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# Alteration of Fractionation and Bioavailability of Arsenic (As) in Paddy Soil under Transition from Aerobic to Anaerobic Conditions

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## ABSTRACT

The impact of the change from aerobic to anaerobic immersed soil conditions on arsenic (As) fractionation (Tessier's method) and its bioavailability (ethylene diamine tetraacetic acid extractable) were assessed. As-contaminated paddy soils were tested by laboratory simulation experiments. The samples were aerobic, with 35-49 mg/kg of As at low bioavailability (<2%). Most As was distributed in the stable fraction (77%), followed by As bound to ferric and manganese oxide (17%) and organic compounds (5%), while the mobile fraction (exchangeable and mildly acid-soluble) was limited (1%). After one month under anaerobic simulation, redox potential reduced to less than zero (-32 to -124 mV). The stable fraction of As decreased (-17%), while the mobile fraction increased (+16%) and As bioavailability also increased (+26% total As). Increase in the As mobile fraction was associated with freshly precipitated compounds. The As content in the soil altered from a stable fraction to an available fraction when confined in an anaerobic environment for a long period. Results indicated that agricultural methods which promoted anaerobic conditions in As-contaminated soil should be avoided.

## 1. INTRODUCTION

Arsenic (As) is a highly toxic and carcinogenic metalloid that occurs naturally as a component of several minerals in the Earth's crust. The geochemical map published by Thailand's Department of Mineral Resources shows that As is found at >0.5 mg/kg in rocks covering more than one-third of the land surface area. The background concentration (95th percentile) of As in soil in Thailand was determined at <26 mg/kg by the Department of Land Development (2015).

In Khao Luang, a sub-district of Loei Province in Northeastern Thailand, 70% of the land area is used for agriculture. The main crops are rice, corn, sugar cane, soybean and para rubber trees. The As background geochemical value in this province is higher than in the surrounding areas. Comprehensive measurements around Khao Luang determined As concentrations in the soil ranging between 2 and 95 mg/kg (Department of Primary Industries and Mines, 2012). Consumption of food contaminated with As via soil-plant transfer is considered to be the major pathway of human exposure to toxic elements (Foucault et al., 2013), suggesting that ingesting agricultural products from this area is risky. Arsenic that can be readily transported from soil to agricultural plants is known as bioavailable As. The bioavailability of As in soil depends on how its chemical speciation and fractionation are altered by several dynamic biogeological soil processes (Khalid et al., 2017). Hartley and Dickinson (2010) demonstrated that the mobility of As in sediments was higher when the redox potential (Eh) was less than 100 mV. Rice farming has the potential to turn soil conditions anaerobic due to waterlogging and microbial activity. The Eh of paddy soil can be as low as -200 mV (Yu et al., 2016), thereby promoting the mobility of As and increasing its bioavailability. Water management in paddy fields reduced the Eh of paddy soil, resulting in higher As uptake in the rice (Zia et al., 2017; Cao et al., 2020). Wang et al. (2015) and Liu et al. (2015) demonstrated that the bioavailable fraction of As was not associated

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with ferric hydrous oxide or As-bearing minerals, while Yu et al. (2016) demonstrated that reductive release of As was related to the As fraction bound to iron compounds in the soil and linked to iron redox cycling induced by wet/dry paddy cycles.

Sequential extraction is a method that can be applied to indicate stability or mobility of As in the soil, and classifying the fractionation of As would help to understand the mechanisms of its behavior. Thus, our research interest involved investigating the changes when paddy soil ambient condition was changed from an aerobic to an anaerobic environment. Anaerobic conditions were induced in the laboratory using As-containing paddy soil as the sample. Soil properties, As fractionation and its bioavailability were quantified to assess the consequences of lowering soil Eh in the context of As paddy soil mobility. Our results will improve the current understanding of As movement in soil under strong anaerobic environmental conditions.

## 2. METHODOLOGY

### 2.1 Sample collection and preparation

The study area is located in Khao Luang sub-district, Loei Province, Thailand. The soil in this area is classified as Loei-soil series that are formed from residuum and local granite colluvium. The soil is clay or clay loam with a dark reddish-brown color. Field pH of the topsoil of this soil series was 6.0 (Malairotsiri et al., 2004). Four composite soil samples were collected from four paddies (A, B, C, and D) in the Nam-Huai Canal area during July 2017. In each paddy, four topsoil samples (0-20 cm) were collected using a shovel and kept in Ziplock bags in cool conditions before delivery to the laboratory. The soil samples were separated into two parts. One part was dried at 40°C and kept as a dry sample in a desiccator. Dry samples were used to analyze organic matter (OM) and total concentration of As and Fe. The other part of the composite sample was kept in a freezer as a field moist sample. Field moist samples were used to analyze soil pH, redox potential (Eh), acid volatile sulfide (AVS), fractionation and bioavailability of As to minimize the phase transition of metals in the sample (Qi et al., 2014).

### 2.2 Experiment

The soil samples were analyzed in a laboratory for pH, Eh, OM, AVS, total As, and Fe, fractionation of As and bioavailable As before undergoing a simulation experiment. Two simulation reactors were used for each paddy. The experiment was designed to observe changes in As fractionation and bioavailability, assuming that the paddy soil was in an immersed and strongly-reduced condition for one month. The goal of this simulation was to measure the negative Eh value and AVS that developed in a highly-reduced paddy soil environment (Yu et al., 2016).

The anaerobic simulation was conducted in batch mode. A 500-mL amber glass bottle was used as the reactor and filled with a 300 g dry soil sample from each paddy. Then, 350 mL of Nutrient Broth (MP Biomedicals) with solution strength equivalent to 5,000 mg/L of COD was applied to induce biogeochemical activity. An aliquot of 1.329 g of sodium sulfate ($Na_2SO_4$) (99%, Merck) was added as the seed for sulfide development, equivalent to 1,000 mg/kg of sulfide as the range of AVS in natural anaerobic soil (30-1,600 mg/kg, Essington, 2004; Kabata-Pendias, 2001). Each reactor bottle was tightly capped and kept in the dark for one month at room temperature.

### 2.3 Sample analysis

Total As and Fe, pH, Eh, AVS and OM were quantified. Tessier's sequential extraction method (Tessier et al., 1979) was applied to observe the fractionation of As in the soil. EDTA (ethylene diamine tetraacetic acid, 100%, Carlo Erba) extraction was used to determine the bioavailability of As in the soil as this expressed a good relationship to As in plants (Gregori et al., 2004). All analyses and measurements were performed in triplicate.

Details of the analytical methods are described in the Supplementary Material. Briefly, pH and Eh of the soil samples were determined by soaking a pH and ORP probe in the soil suspension (soil:water, 1:1 w/v) (U.S. EPA 9045D for pH and ASTM G200-09 for ORP). OM was quantified by the Walkley-Black modified acid-dichromate digestion-$FeSO_4$ titration method. The AVS in the sample was quantified by a method modified from van Griethuysen et al. (2002). Microwave digestion was applied to prepare the sample for total concentration measurement of As and Fe, while inductively coupled plasma optical emission spectroscopy was used to quantify As and Fe. The recovery of As was tested using European Reference Material (ERM-CC141), ranging from 91% to 103%.

The Tessier extraction procedure was applied for sequential extraction because this method interrelated well with the environmental conditions. Soil digestion using nitric acid and hydrochloric acid was applied for the final extraction step, and extractant from each step was measured for As. Following this approach, the As in the soil was divided into five fractions as exchangeable fraction (F1), mild acid-soluble fraction (F2), reducible fraction (F3), oxidizable fraction (F4) and residual fraction (F5). Pseudo-total concentrations of As were evaluated as the sum of every fraction, with recovery checked against total concentration and ranging from 93% to 130%.

### 2.4 Data analysis

Statistical analyses were conducted using the SPSS program (ver. 22). The t-test was applied to examine differences between As fractionation before and after anaerobic simulation, and p-value 0.05 was used to determine significance.

## 3. RESULTS

### 3.1 Characteristics of paddy soil samples

The collected topsoil samples comprised brown clay loam with a natural soil smell. Soil characteristics are shown in Table 1. Sample pH values were similar to area background values and classified as fertile paddy soil (Rice Research Institute, 2004) by their OM content. Eh values of the soil samples were greater than +400 mV, indicating aerobic condition that oxygen was used as a terminal electron acceptor in biological transformation (Mitsch and Gosselink, 2015). AVS content was not detected (<100 mg/kg) consistent with the high Eh values.

Arsenic concentrations in the soil samples varied from 35 to 49 mg/kg, concurring with previous reports (Department of Primary Industries and Mines, 2012), while bioavailable As ranged 0.66-0.79 mg/kg (1.6-2.1% of total As) (Figure 1). The Tessier fractionation of As in soil samples is shown in Figure 2. Fractionation of As in all initial paddy soils was similar and mainly distributed in F5 (77±5%), followed by F3 (17±5%), F4 (5.0±1.3%), F1 (0.050±0.048%) and F2 (0.56±0.62%).

### 3.2 Characteristics of paddy soil samples after anaerobic simulation

After one month of simulation, soil conditions in each reactor changed as expected (Table 1). The Eh values of the systems decreased to less than zero and AVS developed as the goal of this simulation. The pH values changed from acid to neutral, concurring with other research studies (Sahrawat, 2015; Yamaguchi et al., 2011). The OM content did not change significantly (2.21-2.36%).

**Table 1.** Characteristics of paddy soil samples before and after anaerobic simulation

| Initial paddy soil | | | | |
|---|---|---|---|---|
| Characteristic | A | B | C | D |
| Total As (mg/kg) | 40.95±0.61 | 36.59±2.75 | 48.65±0.47 | 35.14±1.46 |
| Total Fe (g/kg) | 33.13±1.56 | 27.20±2.07 | 29.56±1.32 | 22.94±1.95 |
| Eh (mV) | 614±9 | 560±2 | 676±9 | 595±13 |
| pH | 5.28±0.01 | 6.00±0.02 | 5.59±0.06 | 5.93±0.16 |
| AVS (mg/kg) | nd | nd | nd | nd |
| OM (%) | 2.60±0.16 | 2.07±0.12 | 2.18±0.08 | 2.31±0.18 |
| Bioavailable As (mg/kg) | 0.79±0.02 | 0.66±0.01 | 0.76±0.01 | 0.73±0.03 |
| Paddy soil after anaerobic simulation | | | | |
| Characteristic | A | B | C | D |
| Total As (mg/kg) | 39.49±3.14 | 33.19±1.90 | 40.42±5.73 | 36.66±1.45 |
| Total Fe (g/kg) | 33.70±2.97 | 26.05±1.94 | 30.40±2.12 | 22.81±2.68 |
| Eh (mV) | -124±9 | -95±12 | -32±14 | -69±32 |
| pH | 7.28±0.01 | 7.37±0.05 | 7.55±0.02 | 7.34±0.02 |
| AVS (mg/kg) | 559±181 | 662±268 | 1,136±170 | 962±184 |
| OM (%) | 2.29±0.04 | 2.21±0.14 | 2.22±0.10 | 2.36±0.04 |
| Bioavailable As (mg/kg) | 11.83±2.85 | 9.90±1.55 | 11.24±1.38 | 11.47±1.34 |

Note: nd=not detected. Limit of quantification for AVS was 100 mg/kg.

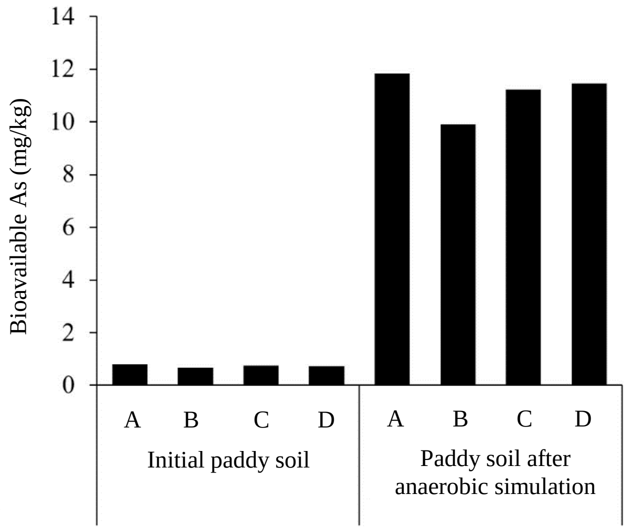

**Figure 1.** Bioavailability of Arsenic in paddy soil samples before and after anaerobic simulation

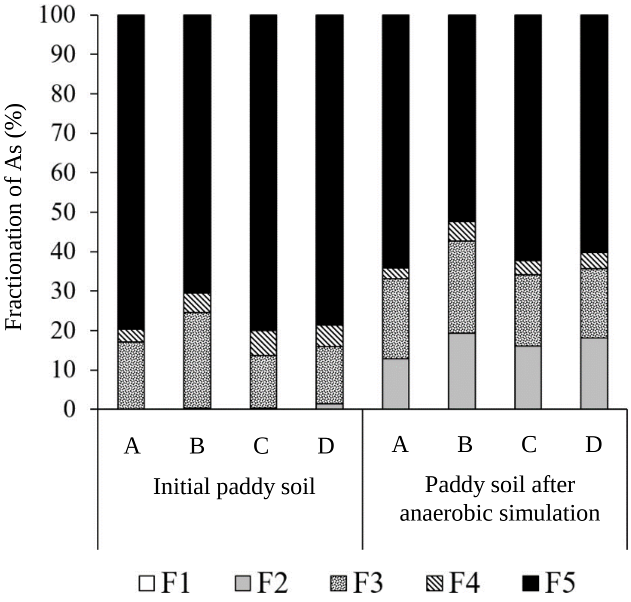

**Figure 2.** Fractionation of Arsenic in paddy soil samples before and after anaerobic simulation

Concentrations of As and Fe in the soil before and after the simulation were similar, while As in the supernatant was not measurable (<0.005 mg/L). Bioavailable As of the anaerobic paddy soil samples increased markedly from 0.66-0.79 mg/kg to 9.9-11.8 mg/kg (23-32% of total As) (Figure 1). The F2 and F5 values of As in paddy soil altered remarkably, while F1, F3, and F4 did not change significantly (p>0.05). The As content in F5 decreased from 77±5% to 60±5%, whereas As in F2 increased from 0.56±0.62% to 17±3% (Figure 2).

## 4. DISCUSSION

In the soil samples, As was mainly distributed in F5, indicating the geogenic origin of As in this area (Fu et al., 2011; Wang et al., 2015), which was considered stable. Due to the As in mobile forms, As bound to amorphous oxides and bound to organic matters were eliminated during the 1st to 4th extraction step. The As content in F5 of Tessier's fractionation likely comprised of As-sulfide minerals and well-crystallized forms of Fe and As. Drahota and Filippi (2009) and Kim et al. (2014) found that the major As-sulfide minerals in the mining area were arsenopyrite, orpiment and arsenic-bearing pyrite, while their main weathered minerals were well-crystalized Fe arsenates (scorodite) and As associated with well-crystalized Fe oxides generally found in naturally As-rich soil. In this study, based on the soil sample characteristics, we presumed that As in F5 was likely predominated by well-crystalized weathered minerals.

Similar to other studies, despite the stable form of As in the soil, As bound to amorphous ferric oxide formed the major fraction of As (Baig et al., 2009; Farooq et al., 2010; Devesa-Rey et al., 2008; Hsu et al., 2012; Kim et al., 2014; Wang et al., 2015). Natural soil generally contains high amounts of ferric oxide that can adsorb various trace elements by various mechanisms (Hooda, 2010). The distribution of As in our paddy soil samples conformed to trends that As in F3 was the second major fraction.

The As in F4 was released from oxidizable components in the soil as organic compounds and some sulfide compounds. However, the AVS in this soil sample was not detectable. As content in F4 was believed to be bound to organic compounds. Similar to other studies, the As content in F4 was a minor fraction (Baig et al., 2009; Farooq et al., 2010; Fu et al., 2011). The As in F1 was the exchangeable fraction, while F2 contained As that was readily releasable by mild acid conditions, such as As bound to carbonate compounds or fresh precipitates. Both these fractions contributed less than 1% of the total As in the soil samples.

After one month of anaerobic induction by microbial activity, only As in F5 (stable fraction) and F2 (mild acid-soluble fraction) changed. A 17% decrease of As in F5 was observed, responding to a 16% increase of As in F2. This change of As fractionation indicated the release and resorption of As among the soil materials. However, we did not anticipate any change of As in F5 but expected F3 (bound to amorphous Fe oxide) to decrease via the iron reduction process.

Some toxic heavy metals become non-mobile under anaerobic environmental conditions via sulfidation (Hartley and Dickinson, 2010), but As can be altered to mobile forms (Kumarathilaka et al., 2018) or released to an aqueous solution (Hartley and Dickinson, 2010; Xue et al., 2020; Zhang et al., 2019). The released As content correlated well with released iron as ferrous (Xue et al., 2020). The iron redox cycle is governed by wet/dry cycles in paddy fields. The general explanation is that the source of released As is associated with ferric hydrous oxides in the soil (Hashimoto and Kanke, 2018; Rinklebe et al., 2016; Xue et al., 2020; Yamaguchi et al., 2011**)**. Amorphous ferric hydrous oxide plays a much larger role in this dynamic environment than well-crystallized forms, with greater sensitivity toward released/resorbed ions in soil-water systems due to greater surface area and surface activity (Yang et al., 2020). It is reductively dissolute during wet periods and oxidatively re-formed to be the amorphous ferric hydrous oxide state during dry periods (Winkler et al., 2018). Well-crystallized forms of ferric hydrous oxide are less sensitive to both redox reactions and release/resorption. Once dissolute, it cannot easily reform under general paddy conditions (Yang et al., 2020). Therefore, major As contents released and resorbed normally relate to amorphous ferric hydrous oxides. Nevertheless, only a small fraction of ferric hydrous oxide in the soil (e.g., surface of materials) contributes to this redox cycle, while most remains to resorb released ions. Fractionation of metals (and metalloids) associated with ferric hydrous oxide decreases, remains unchanged or increases when immersed in anaerobic conditions, depending on complex interactions between the many ions in the soil (Damrongsiri, 2018). This might explain why the F3 As content did not change significantly.

The stable form of As is less sensitive to environmental degradation. A recent study on the effects of farming on As-contaminated land by Yang et al. (2020) pointed out that scorodite can be reductively dissolved in the paddy environment. Arsenopyrites, which are stable, were can be significantly reduced and dissolved under anaerobic conditions (Kawa et al., 2019). Therefore, we presumed that both well-crystallized forms of As and Fe and arsenic sulfide minerals could be reductively dissolved, possibly explaining why the As content in F5 decreased during anaerobic incubation.

Once released, As is available for further reaction in anaerobic soil-water systems and re-associates with other soil materials. These resorbed materials and co-precipitates of As in soil initially appear as an early fraction, having loosely bound (Lu et al., 2019; Zhang et al., 2019). In our Eh-pH anaerobic system, dissolved As was near the theoretical transition line of $H_2AsO_4^-$, $HAsO_4^{2-}$, $As_2O_3$, and $As_2S_3$ in As-S-O-H systems (Brookins, 1988) and trending to become $HAsO_4^{2-}$ (As(V)); however, the speciation of As in soil is far more complex. In soil under anaerobic conditions, As(V) is released when As-bearing ferric hydrous oxide is dissolute. Some released As(V) is reduced to As(III) by microorganisms that can reduce both ferric materials and As(V) (Yu et al., 2016). Therefore, in an anaerobic paddy soil system, a large amount of As is in form of As(III) (Yamaguchi et al., 2011; Xu et al., 2016).

Under an anaerobic water-soil system, As(V) precipitates as ferric arsenate and ferrous arsenate (Fan et al., 2014). Beside, both As(V) and As(III) sorb to remaining ferric hydrous oxide and many freshly formed solids (Coker et al., 2006; Fan et al., 2014; Khalid et al., 2017). As could adsorbed to siderite (ferrous carbonate, $FeCO_3$) (Guo et al., 2013), troilite (ferrous sulfide, FeS) and pyrite (ferrous disulfide, $FeS_2$) (Bostick and Fendorf, 2003). In our anaerobic induction experiment, released As was reformed into F2 that was likely combination of these reviewed forms.

The very low bioavailability of As in our collected soil samples may relate to the very low distribution of As in F1 and F2. After one month of anaerobic induction, only As in F2 increased, responding directly to the increased bioavailability of As in the soil. In general, the bioavailability of elements relates to their mobile fraction (e.g., F1 and F2 of Tessier's fractionation). Liu et al. (2015) investigated the relationship between As in rice and the fractionation of As in soil. They found a positive correlation between As in rice and F1 and F2 of Wenzel's fractionation (non-specifically sorbed fraction and specifically sorbed fraction) of As in soil but a negative correlation with As bound to amorphous and crystallized iron oxide fraction and stable fraction. A similar result was shown by Wang et al. (2015). They used $NaHCO_3$ extractable As to indicate As bioavailability and investigated its relationship with the fractionation of As in soil, while Yu et al. (2017) demonstrated that the application of iron compounds in soil significantly decreased As accumulation in rice. These studies confirmed that, under anaerobic condition, bioavailable As in soil was freshly precipitated As compounds, As re-adsorbed onto soil materials and As associated with freshly formed solids.

## 5. CONCLUSION

Samples of As-contaminated soil were collected and used in a simulation experiment. The soil samples exhibited aerobic conditions with 35-49 mg/kg As. The As was mainly distributed as a stable fraction, with a limited mobile fraction (<1%). The bioavailability of As was also very low (<2%). After one month of anaerobic incubation, the stable fraction of As (F5) in soil decreased significantly and reformed as As in the mobile fraction (F2), corresponding to an increase in bioavailability. Results indicated that the stable form of As in soil was released under an anaerobic environment. The released As then precipitated, bounded to soil materials or bounded to freshly-precipitated compounds. Results indicated that the bioavailability of As in paddy soil increased when the soil was subjected to anaerobic conditions for a long period. Therefore, agricultural activity that causes anaerobic induction in As-contaminated soil should be avoided. Cultivation of other crops without a waterlogging period or non-food plants should be considered.

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