compulsion was obviously dependent on the quantity of remaining acidic groups not accessible to folded alkali (Table 3). The effect was governed by the “steric hindrance” related with a specific conformation of such molecules owing to their coiled structure (Ghosh et al., 2012). The time necessary to achieve a stable higher pH (65–112 h) (Table 3) can be determined by the ease of decoiling, and is usually inversely proportional to intra-molecular H-bonding. The R-group of the amino acid unit, which determined the “flexibility” of the molecules, frequently interrupted the formation of such bonds (Mukhopadhyay and Sanyal, 2004). In spite of consuming less fresh alkali, the HA samples isolated from the enriched vermicompost samples, HA-V₄, HA-V₅, and HA-V₆, took longer to reach the steady higher pH. This could be due to the HAs’ highly coiled polycondensed structure, as well as their substantially larger molecular weight. Because such coil formation did not necessitate the formation of intramolecular hydrogen bonds by the –COOH and phenolic –OH groups, little ‘extra acidity’ was trapped inside the coils. The existence of the small ‘R’ group of other HA/FA samples, namely HA-V₄ and HA-V₅, in comparison to the HA samples, derived from the enhanced vermicompost samples, may have been accountable for the considerably shorter time to reach the stable higher pH.

3.3.2. Viscometric measurement of the HA/FAs extracted from enriched vermicompost

Table 4 showed the experimental B values ($B_{\text{expt.}}$) and molecular weight (M) of specified HA/FA samples. The data for the current HA/FA samples pointed to a strengthening of the ‘local’ water structure, resulting in an increase in HA/FA solution viscosity (relative to water) and a positive viscosity B co-efficient. Mandal and Sanyal (1984) proposed a method for determining the folded shape of HA by comparing

the experimental B coefficient ($B_{\text{expt.}}$) of the Jones-Dole equation with the additive value, B, calculated by adding the B contributions attributed to the prevalence of various hydrophilic functional groups in HA molecules. With the exception of FA and HA of V₃, the HA/FA samples extracted from vermicomposts had a higher $B_{\text{expt.}}$ value than the HA/FA samples isolated from soil. This could be due to the rise of $B_{\text{expt.}}$ values of HA/FA due to increase of the carbon number in acid molecules. The disperse phase or solute-solute interactions were negligible as long as the suspensions were dilute i.e., the A coefficient of Jones-Dole equation was negligibly small (Mukhopadhyay and Sanyal, 2004). At the same time, the coiled character of HA/FA samples could be the reason for the $B_{\text{expt.}}$ value variations (Sinha and Bhattacharyya, 2011; Ghosh et al., 2012).

The higher $B_{\text{expt.}}$ of HA/FA indicated above could be due to the stable coiling (higher degree) therefore larger molecular weight of HA/FA samples recovered from organics versus HA/FA samples isolated from soil. The modified Staudinger’s equation was used to calculate average molecular weight (M) using intrinsic viscosities (η) calculated from $B_{\text{expt.}}$ values (McBain, 1950; Chen and Schnizer, 1976); where a greater $B_{\text{expt.}}$ value corresponds to a higher molecular weight (Sinha and Bhattacharyya, 2011; Ghosh et al., 2012). The HA/FA extracted from given vermicompost samples showed a much higher molecular weight, in the order: HA-V₄ > HA-V₅ > HA-V₆ > HA-V₁ > HA-V₇ > HA-V₂ > HA-soil > FA-V₂ > FA-V₆ > FA-V₁ > FA-V₇ > FA-V₅ > FA-V₄ > FA-soil > HA-V₃ > FA-V₃.

3.3.3. Spectrophotometric measurement of the HA/FAs extracted from enriched vermicompost

The results of Table 4 showed the E₄/E₆ values of selected HA/FA samples at two different pH values (pH 7.0 and 9.0). The E₄/E₆ ratio always was higher than unity, with FA fractions exhibiting higher value than the corresponding HA fractions. The ratio of optical densities or absorbance of solutions of dilute aqueous HA and FA at 465 and 665 nm has been widely used for the characterization of these materials. This E₄/E₆ ratio is inversely related to the degree of condensation of the aromatic network in humic substances. The ratio is primarily governed by the particle size, molecular weight and degree of aromaticity (Kononova, 1969), so that a high ratio would reflect a low degree of aromatic condensation and the presence of relatively large proportions of aliphatic structures (Ghosh et al., 2012). Fulvic colloids had a larger E₄/E₆ ratio because they were less condensed and aromatic (Sanyal, 2002). Table 4 also recorded the $\Delta E_4/E_6$ ratio, which can be used as a

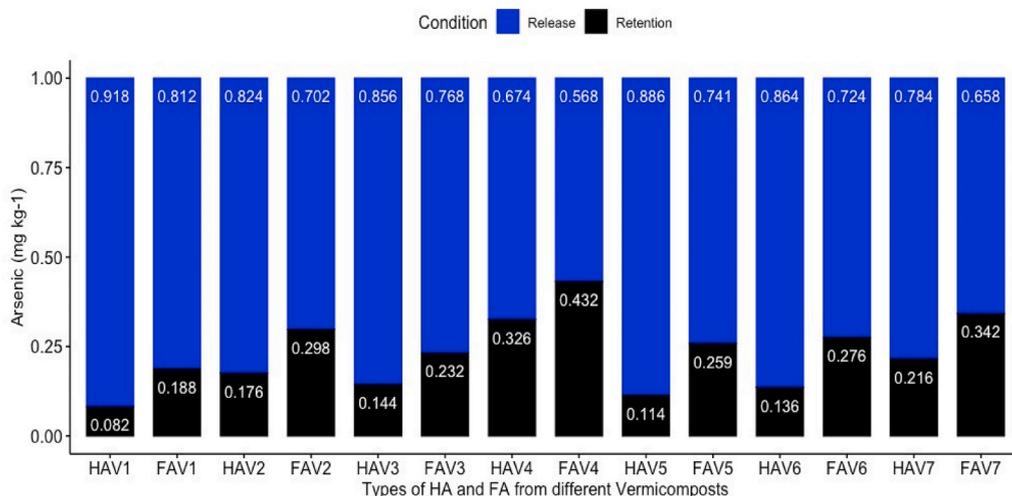


Fig. 3. Efficiency of organo-As complexes of the different types of vermicompost on retention and release of As.

“index of flexibility” of the HA/FA molecules because the addition of alkali (NaOH) rendered the molecules significantly unfolded “under thermodynamic compulsion” (Sanyal, 2002; Sinha and Bhattacharyya, 2011). For the supplied HA/FA samples, the E₄/E₆ ratio was determined to be negative. This could be owing to the increased degree of aromatic ring condensation in these samples.

The degree of compression of the aromatic network in humic compounds was inversely related to the E₄/E₆ ratio. Because the aromatic moiety has been the most hydrophobic, it tends to conceal itself in the

coil’s innermost core, leaving the aliphatic moiety to form the coil’s outer periphery. Due to a specific structural arrangement of the molecule, the aliphatic component (i.e., absorbance at 465 nm, E₄) absorbed more light than the aromatic part (i.e., absorbance at 665 nm, E₆) (Sinha and Bhattacharyya, 2011).

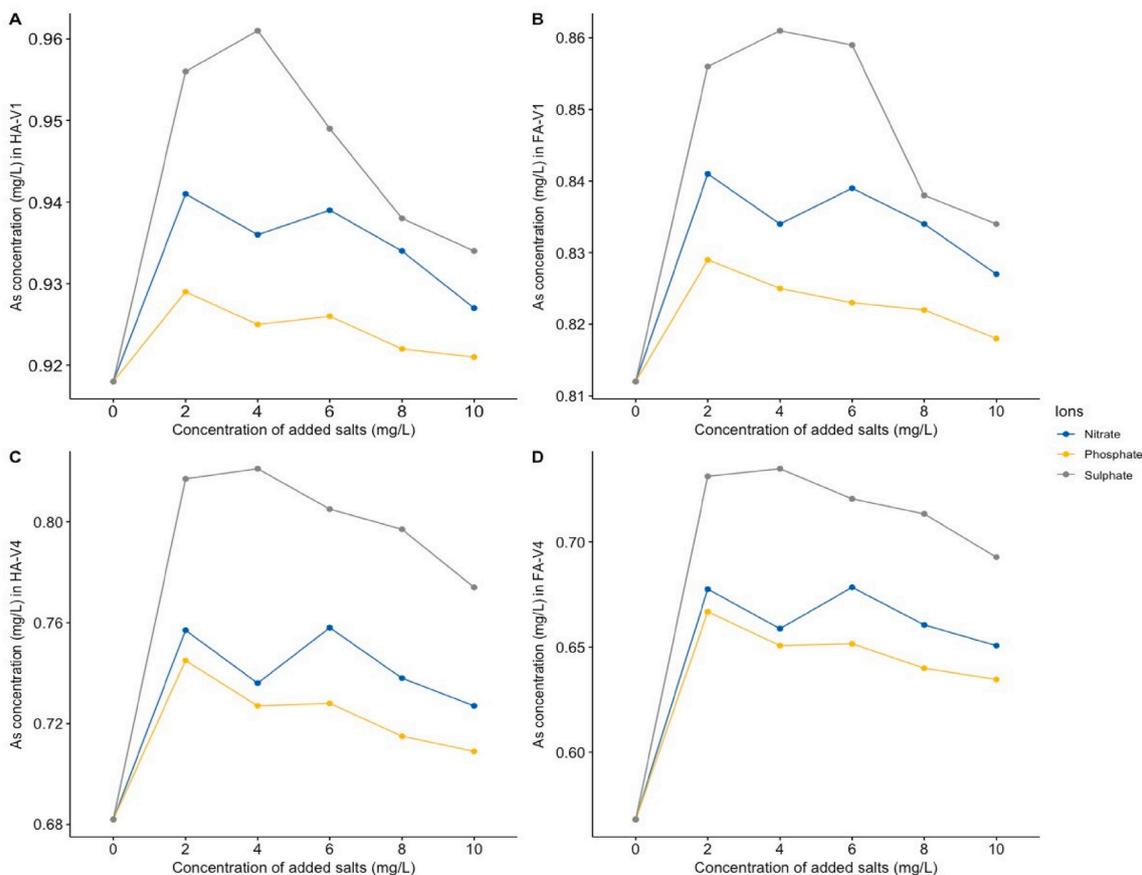


Fig. 4. Release of As from organo-As complex (HA/FA) in presence of different oxy-anions (nitrate, phosphate and sulphate) of V₁ as non-enriched vermicompost (A: HA bound As, B: FA bound As) and V₄ as Zn and Fe enriched vermicompost (C: HA bound As, D: FA bound As).

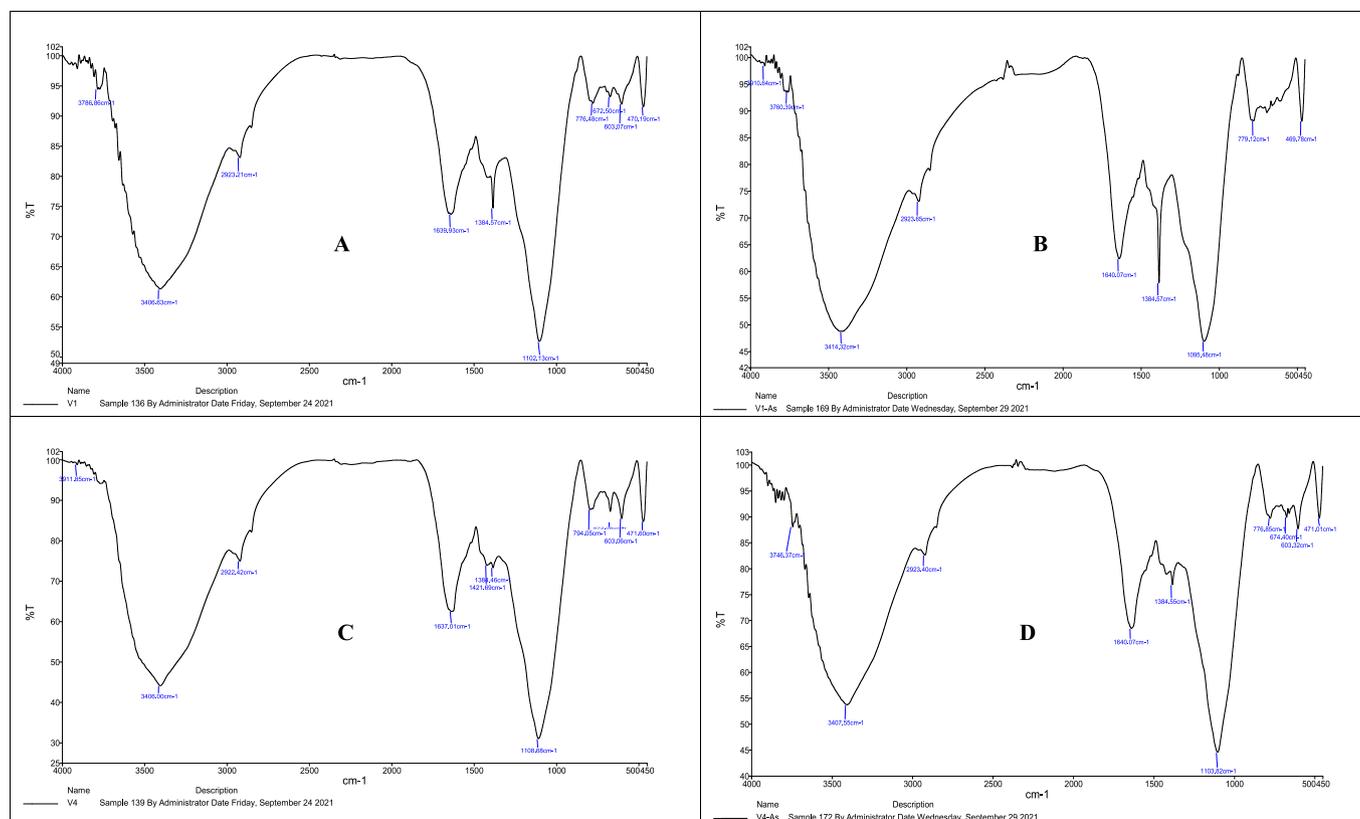


Fig. 5. FT-IR spectroscopic images of vermicompost samples: (A) V₁, (B) V₁ with As spiking, (C) V₄ and (D) V₄ with As spiking.

3.4. Determination of stability constant of organo-arsenic complexes obtained by the ion-exchange method

The values of x (the number of mol HA/FA that join with 1 mol As) and $\log K$ (the stability constant of As–HA/FA complexes) have been shown in Table 4. The stability constant of As–humate/fulvate complexes ranged from 2.70 to 10.20 at pH 5.0, with FA–V₄ having the greatest $\log K$ and x values. The $\log K$ values were similar to those reported for heavy metal–humic complexes in general (Sinha and Bhattacharyya, 2011; Ghosh et al., 2012; Mandal et al., 2019a; Kumar et al., 2021). Furthermore, larger molecular weight HA presumably bind heavy metals more effectively than lower molecular weight HA (Mukhopadhyay and Sanyal, 2004). The $\log K$ values in the current study followed the order: FA–V₄ > FA–V₇ > HA–V₄ > FA–V₂ > FA–V₆ > FA–V₅ > FA–V₃ > HA–V₇ > FA–V₁ > HA–V₂ > HA–V₃ > HA–V₆ > HA–V₅ > FA–soil > HA–V₁ > HA–soil.

A significant and positive correlation of $\log K$ values was observed with overall acidity ($r = 0.67^{**}$), but not with molecular weight ($r = -0.31$), (E_4/E_6) at pH 7.0 ($r = 0.15$), or excess time to reach the stable higher pH ($r = -0.144$) of the HA/FA component (Fig. 2). As previously stated, HA/FA hydrophobicity projected to rise with molecular weight, resulting in an increase of surface excess in water and the time required to achieve a stable higher pH during potentiometric titration of the HA/FA samples. It is possible that deprotonation of the carboxyl groups and phenolic and aliphatic OH groups occurred under the given pH conditions. As a result, ligand exchange was thought to play a smaller role in sorption of As on HA, while van der Waals forces and cation bridges were thought to play a bigger role (Mandal et al., 2019a; Kumar et al., 2021). Based on the relatively large positive correlation of $\log K$ with total acidity of the HA/FA samples (Fig. 2) it can be concluded that the carboxylic and phenolic groups, may be engaged in the formation of complexes with As in aqueous phase (Mandal et al., 2019a; Kumar et al., 2021).

3.5. Retention and release pattern of arsenic from HA/FAs of enriched vermicompost

Apart from this, the efficiency of organo-arsenic complexes of the different types of vermicompost used in the current study was evaluated based upon the retention and release phenomenon of arsenic. The results as evident from Fig. 3 revealed that replacement of As were lower with the HA/FA samples extracted from enriched than non-enriched vermicompost in the order: HA–V₁ > HA–V₅ > HA–V₆ > HA–V₃ > HA–V₂ > FA–V₁ > HA–V₇ > FA–V₃ > FA–V₅ > FA–V₆ > FA–V₂ > HA–V₄ > FA–V₇ > FA–V₄. It is evident that for each kind of vermicompost samples, FAs usually had lower estimates of release of As from organo-As complexes than their corresponding HAs. The lowest release has been associated with FA–V₄ which might be due to the fact that it had a greater ability to retain the As (1.36 mol of HA/FA bind with 1 mol of arsenic, as evident from mole ratio), thereby reducing the bioavailability of As. Sinha and Bhattacharyya (2011) earlier revealed that HA and FA isolated from vermicompost have the ability to bind As (1.15 mol of FA binds with 1 mol of As). In another study, Mandal et al. (2019a) reported a mole ratio of 1.12 for FA extracted from vermicompost. In this current work, the higher mole ratio justifies the fact that Fe and Zn enriched vermicomposts have an advantage over non-enriched vermicomposts in terms of complexing ability.

3.6. Release isotherms of arsenic from organo-arsenic complexes

As release isotherms from HA/FA complexes in presence of competing ions like phosphate, nitrate, and sulphate salts have been shown in Fig. 4 (A, B, C, D). The ion release isotherms indicated that the exchange took place in two or three steps. The initial extent of equilibrium exchange was rather high, followed by a limiting equilibrium exchange. The figures suggested that among the three competitive oxy-anions, the release isotherms followed the general trend of sulphate >

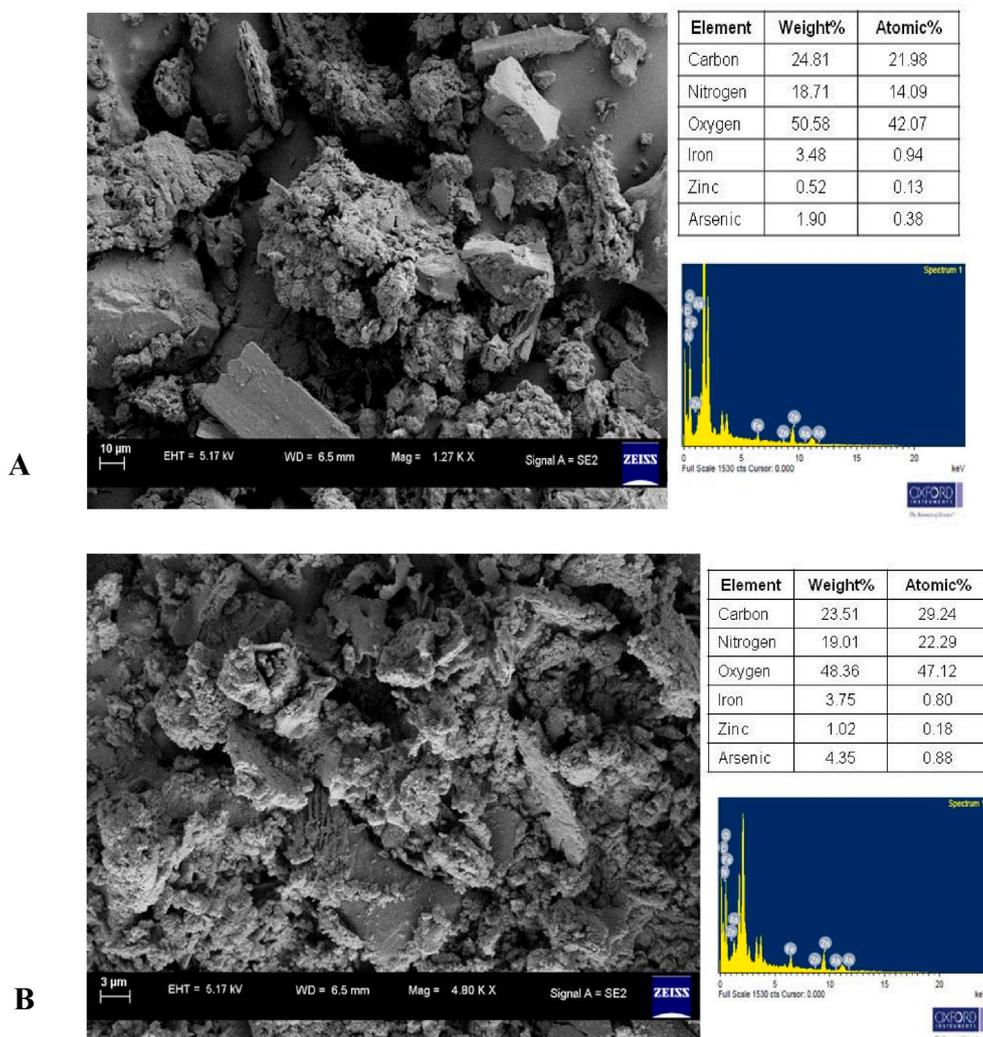


Fig. 6. SEM image of (A) non-enriched vermicompost (V_1) and (B) enriched vermicompost (V_4) with As spiking.

nitrate > phosphate for both HA and FA in most of the vermicompost samples.

Two general surface complexes were influenced to govern the adsorbate and adsorbent namely the inner- and outer-sphere surface complexes, which are distinguished by the presence or absence of the adsorbate molecule's hydration sphere during contact (Mukhopadhyay and Sanyal, 2004; Ghosh et al., 2012). The surface complex is called outer-sphere when at least one water molecule from the hydration sphere is maintained after sorption. An inner-sphere complex is created when an ion is attached directly to the adsorbent without the presence of the hydration sphere. It was observed that SO_4^{2-} and NO_3^- have a higher tendency than other anions to dislodge As from complexes (Borda and Sparks, 2008; Sinha and Bhattacharyya, 2011).

3.7. Fourier-transform infrared (FT-IR) spectroscopy

In the current study we have tried to investigate whether the interaction of As with vermicompost either enriched with Zn and Fe, or not, was owing to sorption process of As ions with functional groups of vermicompost. To effectuate such study, an FTIR analysis was carried out. Based on principal of IR (Infra-red) bands, each peak's absorptions for certain functional groups were compared to the corresponding functional groups in normal vermicompost, where the wave numbers at $3300\text{--}3500\text{ cm}^{-1}$ (O-H), $1570\text{--}1550\text{ cm}^{-1}$ (N-H deformation), $2925\text{--}2850\text{ cm}^{-1}$ (C-H bonds), showed a slight shift in the presence of As in

vermicompost (Fig. 5). The FTIR bands assigned an asymmetric stretch of the NH_2 , N-H wag, and S-O bends at the $850\text{--}750\text{ cm}^{-1}$, $750\text{--}700\text{ cm}^{-1}$ and $680\text{--}610\text{ cm}^{-1}$ respectively for different Zn and Fe enriched vermicompost. Finally, a significant displacement of FTIR bands corresponding to different chemical groups was observed when As was spiked on these vermicompost samples. The enriched vermicompost (especially V_4 and V_7) showed a greater tendency of such shift in As spiking attributing its greater stability and complexity. Such kind of shifting mechanism with organic or inorganic toxic components is not uncommon and has been widely reported by Das et al. (2016) and Bhat et al. (2017).

3.8. Scanning Electron Microscopy (SEM)

The SEM imagery revealed significant differences between non-enriched vermicompost and enriched vermicompost samples. Again when As spiked vermicompost samples were subjected to SEM analysis there was significant variations as compared to samples without As spiking (Fig. 6). In almost all vermicompost samples As spiking was associated with aggregation of granular structure of vermicompost. The Energy Dispersive X-Ray Analysis (EDX) analysis showed a distinct signal corresponding to As peak in presence of As treated vermicompost which was not present in samples without As spiking. Interestingly, such distinct EDX signal and peak was more pronounced in case of Zn and Fe enriched vermicompost samples especially V_4 and V_7 than the non-

enriched vermicompost. The quantification of percent content of different elements in the vermicompost samples reveal a concomitant increase in As sorption when the Zn and Fe content of the vermicompost samples increased. This can clearly give an insight that Zn and Fe enriched vermicompost had greater capacity of sequestering As and thus holistically provide a solution to curb As toxicity in rice system. This might possibly arise due to complexation and adsorption of As with the carboxylic and phenolic functional groups leading to the aggregation or the formation of natural adducts (Ghosh et al., 2012; Mandal et al., 2019a).

4. Conclusion

From our findings it can be concluded that the complexes formed with As in the aqueous phase indicated that the stability of FA extracted from enriched vermicompost V₄ (Zn and Fe sulphate @ 10% w/w dry weight basis of composting substrates before application of vermiforms) had the maximum log K of 10.20 and a mole ratio (x) value of 1.36. The findings of the analysis on arsenic HA/FA complexation equilibria are expected to shed light on the stability of these complexes, demonstrating their reliance on the properties and behavior of the humic polymer, which, in turn, can affect the retention/release of such a toxic metal in soil matrix, particularly when Zn and Fe enriched vermicompost has been applied. Arsenic release isotherms from HA and FA complexes after treatment with sulphate, phosphate, and nitrate salts revealed that sulphate had a higher degree of exchangeability than other oxyanions from those kind of organo-arsenic complexes. The findings reveals the ability of the native soil organic fraction to sorb arsenic, reducing its toxicity in the soil/crop system and, as a result, its entry into the food chain. As the tropical soils are poor in soil organic matter, it may be possible to improve soil organic matter stock and hence arsenic retention in arsenic-affected soils using appropriate management measures (such as agricultural waste recycling, organic manure inclusion, and so on).

Credit author statement

Sudip Sengupta: Methodology, Investigation, Data curation, Software and Writing- Original draft preparation; **Kallol Bhattacharyya:** Conceptualization, Methodology, Supervision; **Jajati Mandal:** Data curation, Visualization, Software, Writing- Reviewing and Editing; **Asoke Prasun Chattopadhyay:** Methodology, Validation, Supervision.

Research involving human participants and/or animals

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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.

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References

- Abad, M., Noguera, P., Puchades, R., Maquieira, A., Noguera, V., 2002. Physico-chemical and chemical properties of some coconut coir dusts for use as a peat substitute for containerised ornamental plants. *Bioresour. Technol.* 82 (3), 241–245.
- Ahn, H.K., Richard, T.L., Glanville, T.D., 2008. Optimum moisture levels for biodegradation of mortality composting envelope materials. *Waste Manag.* 28 (8), 1411–1416.
- Alef, K., 1995. Enrichment, isolation and counting of soil microorganisms. In: *Methods in Applied Soil Microbiology and Biochemistry*. Academic press, pp. 123–191.
- ASAE, 1998. Soil moisture. In: *Transactions of the ASAE*. <https://elibrary.asabe.org/>.
- Atiyeh, R.M., Subler, S., Edwards, C.A., Bachman, G., Metzger, J.D., Shuster, W., 2000. Effects of vermicomposts and composts on plant growth in horticultural container media and soil. *Pedobiologia* 44 (5), 579–590.
- Bachman, G.R., Metzger, J.D., 2007. Physical and chemical characteristics of a commercial potting substrate amended with vermicompost produced from two different manure sources. *HortTechnology* 17 (3), 336–340.
- Bandyopadhyay, B.K., Sarkar, P., Sen, H.S., Sanyal, S.K., 2004. Influence of soil properties on arsenic availability in soil. *Indian Soc. Soil Sci.* 52 (1), 50–55.
- Bhat, S.A., Singh, J., Vig, A.P., 2017. Instrumental characterization of organic wastes for evaluation of vermicompost maturity. *J. Anal. Sci. Technol.* 8 (1), 1–12.
- Bhattacharyya, K., Sengupta, S., Pari, A., Halder, S., Bhattacharyya, P., Pandian, B.J., Chinchmalapure, A.R., 2021. Characterization and risk assessment of arsenic contamination in soil–plant (vegetable) system and its mitigation through water harvesting and organic amendment. *Environ. Geochem. Health* 43, 2819–2834.
- Borda, M.J., Sparks, D.L., 2008. Kinetics and mechanisms of sorption–desorption in soils: a multiscale assessment. *Biophysico-chemical processes of heavy metals and metalloids in soil environments* 97–124.
- Buschmann, J., Kappeler, A., Lindauer, U., Kistler, D., Berg, M., Sigg, L., 2006. Arsenite and arsenate binding to dissolved humic acids: influence of pH, type of humic acid, and aluminum. *Environ. Sci. Technol.* 40 (19), 6015–6020.
- Casida Jr., L.E., Klein, D.A., Santoro, T., 1964. Soil dehydrogenase activity. *Soil Sci.* 98 (6), 371–376.
- Chen, Y., Schnitzer, M., 1976. Scanning electron microscopy of a humic acid and of a fulvic acid and its metal and clay complexes. *Soil Sci. Soc. Am. J.* 40 (5), 682–686.
- Costa, A.S., Nascimento, A.L., Botero, W.G., Carvalho, C.M., Tonholo, J., Santos, J.C., Anunciação, D.S., 2022. *Sci. Total Environ.* 802, 149779.
- Craw, D., Chappell, D.A., 2000. Metal redistribution in historic mine wastes, coromandel peninsula, New Zealand. *N. Z. J. Geol. Geophys.* 43, 187–198.
- Das, S., Deka, P., Goswami, L., Sahariah, B., Hussain, N., Bhattacharyya, S.S., 2016. Vermiremediation of toxic jute mill waste employing *Metaphire* posthuma. *Environ. Sci. Pollut. Control Ser.* 23 (15), 15418–15431.
- Domínguez, J.J., Edwards, C.A., 2011. Biology and ecology of vermiforms species used for vermicomposting. In: *Edwards, C.A., Arancon, N.Q., Sherman, R.L. (Eds.), Vermiculture Technology: Vermiforms, Organic Waste and Environmental Management*. CRC Press, Boca Raton, pp. 27–40. <https://www.taylorfrancis.com>.
- Domínguez, J., Aira, M., Kolbe, A.R., Gómez-Brandón, M., Pérez-Losada, M., 2019. Changes in the composition and function of bacterial communities during vermicomposting may explain beneficial properties of vermicompost. *Sci. Rep.* 9 (1), 1–11.
- Elbl, J., Maková, J., Javoreková, S., Medo, J., Kintl, A., Lošák, T., Lukas, V., 2019. Response of microbial activities in soil to various organic and mineral amendments as an indicator of soil quality. *Agronomy* 9 (9), 485.
- Fertilizer (Control) Order, Dept of Agriculture Cooperation, Government of India, <https://fert.nic.in> (accessed on 01.11.2021).
- Geiklooi, A., Shirmohammadi, E., 2013. Effect of enriched vermicompost manure in improve of iron and zinc deficiencies and quality characteristics of peach trees. *Int. J. Farm. Allied Sci.* 2 (21), 930–934.
- Ghosh, K., Das, I., Das, D.K., Sanyal, S.K., 2012. Evaluation of humic and fulvic acid extracts of compost, oilcake, and soils on complex formation with arsenic. *Soil Res.* 50 (3), 239–248.
- Goswami, L., Pratihari, S., Dasgupta, S., Bhattacharyya, P., Mudoj, P., Bora, J., Bhattacharyya, S.S., Kim, K.H., 2016. Exploring metal detoxification and accumulation potential during vermicomposting of Tea factory coal ash: sequential extraction and fluorescence probe analysis. *Sci. Rep.* 6 (1), 1–13.
- Hansen, E.H., Schnitzer, M., 1969. Molecular weight measurements of polycarboxylic acids in water by vapor pressure osmometry. *Anal. Chim. Acta* 46 (2), 247–254.
- Hashemimajd, K., Jamaati-e-Somarin, S., 2011. Investigating the effect of iron and zinc enriched vermicompost on growth and nutritional status of peach trees. *Sci. Res. Essays* 6 (23), 5004–5007.
- Hashemimajd, K., Kalbasi, M., Golchin, A., Shariatmadari, H., 2004. Comparison of vermicompost and composts as potting media for growth of tomatoes. *J. Plant Nutr.* 27 (6), 1107–1123.
- Hussain, M.M., Bibi, I., Niazi, N.K., Shahid, M., Iqbal, J., Shakoor, M.B., Ahmad, A., Shah, N.S., Bhattacharyya, P., Mao, K., Bundschuh, J., 2021. Arsenic Biogeochemical Cycling in Paddy Soil-Rice System: Interaction with Various Factors, Amendments and Mineral Nutrients. *Science of the Total Environment*, 145040.
- Jackson, H.C., 1973. *Soil Chemical Analysis*, Pub. Prentice Hall of India Private Limited, New Delhi, India.
- Jackson, M.L., 1967. *Soil Chemical Analysis*. Prentice-Hall of India Pvt. Ltd., New Delhi, p. 498.
- Karmakar, S., Prakash, P., Chattopadhyay, A., Dutta, D., 2021. Zinc sulphate and vermicompost mitigate phytotoxic effects of arsenic by altering arsenic uptake, biochemical and antioxidant enzyme activities in wheat (*Triticum aestivum* L.). *Russ. J. Plant Physiol.* 68 (1), S72–S81.

- Kaushik, P., Garg, V.K., 2003. Vermicomposting of mixed solid textile mill sludge and cow dung with the epigeic vermiform *Eisenia foetida*. *Bioresour. Technol.* 90 (3), 311–316.
- Khater, E.S.G., 2015. Some physical and chemical properties of compost. *Int. J. Waste Resour.* 5 (1), 172.
- Kononova, M.M., Newman, A.C.D., 1969. *Soil Organic Matter*. Pergamon Press, NY, USA.
- Kumar, S.N., Mishra, B.B., Kumar, S., Mandal, J., 2021. Organo-arsenic complexation studies explaining the reduction of uptake of arsenic in wheat grown with contaminated irrigation water and organic amendments. *Water, Air, Soil Pollut.* 232 (3), 1–10.
- Kumari, P.B., Singh, Y.K., Mandal, J., Shambhavi, S., Sadhu, S.K., Kumar, R., Ghosh, M., Raj, A., Singh, M., 2021. Determination of safe limit for arsenic contaminated irrigation water using solubility free ion activity model (FIAM) and Tobit Regression Model. *Chemosphere* 270, 128630.
- Lin, H.T., Wang, M.C., Li, G.C., 2004. Complexation of arsenate with humic substance in water extract of compost. *Chemosphere* 56 (11), 1105–1112.
- Liu, C., Yu, H.Y., Liu, C., Li, F., Xu, X., Wang, Q., 2015. Arsenic availability in rice from a mining area: is amorphous iron oxide-bound arsenic a source or sink? *Environ. Pollut.* 199, 95–101.
- Mandal, J., Golui, D., Datta, S.P., 2019a. Assessing equilibria of organo-arsenic complexes and predicting uptake of arsenic by wheat grain from organic matter amended soils. *Chemosphere* 234, 419–426.
- Mandal, J., Golui, D., Raj, A., Ganguly, P., 2019b. Risk assessment of arsenic in wheat and maize grown in organic matter amended soils of Indo-Gangetic plain of Bihar, India. *Soil and Sediment Contamination*. *Int. J.* 28 (8), 757–772.
- Mandal, J., Sengupta, S., Sarkar, S., Mukherjee, A., Wood, M., Hutchinson, S.M., Mondal, D., 2021. Meta-analysis enables prediction of the maximum permissible arsenic concentration in Asian paddy soil. *Front. Environ. Sci.* 9, 760125.
- Mandal, S.K., Sanyal, S.K., 1984. Shape of humic acid (a natural polymer) from viscosity studies. *J. Indian Soc. Soil Sci.* 32 (2), 224–229.
- Manyuchi, M.M., Whingiri, E., 2014. Effect of vermicomposting period, substrate quantity, cow dung composition and their interactions on *Eisenia fetida* during vermicomposting. *Int. J. Curr. Microbiol. Appl. Sci.* 3 (8), 1021–1028.
- Marzi, M., Shahbazi, K., Kharazi, N., Rezaei, M., 2020. The influence of organic amendment source on carbon and nitrogen mineralization in different soils. *J. Soil Sci. Plant Nutr.* 20 (1), 177–191.
- Matsumoto, S., Kasugaa, J., Taikib, N., Makinoc, T., Araoc, T., 2015. Reduction of the risk of arsenic accumulation in rice by the water management and material application in relation to phosphate status. *J. Plant Interact.* 10 (1), 65–74.
- McBain, J.W., 1950. Colloid science. *Soil Sci.* 70 (5), 412.
- Mondal, A., Goswami, L., Hussain, N., Barman, S., Kalita, E., Bhattacharyya, P., Bhattacharya, S.S., 2020. Detoxification and eco-friendly recycling of brick kiln coal ash using *Eisenia fetida*: a clean approach through vermitechnology. *Chemosphere* 244, 125470.
- Mukhopadhyay, D., Sanyal, S.K., 2004. Complexation and release isotherm of arsenic in arsenic-humic/fulvic equilibrium study. *Aust. J. Soil Res.* 42, 815–824.
- Nikaen, M., Nafez, A.H., Bina, B., Nabavi, B.F., Hassanzadeh, A., 2015. Respiration and enzymatic activities as indicators of stabilization of sewage sludge composting. *Waste Manag.* 39, 104–110.
- Pączka, G., Mazur-Pączka, A., Garczyńska, M., Kostecka, J., Butt, K.R., 2020. Effects of vermireactor modifications on the welfare of vermiforms *Eisenia fetida* (Sav.) and properties of vermicomposts. *Agriculture* 10 (10), 481.
- Pal, R., Bhattacharyya, P., Das, P., Chakrabarti, K., Chakraborty, A., Kim, K., 2007. Relationship between acidity and microbiological properties in some tea soils. *Biol. Fertil. Soils* 44 (2), 399–404.
- Pandey, A.K., Pandey, S.D., Misra, V., 2000. Stability constants of metal-humic acid complexes and its role in environmental detoxification. *Ecotoxicol. Environ. Saf.* 47 (2), 195–200.
- Paul, S., Das, S., Raul, P., Bhattacharya, S.S., 2018. Vermi-sanitization of toxic silk industry waste employing *Eisenia fetida* and *Eudrilus eugeniae*: substrate compatibility, nutrient enrichment and metal accumulation dynamics. *Bioresour. Technol.* 266, 267–274.
- Qiao, J.T., Liu, T.X., Wang, X.Q., Li, F.B., Lv, Y.H., Cui, J.H., Zeng, X.D., Yuan, Y.Z., Liu, C.P., 2018. Simultaneous alleviation of cadmium and arsenic accumulation in rice by applying zero-valent iron and biochar to contaminated paddy soils. *Chemosphere* 195, 260–271.
- Ramnarain, Y.I., Ansari, A.A., Ori, L., 2019. Vermicomposting of different organic materials using the epigeic vermiform *Eisenia foetida*. *Int. J. Recycl. Org. Waste Agric.* 8 (1), 23–36.
- Relan, P.S., Girdhar, K.K., Khanna, S.S., 1984. Molecular configuration of compost's humic acid by viscometric studies. *Plant Soil* 81 (2), 203–208.
- Roy, S., Sarkar, D., Datta, R., Bhattacharya, S.S., Bhattacharyya, P., 2022. Assessing the arsenic-saturated biochar recycling potential of vermitechnology: insights on nutrient recovery, metal benignity, and microbial activity. *Chemosphere* 286, 131660.
- Saada, A., Breeze, D., Crouzet, C., Cornu, S., Baranger, P., 2003. Adsorption of arsenic (V) on kaolinite and on kaolinite-humic acid complexes: role of humic acid nitrogen groups. *Chemosphere* 51 (8), 757–763.
- Sadet-Bourgeteau, S., Houot, S., Dequiedt, S., Nowak, V., Tardy, V., Terrat, S., Montenach, D., Mercier, V., Karimi, B., Prévost-Bouré, N.C., Maron, P.A., 2018. Lasting effect of repeated application of organic waste products on microbial communities in arable soils. *Appl. Soil Ecol.* 125, 278–287.
- Sahariah, B., Goswami, L., Kim, K.H., Bhattacharyya, P., Bhattacharya, S.S., 2015. Metal remediation and biodegradation potential of vermiform species on municipal solid waste: a parallel analysis between *Metaphire posthuma* and *Eisenia fetida*. *Bioresour. Technol.* 180, 230–236.
- Sanyal, S.K., 2002. Colloid chemical properties of soil humic substances: a relook. In: *Bulletin of the Indian Soc. vol. 21. Soil Sci.*, New Delhi, pp. 537–569.
- Sanyal, S.K., Mandal, S.K., 1983. Viscosity B coefficients of alkyl carboxylates. *Electrochim. Acta* 28 (12), 1875–1876.
- Schaub-Szabo, S.M., Leonard, J.J., 1999. Characterizing the bulk density of compost. *Compost Sci. Util.* 7 (4), 15–24.
- Schnitzer, M., Gupta, U.C., 1964. Some chemical characteristics of the organic matter extracted from the O and B2 horizons of a gray wooded soil. *Soil Sci. Soc. Am. J.* 28 (3), 374–377.
- Schnitzer, M., Khan, S.U., 1972. *Humic Substances in the Environment* (No. 631.417). M. Dekker, New York.
- Schnitzer, M., Skinner, S.I.M., 1966. Organo-metallic interactions in soils: 5. Stability constants of Cu⁺⁺, Fe⁺⁺⁺, and Zn⁺⁺⁺-fulvic acid complexes. *Soil Sci.* 102 (6), 361–365.
- Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl. Environ. Microbiol.* 43 (6), 1256–1261.
- Sengupta, S., Bhattacharyya, K., Mandal, J., Bhattacharya, P., Halder, S., Pari, A., 2021. Deficit irrigation and organic amendments can reduce dietary arsenic risk from rice: introducing machine learning-based prediction models from field data. *Agric. Ecosyst. Environ.* 319, 107516.
- Shakoor, M.B., Nawaz, R., Hussain, F., Raza, M., Ali, S., Rizwan, M., Oh, S.E., Ahmad, S., 2017. Human health implications, risk assessment and remediation of As-contaminated water: a critical review. *Sci. Total Environ.* 601, 756–769.
- Sinha, B., Bhattacharyya, K., 2011. Retention and release isotherm of arsenic in arsenic-humic/fulvic equilibrium study. *Biol. Fertil. Soils* 47 (7), 815–822.
- Sinha, B., Bhattacharyya, K., Giri, P.K., Sarkar, S., 2011. Arsenic contamination in sesame and possible mitigation through organic interventions in the lower Gangetic Plain of West Bengal, India. *J. Sci. Food Agric.* 91 (15), 2762–2767.
- Sivakumar, S., 2015. Effects of metals on earthworm life cycles: a review. *Environ. Monit. Assess.* 187 (8), 1–16.
- Stevenson, F.J., 1994. *Humus Chemistry: Genesis, Composition, Reactions*. John Wiley & Sons.
- Stokes, R.H., Mills, R., 1965. *Viscosity of Electrolytes and Related Properties* (No. 532.58). Pergamon Press.
- Sturrock, P.E., 1968. Equations for potentiometric titration (the author replies). *J. Chem. Educ.* 45 (9), 621.
- Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1 (4), 301–307.
- Tian, W., Wang, L., Li, Y., Zhuang, K., Li, G., Zhang, J., Xiao, X., Xi, Y., 2015. Responses of microbial activity, abundance, and community in wheat soil after three years of heavy fertilization with manure-based compost and inorganic nitrogen. *Agric. Ecosyst. Environ.* 213, 219–227.
- Tiessen, H., Moir, J.O., 1993. Total and organic carbon. In: Carter, M.E. (Ed.), *Soil Sampling and Methods of Analysis*. Lewis Publishers, Ann Arbor, MI, pp. 187–211.
- Tripathy, S., Bhattacharyya, P., Mohapatra, R., Som, A., Chowdhury, D., 2014. Influence of different fractions of heavy metals on microbial ecophysiological indicators and enzyme activities in century old municipal solid waste amended soil. *Ecol. Eng.* 70, 25–34.
- Wang, H., Liang, D., Wang, Y.N., Sun, Y., Li, W., Zhang, D., Tsang, Y.F., Pan, X., 2019. Fabricating biogenic Fe (III) flocs from municipal sewage sludge using NAFO processes: characterization and arsenic removal ability. *J. Environ. Manag.* 231, 268–274.
- Yu, Z., Qiu, W., Wang, F., Lei, M., Wang, D., Song, Z., 2017. Effects of manganese oxide-modified biochar composites on arsenic speciation and accumulation in an indica rice (*Oryza sativa* L.) cultivar. *Chemosphere* 168, 341–349.
- Zhu, X., Chen, B., Zhu, L., Xing, B., 2017. Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: a review. *Environ. Pollut.* 227, 98–115.