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1 **Title Page**

2 **Manuscript Title:**

3 ORGANO ARSENIC COMPLEXATION STUDIES EXPLAINING THE REDUCTION OF UPTAKE OF
4 ARSENIC IN WHEAT GROWN WITH CONTAMINATED IRRIGATION WATER AND ORGANIC
5 AMENDMENTS

6 **Authors Name:**

7 Shyam Nandan Kumar, Bipin Bihari Mishra, Sunil Kumar and Jajati Mandal^{1*}

8 **Author(s) affiliations:**

9 *Department of Soil Science and Agricultural Chemistry, Bihar Agricultural University, Sabour-813210*

10 *¹School of Science Engineering and Environment, University of Salford, Salford, UK*

11

12 **Corresponding author:**

13 Jajati Mandal, e-mail: jajati.bckv@gmail.com , Mobile: +91-8709547218

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Abstract

31 Application of organics in soil can reduce uptake of arsenic (As) in food crops grown on soil spiked with As.
32 However, the results may be different if the crop is grown with As contaminated irrigation water. In this study,
33 a modest initiative has been undertaken to assess the organo-arsenic equilibria using the fulvic and humic
34 extracted from paddy husk (PH), vermicompost (VC) and soil. The fulvic and humic acids were found to be
35 polyprotic in nature. Fulvic acid extracted from paddy husk have the highest mole ratio or combining ability of
36 1.88 ± 0.59 and stability constant ($\log K$) of 8.96 ± 0.21 . Based on the above findings, a greenhouse experiment
37 with wheat crop was conducted using different concentrations of As in irrigation water (1, 2, 4 and 8 mg L⁻¹),
38 irrigated 5 times throughout the crop growth period and 3 levels of amendments (No amendment, VC and PH @
39 10 t ha⁻¹ each). Paddy husk and also vermicompost can be effectively used as an amendment to trim down the
40 bioavailability of arsenic in the wheat grain provided the As content in irrigation water is < 2 mg L⁻¹. Risk
41 assessment of As spiked soils was carried out and expressed in hazard quotient as per USEPA guideline.
42 Solubility free ion activity model was also used to forecast the transfer of As from As spiked soil to wheat grain.

43

44 **Keywords:** Arsenic–humic and fulvic complex, wheat, stability constant, Solubility and Free Ion Activity
45 Model (FIAM), hazard quotient (HQ)

46 Introduction

47 Contamination of arsenic (As) in soil and groundwater is a huge environmental apprehension worldwide
48 (Mandal et al. 2019a). Elevated concentrations of As in groundwater has been narrated from several countries
49 across the globe, including the Gangetic plains of India (Sanyal et al. 2015). Drinking water is considered as the
50 prime source for As exposure, there are other sources like irrigation with contaminated water that leads towards
51 arsenic exposure through soil-crop-food transfer. Presence of As in rice grain grown mainly in South East Asia
52 have created a cataclysm, as rice is considered as one of the important component in the human diet of the
53 region (Meharg and Rahman 2003; Meharg 2004). Golui et al. (2017) narrated that concentration of As in rice
54 grain grown on As polluted soils of West Bengal, India ranged from 2-1260 µg kg⁻¹ Similarly, elevated
55 intensities of As in soil and crop plants also reported from As polluted zones of Patna district of Bihar, India
56 (Mandal et al. 2019a). Bihar lying in the Arsenic contaminated Indo-Gangetic Plain leading to toxicities in
57 human *via* food chain principally through cereal crops portrayed due attention from investigators recently. A lot
58 of information is available regarding As loading in rice crop due to application of contaminated irrigation water
59 and their mitigation through management strategies has been reported (Sarkar et al. 2012; Rahaman et al. 2014;
60 Das et al. 2016; Shah et al. 2016; Mukherjee et al. 2017; Shrivastava et al. 2020) In case to wheat the
61 information is scanty. As content in wheat grains to the tune of 43.64 ± 48.19 mg kg⁻¹ collected from 77
62 households across 19 villages of Bihar was reported by (Suman et al. 2020). (Mandal et al. 2019b) reported the
63 loading of As in wheat, grown with contaminated irrigation water in field condition. Wheat holds a significant
64 share of the cereal basket of India. It is also one of the prime sources of cereals for dietary intake which are
65 cultivated extensively in the Indo-Gangetic plain of India. Hence mitigation strategies like application of organic
66 amendments should be explored for reducing the As content in wheat grain. Organic amendments applied in soil
67 after decomposition produces phenols, amino acids, sugar acids and simple aliphatic acids. It consist of fulvic
68 acids (FA) and humic acids (HA) as decomposition products varying in molecular weight and chemical

69 structures consisting of several functional groups with variable properties. The mobility of As in soil is limited
70 due to adsorption by various functional groups present in HAs and FAs (Sinha et al. 2011b; Mandal et al.
71 2019a). Application of organic amendments (vermicompost, phosphocompost, mustard cake and farm yard
72 manure) to reduce the As content in sesame grown in contaminated fields with As (0.32 mg L^{-1}) laden irrigation
73 water has been reported by (Sinha et al. 2011a). The efficacy of organic amendments in reducing As content in
74 wheat grown in As spiked soil has been conveyed by (Mandal et al. 2019a). To mitigate As contamination in
75 wheat-maize cropping system practiced in As contaminated fields of Bihar irrigated with As (0.18 mg L^{-1})
76 contaminated irrigation water, sugarcane baggasse, rice straw and paddy husk has been used as amendments as
77 reported by (Mandal et al. 2019b).

78 In all these studies the level of contamination in irrigation water was fixed at a certain level. So the
79 efficacy of these organic amendments and also use of new amendments like paddy husk (PH) is needed to be
80 assessed when crops are grown with varying levels of contaminated irrigation water to ensure the aptness of
81 application of organics for mitigation of As in field conditions where the levels of As in irrigation water varies
82 widely. Herein lies the novelty of this study.

83 There are various models which are helpful in predicting the bio-uptake and the ecotoxicity, like FIAM
84 and Biotic Ligand Model (BLM) as reported by (Jopony and Young 1994; Datta and Young 2005; Golui et al.
85 2020). Among this, Windermere Humic Aqueous Model (WHAM VII) mainly focuses on the behavior of the
86 metals in soil and water through simulation (Lofts and Tipping 2011). Most of the predictability models
87 mentioned above require a lot of parameters as input variables to achieve the desired output and hence tedious
88 laboratory analysis of the various parameters are needed to be undertaken. In this regard, a simple predictability
89 model like the solubility free ion activity model (FIAM) to forecast the As uptake by the crops would be
90 beneficial. The FIAM predicts only with soil pH, organic carbon content and extractable As, which can be easily
91 analysed in laboratory condition. Previously, efficacious prediction of lead, cadmium, chromium, As uptake by
92 the plants has been recounted by several authors (Meena et al. 2016; Golui et al. 2017; Mandal et al. 2019a and
93 2019b). This model will help in fruitful forecasting of As uptake by plant which will further support in regular
94 risk evaluation of contaminated soil and will open up a new avenue of research area for remediation of As
95 contaminated soils.

96 With this background information, a modest initiative was undertaken (i) to examine the properties of humic and
97 fulvic acid extracted from the vermicompost (VC) and decomposed paddy husk (PH) and As-HA/FA
98 complexation equilibria. (ii) to evaluate the effectiveness of organics on As content in wheat grain grown with
99 different levels of contaminated irrigation water and (iii) to quantify the predictability of FIAM.

100 **Materials and Methods**

101 *Soil sample collection*

102 Surface soil (0-15 cm) was collected for conducting the pot experiment from Bihar Agricultural University
103 farm, Sabour ($24^{\circ}14'N$, $87^{\circ}2'E$ and 37.9 msl). The soil samples were air dried, grinded and sieved through a 2
104 mm sieve for laboratory analysis.

105 *Extraction of Humic Acid (HA) and Fulvic Acid (FA)*

106 HA and FA were extracted from decomposed paddy husk (PH), vermicompost (VC), and experimental soil
 107 sample as per the methods outlined by (Kononova 1966; Mandal et al. 2019a). The HA/FA extracted is being
 108 represented as follows:

Humic/Fulvic	Amendments
HA/VC (HA1)	Humic acid from vermicompost
FA/VC (FA1)	Fulvic acid from vermicompost
HA/SB (HA2)	Humic acid from decomposed Paddy Husk
FA/SB (FA2)	Fulvic acid from decomposed Paddy Husk
HA/S	Humic acid from experimental soil
FA/S	Fulvic acid from experimental soil

109

110 *Determination of Carboxylic and Phenolic (OH)*

111 The carboxylic and phenolic (OH) was determined by potentiometric titration as per (Mandal et al. 2019a)

112 *Visible spectrophotometric measurement of HA and FA*

113 The absorbance at 465 nm (E4) and 665 nm (E6) for the dilute solutions of HA and FA (Na form) were
 114 recorded by a SYSTRONICS 117 spectrophotometer at pH 7.0 and 9.0 (Chen et al. 2002) .

115 *Determination of Stability Constant*

116 The standard method as outlined by Schnitzer and Skinner (1966) was followed to determine the stability
 117 constants of the As-HA/FA complexes. Aliquots of 0 - 10 ml of HA or FA solution were taken into 50 ml
 118 volumetric flasks and diluted up to 30 ml with distilled water. Then 5 ml of 1 M NaCl solution were added to
 119 each volumetric flask, subsequently by HA or FA solution were equilibrated with 100 mg As L⁻¹ by adding 6 ml
 120 of aqueous solution of NaAsO₂. The pH was adjusted to 5.0 by adding a few drops of 0.1 M NaOH or 0.1 M
 121 HCl solution. Finally, the volume of each flask was made up to 50 ml with distilled water. This solution of As-
 122 HA or FA solution were transferred to a 250 ml conical flask along with 0.5 g of chloride-saturated anion
 123 exchange resin and shaken for 1 h so that equilibrium was reached within this time. The exchange resin was
 124 then removed by filtration and the filtrate plus washings were dried on a hot water bath and then digested with
 125 5:1:: HNO₃:H₂SO₄ on a hot plate Schnitzer and Skinner (1966). Each digest was diluted with distilled water to
 126 100 ml, and the concentrations of As was then measured by atomic absorption spectrophotometry (Agilent 240
 127 FS) coupled with Vapour Generation Accessory (VGA 77). The logarithm of the stability constant of the As-
 128 humate or fulvate complex (log K) was determined by using the following relationship:

129
$$\log[\lambda_0/\lambda-1] = \log K + x\log[HA] \quad (1)$$

130 Lamda (λ) is the distribution constant in the presence of HA/FA, λ_0 was determined from the following
 131 expression: $\alpha_0 V / (100 - \alpha_0)g$; where α_0 is the percent of total As bound to the exchange resin, $(100 - \alpha_0)$ is the
 132 percent of total As remaining in the solution, and V is the volume of solution (100 ml) and g is the weight of
 133 exchange resin (0.5 g). The slope of the best fit linear plot of $\log[\lambda_0/\lambda-1]$ vs. the HA/FA concentrations denoted
 134 the value of x, while the intercept furnished the value of log K (Eq. (1); Schnitzer and Skinner, 1966).

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138 *Pot experiment*

139 Pots (plastic) filled with 10 Kg of soil in the winter season of 2018 and 2019. The pots were irrigated with 4
140 levels of As contaminated irrigation water (1, 2, 4 and 8 mg L⁻¹). Sodium arsenate (Na₂ HAsO₄, 7H₂O) was
141 used as the source of As in the irrigation water. Altogether 6 irrigations were applied having a volume of 800
142 mL at each time throughout the crop growth period. Three levels of organic amendments viz. control (no
143 amendment), Vermicompost (VC) and decomposed Paddy Husk (PH) @ 44.64 g kg⁻¹ of soil (10 t ha⁻¹).
144 Reagent grade Urea, Monopotassium phosphate and Potassium chloride were used as a source to provide the
145 recommended doses of Nitrogen, Phosphorus and Potassium (NPK) (at the rate of 150, 60 and 40 kg ha⁻¹).
146 Wheat (Variety:HD2967) was sown and 5 plants plot⁻¹ was maintained throughout the experiment. The study
147 was performed in Completely Randomized Design (CRD) with three replications using 36 (4X3X3) pots. Wheat
148 grains were harvested after full maturity.

149 *Analysis of organic amendments soil and plant samples*

150 The organic amendments after drying were grinded and sieved with 2mm sieve for analysis. Total Carbon and
151 Nitrogen was determined with CHNS-Analyzer (Make: Euro Vector, Model: Euro EZ 3000). The Phosphorus
152 (P) and Potassium (K) content was determined after di-acid digestion (9:4 mixture of HNO₃:HClO₄) of the
153 samples. P was estimated by the vanadate-molybdate yellow colour method and K was estimated by flame
154 photometry (Jackson,1973). The soil pH was determined by (1:2.5 soil-to-water ratio), Eh was estimated by
155 portable ORP meter, oxidisable organic Carbon by (Walkley and Black 1934) soil texture by (International
156 pipette method), available N was determined by (Subbiah and Asija 1956), P by (Olsen et al.1954) and K by
157 (Jackson, 1973). The extractable As and total As was analysed with 0.5 M NaHCO₃, pH 8.5 and aqua-regia
158 (Sparks et al. 2003) respectively. Plant samples were digested with HNO₃, HClO₄ and H₂SO₄, 10:4:1 (v/v/v) for
159 total As measurement. The soil extract and the digested plant samples were prepared as per the methods outlined
160 by (Mandal et al. 2019a). The resultant solution was analyzed in an Agilent 240 FS AAS with Vapor Generation
161 Accessory (VGA 77) at λ_{max}~ 193.7 nm. In every set of 25 samples two reagents blank and one reference
162 materials of rice (SRM1568a), prepared by National Institute of Standards and Technology (NIST) were used.
163 The endorsed value of the reference material is 290 ± 30 µgkg⁻¹. The reference material was analyzed thrice and
164 the As recovery was 284 ± 12.3µg kg⁻¹.

165 *Prediction of As in wheat*

166 For prediction of As, FIAM was used (Dutta and Young 2005) with the assumption that it is governed by the
167 activity of As in soil water. Here the TF is enunciated as the relationship of As content in plant [M_{plant}] to the
168 activity of ion motion in soil water (Mⁿ⁻) as follows, (Mirecki et al. 2015) :

169
$$TF = \frac{\log [M_{plant}]}{(M^{n-})} \quad (1)$$

170 To predict the free ion activity of As a Freundlich equation which is pH dependent was used as proposed by
171 (Dutta and Young 2005 and Pierzynski et al. 2000) which is as follows:

172
$$p(M^{n-1}) = \frac{p[M_C] + k_1 + k_2 pH}{n_F} \quad (2)$$

173 Where (Mⁿ⁻) --- As activity in soil solution;

174 M_C --- labile pool of As in soil
 175 k_1 and k_2 --- empirical constants
 176 n_F --- power term from the Freundlich equation
 177 $p [M_{plant}] = C + \beta_1 p[M_C] + \beta_2 pH$ (3)Combining equation 1 and 2
 178 Where

179
$$C = \frac{k_1}{n_F} - TF, \beta_1 = \frac{1}{n_F}, \beta_2 = \frac{k_2}{n_F}$$

180 where C is the coefficient for As, β_1 and β_2 are the plant- specific coefficient. Eq. (3) was calculated by the
 181 “SOLVER” feature of Microsoft Excel 2010.

182 *Risk assessment*

183 The hazard quotient (HQ), for assessing the risk was calculated following the protocol outlined by (Mandal et al.
 184 2019a). The PMTDI (Provisional Maximum Tolerable Daily Intake) for As was considered as per (WHO, 1996)
 185 was considered as 2.1 $\mu\text{g As kg body weight}^{-1}\text{day}^{-1}$. The Average Daily Dose (ADD) of As exceeds the R_fD ,
 186 reference dose (R_fD ; $\text{mg. kg body weight}^{-1}\text{day}^{-1}$) when the $HQ > 1$ which indicates a risk in consumption. The
 187 daily consumption of wheat was considered 200 g day^{-1} and the adult body weight was considered as 68 kg,
 188 (Rahman et al. 2013). For an adult the HQ was calculated as:

189
$$HQ = \frac{M_{plant} \times W}{R_fD \times 68}$$

190 Where M_{plant} is As content (mg kg^{-1}) in the grain of wheat grown with As laden irrigation water; W is the daily
 191 intake of grain of wheat. Here the perilous value of HQ was considered as 0.5 as the contribution from the food
 192 source is only taken into account.

193 *Statistical Analysis*

194 For data analysis facility of Microsoft excel 2010 and SPSS 16.0 was used.

195 **Results and Discussion**

196 The characteristics of the organic amendments used has been depicted in Table 1. The carbon (C) content was
 197 217 and 383 g Kg^{-1} for vermicompost and paddy husk respectively. The nitrogen (N) content was 18 for
 198 vermicompost and 23 for paddy husk. The phosphorus (P) and Potassium (K) were found to be 7 and 9 g kg^{-1}
 199 for vermicompost and 5 and 6 g kg^{-1} for paddy husk. Potentiometric titration of the HA/FA separated from the
 200 organics and soil for determination of carboxylic and phenolic groups revealed the presence of these groups in
 201 higher quantities (Table 2). The maximum total acidity of 407.34 cmol Kg^{-1} was observed with respect to FA
 202 extracted from paddy husk. The total acidity the HA and FA abide by the order:
 203 $FA_2 > HA_2 > FA_1 > HA_1 > HA/S > FA/S$ which were as per the findings reported by (Mukhopadhyay and Sanyal
 204 2004; Mandal et al. 2019a). The polyprotic nature of the HA and FA and also the 1st and final inflexion point
 205 can be visualised from Table 3. ΔpH is the difference of pH between the pH values at 1/4 and 3/4 of the pH at
 206 the final inflexion points. It was observed that the ΔpH values of HA and FA were more than 0.954 which
 207 braced the fact that they all are polyprotic in nature as already reported by (Mandal et al. 2019a). The values of
 208 (E_4/E_6) that is the absorption at 445 nm (denoted by E_4) and absorption at 665 nm (denoted by E_6) are given in

209 Table 4. It shows the prevalence of aliphatic and aromatic groups in the given HA or FA molecule respectively.
210 The ratio of E_4 and E_6 values provides a better index of the aliphatic and aromatic group preponderance in HA
211 and FA. Higher absorption at longer wavelengths is due to the escalation of mobility of delocalized π electron
212 clouds over aromatic carbon nuclei and hence HAs register higher E_6 (lower E_4) values corresponding to the
213 FAs. Similar findings have been previously reported by (Kar and Sanyal 1988; Datta et al. 2001; Mandal et al.
214 2019a). As the (E_4/E_6) ratio is more than unity at both pH 7.0 and 9.0 is in accordance to the above mentioned
215 hypothesis. The (E_4/E_6) ratio of the FA of all amendments is more than that of the HA which support the fact of
216 aromatic-aliphatic balance in those fractions.

217 The combining capacity of HA and FA has been depicted in Table 5. The (x) denotes mole ratio that indicates
218 the moles of HA or FA that mingle with unit mole of As and the stability constant is signified by log K of As-
219 HA/FA complexes. Stability constant values ranged from 3.61 to 8.96 at pH 5.0. The highest log K value of
220 8.96 was observed with respect to FA2 i.e. FA extracted from decomposed paddy husk and the corresponding
221 mole ratio was 1.88. The log K values followed the order: FA2>FA1>HA1>HA2>FA/S>HA/S. A noteworthy
222 and affirmative correlation between Log K values and total acidity ($r=0.64$) establishes the fact of dependency
223 stability constant with the active functional groups of the HA and FA. Similar findings were reported by (Sinha
224 et al. 2011b and Mandal et al. 2019a). The binding of As both in trivalent and pentavalent form with HA and FA
225 in water and also in natural organic matter, complexing with two classes of binding sites has been reported by
226 (Fakour et al. 2014). The geometrical alignment of the adsorbate at the adsorbent surface is governed by two
227 types of surface complexes. The inner-sphere complex having the presence of the hydration sphere and the
228 outer-sphere complex is devoid of hydration sphere. In case of As, having the two-phase exchange the early
229 exchange is from the loosely held positions of the outer-sphere complexation sites and subsequently from
230 comparatively strongly bonded inner sphere complexation positions. Decomposed PH having a higher binding
231 capacity compared to VC is mainly due to its chemical configuration. Complex formation on the surface is the
232 mechanism by which the metal ions gets adsorbed on PH. The hydrolysis of SiO_2 present in PH leads to the
233 production of SiOH, a hydroxide surface group. This SiOH that is the silanol group is responsible for the
234 development of a silica surface that is weakly acidic in nature. Apart from silicon dioxide the cellulose present
235 in PH also contributes to the adsorption of As. The cellulose consist of -COOH, -SH etc. groups that undergoes
236 complexation of As on their surface mainly through the dissociation of protons. The combined effect of ion
237 exchange and surface complexation results in the binding of As by PH as reported by (Khalid et al.1998).

238 As per the findings of As-HA/FA equilibrium study and to quantify the efficacy of the organics pot experiment
239 was conducted on availability of As for crop uptake. The physico-chemical properties of the experimental soil
240 can be visualized in Table 6. The soil have pH 7.62 which is in the neutral range, Eh 326 mV, low in organic
241 Carbon content of 0.39 %, silty clay in texture , low in N (210 Kg ha^{-1}) and P (16 Kg ha^{-1}) and high in K (340
242 Kg ha^{-1}) content. The extractable As was (0.06 mg Kg^{-1}) and total As (1.13 mg Kg^{-1}) respectively. The pH,
243 redox potential (Eh), organic carbon, available As and As content in wheat grain has been depicted in Table 7.
244 The pH and Eh ranged from 7.57 to 7.62 and from 326 to 330 mV respectively and was observed to be
245 statistically non-significant. The oxidizable organic carbon (OC) content ranged between 0.41-0.68% and was
246 found to be statistically significant. The increase in OC content may be attributed to the decomposition of
247 vermicompost and paddy husk. In wheat grain As ranged from 0.09 to 0.64 mg kg^{-1} which was statistically

248 significant. An increase in grain As content was observed in this study in comparison to wheat grown in As
249 spiked soil as reported by (Mandal et al. 2019a) where it ranged from 0.011-0.085 mg kg⁻¹. This is
250 predominately for the repeated loading of As in soil from irrigation water and that has been reflected in
251 available As and which in turn contributed to grain As content. The bioavailable As content in soil varied from
252 0.63 to 3.12 mg kg⁻¹ and was statistically significant. As per the pH and Eh of the soil is concerned pentavalent
253 As should predominate within the rhizosphere. At pH 6-8, and in an aerobic oxidized environment (redox
254 potential, Eh= 0.2-0.5V), As acid species and arsenate oxyanions (pentavalent As forms) occur in considerable
255 proportions as reported by (Sanyal et al. 2015). With the escalation of As content in the irrigation water the soil
256 available As also got amplified. The organics applied, notably reduced the As content of wheat grain
257 irrespective of the different levels of As being added to the soil through irrigation water. The effectiveness of
258 organic amendments followed the order PH>VC>Control. The results of the pot experiment supported the
259 findings of equilibrium studies regarding the complexing ability of the HA and FAs. Stable complexes are
260 formed due to the existence of humic substances that reduces the bioavailability of metals and metalloids. HA
261 and FA having larger molecular structure than the soil pore size and hence reduces its downward freedom of
262 movement. The binding of As by organic amendments by complexation has been narrated by (Sinha et al.
263 2011b; Fakour et al. 2014; Mandal et al. 2019a). The binding of different species of As viz. arsenate, arsenite
264 and arsenate oxyanions to the active positions of the HA and FA are governed by primarily three processes.
265 Firstly, covalent bond interaction with active sites was insinuated by (Buschmann et al. 2006), secondly, ionic
266 associations was proposed by (Saada et al. 2003) and cationic bridging complexes by Lin et al. (2004).

267 The As and the model coefficients (C, β_1 and β_2) of the FIAM are depicted in Table 8. The values of C,
268 β_1 and β_2 were -15.104, 1.739 and 1.048, respectively. The variation of As content in grain pertaining to soil pH
269 and organic carbon was observed to be as high as 97 %. Risk assessment in terms of Hazard Quotient (HQ) is
270 depicted in Table 7. The results indicate that wheat grain subjected to application of As contaminated irrigation
271 water above 2 mg L⁻¹ will impart health risk to humans as far as its As content is concerned (HQ>0.5) regardless
272 of application of organic amendments. It is observed that the amendments were effective in lowering the As
273 content in grain at all the four levels of contaminated irrigation water applied. In terms of HQ the consumption
274 of wheat for human is safe, up to application of 2 mg L⁻¹ As contaminated irrigation water along with organic
275 amendments. An attempt has been made to compare the actual and predicted values of As concentration in
276 wheat grain as can be visualized in Figure 1 and also between observed and predicted HQ values as revealed in
277 Figure 2. As observed in the figures it can be concluded that assessed As concentration and HQ were in close
278 concordance with the modelled values. Similar findings was reported by (Mandal et.al 2019a and 2019b) in case
279 of wheat and by (Kumari et.al. 2020) in case of rice crop. Hence, the model can be exercised as an instrument
280 for risk assessment prediction after proper validation and calibration.

281 **Conclusion**

282 The above study revealed the polyprotic character of the HA and FA extricated from the organics and higher
283 aliphatic-aromatic balance was observed in FA with respect to the HA. The complexing capability of FA with
284 As is higher contrast to HA and organic fractions from decomposed paddy husk proved be a prospective
285 complexing agent of As. From the pot experiment it can be concluded that the organic amendments reduced the
286 uptake of As and the order of efficacy is as follows: PH>VC>Control and it can be a viable option for mitigation
287 of As contamination. Hence decomposed paddy husk can be effectively used as an amendment to lower the

288 bioavailability of As in the crops. Risk assessment in terms of HQ and also predicting As content with the help
289 of FIAM was found to be promising.

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297 **Conflicts of interest/Competing interests:** The authors declare no conflicts of interest.

298 **Availability of data and material:** Data generated from the experiment

299 **Code availability:** Not applicable

300 **Authors' contributions:** All authors contributed significantly towards the final make-up of the paper.
301 Expressing in terms of author initials, JM and BBM conceived the idea of the experiment; SNK carried out the
302 experiment and SK statistical computations; JM, SNK and SK contributed in analysis; BBM provided valuable
303 feedbacks after proofreading; JM and BBM prepared the original draft, edited and compiled the manuscript.

304 **References**

305 Buschmann, J., Kappeler, A., Lindauer, U., Kistler, D., Berg, M., & Sigg, L. (2006). Arsenite and arsenate
306 binding to dissolved humic acids: influence of pH, type of humic acid, and aluminum. *Environmental Science
307 and Technology*, 40, 6015–6020.

308 Chen, J., Gu, B., Leboeuf, E.J., Pan, H., & Dai, S. (2002). Spectroscopic characterization of the structural and
309 functional properties of natural organic matter fractions. *Chemosphere*, 48, 59–68.

310 Das, S., Chou, L.M., Jean, J.S., Liu, C.C., & Yang, H.J. (2016). Water management impact on arsenic behaviour
311 and rhizosphere bacterial communities and activities in a rice agro-ecosystem. *Science of the Total Environment*,
312 542, 442-452.

313 Datta, A., Sanyal, S.K., & Saha, S. (2001). A study on natural and synthetic humic acids and their complexing
314 ability towards cadmium. *Plant and Soil*, 235,115–125.

315 Datta, S. P., & Young, S. D. (2005). Predicting metal uptake and risk to human food chain from leaf vegetables
316 grown on soil amended by long term application of sewage sludge. *Water Air and Soil. Pollution*, 163, 119-136.

317 Fakour, H., Lin, T.F. (2014). Experimental determination and modeling of arsenic complexation with humic and
318 fulvic acids. *Journal of Hazardous Materials*, 279, 569–578

319 Golui, D., Guha Mazumdar, D. N., Sanyal, S. K., Datta, S. P., Ray, P., Patra, P. K., Sarkar, S., & Bhattacharya,
320 K.(2017). Safe limit of arsenic in soil in relation to dietary exposure of arsenicosis patient from Malda district
321 West Bengal – A case study. *Ecotoxicology and Environmental Safety*, 144, 227-235.

322 Jackson, M.L. (1973) *Soil Chemical Analysis*, Prentice Hall India Pvt.Ltd., New Delhi

323 Kar, R. & Sanyal, S.K. (1988). A study on synthetic humic acids. *Journal of Indian Chemical Society*, 65, 834–
324 837.

325 Khalid, N., Ahmad, S., & Toheed, A. (1998). Immobilization of arsenic on rice husk. *Adsorption Science
326 Technology*, 16(8), 655-666

327 Kononova, M.M.1966. *Soil organic matter. Its nature, its role in soil formation and in soil fertility* (2nd English
328 edn, trans from Russian: Nowakowsky TZ, Newman ACD). Pergamon, Oxford, p 544.

329 Kumari, P.B., Singh, Y.K., Mandal, J., Shambhavi, S., Sadhu, S.K., Kumar, R., Ghosh, M., Raj, A., & Singh,
330 M.(2020). Determination of safe limit for arsenic contaminated irrigation water using solubility free ion activity
331 model (FIAM) and Tobit Regression Model. *Chemosphere*. <https://doi.org/10.1016/j.chemosphere.2020.128630>.

332 Lin, H.T., Wang, M.C., & Li, G.C. (2004).Complexation of arsenate with humic substance in water extract of
333 compost. *Chemosphere*, 56, 1105–1112.

334 Lofts, S., & E. Tipping.(2011) Assessing WHAM/Model VII against field measurements of free metal ion
335 concentrations: model performance and the role of uncertainty in parameters and inputs. *Environmental*
336 *Chemistry*, 8(5), 501-516.

337 Mandal J., Golui D., & Datta S.P. (2019a). Assessing equilibria of organo-arsenic complexes and predicting
338 uptake of arsenic by wheat grain from organic matter amended soils. *Chemosphere*, 234, 419-426.

339 Mandal J., Golui D., Akanksha., & Ganguly P. (2019b). Risk assessment of Arsenic in Wheat and Maize grown
340 in organic matter amended soils of Indo-Gangetic plain of Bihar, India. *Soil and Sediment Contamination: An*
341 *International Journal*, 28 (8), 757-772.

342 Meena, R., Datta, S. P., Golui, D., Dwivedi, B. S., & Meena, M. C. (2016). Long term impact of sewage
343 irrigation on soil properties and assessing risk in relation to transfer of metals to human food chain.
344 *Environmental Science and Pollution Research*, 23,14269–14283.

345 Meharg, A. A. (2004). Arsenic in rice-understanding a new disaster for South-East Asia. *Trends in Plant*
346 *Science*, 9, 415–417.

347 Meharg, A. A., & Rahman, M. M. (2003). Arsenic contamination of Bangladesh paddy field soils: implications
348 for rice contribution to arsenic consumption. *Environmental Science and Technology*, 37, 229–234.

349 Mirecki, N., Agic, R., Sunic, L., Milenkovic, L., & Ilic, Z. S. (2015). Transfer factor As indicator Of heavy
350 metals content In plants. *Fresenius Environmental Bulletin*, 24, 4212–4219.

351 Mukherjee, A., Kundu, M., Basu, B., Sinha, B., Chatterjee, M., Bairagya, M.D., Singh, U.K., & Sarkar,
352 S.(2017). Arsenic load in rice ecosystem and its mitigation through deficit irrigation. *Journal of Environmental*
353 *Management*, 197, 89-95.

354 Mukhopadhyay, D., & Sanyal, S.K. (2004). Complexation and release isotherm of arsenic in arsenic-
355 humic/fulvic equilibrium study. *Australian Journal of Soil Research*, 42, 815–824.

356 Olsen, S.R.: Estimation of available phosphorus in soils by extraction with sodium bicarbonate (No. 939). US
357 Department of Agriculture, Washington DC, (1954).

358 Pierzynski, G. M., Sims, J. T., & Vance, G. F. (2000). Soil phosphorus and environmental quality p. 155 207. In
359 Boca Raton (Ed.), *Soils Environ Quality* (pp.155-207). CRC Press: FL.

360 Rahaman, S., Sinha, A. C., Pati, R., & Mukhopadhyay, D. (2013). Arsenic contamination: a potential hazard to
361 the affected areas of West Bengal, India. *Environment Geochemistry and Health*, 35, 119–132.

362 Saada, A., Breeze, D., Crouzet, C., Cornu, S., & Baranger, P.(2003). Adsorption of arsenic (V) on kaolinite and
363 on kaolinite–humic acid complexes — role of humic acid nitrogen groups. *Chemosphere*, 51, 757–763.

364 Sanyal, S. K., Gupta, S. K., Kukal, S. S., & Rao, J. K. (2015). Soil degradation, pollution and amelioration. In:
365 H. Pathak, S. K. Sanyal and P. N. Takkar (Eds.) *State of Indian Agriculture-Soil*, (pp. 234-266). National
366 Academy of Agricultural Sciences, New Delhi.

367 Sarkar, S., Basu, B., Kundu, C.K., & Patra, P.K. (2012). Deficit irrigation: An option to mitigate arsenic load of
368 rice grain in West Bengal, India. *Agriculture, Ecosystems and Environment*, 146, 147–152.

369 Schnitzer, M., & Skinner, SIM. (1966). Organo-metallic interactions in soils: 5. Stability constants of Cu²⁺, Fe²⁺
370 and Zn²⁺ fulvic acid complexes. *Soil Science*, 102, 361–365

371 Shah, A.L., Naher, U.A., Hasan, Z., Islam, S.M.M., Rahman, M.S., Panhwar, Q.A. & Shamshuddin, J. (2016).
372 Arsenic management in contaminated irrigation water for rice cultivation. *Tropical Agricultural Science*, 39,
373 155-166.

374 Shrivastava, A., Barla, A., Majumder A., Singh S., & Bose, S. (2020). Arsenic mitigation in rice grain loading
375 via alternative irrigation by proposed water management practices. *Chemosphere*, 238, 124988.

376 Sinha, B., & Bhattacharyya, K. (2011b). Retention and release isotherm of arsenic in arsenic–humic/fulvic
377 equilibrium study. *Biology and Fertility of Soils*, 47, 815–822

378 Sinha, B., Bhattacharyya, K., Giri, P. K., & Sarkar, S. (2011a). Arsenic contamination in sesame and possible
379 mitigation through organic interventions in the lower Gangetic Plain of West Bengal, India. *Journal of Science*
380 *Food and Agriculture*, 91, 2762-2767.

381 Sparks, D.L. (2003). *Environmental soil chemistry*, 2nd edn. Academic, San Diego

382 Subbiah, B.V., & Asija, G.L. (1956). A rapid procedure for the determination of available nitrogen in soils.
383 *Current Science*, 25, 259–260.

384 Walkley, A., & I.A Black .(1934).An examination of the Degtjareff method for determining soil organic matter,
385 and a proposed modification of the chromic acid titration method. *Soil Science*, 37(1), 29-38.

386 WHO.1996. Trace elements in human nutrition and health, Geneva.

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412 Table 1. Properties of Vermicompost and Paddy Husk

Properties	Vermicompost	Paddy Husk
C (g kg ⁻¹)	217	383
N (g kg ⁻¹)	18.0	23.0
P (g kg ⁻¹)	7.00	5.0
K (g kg ⁻¹)	9.00	6.0

413

414 Table 2. COOH and Phenolic (OH) groups of HA and FA samples

HA/FA Samples	Amount of COOH group (cmol Kg ⁻¹)	Amount of Phenolic (OH) group (cmol Kg ⁻¹)	Total Acidity (cmol Kg ⁻¹)
HA/VC (HA1)	78.33±3.21	44.96±3.37	123.29±5.03
FA/VC (FA1)	140±6.78	103.00±7.94	243.23±2.52
HA/PH (HA2)	198.90±5.93	112.84±4.25	311.74±10.18
FA/PH (FA2)	220.38±11.05	180.96±3.45	401.34±11.03
HA/S	54.51±3.29	51.18±4.28	105.69±7.54
FA/S	45.10±2.09	37.67±2.52	82.77±2.12

415

416 Table 3. Polyprotic nature of HA and FA samples by potentiometric titration

HA/FA Samples	pH at 1 st inflexion	pH at final inflexion	$\Delta\text{pH} = \text{pH}_{(3/4)} - \text{pH}_{(1/4)}$	Conclusion
HA/VC (HA1)	9.46±0.07	9.71±0.06	4.85±0.02	Polyprotic
FA/VC (FA1)	4.91±0.04	6.44±0.07	3.22±0.02	Polyprotic
HA/PH (HA2)	8.50±0.19	9.89±0.08	4.95±0.03	Polyprotic
FA/PH (FA2)	4.39±0.13	7.14±0.06	3.57±0.02	Polyprotic
HA/S	8.45±0.14	9.11±0.08	4.56±0.03	Polyprotic
FA/S	6.59±0.13	7.66±0.09	3.83±0.03	Polyprotic

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418 Table 4. Absorption of HA and FA samples at 465 and 665 nm at pH 7.0 and 9.0

HA/FA Samples	E ₄ /E ₆ ratio at pH 7.0	E ₄ /E ₆ ratio at pH 9.0
HA/VC (HA1)	6.23±0.91	6.31±1.32
FA/VC (FA1)	6.93±1.12	6.11±1.14
HA/PH (HA2)	5.41±1.02	6.15±0.83
FA/PH (FA2)	6.32±0.94	5.78±1.17
HA/S	6.84±0.92	6.51±1.21
FA/S	8.47±1.03	7.24±1.31

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420 Table 5. Mole ratio (x) and stability constant (log K) of the complexes

HA/FA Samples	Mole ratio (x)	Stability Constant (log K)
HA/VC (HA1)	1.34±0.11	5.94±0.02
FA/VC (FA1)	1.71±0.50	7.79±0.17
HA/PH (HA2)	1.07±0.09	5.73±0.03
FA/PH (FA2)	1.88±0.59	8.96±0.21
HA/S	0.95±0.03	3.61±0.03
FA/S	1.05±0.15	4.74±0.10

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422 Table 6. Physicochemical properties of the experimental soil

pH	OC (%)	Sand (%)	Silt (%)	Clay (%)	Available N (Kgha ⁻¹)	Available P ₂ O ₅ (Kgha ⁻¹)	Available K ₂ O (Kgha ⁻¹)	Available Arsenic (mg Kg ⁻¹)	Total Arsenic (mg Kg ⁻¹)
7.62	0.39	9.6	34.8	55.6	210	16	342	0.06	1.13

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424 Table 7. Arsenic content in organic amended soil and grain of Wheat and corresponding HQ (Mean data of two
425 years).

Treatment Combinations	pH	Eh (mV)	OC (%)	Available As (mg kg ⁻¹)	As content in Wheat (mg kg ⁻¹)	Hazard Quotient (HQ)
T1(1+0)	7.62 ^a	328 ^a	0.43 ^a	0.87 ^a	0.16 ^a	0.2
T2(2+0)	7.59 ^a	326 ^a	0.41 ^a	1.62 ^b	0.38 ^b	0.5
T3(4+0)	7.61 ^a	329 ^a	0.42 ^a	2.51 ^c	0.59 ^c	0.8
T4(8+0)	7.61 ^a	330 ^a	0.42 ^a	3.47 ^d	0.74 ^d	1.0
T5(1+PH)	7.59 ^a	327 ^a	0.68 ^b	0.63 ^e	0.09 ^e	0.1
T6(1+VER)	7.57 ^a	329 ^a	0.62 ^c	0.79 ^f	0.13 ^f	0.1
T7(2+PH)	7.61 ^a	330 ^a	0.67 ^b	1.08 ^g	0.24 ^g	0.3
T8(2+VER)	7.57 ^a	326 ^a	0.63 ^c	1.43 ^h	0.32 ^h	0.4
T9(4+PH)	7.57 ^a	327 ^a	0.67 ^b	2.02 ⁱ	0.43 ⁱ	0.6
T10(4+VER)	7.58 ^a	329 ^a	0.63 ^c	2.27 ^j	0.48 ^j	0.6
T11(8+PH)	7.57 ^a	328 ^a	0.68 ^b	2.96 ^k	0.58 ^k	0.8
T12(8+VER)	7.61 ^a	329 ^a	0.62 ^c	3.12 ^l	0.64 ^l	0.8

426 Means with same letter are not significantly different.

427 PH: Paddy Husk, VER: Vermicompost

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431 Table 8. Model parameters for predicting uptake of As by Wheat as a function of pH, oxidisable Organic
 432 Carbon and Olsen extractable As

Element	Model Parameters			
	C	β_1	β_2	R ²
As	-15.10	1.73	1.04	0.97

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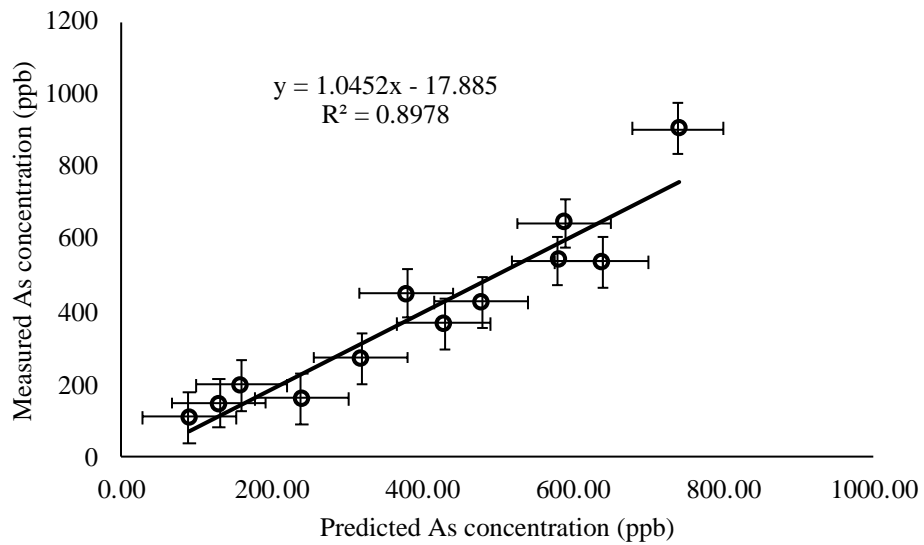
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Figure 1. Correlation between measured and predicted As content in wheat

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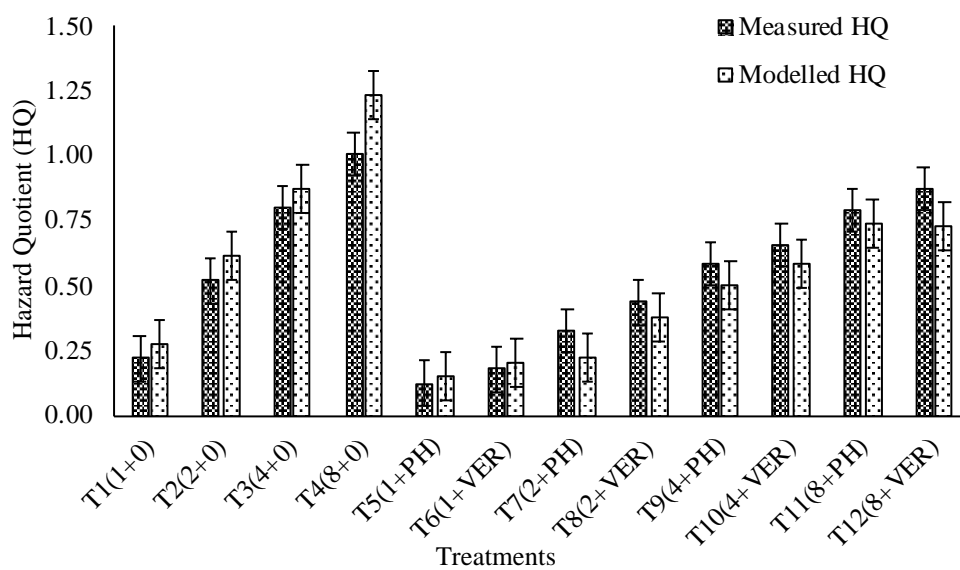
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Figure 2. Comparison between measured and predicted Hazard Quotient (HQ)