Evaluation of different extractants to estimate bioavailable arsenic in soil

Rahul Mishra
Indian Institute of Soil Science
Siba Prasad Datta (profssac2017@gmail.com)
IARI: Indian Agricultural Research Institute
Debasis Golui
North Dakota State University
Mahesh Chand Meena
Indian Agricultural Research Institute
Brahma Swaroop Dwivedi
National Bureau of Soil Survey and Land Use Planning
Mohammad Mahmudur Rahman
The University of Newcastle
Kali Kinkar Bandyopadhyay
Indian Agricultural Research Institute
Arti Bhatia
Indian Agricultural Research Institute
Punyavrat S Pandey
Indian Council of Agricultural Research

Research Article

Keywords: Arsenic, extractants, acid, salt, ICP-MS, plant arsenic

Posted Date: September 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2046165/v1

Abstract

Owing to the similar chemistry of phosphorus (P) and arsenic (As), sodium bicarbonate (0.5 N $NaHCO_3$) is commonly used to extract plant accessible As in soil. However, 0.5 N $NaHCO_3$ is not compatible with the ICP-MS measurement due to the large amount of dissolved solids. This investigation set out to devise a suitable extractant for determining extractable As in soil and measured by the ICP-MS. Paired soil and plant samples were collected from paddy fields in West Bengal, India. Soil was extracted with 0.5 *M* $NaHCO_3$, 0.03 *M (0.1 N)* and 0.17 *M (0.5 N)* phosphoric acid (H_3PO_4), 0.05 *M* (0.1 N) and 0.25 *M (0.5 N)* sulfuric acid (H_2SO_4), and 0.01 M calcium chloride ($CaCl_2$). This made it possible to measure As by hydride generation-atomic absorption spectrophotometer (HG-AAS), while ICP-MS was used for the determination of As extracted from soil with different concentrations (0.1-1.5*M*) of HNO_3. The $NaHCO_3$ extractable As was 1.45 and 1.23 mg kg^{-1} for soil to extractant ratio of 1:20 and 1:5, respectively. Of these extractants, 1.5 N HNO_3 extractable As had the best correlation with As content in rice grain (r = 0.45**) and straw (r = 0.71**), comparable with standard extractant i.e. 0.5 N $NaHCO_3$ (r = 0.47** and r = 0.64** in case of grain and straw, respectively). A significant positive relationship of 1.5 N HNO_3 was obtained with 0.5 N $NaHCO_3$. Thus, 1.5 N HNO_3 may serve as an extractant for soil As, which is compatible with ICP-MS analysis.

Introduction

Arsenic (As) is not an essential element for plant, animal, or human life but it does have a wide range of biological outcomes. If the concentration of As in soil is higher than a certain threshold limit it can be toxic to humans, flora and fauna. Rahaman et al. (2013) suggested that total As concentration in soil ranging from 10 to 20 mg kg^{-1} is a reliable indicator of As pollution. A meta-analysis conducted by Mandal et al. (2021) suggested that the maximum permissible limit of As content in rice grain exceeded the threshold value of 200 µg kg^{-1}. This was especially the case if more than 14 mg kg^{-1} As concentration in paddy soil of the Asian region was recorded. Further studies suggested that total As content in soils does not always reflect its bioavailability or potential toxicity measurements, which are far more essential for determining potential environmental implications. The forms of As that are physiologically accessible or bioavailable for plants uptake, pose the most danger due to further biomagnification. The quantity, chemical form, and strength of bonding with soil particles all influence plant availability (Chen et al., 2020). Various soil chemical techniques can extract distinct amounts of soil As, including sequential and single chemical extraction methods (Kabata-Pendias, 2004), yet their capacity to quantify the amount of plant-accessible As from soil is not well understood.

Some reports have correlated bioaccessible As with in-vitro analysis or human oral bioaccessibility of As (Bari et al., 2021; Li et al., 2015; Rodrigues et al., 2018; Fernández-Caliani et al., 2019). A few studies have set out to derive relationships between forms of As in soils and plant As content (Raj et al., 2021; Yu et al., 2016). Therefore, a suitable analytical approach is necessary to assess readily available forms of As in soil. No such study has correlated bioavailable As and actual plant As uptake in field conditions. Hence this study will provide useful information to advance existing knowledge. The ideal chemical extractant and form of As in soils must be determined to assess the element's availability, solubility, and bioavailability for plant absorption (Martinez-Sánchez et al., 2011). Many extractants are available for extraction of As from soil, i.e. bioavailable form. For neutral soil, 1.0 N NH_4Cl, 0.5 N NH_4F, saturated NaCl and 0.5 N H_2SO_4 were employed to extract As. Of these extractants 0.5 N NH_4F was the best one (Johnston and Barnard, 1979). Furthermore, when 0.2 M NH_4-oxalate was tested with different shaking periods to determine As in soil, 0.17 h emerged as the best (Wenzel et al., 2001). Trang Hoang and Hahn (2015) used extractants such as 0.05 M $(NH_4)_2SO_4$, 0.05 M $(NH_4)_2HPO_4$, 0.2 M NH_4-oxalate buffer, 0.2 M NH_4-oxalate buffer + 0.1 M ascorbic acid (pH = 3.25) and HNO_3/ H_2O_2 to investigate As fractionation in Vietnamese agricultural soils. Extractants 0.05 M $(NH_4)_2SO_4$ and 0.05 M $(NH_4)_2HPO_4$ bsically reflect easily available fractions of As in soil that constitute only 7.0% of total As in soil.

In a sequential extraction experiment, Nguyen Van et al. (2017) evaluated extractants, namely 0.05 M $(NH_4)_2SO_4$ and 0.05 M (NH_4) H_2PO_4 to extract non-specifically and specifically held As in soil and suggested these fractions reflect plant bioavailability. Raj et al. (2021) evaluated six extractants – 0.2 M NH_4-oxalate, 0.05 N HCl + 0.025 N H_2SO_4, 0.5 M KH_2PO_4, 0.5 N NH_4F, 0.5 M $NaHCO_3$ and 0.5 M EDTA - and suggested that 0.5 M KH_2PO_4 have the best correlation with plant As. In most cases the extractant used for extracting bioavailable As from soil is 0.5 N $NaHCO_3$ (pH = 8.5) (Ghosh et al., 2021; Golui et al., 2017; Das et al., 2016). The amount of As extracted depends on the strength of the chemical used. For a suitable extractant, the amount of extracted As must be reflected in plant uptake. Therefore, when evaluating the extractant's suitability, plant uptake studies should be combined to select the best one. Correlation between soil parameters and plant As content is essential to choose the best extractant. Suitable soil analysis for calculating extractable As must account for important soil properties such as pH and organic carbon which is simple to do in a laboratory. Total and extractable As account for 32.2% and 22.2% of the variation in grain As content while soil pH, organic matter (OM) and clay contents describe 13.1%, 7.9%, and 5.3% of the variation, respectively (Yao et al., 2021). Bari et al. (2021) reported that partical size is one important aspect which affects bioassessible As. This study revealed a strong correlation between in-vitro assay of As and 0.43 M HNO_3 extractable As in finer fractions of soil. Also of note is that a single test cannot be suggested for all soils, so choosing an extractant that can reflect As availability in a specific soil group is critical (Anawar et al., 2008).

A variety of analytical instruments are used for examining As in solution such as graphite furnace atomic absorption spectrometry (GF-AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) coupled with a hydride generator (Mucci et al., 2003; Van Herreweghe et al., 2003). Flame AAS is not widely used for As determination in solution due to poor detection limit (< 1 mg L^{-1}) and interference from other elements in the flame. In the case of Flame AAS, a hydride generator (HG) system is combined which converts metalloids such as As, Bi, Sb, Se, Te, Ge, and Sn into their respective hydride form in the presence of strong reducing agents and improves the detection limit up to 100 times (Van Herreweghe et al., 2003). For conversion of metalloids into a volatile hydride form, many chemicals are needed, for instance hydrochloric acid, potassium iodide, ascorbic acid, sodium borohydride, etc. This process also requires time for reduction and more glassware, which leads to possible contamination and further dilution of metalloids in samples. Basically, analysis of As in HG-AAS is expensive, time-consuming and extensive labour is involved. Another limitation in As determination with HG-AAS is that all the As species do not have equal affinity for hydride formation (Welna et al. 2020).

Advances in automation and new generations of multi-element analytical equipment, such as inductively coupled plasma mass spectrometer (ICP-MS), have substantially diminished analytical times and enabled the simultaneous determination of a large number of elements. For the analysis of As-bearing extracts, ICP-MS is widely utilized (Hudson-Edwards et al., 2003). However, one of the limitations of ICP-MS instruments is handling high salt matrices and identifying components at ultra-trace levels, so a pre-treatment phase is required (O'Sullivan et al., 2013). In ICP-MS, a sample with a total dissolved solids (TDS) concentration of less than 0.2% (2 g/L) is typically advised to reduce sample-specific matrix effects and the risk of nebulizer clogging (Wilschefski and Baxter, 2019). Another limitation with ICP-MS for the determination of As in extract is if samples with high chloride (Cl) concentrations lead to molecular interferences (Hwang et al., 2021). Considering the above, in this present investigation, an effort was made to evaluate the feasibility of several extractants for plant-available As in relation to soil parameters and plant uptake of As. In this study, we assume that As extracted with a smaller concentration of acids may reflect the bioavailable fraction of As in soil, which has a better correlation with plant As content.

Materials And Methods

Soil sampling

In this study we selected one As affected district (Nadia) in West Bengal, and it is located between latitude 22º 53"-24º 11" North and 88º 09" and 88º 48" East longitude. Paired soil and rice plant samples (201, GPS added) were collected at a depth of 0–15 cm in such a way to accommodate maximum variability in As content of soils (sampling location presented in Fig. 1). The collected samples were air-dried at room temperature, processed and passed through 2.0 mm sieves and thoroughly mixed. These processed soil samples were used for the analysis of pH (soil: water = 1:2.5) and organic carbon (OC) as outlined by Jackson (1973) and Walkley and Black (1934), respectively.

Soil extraction procedures and analysis of As

A series of batch extraction experiments were conducted using a naturally contaminated soil with different extracting solutions, namely sodium bicarbonate ($NaHCO_3$), phosphoric acid (H_3PO_4), sulfuric acid (H_2SO_4), nitric acid (HNO_3) and calcium chloride ($CaCl_2$) in various concentration like 0.5 *M* $NaHCO_3$ (1:20), 0.5 *M* $NaHCO_3$ (1:5), 0.03 M (0.1 N) and 0.17 M (0.5 N) concentration of H_3PO_4, 0.05 M (0.1 N) and 0.25 M (0.5 N) concentration of H_2SO_4, 0.1 *M* (0.1 N) and 0.5 *M* (0.5 N) concentration of HNO_3 with soil-to-solution ratio (1:10) and 0.01 M $CaCl_2$ (1:10). In total, the collected 201 samples were extracted with the above-mentioned extractants. For this purpose, 10 g of soil samples extracted with different reagents (chemicals used here were of Merck emsure) *viz.* 0.5 N $NaHCO_3$ with soil-to-solution ratio (1:20 and 1:5) (Olsen et al., 1954), 0.1 N and 0.5 N concentrations of H_3PO_4, H_2SO_4, and HNO_3 with soil-to-solution ratio (1:10) and 0.01 M $CaCl_2$ (1:10) (Spark, 1996). Extraction was done at room temperature at 180 rpm and shaking time lasted 1 hr except for 0.5 N $NaHCO_3$ which was 30 min. A further 38 soil samples were extracted with 1.0 N and 1.5 N concentrations of HNO_3 with soil-to-solution ratio (1:10) under similar conditions (shaking time 1 hr at 180 rpm). Of these 201 samples, 38 were selected based on As content as extracted with 0.5 N $NaHCO_3$ (1:20). Arsenic content in the extract of HNO_3 was determined in ICP-MS (Nexlon 300X, Perkin Elmer, USA) while amounts of As in other extractants were determined through HG-AAS (Z-Xpress 8000, Spectrum, China).

Plant digestion procedure and determination of As

For determining As in rice grain and straw, the collected grain and straw samples were digested in HNO_3 (suprapur grade acids) using a microwave digestion system (Multiwave ECO, Anton Paar). For this purpose, 0.5 g of samples were pre-digested overnight with 7 ml of HNO_3 in a PTEF-TFM vessel and digested in a microwave digestion system as per the equipment's operating program. Arsenic content in aliquot was determined with ICP-MS (Nexlon 300 ×, Perkin Elmer, USA). For quality control, standard reference material (Tomato leaves, 1573a) from NIST was employed.

Statistical analysis

Descriptive statistics parameters for different soil properties, plant As content, and As extracted by different extractants were obtained using SAS software (SAS, 2011). To visualize the link between soil properties, plant As content and extractable As, Pearson's correlation matrix was utilized. Further, a simple regression analysis (n = 38) was carried out to explain the contribution of extractable As to straw and grain As content.

Results And Discussion

Characterization of collected soil and plant samples

The pH value of the soil samples (N = 201) collected from the study area varied from 5.38 to 8.05 with an average value of 7.56. Soil reaction in the area varies from acidic to alkaline in nature. The OC content varies from 0.59 to 1.67%. This indicated that soil belongs to the medium to high carbon content category. Further analysis of selected 38 samples, pH of samples ranged from 6.72 to 7.96 (mean pH 7.59); while OC content of soil varied from 0.61 to 1.37% (mean OC = 0.98%). Arsenic content in rice straw (N = 201) ranged from 0.88 to 11.1 mg kg^{-1} with a mean value of 3.48 mg kg^{-1}, whereas As content in rice grain ranged from 0.20 to 0.61 mg kg^{-1} with an average value of 0.43 mg kg^{-1}. Further analysis of selected 38 samples showed that As content in rice straw was 3.63 mg kg^{-1} (1.75 to 11.1 mg kg^{-1}), while grain As content amounted to 0.41 mg kg^{-1} (0.20 to 0.59 mg kg^{-1}). Generally, the distribution patterns of metals and metalloids are not regular and highly diverse patterns were obtained.

Some studies conducted in blocks of Nadia reported average extractable As ranged from 0.86 to 7.62 mg kg^{-1} (Ghosh et al., 2021). For example, in Malda district, Olsen extractable As in soil ranged from 0.01 to 0.62 mg kg^{-1}. Higher extractable As content in soil may be due to excessive use of As contaminated water over a longer period of time. More or less selected samples, out of a total of 201, had similar soil properties and As content in plants. Total As content in

soil is not reflected well in terms of plant As uptake and food chain contamination. No extractable As-based maximum permissible limit has been documented anywhere in the world. Arsenic sorption or desorption on soil mineral surfaces is influenced by soil pH, with higher pH values promoting As release (Zhu et al., 2014). In this study, diversity in soil properties has been selected for designing new extractant which reflects a bioavailable fraction of As in soil. Absorption and accumulation of As by plants are significantly influenced by the bioavailability of As in soil (Stroud et al., 2011). Accuracy in measurement of bioavailable fraction of As in soil improves ecological risk assessment of As in conjunction with total As content (Chen et al., 2020).

Arsenic extracted with different extractants

Result indicated that $NaHCO_3$ (1:20) extractable As was 1.45 mg kg^{-1} (0.48–3.57 mg kg^{-1}), As extracted with $NaHCO_3$ (1:5) was 1.23 mg kg^{-1} (0.28–4.11 mg kg^{-1}), 0.1 N and 0.5 N H_3PO_4 extractable As was 1.43 and 2.85 mg kg^{-1}, respectively, 0.1 N and 0.5 N H_2SO_4 extractable As was 0.57 and 4.51 mg kg^{-1}, respectively (Table 1). Arsenic extracted with 0.1 N and 0.5 N HNO_3 was 0.20 and 0.94 mg kg^{-1}, respectively, while, 0.01 M $CaCl_2$ extractable As was 0.10 mg kg^{-1} (0.01–0.55 mg kg^{-1}) (Table 1). Based on analysis of 201 samples, the trend of As extracted by different reagents was as follows: 0.5 N H_2SO_4 > 0.5 N H_3PO_4 > 0.5 N $NaHCO_3$ (1:20) > 0.1 N H_3PO_4 > 0.5 N $NaHCO_3$ (1:5) > 0.5 N HNO_3 > 0.1 N H_2SO_4 > 0.1 N HNO_3 > 0.01 M $CaCl_2$. A further 38 samples were analyzed with a larger concentration of HNO_3, and results revealed that the mean values of 1 N HNO_3, and 1.5 N HNO_3 extractable As were 0.97 and 1.42 mg kg^{-1}, respectively (Table 2). Current assessments of the bioavailability of As in soils often employ the single-step extraction strategy, which is based on the equilibrium distribution principle (Rodrigues et al., 2018). Common extractants use of extraction of plant available nutrients viz. Mehlich-3 and Bray P-1, have a soil-to-solution ratio of 1: 10 that correlated better with plant nutrient content or uptake (Shahandeh et al., 2017; Gutierrez Boem et al., 2011).

Table 1
Arsenic extracted by different extractants

	$NaHCO_3$ (1:20)	$NaHCO_3$ (1:5)	0.1 N H_3PO_4	0.5N H_3PO_4	0.1 N H_2SO_4	0.5 N H_2SO_4	0.1 N HNO_3	0.5 N HNO_3	0.01 M $CaCl_2$
Mean#	1.45 ± 0.58	1.23 ± 0.71	1.43 ± 0.58	2.85 ± 1.35	0.57 ± 0.72	4.51 ± 1.76	0.20 ± 0.34	0.94 ± 0.55	0.10 ± 0.07
Minimum	0.48	0.28	0.40	0.65	0.01	1.00	0.01	0.24	0.01
Maximum	3.57	4.11	4.82	7.84	5.53	10.1	2.91	3.25	0.55
Variance	0.33	0.50	0.34	1.82	0.52	3.09	0.12	0.31	0.005
Kurtosis	0.99	2.26	7.14	1.43	17.3	0.07	39.0	4.63	8.21
Skewness	0.90	1.30	1.63	0.93	3.56	0.44	5.73	1.97	1.88

#Mean ± SD, *****All the value of As extracted by different extractants is in mg kg^{-1} (Total soil samples analyzed = 201)

Table 2
pH, organic carbon, straw As, grain As and As extracted by different extractants on selected soil samples (N = 38)

	pH	OC %	$NaHCO_3$ (1:20)	$NaHCO_3$ (1:5)	0.1 N H_3PO_4	0.5N H_3PO_4	0.1 N H_2SO_4	0.5 N H_2SO_4	0.1 N HNO_3	0.5 N HNO_3	1N HNO_3	1.5 N HNO_3	0.01 M $CaCl_2$	Straw As	Grain As
Mean	7.59	0.98	1.38	1.33	1.67	2.89	0.72	4.26	0.30	1.14	0.97	1.42	0.11	3.63	0.41
Median	7.69	0.95	1.31	1.12	1.44	2.56	0.52	4.11	0.15	0.99	0.82	1.40	0.08	3.11	0.44
Minimum	6.72	0.61	0.53	0.31	0.46	0.69	0.00	1.95	0.01	0.24	0.21	0.42	0.01	1.75	0.20
Maximum	7.96	1.37	3.38	4.11	4.82	6.61	4.36	7.62	2.91	2.87	2.84	3.24	0.55	11.13	0.59
Standard Deviation	0.27	0.20	0.60	0.86	0.82	1.12	0.76	1.42	0.50	0.65	0.58	0.65	0.09	2.05	0.12
Variance	0.07	0.04	0.36	0.75	0.68	1.26	0.58	2.03	0.25	0.42	0.33	0.42	0.01	4.20	0.01
Kurtosis	1.62	-0.94	0.53	4.56	5.54	1.99	14.0	0.08	21.2	1.68	2.54	0.74	12.9	5.07	-0.97
Skewness	-1.05	-0.08	3.38	2.07	2.04	0.98	3.20	0.76	4.27	1.40	1.51	0.82	3.01	2.24	-0.26

*****All the value of As extracted by different extractants is in mg kg^{-1} including straw and grain As ; As = arsenic

Thus in this study, the same ratio of soil and extractant was chosen for the extraction of soil As which reflects the bioavailable form of As in soil. In metal contaminated soils, bioavailable fractions of metal extracted with 0.05 M EDTA, a well-established extraction procedure where 1:10 soil-to-solution ratio and shaking time of 1 hr was selected which reflected an easily extractable pool of metals. With this analogy, 1 hr shaking time was selected for extraction of soil As (Quevauviller, 1998). Compared to other extractants, H_3PO_4 and H_2SO_4 have higher extractability of soil As, which may be due to their ability to extract As from different fractions such as non-specifically and specifically sorbed fractions (Datta et al., 2018). Another reason may be that due to its higher acidity and chelation capacity, it dissolves metal oxides and releases As from them. In another study extraction of specifically sorbed As, different salts of phosphate such as NaH_2PO_4, $NH_4H_2PO_4$, and KH_2PO_4 were employed. The higher extractability of phosphatic salts was also reported by Raj et al. (2021). The basis of extraction of this fraction is the competitive exchange between phosphate (PO_4^{3-}) and arsenate (AsO_4^{3-}) in soils, where arsenate is preferentially desorbed to phosphate given its smaller size and higher charge density (Wenzel et al. 2001; Cai et al. 2002).

Evaluating the suitability of extractants with ICP-MS for As determination

In this study we evaluated $NaHCO_3$,$CaCl_2$, H_3PO_4, HNO_3, and H_2SO_4 extractable soil As in various concentrations concerning plant As and their compatibility with ICP-MS. Literature suggested that using $NaHCO_3$-based matrix for determination of As with ICP-MS are not advisable due to high TDS. In ICP-MS, a sample with a total dissolved solids (TDS) concentration of less than 0.2% (2 g/L) is typically advised to curtail sample-specific matrix effects and the risk of nebulizer clogging (Wilschefski and Baxter, 2019). The problem with $CaCl_2$ is the presence of chloride. The existence of chloride (Cl) in final prepared samples leads to polyatomic ions $^{40}Ar^{35}Cl$ and $^{35}Cl^{16}O$ formation in a smaller concentration of As in samples and hamper its determination in ICP-MS. Thus an acid-based extractant may be a suitable option for As determination in ICP-MS. Further, some studies suggested that due to higher viscosity and variation in surface tension of H_3PO_4 and H_2SO_4, changes in droplet size distribution and nebulization efficiency reduce the signal intensity in ICP-OES (Yoshimura et al., 1990). For this reason, H_3PO_4 and H_2SO_4 have not been suitable extractants for As determination by ICP-MS. Another potential extractant is HNO_3, which was evaluated in concentration viz. 0.1 N, 0.5N, 1.0 N, and 1.5 N. Their results revealed that 1.5 N HNO_3 with soil-to-solution ratio (1:10) and shaking time 1 hr had a better correlation with grain and straw As content. A larger concentration of HNO_3 was not evaluated because a high concentration of acids may cause problems in instruments; reports suggested that a high concentration of HNO_3 reduces signal intensity in ICP-OES (Yoshimura et al., 1990).

Most researchers prefer the HNO_3 matrix for the determination of elements in ICP-MS due to its strong oxidizing ability, high solubility of nitrates after dissociation of HNO_3, and relative lack of chemical and spectral interferences as compared to acids containing Cl, S, F, or P (Gaines, 2011). Nitric acid is compatible with ICP-MS and a strong oxidizing agent that dissociated into hydrated H^+ and NO_3^-. In our examined soils, As was mostly linked with amorphous Fe and Mn oxides so that an increase in acidity enhances the extractability of HNO_3. The As extraction efficiency significantly varied with HNO_3 concentrations. In the absence of acid or alkali, a control experiment revealed only around 3% extraction, meaning that the majority of As extractions are attributable to acid or alkali extraction. However, strong acids like $HClO_4$ only extract 51% of total As at 10% of acid concentration (Alam and Tokunaga, 2006). When HNO_3 is used as extractant for soil As it creates a problem in the redox conversion of inorganic As species because it converts arsenite into arsenate under an oxidizing environment. Therefore, it will not be suitable for a speciation study in soil. Alam and Tokunaga (2006) argued that extraction of arsenite abates to 0.4% with increasing concentration of HNO_3 from 5.0–40%. A higher concentration of nitric acid was not used because it extracts As from other pools that are not available for plant uptake in immediate effects, and may reduce signal intensity during determination.

Correlation among soil properties, soil extractable As, and plant As

For this purpose, a total of 201 samples were analyzed, and a correlation was drawn among all the parameters (Table 3). A significant positive correlation was observed among pH, 0.1 N H_3PO_4 (r = 0.16*) and 0.5 N H_3PO_4 (r = 0.17*) extractable As. Meanwhile no significant positive correlation was observed with pH and H_2SO_4, HNO_3, $CaCl_2$ extractable As, and plant As. The OC had a significant negative correlation with 0.1 N HNO_3 (-0.16*) and grain As content (-0.10*) while other extractants and plant As did not attain a level of significance. Sodium bicarbonate (1:20) exhibited a significant positive correlation with $NaHCO_3$ (1:5) (0.54**), 0.1 N H_3PO_4 (0.59**), 0.5 N H_3PO_4 (0.47**), 0.5 N H_2SO_4 (0.48**), 0.1 N HNO_3 (0.14*), 0.5 N HNO_3 (0.42**), 0.01 M $CaCl_2$ (0.18*) extractable As, straw (0.66**) and grain As (0.53**) (Table 3). Sodium bicarbonate (1:5) extractable As had a significant positive correlation with all extractants as well as grain and straw As content. However, a relatively lower correlation was observed with grain and straw As content when compared to $NaHCO_3$ (1:20) (Table 3).

Table 3
Correlation among soil properties, extractable As, straw and grain As content (N = 201)

	pH	OC %	$NaHCO_3$ (1:20)	$NaHCO_3$ (1:5)	0.1 N H_3PO_4	0.5N H_3PO_4	0.1 N H_2SO_4	0.5 N H_2SO_4	0.1 N HNO_3	0.5 N HNO_3	0.01 M $CaCl_2$	Straw As	Grain As
pH	1												
OC %	0.01	1											
$NaHCO_3$ (1:20)	0.11	0.01	1										
$NaHCO_3$ (1:5)	0.09	0.01	0.54**	1									
0.1 N H_3PO_4	0.16*	-0.10	0.59**	0.55**	1								
0.5N H_3PO_4	0.17*	-0.07	0.47**	0.52**	0.66**	1							
0.1 N H_2SO_4	0.06	0.10	0.11	0.214**	0.28**	0.12	1						
0.5 N H_2SO_4	0.12	0.02	0.48**	0.48**	0.46**	0.54**	0.09	1					
0.1 N HNO_3	0.03	-0.16*	0.14*	0.21**	0.42**	0.13	0.25**	0.1	1				
0.5 N HNO_3	0.03	0.03	0.42**	0.40**	0.55**	0.36**	0.33**	0.29**	0.28**	1			
0.01 M $CaCl_2$	0.11	-0.09	0.18*	0.27**	0.29**	0.14*	0.20**	0.23**	0.28**	0.26**	1		
Straw As	0.01	-0.04	0.66**	0.51**	0.65**	0.40**	0.13	0.34**	0.34**	0.50**	0.24**	1	
Grain As	0.12	-0.10	0.53**	0.22**	0.28**	0.16*	0.08	0.19**	0.11	0.28**	0.25**	0.33**	1
*** Correlation is significant at the 0.05 level**													
**** Correlation is significant at the 0.01 level**													

A significant positive correlation with all the extractants, straw, and grain As was recorded with 0.1 N H_3PO_4, this extractant had a comparable correlation with $NaHCO_3$ (1:20) extractable As and straw As but a poor correlation was observed with grain As content. A relatively poorer correlation was observed with all the extractants, straw, and grain As content for 0.5 N H_3PO_4 extractable As. Arsenic extracted with 0.1 N H_2SO_4 was positively correlated with 0.1 N, 0.5 N HNO_3, and 0.01 M $CaCl_2$ extractable As but did not attain a level of significance with straw and grain As content (Table 3). Arsenic extracted with 0.1 N HNO_3 and 0.5 N HNO_3 wielded a significant positive correlation with straw and grain As content but did attain a higher level of correlation in comparison to $NaHCO_3$ (1:20) (Table 3). Arsenic extracted with 0.01 M $CaCl_2$ was significantly correlated with grain As (0.25**) content and straw As content (0.24**). A further 38 samples were selected based on $NaHCO_3$ (1:20) extractable As and analyzed with a higher concentration of HNO_3 viz. 1.0 N and 1.5 N with the objective of finding out if a higher concentration of HNO_3 is better correlated with grain and straw As content. The result indicated that all the extractants had a better correlation with grain and straw As content (Table 4). Results revealed that 1.0 N HNO_3 had a better correlation with straw (0.70**) and grain (0.34**) As content than As extracted with a smaller concentration of HNO_3 (0.1 N and 0.5 N HNO_3) (Table 4).

Table 4
Correlation among soil properties, extractable As, straw and grain As content of selected samples (N = 38)

	pH	OC %	$NaHCO_3$ (1:20)	$NaHCO_3$ (1:5)	0.1 N H_3PO_4	0.5N H_3PO_4	0.1 N H_2SO_4	0.5 N H_2SO_4	0.1 N HNO_3	0.5 N HNO_3	1N HNO_3	1.5 N HNO_3	0.01 M $CaCl_2$	Straw As	Grain As
pH	1.00														
OC %	0.18	1.00													
$NaHCO_3$ (1:20)	0.09	-0.21	1.00												
$NaHCO_3$ (1:5)	0.25	-0.26	0.50**	1.00											
0.1 N H_3PO_4	0.10	-0.28	0.55**	0.72**	1.00										
0.5N H_3PO_4	0.09	-0.15	0.46**	0.53**	0.71**	1.00									
0.1 N H_2SO_4	0.12	0.08	0.53**	0.25	0.38*	0.25	1.00								
0.5 N H_2SO_4	0.09	-0.08	0.35*	0.56**	0.47**	0.37*	0.17	1.00							
0.1 N HNO_3	0.14	-0.25	0.29	0.59**	0.64**	0.25	0.48**	0.36*	1.00						
0.5 N HNO_3	0.14	-0.16	0.49**	0.27	0.32*	0.25	0.40*	0.30	0.10	1.00					
1N HNO_3	0.10	-0.37*	0.58**	0.68**	0.71**	0.44**	0.11	0.64**	0.34*	0.40*	1.00				
1.5 N HNO_3	0.14	-0.28	0.86**	0.55**	0.63**	0.42**	0.47**	0.43**	0.41**	0.55**	0.77**	1.00			
0.01 M $CaCl_2$	0.04	-0.28	0.20	0.40*	0.57**	0.28	0.42**	0.30	0.76**	0.04	0.27	0.27	1.00		
Straw As	0.12	-0.32	0.64**	0.70**	0.89**	0.57**	0.50**	0.42**	0.68**	0.50**	0.70**	0.71**	0.55**	1.00	
Grain As	0.02	-0.27	0.47**	0.15	0.49**	0.20	0.43**	0.08	0.27	0.35*	0.34*	0.45**	0.34*	0.41*	1.00
*** Correlation is significant at the 0.05 level**															
**** Correlation is significant at the 0.01 level**															

With increasing concentration of HNO_3 it was evident that 1.5 N HNO_3 result strongly suggested that a better correlation with grain (0.45**) and straw (0.71**) As content was observed. 1.5 N HNO_3 extractable As was better correlated with 0.5 N $NaHCO_3$ (1:20) extractable As (0.86**) (Table 4). Thus 1.5 N HNO_3 may function as a suitable extractant for soil As which is compatible with ICP-MS analysis. Howver, it should be noted that this extractant needs to be evaluated in diverse soil orders and conditions. Partial regression analysis of the selected 38 samples suggested that 0.5 N $NaHCO_3$ (1:20) extractable As explains 41% variability in straw As content while 0.5 N $NaHCO_3$ (1:5) extractable As explains 49% variability (Fig. 2a). Other extractants like 0.1 N H_3PO_4, 0.5 N H_3PO_4, 0.1 N H_2SO_4, 0.5 N H_2SO_4, 0.1 N HNO_3, 0.5 N HNO_3, 1.0 N HNO_3, 1.5 N HNO_3 and 0.01 M $CaCl_2$ extractable explain 79%, 32%, 25%, 18%, 46%, 28%, 48%, 51% and 30% variability in straw As content, respectively (Fig. 2a and 2b).

The corresponding values for grain As content were 23%, 2.1%, 24%, 4.0%, 18%, 1.0%, 10%, 11%, 12%, 20% and 11%, respectively (Fig. 3a and 3b). Step-wise multiple regression equation analysis suggested that straw arsenic content was better predicted when combining two acids with the concentration of 0.1 N H_3PO_4 and 1.5 N HNO_3 (Fig. 3b-f). Giri et al. (2012) reported that a positive correlation between available As and pH may be due to a reduction in As^{5+} adsorption with rising pH above 8.5 while the reverse trend was observed in the case of As^{3+}. When an increase soil organic carbon occurred, As extracted with different extractants fell and this may be due to the presence of humic acid that binds As. Organic matter can compete with As for sorption sites in soil, and dissolved organic matter can also inhibit As sorption to soil particles through redox reactions and the formation of soluble complexes with As (Kumar et al., 2021; Mahimairaja et al., 2005). There was a positive correlation among different extractants indicating that more or less all the extractants extract As from comparable pools.

Conclusions

The extractants to remove bioavailable As from soil is 0.5 N $NaHCO_3$ (1:20) but it is not compatible with ICP-MS estimation of As due to its high salt content. This study evaluated soil properties, different acids and salt extractants, and their correlation with plant As content. Arsenic extracted with most common extractant 0.5 N $NaHCO_3$ (1:20) was 1.45 mg kg^{-1} while 1.5 N HNO_3 extractable As was 1.42 mg kg^{-1}. A more or less similar amount of As is extracted from soil by both extractants. Further correlation analysis revealed that pH has a positive correlation while OC content has a negative correlation with grain and straw As content. A relatively higher correlation was observed with grain and straw As content with 0.5 N $NaHCO_3$ (1:20), followed by 1.5 N HNO_3. The highest correlation was observed between 0.5 N $NaHCO_3$ (1:20) extractable As and 1.5 N HNO_3 extractable As. Nitric acid is relatively compatible with ICP-MS. Thus, 1.5 N HNO_3 may be used as a suitable extractant for extracting bioavailable As and subsequent estimation in ICP-MS.

Declarations

Conflicts of interest/Competing interests: 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.

Funding: ICAR-Indian Agricultural Research Institute, New Delhi in form of ICAR-IARI merit scholarships, provides all the laboratory facilities and expenditures.

Availability of data and material: Not applicable

Code availability: Not applicable

Authors' contributions: **Rahul Mishra:** Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing - original draft, Investigation. **S.P. Datta:** Supervision, Resources, Validation, Writing - review & editing. **D. Golui:** Methodology, Data curation, Formal analysis. **M.C. Meena:** Supervision, Resources. **B.S. Dwivedi:** Data curation, Formal analysis, and editing. **M.M. Rahman:** Writing-review and editing. **K.K. Bandyopadhyay:** Review and editing. **A. Bhatia:** Formal analysis, review, and editing. **P.S. Pandey:** Resources, Supervision.

Animal research: Not applicable

Consent to participate: Not applicable

Consent to publish: Not applicable

References

1. Adriano DC (2001) Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals, vol 860. Springer, New York
2. Alam MGM, Tokunaga S (2006) Chemical extraction of arsenic from contaminated soil. J Environ Sci Health A 41(4):631–643
3. Anawar HM, Garcia-Sanchez A, Santa Regina I (2008) Evaluation of various chemical extraction methods to estimate plant-available arsenic in mine soils. Chemosphere 70(8):1459–1467
4. Bari AF, Lamb D, Choppala G, Seshadri B, Islam MR, Sanderson P, Rahman MM (2021) Arsenic bioaccessibility and fractionation in abandoned mine soils from selected sites in New South Wales, Australia and human health risk assessment. Ecotoxicol Environ Saf 223:112611
5. Beauchemin D (2008) Inductively coupled plasma mass spectrometry. Anal Chem 80(12):4455–4486
6. Cai Y, Cabrera JC, Georgiadis M, Jayachandran K (2002) Assessment of arsenic mobility in the soils of some golf courses in South Florida. Sci Total Environ 291(1–3):123–134
7. Chen P, Zhang HM, Yao BM, Chen SC, Sun GX, Zhu YG (2020) Bioavailable arsenic and amorphous iron oxides provide reliable predictions for arsenic transfer in soil-wheat system. J Hazard Mater 383:121160
8. Das B, Pandit MK, Ray K, Bhattacharyya K, Pari A, Sidhya P (2016) Impact of irrigation and organic matter amendments on arsenic accumulation in selected vegetables. Plant Soil Environ 62(6):266–273
9. Datta SP, Meena MC, Barman M, Golui D, Mishra R, Shukla AK (2018) Soil tests for micronutrients: Current status and future thrust. Indian J Ferti 14(5):32–51
10. Falciani R, Novaro E, Marchesini M, Gucciardi M (2000) Multi-element analysis of soil and sediment by ICP-MS after a microwave assisted digestion method. J Anal At Spectrom 15(5):561–565
11. Fernández-Caliani JC, Giráldez MI, Barba-Brioso C (2019) Oral bioaccessibility and human health risk assessment of trace elements in agricultural soils impacted by acid mine drainage. Chemosphere 237:124441
12. Gaines PR (2011) ICP operations guide. A guide for using ICP-OES and ICP-MS.: Inorganic Ventures, pp.4–8
13. Ghosh D, Pal S, Mahanta S, Acharjee P, PATRA P (2021) Distribution and path analysis of arsenic in soil, water and rice in some affected blocks of lower Gangetic plain. J crop weed 17(1):174–182
14. Giri PK, Bhattacharyya K, Sinha B, Mazumdar D (2012) Study of the suitability of selected extractants for determination of plant-available arsenic in some inceptisols of West Bengal, India. Commun Soil Sci Plant Anal 43(19):2449–2466
15. Golui D, Mazumder DG, Sanyal SK, Datta SP, Ray P, Patra PK, Sarkar S, Bhattacharya K (2017) Safe limit of arsenic in soil in relation to dietary exposure of arsenicosis patients from Malda district, West Bengal-A case study. Ecotoxicol Environ Saf 144:227–235
16. Gutierrez Boem FH, Rubio G, Barbero D (2011) Soil phosphorus extracted by Bray 1 and Mehlich 3 soil tests as affected by the soil/solution ratio in Mollisols. Commun Soil Sci Plant Anal 42(2):220–230

17. Hudson-Edwards KA, Houghton SL, Osborn A (2004) Extraction and analysis of arsenic in soils and sediments. Trends Analyt Chem 23(10–11):745–752
18. Hudson-Edwards KA, Macklin MG, Jamieson HE, Brewer PA, Coulthard TJ, Howard AJ, Turner JN (2003) The impact of tailings dam spills and clean-up operations on sediment and water quality in river systems: the Rıos Agrio–Guadiamar. Aznalcóllar Spain Appl Geochem 18(2):221–239
19. Hwang IM, Lee HM, Lee HW, Jung JH, Moon EW, Khan N, Kim SH (2021) Determination of Toxic Elements and Arsenic Species in Salted Foods and Sea Salt by ICP–MS and HPLC–ICP–MS. ACS omega 6(30):19427–19434
20. Jackson ML (1973) Soil Chemical Analysis. Prentice Hall of India Private Limited, New Delhi
21. Johnston SE, Barnard WM (1979) Comparative effectiveness of fourteen solutions for extracting arsenic from four western New York soils. Soil Sci Soc Am J 43(2):304–308
22. Kabata-Pendias A (2004) Soil–plant transfer of trace elements—an environmental issue. Geoderma 122(2–4):143–149
23. Kumar SN, Mishra BB, 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
24. Li SW, Li J, Li HB, Naidu R, Ma LQ (2015) Arsenic bioaccessibility in contaminated soils: Coupling in vitro assays with sequential and HNO3 extraction. J Hazard Mater 295:145–152
25. Maher W, Krikowa F, Kirby J, Townsend AT, Snitch P (2003) Measurement of trace elements in marine environmental samples using solution ICPMS. Current and future applications. Aust J Chem 56(3):103–116
26. Mahimairaja S, Bolan NS, Adriano DC, Robinson B (2005) Arsenic contamination and its risk management in complex environmental settings. Adv Agron 86:1–82
27. Mandal J, Sengupta S, Sarkar S, Mukherjee A, Wood MD, Hutchinson SM, Mondal D (2021) Meta-analysis enables prediction of the maximum permissible arsenic concentration in Asian paddy soil.Front Environ Sci547
28. Martinez-Sánchez MJ, Martínez-López S, García-Lorenzo ML, Martínez-Martínez LB, Pérez-Sirvent C (2011) Evaluation of arsenic in soils and plant uptake using various chemical extraction methods in soils affected by old mining activities. Geoderma 160(3–4):535–541
29. Mucci A, Boudreau B, Guignard C (2003) Diagenetic mobility of trace elements in sediments covered by a flash flood deposit: Mn, Fe and As. Appl Geochem 18(7):1011–1026
30. Nguyen Van T, Osanai Y, Do Nguyen H, Kurosawa K (2017) Arsenic speciation and extraction and the significance of biodegradable acid on arsenic removal—An approach for remediation of arsenic-contaminated soil. Int J Environ Res Public Health 14(9):990
31. Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soil by extraction with sodium bicarbonate. U.S. Department of Agricultural Repot. Circular no. 93
32. O'Sullivan JE, Watson RJ, Butler EC (2013) An ICP-MS procedure to determine Cd, Co, Cu, Ni, Pb and Zn in oceanic waters using in-line flow-injection with solid-phase extraction for preconcentration. Talanta 115:999–1010
33. Quevauviller P (1998) Operationally defined extraction procedures for soil and sediment analysis I. Standardization. Trends Analyt Chem 17(5):289–298
34. Rahaman S, Sinha AC, Pati R, Mukhopadhyay D (2013) Arsenic contamination: a potential hazard to the affected areas of West Bengal, India. Environ Geochem Health 35(1):119–132
35. Raj A, Mandal J, Golui D, Sihi D, Dari B, Kumari PB, Ganguly P (2021) Determination of suitable extractant for estimating plant available arsenic in relation to soil properties and predictability by solubility-FIAM. Water Air and Soil Poll 232(6):1–11
36. Rodrigues SM, Cruz N, Carvalho L, Duarte AC, Pereira E, Boim AGF, Alleoni LRF, Römkens PFAM (2018) Evaluation of a single extraction test to estimate the human oral bioaccessibility of potentially toxic elements in soils: towards more robust risk assessment. Sci Total Environ 635:188–202
37. Rodrigues SM, Cruz N, Carvalho L, Duarte ADC, Pereira E, Boim AG, Alleoni LRF, Römkens PFAM (2018) Evaluation of a single extraction test to estimate the human oral bioaccessibility of potentially toxic elements in soils: Towards more robust risk assessment. Sci Total Environ 635:188–202
38. SAS Institute (2011) The SAS system for Windows. Release 9.2. SAS Inst., Cary, NC
39. Shahandeh H, Hons FM, Provin TL, Pitt JL, Waskom JS (2017) Factors affecting Mehlich III soil test methodology for extractable P. Commun Soil Sci Plant Anal 48(4):423–438
40. Sohrin Y, Bruland KW (2011) Global status of trace elements in the ocean. Trends Analyt Chem 30(8):1291–1307
41. Sparks DL (1996) Methods of soil analysis, part 3 (SSSA Book Series No. 5). SSSA and ASA, Madison, Wisc.
42. Stroud JL, Khan MA, Norton GJ, Islam MR, Dasgupta T, Zhu YG, Price AH, Meharg AA, McGrath SP, Zhao FJ (2011) Assessing the labile arsenic pool in contaminated paddy soils by isotopic dilution techniques and simple extractions. Environ Sci Technol 45:4262–4269
43. Trang Hoang TQ, Hahn C (2015) Arsenic fractionation in agricultural soil in Vietnam using the sequential extraction procedure. In 4th International Conference on Informatics, Environment, Energy and Applications, (Vol. 2015, pp. 2184–2199)
44. Van Herreweghe S, Swennen R, Vandecasteele C, Cappuyns V (2003) Solid phase speciation of arsenic by sequential extraction in standard reference materials and industrially contaminated soil samples. Environ Pollut 122(3):323–342
45. Walkley A, Black IA (1934) An examination of the Degtjareff method for determining Soil Organic matter, and a proposed modification of the Chromic Acid Titration Method. Soil Sci 37:29–38
46. Welna M, Szymczycha-Madeja A, Pohl P (2020) Non-Chromatographic Speciation of As by HG Technique—Analysis of Samples with Different Matrices. Molecules, 25(21): p.4944
47. Wenzel WW, Kirchbaumer N, Prohaska T, Stingeder G, Lombi E, Adriano DC (2001) Arsenic fractionation in soils using an improved sequential extraction procedure. Anal Chim Acta 436(2):309–323

48. Wilschefski SC, Baxter MR (2019) Inductively coupled plasma mass spectrometry: introduction to analytical aspects. Clin Biochem Rev 40(3):115
49. Yao BM, Chen P, Zhang HM, Sun GX (2021) A predictive model for arsenic accumulation in rice grains based on bioavailable arsenic and soil characteristics. J Hazard Mater 412:125131
50. Yoshimura E, Suzuki H, Yamazaki S, Toda S (1990) Interference by mineral acids in inductively coupled plasma atomic emission spectrometry. Analyst 115(2):167–171
51. Yu HY, Ding X, Li F, Wang X, Zhang S, Yi J, Liu C, Xu X, Wang Q (2016) The availabilities of arsenic and cadmium in rice paddy fields from a mining area: The role of soil extractable and plant silicon. Environ Pollut 215:258–265
52. Zhu YG, Yoshinaga M, Zhao FJ, Rosen BP (2014) Earth abides arsenic biotransformations. Annu Rev Earth Planet Sci 42:443–467

Figures

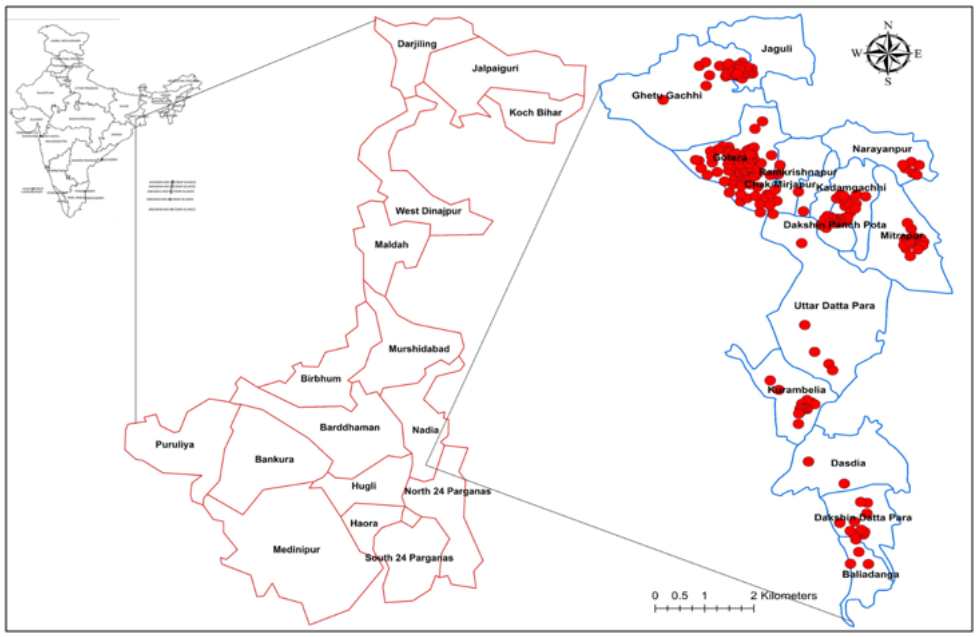

Figure 1

Sampling location (In Nadia District of West Bengal; only for illustration purposes)

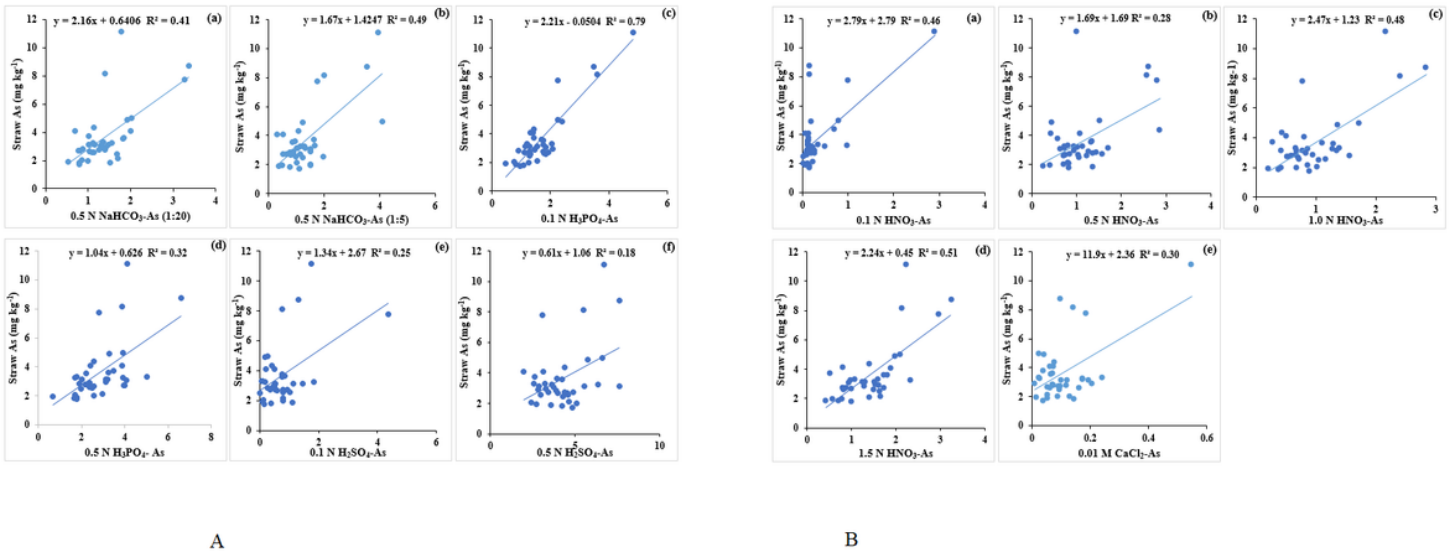

Figure 2

A. Relationship between straw As and (a) 0.5 N NaHCO3 (1:20)-As (b) 0.5 N NaHCO3(1:5)-As (c) 0.1 N H3PO4-As (d) 0.5 N H3PO4-As (e) 0.1 N H2SO4-As (f) 0.5 N H2SO4-As ; (N= 38) **B:** Relationship between straw As and (a) 0.1 N HNO3 -As (b) 0.5 N HNO3 -As (c) 1.0 N HNO3 -As (d) 1.5 N HNO3 -As (e) 0.01 M

CaCl2-As; (N= 38)

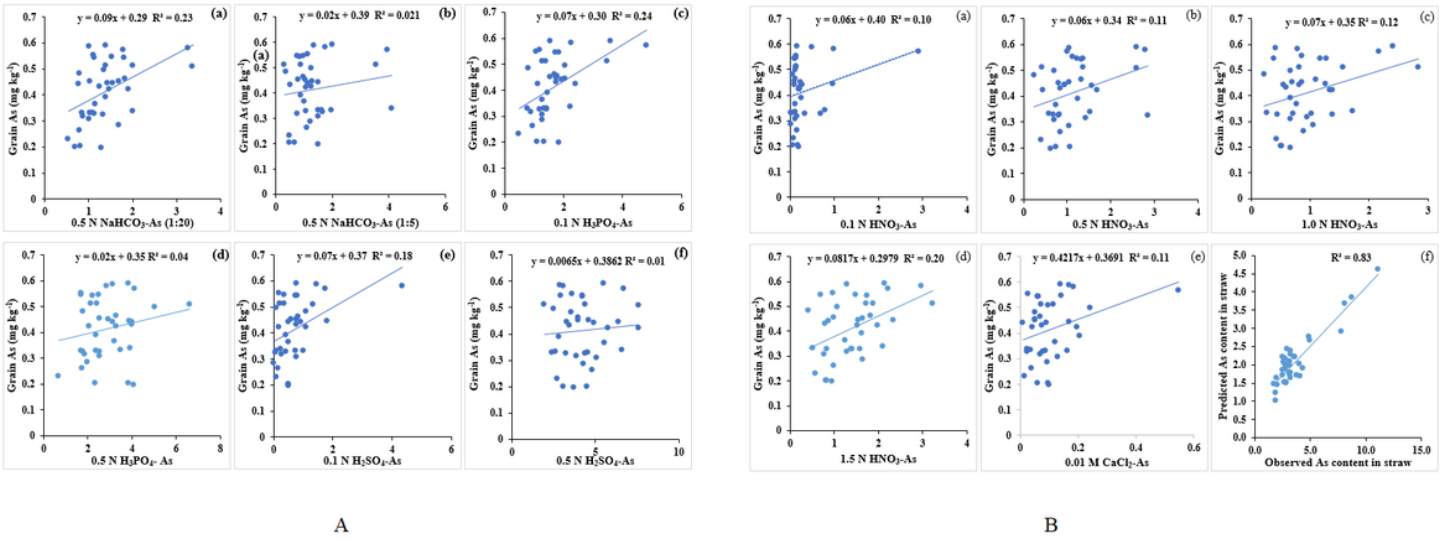

Figure 3

A. Relationship between grain As and (a) 0.5 N NaHCO3 (1:20)-As (b) 0.5 N NaHCO3 (1:5)-As (c) 0.1 N H3PO4-As (d) 0.5 N H3PO4-As (e) 0.1 N H2SO4-As (f) 0.5 N H2SO4-As; (N= 38) **B:** Relationship between grain As and (a) 0.1 N HNO3 -As (b) 0.5 N HNO3 -As (c) 1.0 N HNO3 -As (d) 1.5 N HNO3 -As (e) 0.01 M CaCl2-As (f) Relation between observed As and As derived from stepwise linear regression equation; (N= 38)