Article

Comparison of Various Single Chemical Extraction Methods for Predicting the Bioavailability of Arsenic in Paddy Soils

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The Codex Committee of Contaminants in Food (CCCF) has been discussing a new standard for arsenic (As) in rice since 2010 and a code of practice for the prevention and reduction of As contamination in rice since 2013. Therefore, our current studies focus on setting a maximum level of As in rice and paddy soil by considering bioavailability in the remediation of As contaminated soils. This study aimed to select an appropriate single chemical extractant for evaluating the mobility of As in paddy soil and the bioavailability of As to rice. Nine different extractants, such as deionized water, 0.01 M Ca(NO$_3$)$_2$, 0.1 M HCl, 0.2 M C$_6$H$_8$O$_7$, 0.43 M HNO$_3$, 0.43 M CH$_3$COOH, 0.5 M KH$_2$PO$_4$, 1 M HCl, and 1 M NH$_4$NO$_3$ were used in this study. Total As content in soil was also determined after aqua regia digestion. The As extractability of the was in the order of: Aqua regia > 1 M HCl > 0.5 M KH$_2$PO$_4$ > 0.43 M HNO$_3$ > 0.2 M C$_6$H$_8$O$_7$ > 0.1 M HCl > 0.43 M CH$_3$COOH > deionized water > 1 M NH$_4$NO$_3$ > 0.01 M Ca(NO$_3$)$_2$. Correlation between soil extractants and As content in rice was in the order of : deionized water > 0.01 M Ca(NO$_3$)$_2$ > 0.43 M CH$_3$COOH > 0.1 M HCl > 0.5 M KH$_2$PO$_4$ > 1 M NH$_4$NO$_3$ > 0.2 M C$_6$H$_8$O$_7$ > 0.43 M HNO$_3$ > 1M HCl > Aqua regia. BCF (bioconcentration factor) according to extractants was in the order of : 0.01M Ca(NO$_3$)$_2$ > 1 M NH$_4$NO$_3$ > deionized water > 0.43 M CH$_3$COOH > 0.1 M HCl > 0.43 M HNO$_3$ > 0.2 M C$_6$H$_8$O$_7$ > 0.5 M KH$_2$PO$_4$ > 1 M HCl > Aqua regia. Therefore, 0.01 M Ca(NO$_3$)$_2$ (r=0.78) was proven to have the greatest potential for predicting As bioavailability in soil with higher correlation between As in rice and the extractant.**

Key words: Arsenic, Paddy soil, Bioavailability, Single extraction method

Extraction conditions of the nine extracting solutions for arsenic in soil.

Extraction solution	SSR[a]	Time (hour)	Temp. (℃)	Reference
Deionized water	1:50	16	20	Mackovych et al. (2003)
0.01M Ca(NO$_3$)$_2$	1:2	2	20	Seo et al. (2013)
0.1M HCl	1:5	1	30	MOE (2002)
0.2M C$_6$H$_8$O$_7$	1:5	0.5	20	Amofah et al. (2010)
0.43M HNO$_3$	1:10	1	20	Tippinga et al. (2003)
0.43M CH$_3$COOH	1:40	16	20	Quevauviller et al. (1997)
0.5M KH$_2$PO$_4$	1:20	0.5	20	Giri et al. (2012)
1M HCl	1:5	0.5	30	MOE (2002)
1M NH$_4$NO$_3$	1:2.5	2	20	DIN (1995), Itanna et al. (2008)

[a]Soil to solution ratio

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Introduction

The Ministry of Environment established the criteria of soil contamination for heavy metal(loid)s in the agricultural fields designated by the Soil Environment Conservation Law in 1996 (MOE, 1996). The Ministry of Food and Drug Safety also established the criteria of 0.2 mg/kg for cadmium (Cd) in polished rice in Korea (KFDA, 2000). Recently, our regulations for agricultural environment including soil, irrigation water, agricultural materials (fertilizer and compost etc.), and agricultural products are being gradually reinforced (KFDA, 2011; MOE, 2010a). The CCCF (Codex Committee of Contaminants in Foods) have been under discussion on the maximum levels for arsenic (As) in polished rice since 2010 (FAO/WHO, 2014).

In Korea, the standard method of analyzing heavy metal(loid)s contamination in soils is by aqua regia digestion (MOE, 2010b). This method is acceptable to evaluate the environmental burden of pollutants to the soil and to decide the proper environmental management and human safety. However, this method is not useful for assessing the metal bioavailability to crops. In order to minimize the risk of heavy metal(loid)s to the agricultural environment, crops, livestock, and humans exposed during the agricultural activities, considering bioavailability for the remediation of heavy metal(loid)s is necessary for agro-food safety (Naidu et al., 2003; Heemsbergen et al., 2009; Kim et al., 2009; Salazar et al., 2012; Bolan et al., 2014). Even though the development of analytical methods for measuring the bioavailability of heavy metal(loid)s in agricultural soils was rare in Korea, various assessment techniques were already developed worldwide. Bioavailability of metal(loid)s in agricultural soils was determined mainly by the concentration of metal(loid)s, the species and fractions of a specific metal(loid), and the physico-chemical properties of soils (Ruby et al., 1993; Geebelen et al., 2002; Kim et al., 2012a). Various single and stepwise sequential extraction methods were suggested to estimate the bioavailability of heavy metal(loid)s in agricultural soils. The Soil Environment Conservation Law recommended the use of 0.1 M HCl for cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), nickel (Ni), and zinc (Zn) and 1.0 M HCl for As to evaluate their bioavailability (MOE, 2002). It was reported by regression analysis that the bioavailability of Cd and Zn to rice was higher than those of Cu and Pb. It was also concluded that the 0.1 M HCl extractable heavy metal(loid)s in soil were more closely correlated with heavy metal(loid)s in husked rice than 0.1 M HNO$_3$, 0.005 M DTPA and 0.05 M EDTA extractable heavy metal(loid)s in soil (Jung et al., 2000). It was also reported that the 0.01 M Ca(NO$_3$)$_2$ extraction method was effective than the 1 M NH$_4$NO$_3$ extraction method for the bioavailability of Cd and other metal(loid)s in soils (Seo et al., 2013). However, there are few studies on the As bioavailability in agricultural soils in Korea. Therefore, the objectives

of this study were (i) to propose a suitable single extraction method for assessing the bioavailability of As in soils, (ii) to verify the physico-chemical factors affecting As uptake and its transformation to rice, and (iii) to finally identify and develop the countermeasure techniques to conserve the As contaminated paddy soils.

Materials and Methods

Soil and rice sampling Thirty As contaminated sites with various levels of As in soils were selected based on the result of a detailed survey at the 300 abandoned mines by the Ministry of Environment during 2007~2009 due to high concentration of As (MOE, 2007~2009). Soil and rice samples were collected within a 100~1,000 m distance from each site in 2012. All soil samples comprised of 3~6 sub-samples collected within a depth of 15 cm from the surface in each mine site. Soil samples were air-dried, crushed, passed through a 20-mesh sieve, and ground with a mortar. The harvested rice samples were air-dried, polished with a rice mill (Husked : SYTH88, Ssangyong Instrument, Korea, Polished : McGill miller, HT McGill Inc, USA), and then pulverized with a homogenizer.

Soil properties The soil pH value was measured at the ratio of 1:5 soil:deionized water suspension using pH meter (250A, Thermo Orion, Beverly, MA). The soil organic matter (SOM) was determined by Tyurin method with K$_2$Cr$_2$O$_7$ (Tyurin, 1931). The exchangeable cations, i.e. calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), were measured using 1 N ammonium acetate at pH 7.0, and analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, GBC XMP, Australia) (NIAST, 2000).

Determination of arsenic in soil and rice Standard reference material (SRM; Contaminated soil BAM-U112; BAM Federal Institute for Materials Research and Testing, Berlin, Germany) and 3 g soil samples were acid digested with aqua regia as described by the Ministry of Environment (MOE, 2010b). Aqueous soil samples were filtered with Whatman No. 5B filter, and analyzed for As using hydride generation (HG)-ICP-OES (GBC XMP, Australia). To compare the bioavailability of As in paddy soils, nine different soil extractants such as deionized water, 0.01 M Ca(NO$_3$)$_2$, 0.1 M HCl, 0.2 M C$_6$H$_8$O$_7$, 0.43 M HNO$_3$, 0.43 M CH$_3$COOH, 0.5 M KH$_2$PO$_4$, 1 M HCl, and 1 M NH$_4$NO$_3$ were used (Table 1). These extractants were generally selected on soil extraction efficiency, phytoavailability and soil pollution index. Previous studies have shown that soil extraction or washing process has the potential to remove As from contaminated soils using inorganic phosphate salts (KH$_2$PO$_4$), inorganic acids (HCl and HNO$_3$) and organic acids (CH$_3$COOH, C$_6$H$_8$O$_7$). We

Table 1. Extraction conditions of the nine extracting solutions for arsenic in soil.

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0.5M KH$_2$PO$_4$	1:20	0.5	20	Giri et al. (2012)
1M HCl	1:5	0.5	30	MOE (2002)
1M NH$_4$NO$_3$	1:2.5	2	20	DIN (1995), Itanna et al. (2008)

[a]Soil to solution ratio

selected water and neutral salt extracts (Ca(NO$_3$)$_2$ and NH$_4$NO$_3$) since they are the most widely used, have a small effect on pH, and best correlated with plant uptake. 0.5 M KH$_2$PO$_4$ and 0.43 M HNO$_3$ were also proved to be available to plants. Soil extracts were continuously shaken at 30°C for 1 h, filtered with Whatman No. 5B filter, and analyzed by HG-ICP-OES. The accuracy of As in SRM was 8.41 ± 0.52 with certified values of 10.4 ± 0.4 mg/kg. The recovery values of As was 81.27 ± 4.98. 0.5 g of polished rice samples were transferred into a high pressured-polytetrafluoroethylene (PTFE) vessel and digested with 8 mL of 70% HNO$_3$ and 1 mL H$_2$O$_2$ (Sigma-Aldrich, St. Louise, MO) using microwave digestion system (ETHOS, Milestone, Italy). After cooling to room temperature, the extracts were filtered through a 0.45-μm membrane filter, and adjusted to a final volume of 25 mL. The As contents in polished rice were determined by ICP-MS (Agilent technologies 7500a). The SRM accuracy value of As was 0.29 ± 0.05 with certified value of 0.29 ± 0.03 mg/kg. Extraction efficiencies (%), determined by dividing the extracted As content by total As content were 100.58 ± 17.59.

Calculation of bioconcentration factor (BCF) Bioconcentration factor (BCF, Eq. (1)) is defined as the ratio of the As concentration in crops (mg/kg DW) and soil (mg/kg DW) considering the crop uptake from soils and its transformation to the edible part of crops (Kim et al., 2012b).

$$\text{Bioconcentration factor}\,(BCF) = \frac{As\,conc.\,in\,rice\,(mg/(kg))}{As\,conc.\,in\,soil\,(mg/kg)} \quad (1)$$

Calculation of soil-water partition coefficient for arsenic (Kd) The soil water partition coefficient (Kd, Eq. (2)) describes the partitioning of As over two phases (Krishnamurti and Naidu, 2000; Naidu et al., 1994; Sauve et al., 2000). Equation 3 was used in this study to estimate the As bioavailability in soil.

$$\text{Partitioning coefficient}\,(K_d) = \frac{Solid\,phase\,concentration\,of\,metals}{solution\,phase\,concentration\,of\,metals} \quad (2)$$

$$\text{Partitioning coefficients}\,(K_d) = \frac{Total\,conc.\,of\,As\,in\,soil\,(mg/kg)}{Bioavailable\,As\,content\,in\,soil\,(mg/kg)} \quad (3)$$

Statistical Analysis Single and multiple regression analysis were performed using SPSS statistical program ver. 12.0 (SPSS Co., Chicago, IL) to investigate the influence of soil characteristics, i.e. pH, organic matter, and exchangeable cations on As contents in soil and rice. In regression analysis, the total and extractable As contents in soil, and As concentration in rice were log$_{10}$-transformed to make homogeneous variances.

Results and Discussion

Soil Characteristics The results of characterized soil samples (n=30) are as follows: soil pH ranged from 5.1 to 7.5, SOM contents ranged from 3.79 to 37.85 g/kg dry soil, with a mean value of 22.51 g/kg dry soil. This value was slightly lower than 26.0 g/kg which was the average content of paddy soils in Korea. The concentration of cations ranged between 0.12 - 10.02 cmol$^+$/kg for Ca, 0.21 - 2.05 cmol$^+$/kg for K, 0.20 - 5.27 cmol$^+$/kg for Mg, and 0.21 - 1.65 cmol$^+$/kg for Na (Table 2). Soil pH 7.0 of paddy soils near SS mine was higher than 5.5 of MB mine and 5.6 of TC mine. Ca and Mg content in SS paddy soil were also higher than those of MB and TC mine. It means there was soil reclamation activity for the metal(loid)s contaminated paddy soils near SS mine by the addition of lime material.

Arsenic Contents in Soils and Polished Rice Average content and range of As in the surveyed paddy soils were 26.87 and 10.90 - 88.92 mg/kg, respectively. It was found out that thirteen samples among 30 exceeded the As concern level

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Table 2. Chemical properties of the soils used in this study.

| | pH | SOM | Exchangeable cations | | | |
			Ca	K	Mg	Na
		g/kg	------------------------------ $cmol^+/kg$ ------------------------------			
Ave.	5.78	22.51	4.58	0.62	1.46	0.38
Min.	5.09	3.79	0.12	0.21	0.20	0.21
Max.	7.48	37.85	10.02	2.05	5.27	1.65
Average for paddy soil[a]	5.9	26	5.1	0.30	1.30	0.35
Optimal range[a]	5.5-6.5	25-30	5.0-6.0	0.25-0.30	1.5-2.0	

[a]RDA, 2011.

Table 3. Total arsenic content in the soils and polished rice.

	Total As content in soil	Total As content in rice
	------------------------------ mg/kg ------------------------------	
Ave.	26.87	0.09
Min.	10.90	0.03
Max.	88.92	0.23
Average for paddy soil[a]	7.5	
Concern level in area 1[b]	25	

[a]RDA, 2011.
[b]MOE, 2010a.

Table 4. Arsenic contents with the nine single-extracting solution.

Soil	Deionized water	0.01M $Ca(NO_3)_2$	0.1M HCl	0.2M $C_6H_8O_7$	0.43M HNO_3	0.43M CH_3COOH	0.5M KH_2PO_4	1M HCl	1M NH_4NO_3
	--- mg/kg ---								
Ave.	0.181	0.013	0.665	2.132	2.455	0.497	3.357	5.489	0.018
%[1]	0.67	0.05	2.47	7.93	9.14	1.85	12.49	20.43	0.07
Min.	0.010	0.001	0.040	0.220	0.170	0.020	0.410	0.330	0.001
Max.	1.440	0.112	6.830	11.824	14.25	6.140	14.75	30.04	0.197

[1]Average As content (%) of each extractant against total As in soil

for soil contamination described in the Soil Environment Conservation Act. Average content and range of As in the surveyed rice were 0.09 and 0.03 - 0.23 mg/kg, respectively (Table 3). This value was quite similar with the results surveyed near abandoned mine area in 2000 with an average As content of 0.10 mg/kg and was slightly higher with the results surveyed in the non-contaminated area in 2001 with an average As content of 0.06 mg/kg (Kim et al., 2007). These values were also below the 0.2 mg/kg maximum permitted concentration for inorganic As by Codex Committee of Contaminants on Food (CCCF) considering 76.94% (54.50 - 87.86%) of the ratio of inorganic As against total As in polished rice in Korea (FAO/WHO, 2014; Kim et al., 2013).

Arsenic Contents in Soils using Single Extraction Method
To compare bioavailability of As in paddy soils, 9 different

soil extractants were employed. Extractants of As that have widely been used are deionized water, 0.01 M $Ca(NO_3)_2$, 0.1 M HCl, 0.2 M $C_6H_8O_7$, 0.43 M HNO_3, 0.43 M CH_3COOH, 0.5 M KH_2PO_4, 1 M HCl, 1 M NH_4NO_3 as mentioned in Table 1. Average content and range of As extracted with 9 extractants are shown in Table 4.

The As concentration of soils by using single extraction procedures was in the order of 1 M HCl > 0.5 M KH_2PO_4 > 0.43 M HNO_3 > 0.2 M $C_6H_8O_7$ > 0.1 M HCl > 0.43 M CH_3COOH > deionized water > 1 M NH_4NO_3 > 0.01 M $Ca(NO_3)_2$. 1 M HCl was able to extract As ranging between 3 and 34% where higher extractability was observed for highly contaminated soil. Elliott and Shastri (1999) reported that the overall extent of metal solubilization increased modestly as the system became more acidic. Other researchers suggested low molecular weight organic acid and phosphate salts were more effective in

Table 5. Bioconcentration factor (BCF) of soil to rice according to extracting solution.

Soil	Deionized water	0.01M Ca(NO₃)₂	0.1M HCl	0.2M C₆H₈O₇	0.43M HNO₃	0.43M CH₃COOH	0.5M KH₂PO₄	1M HCl	1M NH₄NO₃	Total As in soil
	--- mg/kg ---									
Ave.	1.036	16.713	0.405	0.094	0.095	0.523	0.041	0.039	13.664	0.004
Min.	0.110	2.075	0.034	0.008	0.006	0.038	0.005	0.003	1.185	0.001
Max.	5.668	94.467	1.540	0.243	0.333	2.834	0.141	0.172	70.850	0.008

Table 6. Regression equation between bioconcentration factor (BCF) of arsenic according to extracting solution and soil chemical properties.

Regression equation	R	P value
Deionized water		
$\quad$ Log(BCF-DW) = 0.091-0.770Log(SOM)	0.52**	<0.003
0.01M Ca(NO₃)₂		
$\quad$ Log(BCF-0.01M Ca(NO₃)₂) = 1.346-1.043Log(SOM)	0.65***	<0.000
$\quad$ Log(BCF-0.01M Ca(NO₃)₂) = 1.561-0.690Log(SOM)+0.649Log(Na)	0.71***	<0.000
0.1M HCl		
$\quad$ Log(BCF-0.1M HCl) = -0.010+1.187Log(Na)	0.60***	<0.000
0.2M C₆H₈O₇		
$\quad$ Log(BCF-0.2M C₆H₈O₇) =-0.660+1.069Log(Na)	0.58**	<0.001
0.43M HNO₃		
$\quad$ Log(BCF-0.43M HNO₃) = -0.667+1.109Log(Na)	0.56**	<0.001
0.43M CH₃COOH		
$\quad$ Log(BCF-0.43M CH₃COOH) = 0.017+0.995Log(Na)	0.53**	<0.002
0.5M KH₂PO₄		
$\quad$ Log(BCF-0.5M KH₂PO₄) = -1.167+0.663Log(Na)	0.47**	<0.009
1M HCl		
$\quad$ Log(BCF-1M HCl) = -1.028+1.184Log(Na)	0.61***	<0.000
$\quad$ Log(BCF-1M HCl) = -1.040+0.763Log(Na)-0.662Log(SOM)	0.68***	<0.000
1M NH₄NO₃		
$\quad$ Log(BCF-1M NH₄NO₃) = 1.511+1.156Log(Na)	0.62***	<0.000

(P<0.01), *(P<0.001)

extracting As, attaining more than 40% extraction in the pH range of 6~8 (Alam et al., 2001; Bhattacharya et al., 2002; Stroud et al., 2011). Bioconcentration factors (BCF) of soil to rice according to various single extracting solutions are shown in Table 5. Crop transformation of As were more affected by the extractable As content than total As in soils which predicts the bioavailability of As in soils (Brun et al., 1998; McLaughlin et al., 2000; Savie et al., 1996). Currently, the BCF calculations established by USEPA (United States Environmental Protection Agency), UKEA (United Kingdom Environment Agency), RIVM (*Rijksinstituut voor Volksgezondheid en Milieu*; Netherland National Institute for Public Health and the Environment) were used for the purpose of environmental risk assessment in Korea (CLEA, 1998; Otte et al., 2001; USEPA, 1992; USEPA, 1996). USEPA reported that the empirical BCF for As in only one sample of grains and cereal, specially sorghum, was 0.026

in the sludge treated soil. This value was absolutely different with 0.004 of rice BCF in this study. It was probably attributed to the varietal and species difference. Kim et al. (2012b) reported that the average transfer coefficient of As to the rice was 0.309 against 0.1 M HCl extractable As in soil. This value was similar with 0.405 value in this study. The BCF value calculated with various extractants by using single extraction procedures was in the order of 0.01 M Ca(NO₃)₂ > 1 M NH₄NO₃ > deionized water > 0.43 M CH₃COOH > 0.1 M HCl > 0.43 M HNO₃ > 0.2 M C₆H₈O₇ > 0.5 M KH₂PO₄ > 1 M HCl. It appeared that 0.01 M Ca(NO₃)₂ extraction was a better option for the determination of bioavailable metal(loid)s in soils. Lee et al. (2012) reported various patterns of crop uptake and transformation of heavy metal(loid)s with different crop species, varieties, parts, growing seasons, plowing, and irrigation methods. Table 6 showed the regression equations

between BCF of As according to extracting solutions and soil chemical properties in order to identify the factors affecting BCF in the presence of different extractants. Deionized water extraction was affected by SOM ($r=0.52^{**}$) while 0.01 M Ca(NO$_3$)$_2$ was affected by both SOM ($r=0.65^{***}$) and exchangeable Na ($r=0.71^{***}$). 1 M HCl extraction was also affected by SOM ($r=0.61^{***}$) and exchangeable Na ($r=0.68^{***}$) with higher coefficients. Most of the extractants were affected by ex-

changeable Na including 0.1 M HCl ($r=0.60^{***}$), 0.2 M C$_6$H$_8$O$_7$ ($r=0.58^{**}$), 0.43 M HNO$_3$ ($r=0.56^{**}$), 0.43 M CH$_3$COOH ($r=0.53^{**}$), 0.5 M KH$_2$PO$_4$ ($r=0.47^{**}$) and 1 M NH$_4$NO$_3$ ($r=0.62^{***}$). In summary, exchangeable Na was the major factor which affected the BCF of various extractants in the current study, however, the chemistry related with Na content on enhanced As uptake revealed by increased BCF is unknown and required further study. SOM was another parameter

Table 7. Partition coefficients (K$_d$) of arsenic according to extracting solution.

Soil	Deionized water	0.01M Ca(NO$_3$)$_2$	0.1M HCl	0.2M C$_6$H$_8$O$_7$	0.43M HNO$_3$	0.43M CH$_3$COOH	0.5M KH$_2$PO$_4$	1M HCl	1M NH$_4$NO$_3$
	-- mg/kg --								
Ave.	279.52	4317.29	112.28	24.91	25.18	148.15	10.39	9.84	3546.75
Min.	42.06	538.86	8.87	4.96	4.25	9.86	4.21	2.62	307.76
Max.	1324.33	22072.22	412.56	60.52	77.90	662.17	26.58	40.13	16554.2

Table 8. Regression equation between partition coefficients (K$_d$) of arsenic according to extracting solution and soil chemical properties.

Regression equation	R	P value
Deionized water		
Log (K$_d$-DW) = 2.103-0.985 Log (K)	0.53**	<0.002
Log (K$_d$-DW) = 2.049-1.051 Log (K)+0.427 Log (Mg)	0.64**	<0.001
0.01M Ca(NO$_3$)$_2$		
Log (K$_d$-0.01M Ca(NO$_3$)$_2$) = 3.783-0.927 Log (SOM)	0.72***	<0.000
Log (K$_d$-0.01M Ca(NO$_3$)$_2$) = 3.612-0.783 Log (SOM)-0.542 Log (K)	0.76***	<0.000
Log (K$_d$-0.01M Ca(NO$_3$)$_2$) = 3.753-0.554 Log (SOM)-0.540 Log (K)+0.422 Log (Na)	0.80***	<0.000
0.1M HCl		
Log (Kd-0.1M HCl) = 2.352+0.966 Log (Na)	0.53**	<0.003
0.2M C$_6$H$_8$O$_7$		
Log (K$_d$-0.2M C$_6$H$_8$O$_7$) = 1.701+0.848 Log (Na)	0.57**	<0.001
Log (K$_d$-0.2M C$_6$H$_8$O$_7$) = 1.469+0.723 Log (Na)-0.726 Log (K)	0.67***	<0.000
0.43M HNO$_3$		
Log (K$_d$-0.43M HNO$_3$) = 1.501-0.828 Log (SOM)	0.54**	<0.002
0.43M CH$_3$COOH		
Log (K$_d$-0.43M CH$_3$COOH) = 2.379+0.774 Log (Na)	0.45*	<0.012
Log (K$_d$-0.43M CH$_3$COOH) = 2.108+0.629 Log (Na)-0.846 Log (K)	0.57**	<0.005
0.5M KH$_2$PO$_4$		
Log (K$_d$-0.5M KH$_2$PO$_4$) = 1.111-0.455 Log (SOM)	0.61***	<0.000
1M HCl		
Log (K$_d$-1M HCl) = 1.143-0.962 Log (SOM)	0.72***	<0.000
Log (K$_d$-1M HCl) = 1.321-0.669 Log (SOM)+0.538 Log (Na)	0.78***	<0.000
1M NH$_4$NO$_3$		
Log (K$_d$-1M NH$_4$NO$_3$) = 3.873+0.936 Log (Na)	0.62***	<0.000
Log (K$_d$-1M NH$_4$NO$_3$) = 3.662+0.822 Log (Na)-0.661 Log (K)	0.69***	<0.000
Log (K$_d$-1M NH$_4$NO$_3$) = 3.096+0.641 Log (Na)-0.869 Log (K)+0.666 Log (Ca)	0.75***	<0.000
Total As in soil		
Log (K$_d$-Total) = 1.280+0.353 Log (SOM)	0.44*	<0.013

*(P<0.05), **(P<0.01), ***(P<0.001)

exhibited significant influence on increased BCF as shown by Gonzaga et al. (2012) previously, which probably due to increased dissolved organic carbon (DOC) in the soil solution sourced from the SOM. In general, DOC induces elevated soluble As concentration through formation of DOC-As complex in the soil pore water.

Calculation of partition coefficients with various extractants
Table 7 shows the partition coefficients (K_d) of arsenic according to single extraction method. The partition coefficients of extractants by using single extraction procedures were in the order of 0.01 M $Ca(NO_3)_2$ > 1 M NH_4NO_3 > deionized water > 0.43 M CH_3COOH > 0.1 M HCl > 0.43 M HNO_3 > 0.2 M $C_6H_8O_7$ > 0.5 M KH_2PO_4 > 1 M HCl. When the partition coefficients are low, the amount of heavy metal(loid)s absorbed on to the soil particle is also low compared with the amount of heavy metal(loid)s in soil solution. Table 8 showed the regression equation between K_d of As according to extracting solution and soil chemical properties in order to identify the factors affecting partition coefficients with different extractants. Deionized water extraction was negatively correlated with exchangeable K ($r=0.53^{**}$) and positively correlated with exchangeable Mg ($r=0.64^{**}$). 0.01 M $Ca(NO_3)_2$ extraction was negatively correlated with exchangeable K ($r=0.76^{***}$) and SOM ($r=0.72^{***}$) and positively correlated with exchangeable Na ($r=0.80^{***}$). 1 M HCl extraction was negatively correlated with SOM ($r=0.72^{***}$) and positively correlated with exchangeable Na ($r=0.78^{***}$).

Therefore, exchangeable K, exchangeable Na and SOM were the major factors affecting the partition coefficients of various extractants similar to the observation made for BCF. Yang et al. (2012) noticed that Kd values varied widely in As-contaminated paddy soils and correlated well with soil pH, SOM and total As. In another study, Fu et al. (2011) reported that grain As concentrations correlated significantly to soil As speciation, SOM and soil P contents.

Table 9 shows the regression equations between As contents in rice, and extracting solutions and soil chemical properties to find the factors affecting As concentration in rice. The As content extracted by single extraction procedures was correlated in the order of deionized water > 0.01 M $Ca(NO_3)_2$ > 0.43 M CH_3COOH > 0.1 M HCl > 0.5 M KH_2PO_4 > 1 M NH_4NO_3 > 0.2 M $C_6H_8O_7$ > 0.43 M HNO_3 > 1 M HCl > Aqua regia. It means that the As uptake to rice was more effective with bioavailable form of As than total As in soil. Among the single extractants, deionized water and 0.01 M $Ca(NO_3)_2$ highly correlated with As concentration in rice and therefore, be a possible extractant to measure the bioavailability of As in soils. By comparing the total amount of rice and extracting solution content, and the relationship between the soil chemical properties, deionized water, 0.01 M $Ca(NO_3)_2$, 0.1 M HCl, 0.2 M $C_6H_8O_7$, 0.43 M CH_3COOH, 0.5 M KH_2PO_4, and 1 M NH_4NO_3 solutions are independent of the substitution and was found to be affected by the exchangeable cation, respectively. Shin (2003) also reported that exchangeable ion, which was

Table 9. Regression equation between arsenic contents in rice, and extractants and soil chemical properties.

Regression equation	R	*P* value
Deionized water		
Log (Total As in rice) = -0.859+0.163 Log (DW-As)+0.309 Log (K)	0.49*	<0.023
Log (Total As in rice) = -0.759+0.197 Log (DW-As)+0.307 Log (K) +0.139 Log (Na)	0.51*	<0.046
0.01M $Ca(NO_3)_2$		
Log (Total As in rice) = -0.323+0.295 Log (0.01M $Ca(NO_3)_2$-As) +0.278 Log (Na)	0.49*	<0.031
Log (Total As in rice) = -0.411+0.227 Log (0.01M $Ca(NO_3)_2$-As)+0.251 Log (Na)+0.296 Log (K)	0.53*	<0.021
0.1M HCl		
Log (Total As in rice) = -0.944+0.115 Log (0.1M HCl-As)+0.366 Log (K)	0.48*	<0.040
Log (Total As in rice) = -0.810+Log (0.1M HCl-As)+0.345 Log (K) +0.220 Log (Na)	0.51*	<0.029
0.2M $C_6H_8O_7$		
Log (Total As in rice) = -0.998+0.088 Log (0.2M $C_6H_8O_7$-As)+0.416 Log (K)	0.44*	<0.048
0.43M CH_3COOH		
Log (Total As in rice) = -0.928+0.122 Log (0.43M CH_3COOH-As)+0.358 Log (K)	0.47*	<0.030
Log (Total As in rice) = -0.815+0.169 Log (0.43M CH_3COOH-As)+0.348 Log (K)+0.177 Log (Na)	0.50*	<0.049
0.5M KH_2PO_4		
Log (Total As in rice) = -1.058+0.164 Log (0.5M KH_2PO_4-As)+0.408 Log (K)	0.48*	<0.026
Log (Total As in rice) = -0.993+0.218 Log (0.5M KH_2PO_4-As)+0.422 Log (K)+0.169 Log (Na)	0.51*	<0.046
1M NH_4NO_3		
Log (Total As in rice) = -0.787+0.102 Log (1M NH_4NO_3-As)+0.401 Log (K)	0.46*	<0.040

*(P<0.05)

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the absorbed form on soil particle and easily exchangeable with other ions, desorbed or changed to ion form in soil solution by pH or the change of surface charge on soil particle. Therefore, exchangeable ion would be a major factor affecting the uptake of heavy metal(loid)s to crop. In conclusion, single extraction using 0.01 M $Ca(NO_3)_2$ was shown to be effective for predicting As bioavailability in soil with higher correlation between As in rice and the extractant.

Conclusions

Development of an appropriate method to determine bio-available As in soil is vital for management of the metal contaminated agricultural soils as As uptake is governed by bioavailable As in soil rather than total As contents. The current study examined nine different single-chemical-extractant to select the most reliable method for determination of available As concentration in the soils through regression analysis between As concentrations measured by each extractant and those in rice. Correlation between soil extractants and As content in rice was in the order of: deionized water > 0.01 M $Ca(NO_3)_2 > 0.43$ M $CH_3COOH > 0.1$ M $HCl > 0.5$ M KH_2PO_4 > 1 M $NH_4NO_3 > 0.2$ M $C_6H_8O_7 > 0.43$ M $HNO_3 > 1$ M $HCl >$ Aqua regia. This result implied that 0.01 M $Ca(NO_3)_2$ single extraction can be a reliable candidate method for determining As bioavailability in the soils.

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