A CONTINUOUS-FLOW SEQUENTIAL EXTRACTION WITH ETAAS DETECTION FOR CHEMICAL SPECIATION OF ARSENIC IN SOIL AND SEDIMENT

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The total arsenic (As) content in a heterogeneous solid material such as soil does not provide adequate information about their potential bioavailability or toxicity. Sequential extraction followed by determination of concentration in each fraction can help identify the proportion of the element in different phases of the material. As fractionation can be used for the prediction of the As bioavailability or toxicity. Sequential extraction using a batch procedure, which is currently widely used is tedious, time consuming and can be erroneous owing to a lengthy process involved.

This work developed a continuous flow sequential extraction system to fractionate arsenic in soil samples into 5 fractions of varying mobility. The extractants used were 1) water 2) 0.5 M $NaHCO_3$ 3) 0.1 M NaOH and 4) 1.0 M HCl. Electrothermal atomic absorption spectrometry was used to determine As in all extracts and residues. The As extracted in water and $NaHCO_3$ can be considered as mobile and highly bioavailable. Arsenic in NaOH fraction is likely to be associated with amorphous Fe and Al minerals in soils and As in the HCl fraction is associated with calcium carbonate. The final fraction is difficult to dissolve and is considered non-bioavailable.

The optimum ETAAS conditions for determination of As in each extract and residues were investigated. The reliability of the method was checked by analysis of the soil and sediment certified reference materials (CRM).

The proposed flow system was assessed by analysing three soil and sediment CRMs (SRM 2704, SRM 2710, and SRM 2711). The summation of concentration of all fractions was found to agree with the certified value. The results of fraction distribution obtained were compared with those of the batch method. The method was also used to analyse the soil samples from Ronphiboon, Thailand and cattle dip sites, Australia. Extractograms obtained were used to evaluate the association of As, Fe, Al and Ca in those samples.

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นวลนาฏ จันทร์เมฆา: การสกัดลำดับขั้นแบบต่อเนื่องและวิเคราะห์ด้วยอะตอมมิคแอบซอร์พชันสเปกโตรเมตรีเพื่อหาสปีชีส์ของสารหนูในดินและดินตะกอน (A CONTINUOUS-FLOW SEQUENTIAL EXTRACTION WITH ETAAS DETECTION FOR CHEMICAL SPECIATION OF ARSENIC IN SOIL AND SEDIMENT) คณะกรรมการควบคุมวิทยานิพนธ์: ยุวดี เชี่ยววัฒนา, Ph.D., ดวงใจ นาคะปรีชา, Ph.D. 92 หน้า. ISBN 974-664-098-4

ค่าความเข้มข้นทั้งหมดของสารหนูในดินเพียงอย่างเดียวไม่สามารถบ่งชี้ถึงอันตรายหรือศักยภาพในการดูดซึมสารหนูของสิ่งมีชีวิต การสกัดลำดับขั้นและหาปริมาณสารหนูที่สกัดได้ด้วยสารสกัดต่างๆสามารถบอกถึงปริมาณสารหนูที่มีอยู่ในรูปฟอร์มต่างๆกันได้ ปริมาณของสารหนูแต่ละรูปฟอร์มที่ได้สามารถใช้ในการทำนายหรือบ่งชี้ถึงอันตรายหรือการดูดซึมสารหนูของสิ่งมีชีวิตได้ การสกัดลำดับขั้นด้วยระบบแบทช์ที่นิยมใช้กันอย่างกว้างขวางในปัจจุบันเป็นวิธีการที่ยุ่งยาก ใช้เวลานานและมีความผิดพลาดได้ง่ายเนื่องจากขั้นตอนในการวิเคราะห์ที่ยาวนาน

งานวิจัยนี้ใช้การสกัดลำดับขั้นแบบต่อเนื่องเพื่อแยกสารหนูในดินออกเป็น 5 ส่วน โดยใช้สารสกัดคือ 1)น้ำ 2)โซเดียมไบคาร์บอเนต 0.5 โมลาร์ 3)โซเดียมไฮดรอกไซด์ 0.1 โมลาร์ และ 4)กรดไฮโดรคลอริก 1.0 โมลาร์ สารหนูที่สกัดได้และสารหนูส่วนที่เหลือนำมาวิเคราะห์ด้วยอิเล็กโตรเทอร์มอลอะตอมมิคแอบซอร์พชั่นสเปกโตรเมตรี (ETAAS) ความเข้มข้นของสารหนูที่สกัดได้ในน้ำและโซเดียมไบคาร์บอเนตเป็นค่าที่บ่งชี้ ถึงปริมาณสารหนูที่มีความเป็นพิษต่อสิ่งแวดล้อมและถูกดูดซึมได้ง่าย สารหนูที่สกัดได้ในโซเดียมไฮดรอกไซด์เป็นสารหนูที่จับกับเหล็กและอลูมิเนียมออกไซด์ในดิน ส่วนสารหนูที่สกัดได้ในกรดไฮโดรคลอริกเป็นสารหนูที่จับกับแคลเซียมคาร์บอเนตในดิน ส่วนที่เหลือสุดท้ายเป็นสารหนูที่ยากต่อการสกัด สารหนูส่วนนี้จึงแทบจะไม่มีอันตรายต่อสิ่งแวดล้อม

งานนี้ได้ศึกษาสภาวะที่เหมาะสมที่สุดในการวิเคราะห์สารหนูที่สกัดได้และสารหนูส่วนที่เหลือจากการสกัดด้วย เครื่อง ETAAS และตรวจสอบความน่าเชื่อถือของวิธีนี้โดยการวิเคราะห์สารอ้างอิงมาตรฐานดินและดินตะกอน (SRM 2704, SRM 2710 และ SRM 2711)

การสกัดลำดับขั้นแบบต่อเนื่องที่พัฒนาขึ้น ได้ทำการตรวจสอบความถูกต้องของเทคนิคนี้โดยนำไปวิเคราะห์กับสารอ้างอิงมาตรฐานดินและดินตะกอน (CRMs) โดยเปรียบเทียบค่าความเข้มข้นรวมของสารหนูจากผลบวกของแต่ละส่วนกับค่ามาตรฐานอ้างอิงพบว่าค่าที่ได้สอดคล้องกัน และนำค่าความเข้มข้นของสารหนูในแต่ละส่วนเปรียบเทียบกับสารหนูที่สกัดได้จากระบบแบทช์ ท้ายสุดได้นำระบบนี้ไปใช้สกัดสารหนูในดินตัวอย่างจาก อ.ร่อนพิบูลย์ ประเทศไทย และ Cattle dip sites, ประเทศออสเตรเลีย Extractogram ที่ได้จากการสกัดสามารถนำมาใช้เพื่อบ่งบอกถึงลักษณะการจับตัวกันของธาตุ สารหนู เหล็ก อลูมิเนียมและแคลเซียมในดิน

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°C	Degree of Celsius
K	Kelvin
mL	Milliliter
µg	Microgram
mg	Milligram
kg	Kilogram
ppm	Part per million
ppb	Part per billion
concen	Concentration
v/v	Volume by volume
w/w	Weight by weight
hr	Hour
FAAS	Flame atomic absorption spectrometry
GFAAS	Graphite furnace atomic absorption spectrometry
SD	Standard deviation
RSD	Relative standard deviation

CHAPTER I

INTRODUCTION

1.1 Arsenic Chemistry in Soil

1.1.1 Source of arsenic in the environment

1.1.1.1 Natural occurrence

Rocks, soils and sediments

Arsenic is widely distributed in a large number of minerals. The highest mineral concentrations generally occur as arsenides of copper, lead, silver, or gold or as the sulfide. Major arsenic containing minerals are arsenopyrite (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3). The arsenic content of the earth's crust is 1.5-2 mg/kg; it ranks 20^{th} in abundance in relation to other elements [1]. Oxidized forms of arsenic are usually found in sedimentary deposits. The elemental oxidation state, though stable in reducing environments, is rarely found. Table 1.1 gives some ranges of the arsenic contents of crustal materials. Although the values shown are generally low, mineralized sulfidic ores may contain much higher concentrations of arsenic.

Table 1.1 Arsenic in crustal materials

Type	As concentration range (mg/kg)
Igneous rocks	
Ultrabasic	0.3-16
Basalts	0.06-113
Andesites	0.5-5.8
Granitic	0.2-13.8
Silicic, Volcanic	0.2-12.2
Sedimentary rocks	
Limestones	0.1-20
Sandstones	0.6-120
Shales and clay	0.3-490
Phosphorites	0.4-188

Uncontaminated soils were found to contain arsenic levels between 0.2 and 40 mg/kg, while arsenic contaminated soils contained up to 550 mg/kg.

The natural level of arsenic in sediments is usually below 10- mg/kg dry weight. Bottom sediments can become substantially contaminated by arsenic from man-made sources. Levels of up to 10000 mg/kg dry weight were found in bottom sediments near a copper smelter in Washington, USA [1].

Arsenic is also present in fossil fuels such as coal, petroleum, and oil shale. The mean arsenic content of U.S. bituminous coals is 150 mg/kg. The

arsenic concentrations in various Finnish peat bogs in the range of 16-340 mg/kg dry peat.

Air

Arsenic enters the atmosphere from natural sources that include volcanic activity, wind erosion, sea spray, forest fires, and low-temperature volatilization. Smelting operations and fossil fuel combustion contribute to anthropogenic sources of arsenic[2]. Arsenic speciation is also complicated by the fact that most of arsenic in the atmosphere is in the form of the particular matter. Less than 10% is present in the vapor phase or on the particles smaller than 0.2 μm. Airborne particulate matter has been shown to contain both inorganic and organic arsenic compounds. Crecelius [3] showed that the concentration of As in rain water was 0.6-1.6 ppb which obtained 35% of the inorganic As in rain water from an urban area was present as arsenite. A studied by Johnson [4]. Showed that methylarsines made up approximately 20% of the total arsenic in ambient air from rural and urban areas.

In unpolluted areas, arsenic concentrations in airborne particulate ranging from less than one to a few nanograms per cubic meter have been reported [2].

Water

The concentration of arsenic in fresh water shows considerable variation with the geological composition of the drainage area and the extent of

anthropogenic activities. The greater density of industrial activity appears to be reflected in slightly higher average arsenic concentration in residential areas. Oceanic constituents tend to be less variable than their freshwater counterparts and the surface water arsenic concentration ranges are subject to some seasonal variations due to biological uptake. These changes will be most important in highly productive coastal waters. Cycling models typically suggest that arsenic enter the ocean from the atmosphere and via rivers as dissolved species and as part of suspended particulate matter. These inputs are offset by removal to the atmosphere as sea salt spray and by precipitation/adsorption to the sediments. Arsenic occurs in both inorganic and organic forms in water. The main organic arsenic species, methylarsonic acid and dimethylarsinic acid, are generally present in smaller amounts than the inorganic forms, arsenite and arsenate. The oxidation state of arsenic in surface waters in various parts of the world remains largely unknown.

The arsenic contents of surface waters in unpolluted areas vary; typical values seem to be a few mg/L or less.

High levels of arsenic have been found in waters from areas of thermal activity. Thermal waters in New Zealand have been shown to contain up to 8.5 mg/L. Geothermal water in Japan contained arsenic levels of 1.8-6.4 mg/L and neighboring streams contained about 0.002 mg/L.

Biota

The concentration of arsenic species in marine and freshwater animal is considerably above background concentrations in surrounding water. The

presence of organoarsenicals in marine organisms is commonly assumed to be due to the accumulation of compounds that have been synthesized from arsenate at low trophic level. Some arsenic concentrations found in marine animals are given in Table 1.2. Living marine organisms have high concentrations of arsenic whereas freshwater fish in uncontaminated waters usually have concentrations of less than 2 μg/g.

Table 1.2 Organoarsenicals isolated from marine animals

Animal	Organ	As concen. range (μg/g)[a]
Shrimp	Meat	5.5-20.8
Lobster	Tail	5.2-39
Crab	Claw, canned, and meat	2.1-8.6
Clam	Gill, midgut gland, siphon, and soft tissue	1.2-5.6
Fish	Muscle	0.55-6
Gastropod	Muscle, midgut gland	3.1-340

[a] wet-weight

Arsenicals have been widely applied in agriculture, starting with the use of arsenite as a weed killer and soil sterilant. Plants grown on arsenic-contaminated soils may contain considerably higher levels, especially in the roots. The arsenic content of plants grown on soils that had never been treated with arsenic-containing pesticides varied from 0.01 to about 5-mg/kg dry weight. Some grasses growing on soils containing high levels of arsenic have been found to elevate arsenic contents.

In humans, arsenic is concentrated in the hair and nails with average concentration of 0.5 mg/kg for hair and the arsenic content in nails in non-exposed individual ranges from 0.5 – 1 mg/kg.

1.1.1.2 Industrial production and uses of arsenic

World production of arsenic in 1973 was about 50000 short tons. Sweden is the leading world supplier, followed by France, South-Western Africa, the Philippines and the USSR [5].

Arsenic trioxide (As_2O_3; synonyms, arsenic acid anhydride and white arsenic) constitutes 97% of arsenic produced that enters end-product manufacturing. Although many of the applications have been abandoned owing to its toxicity, some have continued into the twentieth century.

Agricultural Uses

In agriculture, arsenic is used in the manufacture of insecticides, herbicides, feed additives, wood preservatives and desiccants. The first use of arsenic as an insecticide was in the nineteenth century. Lead arsenate was first used in 1982, primarily as a stomach poison for chewing insects. However, although extensively used in the past, lead arsenate, together with calcium arsenate and Paris green (copper acetoarsenite), is seldom used nowadays.

Sodium arsenite has been used extensively since the 1930s as a livestock dip to control ticks, fleas and lice. Sodium arsenite also has been used

widely as a herbicide, particularly in controlling crabgrass. Newer arsenical herbicides have emerged in the last decade, particularly organic arsenical herbicides such as DSMA (disodium methanearsonate), MSMA (monosodium methanearsonate) and cacodylic acid [$(CH_3)_2AsO(OH)$]. DSMA and MSMA are used as selective herbicides for controlling certain weedy grasses in the turf and the cotton fields in the USA. Cacodylic acid is a contact herbicide that defoliates and desiccates a wide variety of plant species.

Arsenic acid (*m*-arsenic acid, $HAsO_3$; *o*-arsenic acid, $HAsO_4$) has been used as a desiccant for over 20 years. It is extensively used in cotton fields to deplete plants of moisture. The use of arsenic compounds to help preserve wood is still small compared with the use of creosote, petroleum and other preservatives, but chromated copper arsenate has been increasingly employed for this purpose.

Arsenic compounds such as arsanilic acid, 3-nitro-4-hydroxy-phenylarsonic acid, 4-nitro-phenylarsonic acid and 4-ureido-phenylarsonic acid (carbasone), have been used as feed additives since the mid-1940s. They are reported to improve weight, and control enteric diseases in some animals such as pigs and poultry.

Ceramics and Glass

Almost all glass has arsenic in formed of As_2O_3 and As_2O_5 [6] added (0.2-1%). It helps in the formation of the glass and acts as an agent for removing certain gases and oxidizing and reducing trace elements, and as a decolorant [7].

Chemicals

Inorganic arsenic compounds have been used in medicine for centuries. Organic arsenic compounds such as carbasone have been used in intestinal amoebiasis in man and are still being used as anti-parasitic medicines (and food additives) in veterinary medicine.

The arsenic-containing drugs salvarsan, discovered in 1909 by the chemotherapy pioneer Paul Ehrlich, was widely used to treat the organisms which caused amoebic dysentery, syphilis, yaws, relapsing fever, trypanosomiasis and trichomonal vaginitis. Even with the advent of newer miracle drugs, some inorganic and organic arsenicals still find specific applications in human and veterinary medicine.

Arsenic, even though a semi-metal-like element acts as an additive metal under some conditions. For instance, trace amounts of arsenic added to lead-base battery girds and cable sheathing increase their hardness, 0.5-2 %of arsenic added to lead improves the sphericity of lead shot and 3% arsenic added to lead-base bearing alloys improves their mechanical properties. It is also a constituent of gallium arsenide, GaAs, an important semiconductor that may increasingly replace silicon in electronic devices [5].

1.1.2 Chemical behavior of arsenic in soils

Chemical behavior of arsenic is largely similar to that of phosphorus in soils. Important factors effecting these interactions are soil solution chemistry, adsorption and desorption, solid phase formation, effect of redox condition, biological transformations, volatilization and cycling of arsenic in soils. The state of knowledge of these interactions is described in the first three states as follow;

1.1.2.1 Solution chemical of arsenic in soils

The water is trapped in the pores or interstitial sites of soil. It can be separated from the soil by several methods such as using devices to pressurize a diaphram in order to squeeze the soil against a 0.45-μm filter. The chemical extraction method for extracting the exchangeable fraction is usually performed in a neutral salt solution. It would isolate the interstitial water speciation equally well. However, leaching and rapid ion exchange phenomena are known to cause large composition changes.

Total arsenic contamination in soil solution is composed of both organic and inorganic arsenic chemical forms.

Organic complexes of arsenic in soil solution

Organic matter is a chemically reactive component of all soils. Organic molecules generally carry a net negative charge in soil solutions. Arsenic chemical forms in soil solutions are negatively charged oxyanions ($HAsO_4^{2-}$, $H_2AsO_4^-$, $H_2AsO_3^-$, and $HAsO_4^{2-}$). Because of similarity in the nature of charges on both organic molecules and arsenic chemical forms, arsenic has demonstrated a limited affinity for organic complexation in soil. Comparing the chemical behavior of arsenic with that of phosphate in soils, Johnson and Hiltbold [8] commented that one of the striking differences appears to be the inability of arsenic to form organic complexes. It is probable that, within a soil organism, arsenic may bind to organic molecules and be released to soil solution when its tissues are biodegraded. Arsenic has also been applied to several soils as organoarsenical biocides. Arsenic compounds are reduced and methylated species are:

- Methanearsenate [$CH_3AsO_3^{2-}$]
- Dimethylarsonate [$(CH_3)_2AsO_2^-$]
- Trimethylarsineoxide [$(CH_3)_3AsO$]
- Dimethylarsine [$(CH_3)_2AsH$]
- Trimethylarsine [$(CH_3)_3As$]

It has been shown that, in soil solutions, organoarsenicals ultimately transfer to methanearsonic acid. However, systematic field information on the occurrence and persistence of organic arsenic complexes in soil solutions is

limited. It is generally accepted that organoarsenicals complexes constitute a minor fraction of that total dissolved arsenic in soil solution and can be ignored.

Inorganic arsenic chemical forms in soil solution

In general, concentrations of total arsenic, even in the contaminated soil solutions, are expected to be in the range of μgL^{-1} ($\mu g/kg$). Exceptionally high arsenic concentrations may be found in very alkaline or reduced contaminated soils. Information on arsenic concentration in soil solutions is limited. In view of association of arsenic predominantly with the Al, Fe and Mn oxide phase, it might be expected that the interstitial water arsenic would represent only a minor part. This may be true in terms of absolute concentrations, but it is because of this concentration gradient that the pore water is a sensitive indicator of the chemical interactions between the water and the surrounding solid phase of the soil. Furthermore, solution in soil provides the main pathway for the diffusion of dissolved species. Thus it is the fraction most related to mobilization and biological availability [9,10].

Analytical technique to differentiate between As(III), As(V) or organoarsenic are well established. However, further differentiation of As chemical forms at low concentration have not been developed. To overcome the analytical deficiency, the thermodynamic modeling approach is often adopted to show distribution of As chemistry forms in soil solution.

1.1.2.2 Adsorption of arsenic in soil

Knowledge of reaction kinetics at the solid-liquid interface is extremely important in understanding the adsorption/desorption process. Several investigators have attempted to use chemical reaction rate laws and orders to explain the adsorption/desorption of anions at solution/solid interfaces [11]. Adsorption reactions are most confusing and complex and mainly occur on the surfaces of soil colloidal particles. These particles can be clay, oxides or hydroxides surfaces of Al, Fe and Mn, calcium carbonates, and/or organic matter.

Clays

Generally, clays particles are negatively charged silicates minerals and, therefore, preferably adsorb positively charged ions, not As oxyanions, from soil solutions. However, it has been reported that sorption of As oxyanions from soil solution occurs by chemisorption or ligand exchange on clay surfaces, mainly by replacing or competing with phosphate. pH dependent positive charge on clay surfaces may be responsible for the above association between As and clay. In acidic soils where positively charged clay particle (Figure 1.1) exist, As adsorption could be observed [12].

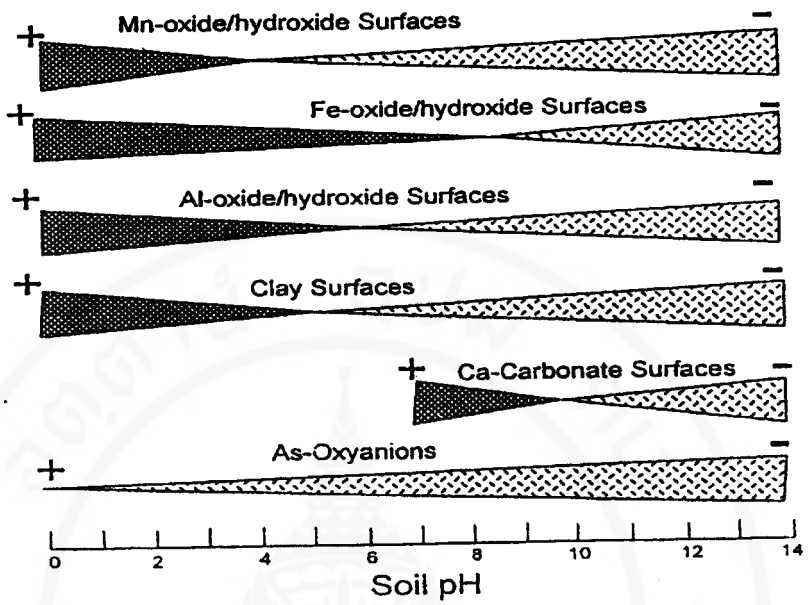


Figure 1.1 Generalized charge distribution on soil colloids [9].

Iron oxide/hydroxide

Iron oxide surfaces are effectively involved in As adsorption in soils. It has been suggested that Fe-oxide/hydroxide surfaces in soil may develop electrical charge due to hydration, specific adsorption, changes in cation coordination, isomorphous replacement, crystallinity, etc. Therefore, adsorption of As on the Fe oxides and hydroxides surfaces can largely be explained on the basis of the type of charges. Hydrous Fe oxides/hydroxides have a zero charge at pH ranging from 7 to 10, with a mean value around 8.5 (Figure 1.1), higher pH favors net negative charge and lower pH enhances net positive charge on these surfaces. Soils are generally below the threshold pH of 8.6 and most of Fe oxide surfaces are expected to be positively charged; suitable for the adsorption of oxyanions of As from the soil solution [13-22].

Al oxide/hydroxide

Adsorption of As on Al oxide surfaces can partially be explained on the basis of charge distribution. The zero charge potential for a variety of Al oxides occurs around pH 6 (Figure 1.1). The Al oxide surfaces carry a net positive charge in acidic soil (pH<6) and net negative charge in near neutral and alkaline soils (pH>6). It may be stated here that, like Fe oxide surface, As may undergo specific adsorption or chemisorption processes on Al oxide surfaces. From the foregoing, it appears that Al oxide surfaces may play a role in As adsorption/chemisorption in acidic soils but a limited role in the near neutral or alkaline soils [17, 21].

Manganese oxide/hydroxide

Manganese oxide/hydroxide surfaces has a zero charge around a pH of about 2 and therefore, carries a net negative surface charge in the soils with pH greater than 5. Because of net negative charge, Mn oxide surfaces are expected to play a limited role in adsorption of As in soils with pH>4 (Figure 1.1). However, ligand exchange or chemisorption of As oxyanions may occur on Mn oxide surfaces.

Carbonate minerals

Carbonate minerals are unstable in acidic soils, but may play a major role in the alkaline soils, particularly in calcareous soils. Woolson et al [32]

conducted an extensive survey of As forms in As-contaminates soils and found that, after the exhaustion of reactive Fe, the dominant forms were controlled by the reactive levels of Ca in calcareous soils. The maximum of As adsorb on calcite around pH 10. Ligand exchange or chemisorption is believed to be responsible for As adsorption on carbonate surfaces in soils. The isoelectric point of Ca carbonates vary between 7 and 10 and depends on mineral type, state of crystallinity, hydration, impurities, soil pH, etc. This mean that carbonate surfaces would have positive charge in soils with pH<9 (Figure 1.1) and thus play an important role in As adsorption in alkaline soils [23].

Organic matter

Biogenic particles, i.e., compounds of biological origin such as organic matter, tend to bind most metals and more strongly than do particles of lithogenic (of rock, etc.) or authigenic (of chemical) origin. Because of a similar type of overall electrical charges on the soils organic matter and As oxyanions, a limited interaction between these two chemical entities is expected.

In addition to soil surfaces, several other factors may affect arsenate adsorption. It appears that the presence of phosphate ions adversely affect arsenate adsorption in soils, especially on clay particles. Soil pH is an important factor in determining arsenic adsorption capacity of soils. Arsenic adsorption increases with a decrease in soil pH and is faster at lower pH [15].

1.1.2.3 Formation of arsenic solid phases in soils

Direct precipitation of As solid phase may not occur except in the As contaminated soils. However, secondary precipitation of As compounds may occur on soil colloid surfaces subsequent to its adsorption. In the secondary precipitation, the first step would probably be the adsorption of dissolved As species on to soil colloidal surfaces as shown in Figure 1.2.

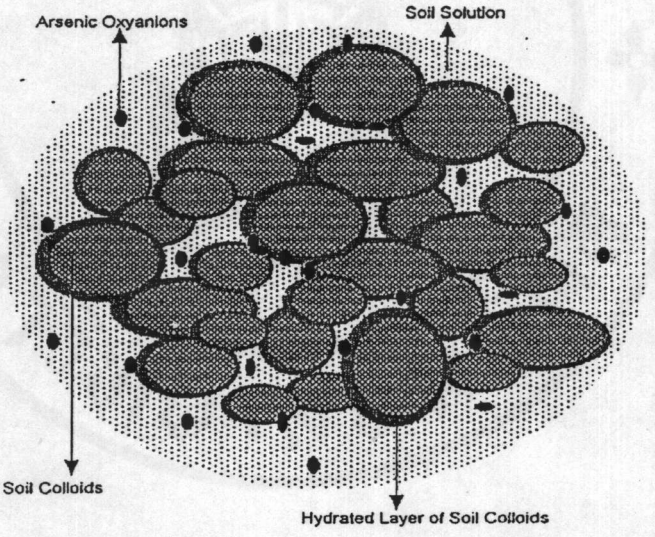


Figure 1.2 Chemisorption of As oxyanions on soil colloids [9].

The adsorbed As ions would gradually and continuously move inside the hydrated layer of colloids. As a result of translocation, As concentrations inside the hydrated mineral layer, with the passage of time may reach to a level to precipitate as an As solid phase. The sorption of As may combine with Fe and Al hydroxides to form new compounds.

Sadiq [9] calculated thermodynamic solubility isotherms of arsenate compounds and plotted (Figure 1.3). In acidic soils, Fe and Al arsenate forms are present in contaminated soils whereas its arsenates are more soluble in alkaline soils. The Ca arsenate behaves differently Fe and Al arsenates. Furthermore, As can form arsenate compounds with Mn in acidic soils. It also showed that $Ca_3(AsO_4)_2$ can dissolve at low soil pH. As a result, As concentration in soil solution from $Ca_3(AsO_4)_2$ dissociation will increase whereas As concentrations from Fe and Al arsenates will decrease.

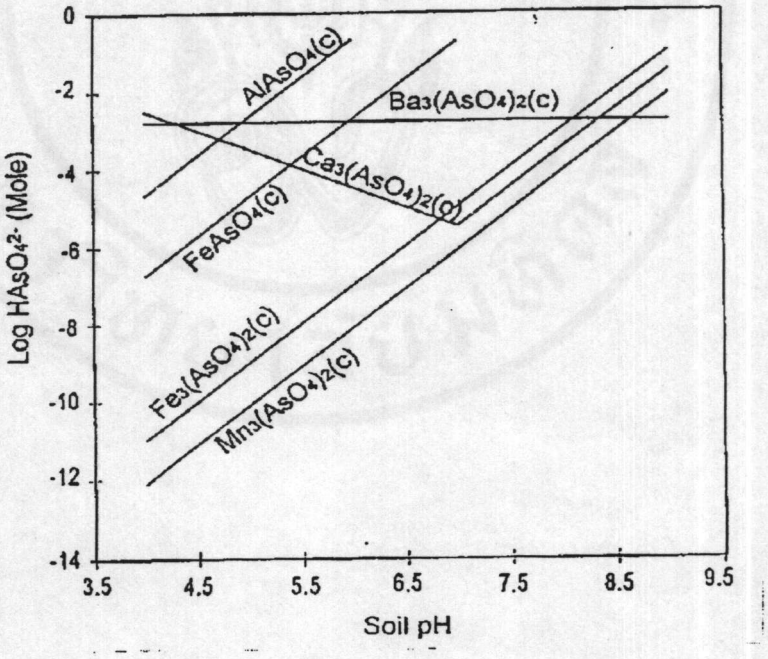


Figure 1.3 Thermodynamic solubility isotherms of arsenate minerals in soils [9].

1.2 Arsenic Speciation

1.2.1 The importance of speciation

The only total concentration of the element can not be used to assess its environmental impact. Since the chemical reactivity of the element depends on the particular species in which it is present, and since often the most abundant species is not the most reactive one. It is not surprising that the behavior of an element in the environment (e.g., bioavailability, toxicity, distribution) on the basis of its total concentration cannot be reliably predicted. In order to comprehend the environmental chemistry of the element it would be necessary to characterize in full the proportions and chemistries of all its various forms under the diverse range of conditions possible in natural systems.

The chemical speciation has been described as [25]:

I. The analysis of species leading to their identification and quantification in a defined area of in a volume (e.g., a sample, species anaylysis).

II. The description of the abundance (or numerical distribution) of species of an element in a volume.

III. The reactivity of a given species.

IV. The transformation of one species into another.

Unfortunately, the identification of individual species and their quantitative determination in the environment is still difficult at present, being

possible only for certain species. Analytical procedures of sufficient sensitivity and specificity are not available to determine the species of many environmentally significant elements. The development of new method for these purposes poses a great challenge to analytical chemists.

1.2.2 Definition of speciation

There is no generally accepted definition of speciation, and various meaning have been attributed to the term by various workers. The species, forms or phases are defined [26];

(I) Functionally defined speciation is exemplified by the plant available species. Other examples of this type of speciation include the use of terms such as biologically active or mobile forms.

(II) Operationally defined speciation, the physical or chemical fractionation process applied to the sample defines the fraction obtained. For example, sequential extraction procedures are commonly chemical fractionation process used to isolate, separate metals associated with the water/acid solution, exchangeable, reducible oxidisable and residual fractions. Physical procedures such as the division of a solid sample into particle size fractions of the isolation of soil solution or sediment pore water by filtration centrifugation or dialysis are also examples of operational speciation.

(III) Specific chemical compounds or oxidation states is the third form of speciation, that in which the precise chemical form of an element is measured or defined, is the hardest to achieve since analytical methodology of great selectivity and sensitivity is required. Specific oxidation state of elements for example, arsenic (III) and arsenic (V) or chromium (III) and chromium (VI) may be distinguished and quantified. For solid sample, this narrowly defined type of speciation is seldom possible since specific extraction procedures tend to change the speciation of this type.

In this study, the operationally defined was chosen for investigation arsenic speciation in soil by used sequential extraction procedures.

1.2.3 Speciation of arsenic in soil by sequential extraction

The total As concentration in soil does not provide information about their potential bioavailability or toxicity. The toxicity effect of As on the plant and human can be different depending on the chemical species. It is always difficult to identify which chemical species of As is present in environment. The sequential extraction techniques and followed by determination of each fraction concentration can help identify the distribution of this element in different phases of the material.

Many studies of metals in sediments and soils have been concerned only with total concentrations of each metal. Metal speciation have become important as a means of understanding the roles played by various species in the environment. Sequential extraction procedure is the method for distinguishing the phases association of metal in sample by treated with a series of chemical of increasing strength. The most important step in obtaining such data is extraction of metal species from the solids by means of chemical extractants. A number of workers have expressed concern about data from sequential extraction. Opinions differ about the kinds and concentrations of the extracting reagents and in the order in which they are applied. In order to do this, well-defined samples must be prepared and analysed. The ability of various extraction reagents to release arsenic in soils depends on their association with particular soil fractions. Several workers have described sequential extraction schemes for soil and sediment as show in table 1.3.

Table 1.3 Sequential extraction schemes for extracting arsenic in soils and sediments

Samples	Extractants	Elements	Workers
Soils	1. 0.1 *N* NH_4Cl (water soluble As) 2. 0.5 *N* NH_4F (Al associated with As) 3. 0.1 *N* NaOH (Fe associated with As) 4. 0.5 *N* H_2SO_4 (Ca associated with As)	As	Johnston et al. [27].
Soils	1. Anion exchange resin (freely exchangeable As) 2. 0.5 *M* $NaHCO_3$ (labile As associated with soil surfaces) 3. 0.1 *M* NaOH (As held by chemisorption to Fe and Al components of soil surfaces) 4. Sonication/ 0.1 *M* NaOH (As held at internal surfaces of soil) 5. 1 *M* HCl (As associated with Ca)	As	Mclaren et al. [28].
Soils and sediments	1. 0.25 *M* HCl and 0.25 *M* H_2NOH-HCl for reductive dissolution 2. (30%) H_2O_2 for oxidative dissolution	As, Se	Gruebel et al. [11].
Sewage Sludge -amended soil	1. 0.5 *M* $Mg(NO_3)_2$ (water soluble As) 2. *M* NaOAc (As bound to Ca) 3. 0.08 *M* H_2NOH-HCl at 96 ° C (As bound to Fe and Mn oxides) 4. 0.02 *M* HNO_3 and H_2O_2 pH 2.0 at 85 ° C (As bound to organic matter and sulfides phases)	As	Carbonell-Barrachina et al. [29].

Table 1.3 Sequential extraction schemes for extracting arsenic in soils and sediments (Continued)

Samples	Extractants	Elements	Workers
Soil and sediment	1. 1.0 *M* CH_3CO_2Na (As bound to Ca) 2. 0.25 *M* H_2NOH-HCl in 0.05 *M* HCl at 60 °C (As bound to amorphous Fe and Mn oxides) 3. 1.0 *M* H_2NOH-HCl in 25% HOAc 90 °C (As bound to crystalline Fe and Mn oxides) 4. 0.75 g $KClO_3$ in 5 ml conc. HCl and 4 M HNO_3 at 90 °C (As bound to organic matter and sulfides phases)	As,Be,Ca, Co,Cr,Cu, Ce,Cd,Ni, P, Ti, V, Pb, Mn, U Zn,Fe,La,Li, Ti	Hall et al. [30].

Two different major schemes for sequential extracting As in soil have been reported. The first scheme used similar reagents as extraction scheme of P in soil because the chemistry of As in soil has apparent similarities with P in soil [16, 28, 31-33].

The other scheme extract elements in soils and sediments into: 1) exchangeable; 2) bound to carbonate and adsorbed; 3) bound to Fe-Mn oxides; 4) bound to organic matter and sulfides and 5) residual forms. This scheme is widely used for metals and transition metals.

In this work, the first group scheme was chosen for extraction As in soil considering the chemistry of As in soil is similar P in soil. The scheme used by McLaren et al. was modified in this work.

The scheme used in this work fractionated arsenic into: labile As (bioavailable As), Fe+Al associated As and Ca associated As. The labile forms of As was extracted with resin and bicarbonate. The rationale for the use of bicarbonate for extraction of available As is provided by the consideration that plant roots produce CO_2 which forms bicarbonate in the soil solution that may solubilize soil P [33, 34]. It is hoped therefore that these extractants somehow simulate the action of plants roots and thus give a more appropriate measure of plant available P. Hydroxides extractable As has lower bioavailability and is associated with amorphous and some crystalline Al and Fe arsenates. HCl dissolves Ca bound As that form stable compounds.

1.3 Continuous-Flow Sequential Extraction System for Speciation of Arsenic in Soil

The sequential extraction of As in soils by a batch system that is widely used can suffer from the problem of readsorption of As during extraction[11]. Our research group has developed a continuous flow system for sequential extraction of heavy metals in soil [35]. This system has shown many advantages over the conventional batch method. It can reduce the effect of pH change during extraction, the varying sample/extractant ratio. Thus in this work, the continuous flow system was applied for As fractionation in soil with subsequent determination of As with ETAAS method.

1.4 Determination of Arsenic by ETAAS

The determination of As in extracts after sequential extraction has to be done. An accurate determination of As is important. The colorimetric method used for determining As has been performed by the classical Gutzeit method for decades [36]. By this method, an As complex with silver diethyldithiocarbamate is formed after the production of the arsine by reduction, and the red complex is measured by molecular absorption at 525 nm [36, 37]. The latter method consists in the use an oxidizing agent like permanganate [38] or iodate [39] to convert all arsenic present in sample to As (V) used to form arseno-molybdenum blue and then measure the absorbance at 865 nm. The colorimetric method are less sensitivity and require long reaction times.

Since atomic absorption techniques was developed, several other analytical AAS techniques have been used for analysis of this element such as hydride generation atomic absorption spectrometry (HGAAS), electrothermal atomic absorption spectrometry (ETAAS). Recently, inductively coupled plasma mass spectrometry (ICP-MS) is allso becoming widely used.

HGAAS is capable of detecting hydride-forming arsenic compounds. Many samples, which contain non hydride-forming arsenicals, must be digested prior to their analysis by HGAAS. Thus incomplete digestion and reduction of arsenical compounds may lead to low recoveries [37, 40, 41]. In addition, the HGAAS method has a tendency to be interfered by coexisting ions [42].

ICP-MS is becoming well-known for determining As. However, this technique has many disadvantages, firstly it requires sample dissolution prior to analysis [43, 44]. When applied to determination of As in the presence of chloride, this technique can suffer from serious spectral interference on monoisotopic $^{75}As^{+}$ arising from the production with in the argon plasma of $^{40}Ar^{35}Cl^{+}$ [43-45].

For these reasons, the techniques that described above are not selected to determine As in the extracts. The ETAAS is used to detect As in this work because it has low detection limits, good precision, simplicity in operation as well as minimum sample pretreatment, which have contributed to the widespread use of the method. Determination of As by ETAAS has been used by many authors in many applications. Takamutsu et al. [46] have reported the development of the determination method for

As compounds such as arsenate, arsenite, monomethylarsonate (MMA) and dimethylarsinate (DMA) in soils by using solvent extraction, anion exchange chromatography and determination of As with ETAAS using $Mg(NO_3)_2$ as modifier.

Kubota et al. [47] has determined As content in natural water by ETAAS after collection as molybdoarsenate on activated carbon (AC). The AC was directly introduced as an AC suspension into a graphite furnace atomizer by using an ultrasonic cleaner for stabilizing the suspension. The Zr solution was added as a matrix modifier.

Paolo [48] showed the determination of urinary arsenic by solvent extraction and ETAAS. The urine samples were acidified with HCl and reduced with KI followed by a stripping step from the organic solvent with 1% HNO_3. By adding Pd as chemical modifier. The same modifier Pd was used in the work of Lian Liang [49] to determine As in ambient water at sub-part-per-trillion levels by ETAAS detection.

The above applications used ETAAS for determination of As by various modifier with good results. The Pd modifier was chosen in this work because Pd has become the most widely used modifier under routine analytical conditions and has been established as modifier with universal applicability. It has been reported that Pd must be in a particular chemical form, such as the metallic form and solid solutions with the elements to be determined in the graphite furnace.

Many workers have studied the effect of Pd modifier and of different matrices for determination of As by ETAAS. Krivan and Arpadajan [50] have studied the influence of the different matrices (HCl, NaCl, HNO_3 and urine) and of various

chemical modifier (W, Pd and a mixture of W+Pd+citric acid) in graphite furnace using radiotracer ^{75}As. Sergio [51] has investigated the effect of concentration of HNO_3, and found that as the HNO_3 concentration increases the As signals decrease, in presence of Pd 5 μg. Furthermore, he has studied the chemical modifiers (Pd and Ni). Pd was selected as modifier for determination of As to avoid Ni contamination because a higher mass of Ni required and since Ni is frequently determined in the laboratory [51]. Furthermore, Vera et al. [52] studied the effect of modifier such as Pd, W and Pd+W. Pd as its nitrate was found to efficiently stabilize each As species upto 1200-1300 °C, whereas Pd as its chloride exhibits effective stabilizing action only for the inorganic As species. The W treatment of graphite tubes in combination with the $Pd(NO_3)_2$ modifier produces excellent stabilizing As action for all the As species.

In this work, the As in the extracts from the sequential extraction and in solid forms were detected by ETAAS. Parameters affecting the determination of As such as amount of Pd modifier and of various media have been investigated.

1.5 Aims of This Work

This work focused on the development of methods for determination of As in each medium by ETAAS and the study of sequential extraction using the continuous flow system with ETAAS detection: Two areas of investigates are as follow.

1.5.1 ETAAS method development includes:

- Study on the effect of each media on As determination by ETAAS.
- Study on the solid sampling introduction ETAAS for measurement of total and residue As in soils.

1.5.2 Study of the continuous flow sequential extraction for As fractionation in soils:

- Study on the effect of the varying sample/extractant ratio.
- Evaluation of the continuous flow sequential extraction system by using certified reference materials (CRMs).
- Application of the continuous flow system to fractionate As in soil samples.

CHAPTER II

EXPERIMENTAL

This chapter contains the description of instrument, operating conditions used in the analytical measurements, chemical reagents, standards and samples preparation. The procedure for determination of total As in soils by slurring sampling ETAAS and fractionations of As by a continuous flow sequential extraction system are also described in this chapter.

2.1 Instrumentation and Equipment

I. Continuous-flow extraction system setup

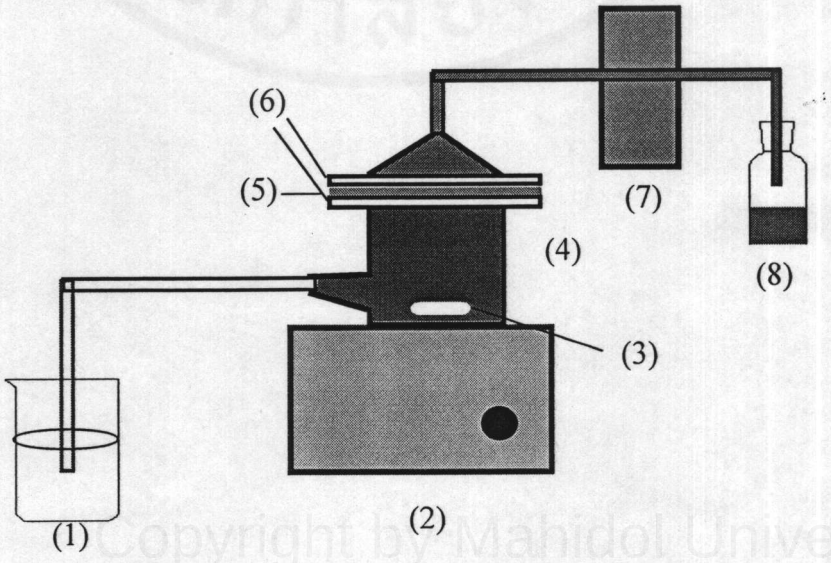


Figure 2.1 A continuous flow sequential extraction system. 1) Extractants 2) Magnetic stirrer 3) Magnetic bar 4) Glass extraction Chamber 5) Glass fiber filter 6) Gasket 7) Peristaltic pump 8) Fraction collector.

A continuous flow sequential extraction system was set up following figure 2.1. An extraction chamber was designed to allow containment and stirring of a weighted sample, and through which extractants could flow sequentially and leach metals from the targeted phases. The outlet of chamber was furnished with a glass filter to allow dissolved matter to flow through. Extractants was pumped through the chamber using a peristaltic pump, and using Tygon pump tube with 2-mm inner diameter. The description of each can present in the extraction system setup is as follow;

(1) Glass extraction chamber

The borosilicate glass extraction chamber for containing the soil sample and flowing extractants previously developed in the Department of Chemistry, Mahidol University [35] was employed.

(2) Magnetic stirrer and magnetic bar

The magnetic stirrer used for mixing during extraction (J. Bibby Science Products, Ltd., Stone Staffordshire, England) was employed.

(3) Peristaltic pump

A peristaltic pump (Ismatec SA, Labortechnik-Analytik, Glattbrugg-Zurich, Switzerland) was used to pump the extractants into the chamber for extraction.

(4) Glass membrane filter

Glass membrane filter (Millipore, Catalog No. APFC02500, pore size 1.2 μm, Ireland) was used for separating the extract from suspension by placing it between the cover and the body of the chamber.

II. Electrothermal Atomic Absorption Spectrometer (ETAAS)

ETAAS measurements were performed with a Perkin-Elmer Analyst 100 (Norwalk, CT, USA) equipped with a deuterium-arc background corrector and an HGA 800 heated graphite atomizer. The sample was introduced into the atomizer by an AS-72 autosampler. The cooling system HGA was also used to allow the temperature of the atomizer to cool down more rapidly. The atomic signals were monitored by a Compaq computer with a Hewlett Packard 870 printer to print out all the analytical data. The radiation source was an arsenic electrodeless discharge lamp operated at 300 mA, and the 193.7 and 197.2 nm wavelength were monitored. The spectral bandwidth used was 0.7 nm. ETAAS is used to determine As, Fe and Al concentrations.

III. Flame atomic absorption spectrometry (FAAS)

FAAS measurements were performed using a Perkin Elmer Model 3100 equipped with deuterium background correction (Connecticut, USA). The FAAS is employed to determine Ca concentration.

IV. Analytical balance

The Precisa balance model 40 SM-200A (Zurich, Switzerland), and model 240A were used to weigh samples or chemical reagents in the preparation of all standard solutions, slurry subsamples and solid samples for the flow sequential extraction.

V. Sieve

The test sieve with the aperture of 2 mm from Endecotts Ltd. (London, England) was used to separate the particle size of soil samples.

VI. Centrifuge and centrifuge tube

The centrifuge (Hettich Universal II Centrifuge, model D 7200, Germany) was used to separate the supernatant from the solid sample. The polypropylene copolymer centrifuge tubes (Nalgene, Rochester, NY, USA) were used for sequential extraction by a batch system.

VII. Vortex mixer

A Vortex-Genie model K-550-GE from Scientific Industries, Inc. (Bohemia, USA) was used for slurry homogenization.

2.2 Chemical Reagents and Gas

I. Chemical Reagents

The chemical reagents in this work were analytical grade and purchased from various sources as indicated in Table 2.1

Table 2.1 List of chemical reagents.

Chemical reagents	Companies
Standard As solution 1000 ppm	Carlo Erba (Milano, Italy)
Standard Fe solution 1000 ppm	E. Merck (Damstadt, Germany)
Standard Al solution 1000 ppm	E. Merck (Damstadt, Germany)
Standard Ca solution 1000 ppm	E. Merck (Damstadt, Germany)
Triton X – 100	Fluka (Switzerland)
Glycerol Hydrate (86-88 % w/w)	Fluka (Switzerland)
Palladium metal	Fluka (Switzerland)
Nitric acid (69-70 % w/w)	J. T. Baker (Phillipsburg, USA)
Hydrochloric acid (36.5-38 % w/w)	J. T. Baker (Phillipsburg, USA)
Hydrofluoric acid (50 % w/w)	J. T. Baker (Phillipsburg, USA)
Sodium bicarbonate	J. T. Baker (Phillipsburg, USA)
Sodium hydroxide	E. Merck (Damstadt, Germany)

II. Gas

High purity grade argon (99.995 %) from Thai Industrial Gas was used as inert gas for protecting the furnace from oxidation during heating and speeding the matrices removal in ashing steps and reducing sensitivity of detection

2.3 Procedure for Preparation of Reagents, Standard Solutions and Samples

Water used for preparation of all solutions is purified water (Milli Q plus, 18.2 M Ω cm^{-1} resistivity)

I. Standard solution

All standard solutions were prepared from 1000 ppm primary solution by diluting with pure water to the desired concentrations.

II. Glycerol hydrate solution

Glycerol hydrate was diluted to give 75 % (v/v) solution, and 0.1%(v/v) Triton X-100.

III. Palladium solution (1000 ppm)

An accurate 0.1g of Pd metal was dissolved in 10 mL of HNO_3 and the minimum volume of HCl. This was then diluted to 100 mL.

IV. Hydrochloric acid 1.0 M

Hydrochloric acid (83.3 mL) was pipetted into a 1000 mL volumetric flask and diluted to 1000 mL.

V. Sodium bicarbonate 0.5 M

Sodium bicarbonate (42.00 g) was dissolved and made up to 1000 mL with purified water.

VI. Sodium hydroxide 0.1 M

Sodium hydroxide (4.00 g) was dissolved and made up to 1000 mL with purified water.

VII. Certified reference material (CRM)

CRMs were purchased from NIST, USA. SRM 2704 is a sediment sample from Buffalo River. SRM 2710 and 2711 are soil samples from Montana.

SRM 2710 contains highly elevated and 2711 contains moderately elevated concentration of metals. The CRMs should be shaken before use.

VIII. Soil samples

Soil samples were from three origins. The first was from Ronphiboon, Nakornsrithamarach province, Thailand. Soil samples were separated into particle size range of < 106, 106-150 and > 150 μm by sieves. The second source was from Yala province, Thailand. The last source was from Australia, the particle size of this soil was < 2 mm. All soils were stored in desiccators.

All glassware, polyethylene bottles and other materials were carefully cleaned by washing with detergent to remove dust, pariculate remnants, then soaked in 10% nitric acid at least overnight and rinsed twice times with purified water.

2.4 Slurry Sample Introduction

The slurry sample introduction method is used for ETAAS determination of total and residual of As in solid soils.

Preparation of slurry

Solid sample was accurately weighed (0.15xx g) in the test tube. After that calculated amount of 75% glycerol (10.00xx g) was added into the test tube and the accurate total weight was measured. Vortex mixer was used to mix the suspension until the slurry was homogenous and transferred to graphite tube. The suspension was then introduced to the graphite tube for ETAAS measurement.

2.5 Sequential Extraction Procedure

The sequential extraction scheme to fractionate As in soil samples into 5 fractions was adapted from that used by McLaren et al. [28] as follow ;

Table 2.2 The sequential extraction scheme for the continuous flow system and the batch system

This work	Scheme used by McLaren
1. Water extractable As	1. Resin extractable As
2. $NaHCO_3$ extractable As	2. $NaHCO_3$ extractable As
3. NaOH extractable As	3. NaOH extractable As
4. HCl extractable As	4. Sonication/NaOH extractable As
5. Residual	5. HCl extractable As
	6. Residual

From Table 2.2, the first step of scheme of McLaren [28] was replaced by H_2O and the 4th step was omitted in this work.

Table 2.3 Sequential extraction scheme for As and fractions separated.

Step	Reagent	Fraction
1	Deionized water	As dissolved in water
2	0.5 M $NaHCO_3$	As associated with soil mineral surfaces
3	0.1 M NaOH	As held to Fe and Al components of soil
4	1.0 M HCl	As associated with Ca compounds
5	HNO_3/HCl/HF	As recalcitrant

2.5.1 Procedure for a batch sequential extraction

Step 1: Water soluble extractable As

A sample of soil (1.00xx g) was suspended in 30 mL of deionized water in a polypropylene centrifuge tube and shaken for 16 h. The soil suspension was centrifuged and the supernatant solution filtered through Whatman no. 42 filter paper.

Step 2: Sodium bicarbonate extractable As

The soil residue from step 1 was resuspended in 30 mL of $NaHCO_3$ (0.5 M) and shaken for 16 h. The soil was then centrifuged and the supernatant solution filtered through Whatman no. 42 filter paper.

Step 3: Sodium hydroxide extractable As

The soil residue from step 2 was resuspended in 30 mL of NaOH (0.1 M) and shaken for 16 h. The soil was then centrifuged and the supernatant solution filtered through Whatman no. 42 filter paper.

Step 4: Hydrochloric acid extractable As

The soil residue from step 3 was resuspended in 30 mL of HCl (1 M) and shaken for 16 h. The soil was then centrifuged and the supernatant solution filtered through Whatman no. 42 filter paper. The soil residue was oven-dried at 60°C for 48 h and then finely ground in agate motar.

Step 5: Residual As

Arsenic in the dried and ground residue from step 4 was determined as for total soil arsenic by solid sampling introduction ETAAS.

2.5.2 Procedure for a continuous flow sequential extraction

Continuous flow sequential extraction was performed by using the setup shown in Figure 2.1 the followings are the described of extraction steps.

Step 1: The accurate weight (0.1000 g) of sample and magnetic bar were placed in the chamber. After the glass filter membrane, gasket and chamber cover were located and the set up was securely clamped, the first extractant (deionized water) was pumped through. The suspension of soil in chamber was mixed by magnetic stirrer. The extractant was separated from solid phase by the glass membrane filter and collected in plastic bottles at 10 or 20 ml intervals to obtain a total volume of 150-200 ml.

Step 2: $NaHCO_3$ was pumped through the extraction chamber. The extractant was collected in the plastic bottles at 10 or 20 ml intervals to obtain a total volume of 150-200 ml.

Step 3: NaOH was pumped through and the extractant was collected as described in the previous steps.

Step 4: HCl was pumped after NaOH was used in the third step and the extractant was collected as described in the previous steps.

Step 5: The solid residue was dried and ground. As in residual fraction was determined by solid sampling introduction ETAAS.

The bottles of the extractants were stored in a refrigerator at 4 °C for subsequent measurement.

2.6 AAS Conditions for Determination of Elements

Concentrations of As, Fe and Al were measured by ETAAS. The graphite furnace operating conditions used were shown in Table 2.4 and 2.5.

Table 2.4 Furnace operating conditions for the analysis of As in the extracts and solid samples by ETAAS

Step	Temperature (°C)	Ramp/Hold time (sec)	Ar gas flow (mL/min)
1. Drying	110	10/5	250
2. Ashing	1200	10/60	250
3. Pre-atomization	200	5/5	250
4. Atomization	2400	0/5	0/50/250
5. Clean up	2500	1/5	250

Table 2.5 Furnace operating conditions for the analysis of Fe and Al in soils by ETAAS

Step	Temperature (^{o}C)	Ramp/Hold time (sec)	Gas flow (mL/min)
1. Drying	110	10/5	Ar 250
2. Ashing	1700[a], 1400[b]	10/30	Ar 250
3. Pre-atomization	200	5/5	Ar 250
4. Atomization	2400[a], 2200[b]	0/5	Ar 250
5. Clean up	2400	1/5	Ar 250

[a] for Aluminium at wavelength 309.3 nm, slit width 0.7 nm

[b] for Iron at wavelength 248.3 nm, slit width 0.2 nm

Concentrations of Ca were measured by FAAS. The Ca operating condition for FAAS measurement was; wavelength 422.7 nm, lamp current 8 mA, slit width 0.7 nm and air-acetylene flame.

CHAPTER III

RESULTS AND DISCUSSION

This chapter presents the results and discussions of this work that can be divided into three parts. Firstly, result of the development of ETAAS method for the determination of As in different media that are $NaHCO_3$, NaOH, and HCl are presented in Section 3.1. The solid sampling ETAAS for determination of total and residual As in solid forms are presented in Section 3.2. Finally, fractionation of As species in soil by using the continuous flow sequential extraction system, followed by ETAAS measurement are described in Section 3.3.

3.1 ETAAS Determination of Arsenic in Various Media

The sequential extraction of As in soil was performed using many extractants. The different reagents used in the extractants may affect the As signals when the extracts were analyzed with ETAAS method. ETAAS was preferred in this work because ETAAS is fast, suitable for routine analysis and has sufficient sensitivity to allow direct determination of As in the extract. The effect of the extracting reagents on As signals was investigated and the effect of glycerol that was used for stabilizing the suspension in the solid sampling ETAAS was also studied.

3.1.1 Effect of Pd and reagents on arsenic signals

Pd modifier has been widely used for determination of As by ETAAS. It enhances the pyrolysis temperature for removing the interference and shifts the atomization peak to higher temperature. In this study, the effect of Pd on peak profiles of arsenic in many media was investigated. Figure 3.1 presents the arsenic signals in 75% glycerol medium and H_2O with varying amount of Pd. Without Pd modifier, the As signal was too low to be detectable.

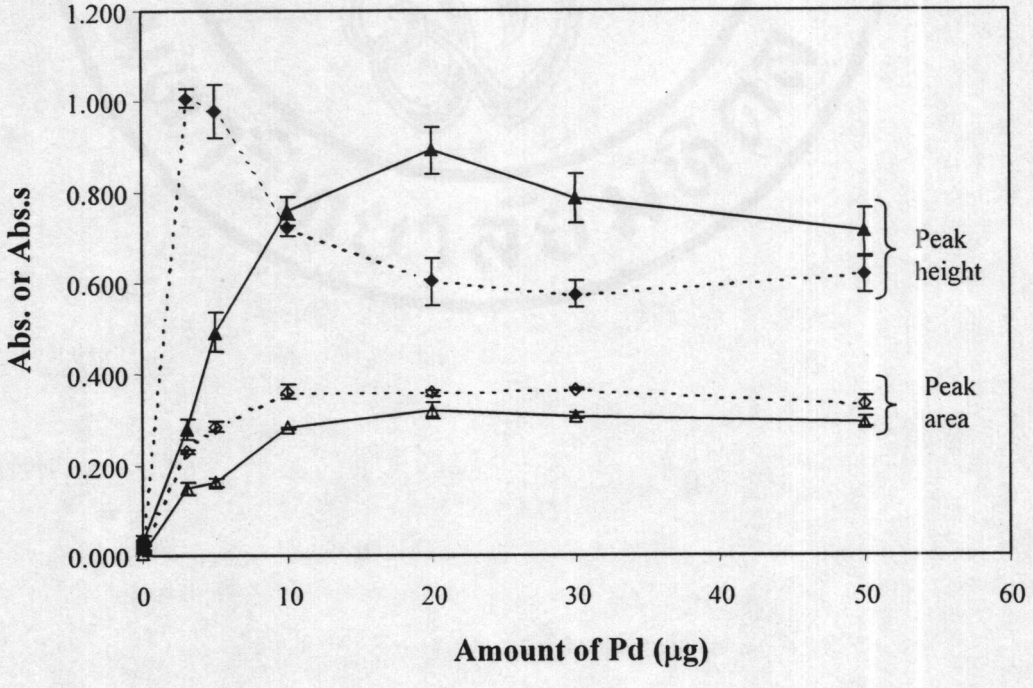


Figure 3.1 Effect of Pd amount on arsenic AA signals in aqueous (◇ ,◆) and glycerol (△ ,▲)

From Figure 3.1 the peak area of arsenic in H_2O and glycerol increases with an increasing amount of Pd and becomes constant at 10 μg. The peak profiles of As at varying Pd amount are shown in Figure 3.2. It can be seen that the peak height of As in glycerol increase with an increase in Pd amount up to 15 μg and it slightly decreases at larger amount of Pd. David et al. [51] have investigated the mechanism of Pd-induced stabilization of As in ETAAS. They reported that arsenic carbide was observed during vacuum vaporization, this contributed to analyte loss only when vaporization occurs in vacuum near 1500 K (arsenic carbide as a species was not found in the atmospheric pressure). The carbide was not formed if sufficient quantity of oxygen or palladium were present. Figure 3.1 and 3.2 showed that for glycerol medium with insufficient Pd (~3 μg), As was lost during atomization stage. The loss of As may have been caused by the forming of arsenic carbide. By increasing Pd amount up to sufficient amount (10 μg), Pd can stabilize As by formation of Pd-As compound.

The peak shapes of As in H_2O showed different tendency from those in glycerol. Significant increasing in peak height of As in H_2O was observed when Pd was increased to 3-5 μg and then decrease in peak height was apparent at 10-50 μg. Thomas et al. [52] reported that the Pd modifier can stabilize As, leading to peak appearance at higher temperature due to formation of Pd-As compound. The reduction in peak height for larger amounts of Pd may be due to a reduction in the analyte generation rate relative to the loss rate at high temperature. So 5 μg of Pd was selected as the modifier for As measurement.

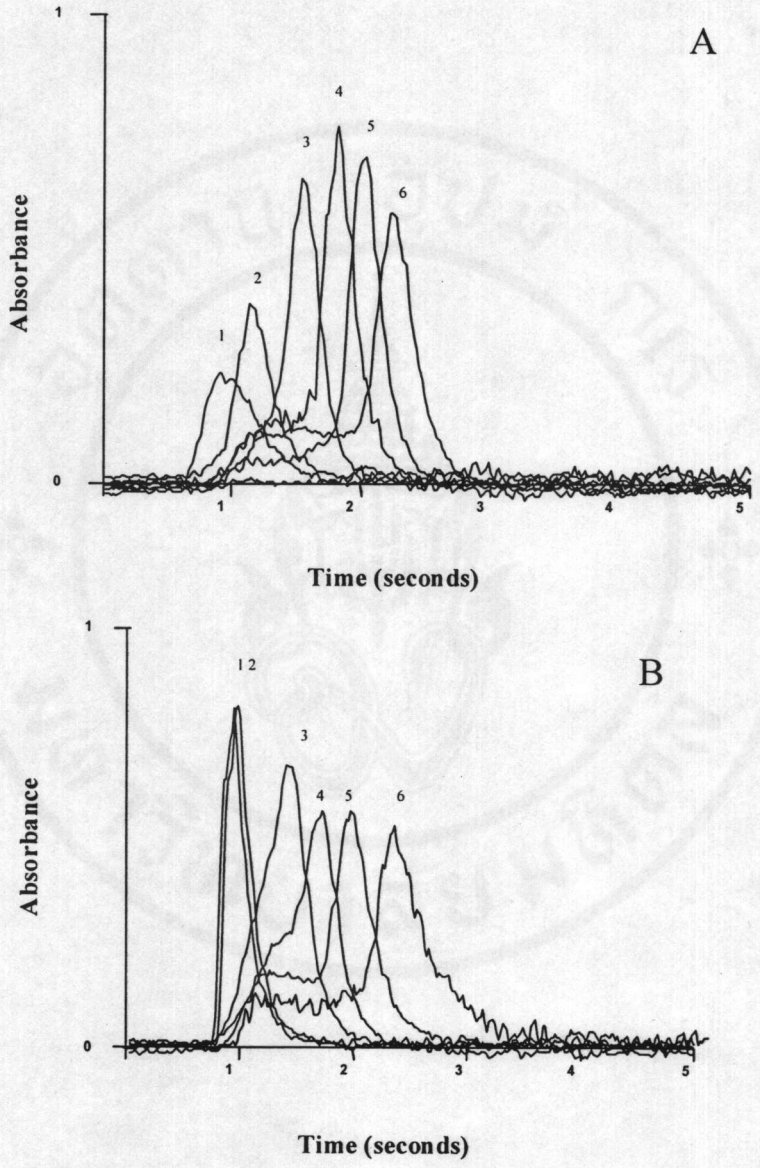


Figure 3.2 Atomic absorption peak of As (0.5 ng) varying Pd amount in glycerol (A) and H_2O (B)

Trace 1: Pd 3 μg, 2: Pd 5 μg, 3: Pd 10 μg, 4: Pd 20 μg , 5: Pd 30 μg, 6: Pd 50 μg

3.1.2 Effect of Pd and extracting reagent on arsenic signals

The sequential extraction of As in soil was performed using many extractants namely; deionized water, 0.5 M $NaHCO_3$, 0.1 M NaOH and 1.0 M HCl. It is important to see the effect of these reagents on ETAAS signal of As. Vera et al. [49] showed that the different matrices such as HCl, HNO_3 and various chemical modifiers have influence on ETAAS signal of As. Furthermore, Sergio [50] also reported that the concentration of matrices (HNO_3) and Pd modifier can affect the As signals. The effect of Pd amount on As signals are also investigated. The results were shown in Figure 3.3.

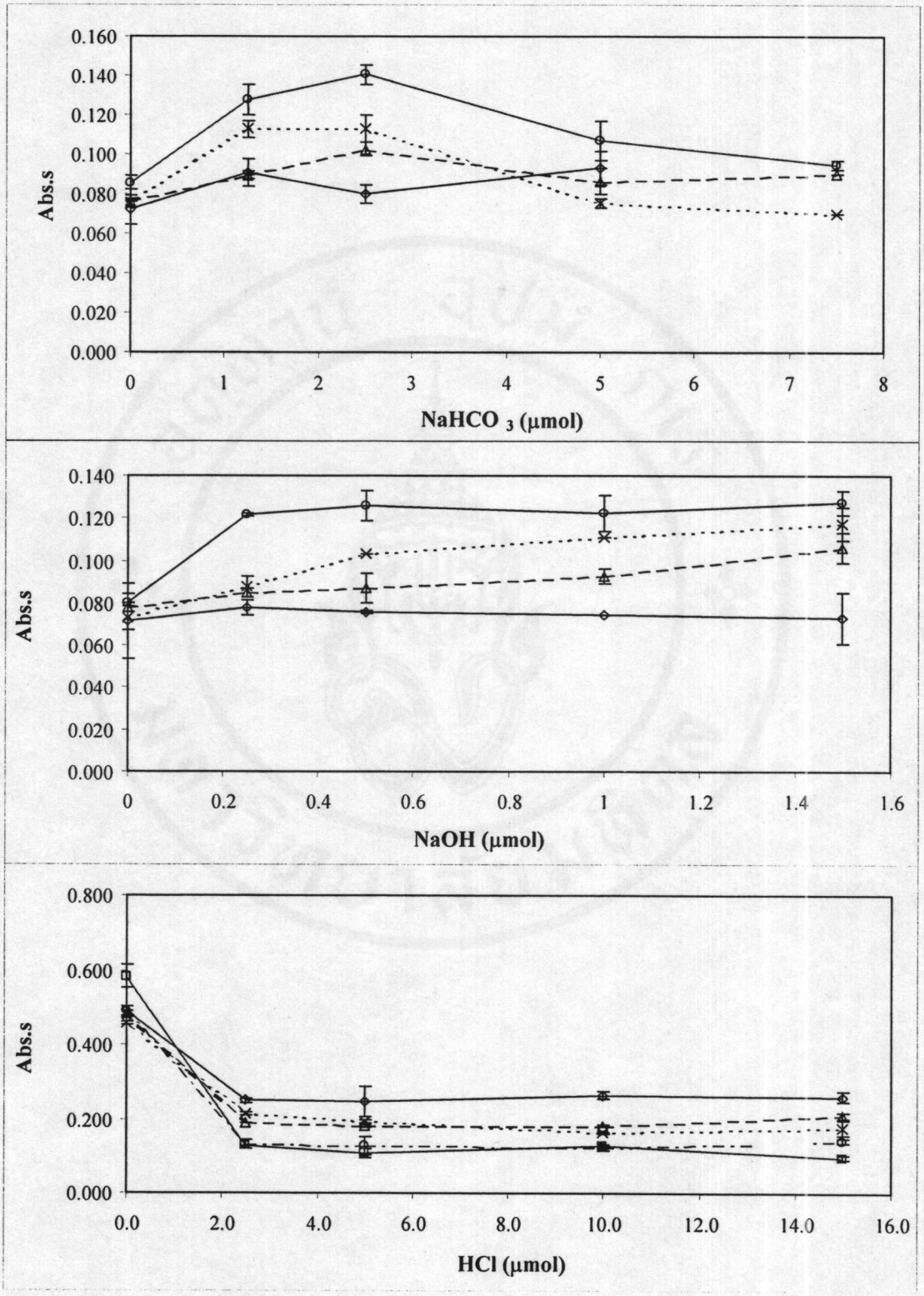


Figure 3.3 Effect of Pd and $NaHCO_3$, NaOH and HCl on atomic absorption signal of As (0.5 ng).
Pd 1 µg (◇), 3 µg (△), 5 µg (x), 10 µg (○) and 25 µg (□)

Figure 3.3 low level of $NaHCO_3$ enhances As signals only slightly at 1-2 μmol of $NaHCO_3$. At increasing amount NaOH, As signals increase slightly at any amount of Pd tested. The As signal decreased sharply in the presence of HCl. This indicated that the action of Pd to stabilize As was not effective in the presence of HCl. Vera et al.[49] reported that the low efficiency of the stabilizing action of Pd as its chloride could be explained by the formation of volatile chloride-containing analyte species, which are not stabilized by the modifier. Thus, residual chloride was expected to have an effect on analyte stabilization and atomization. The decreasing of As signal can be solved by adding a small amount of NaOH as shown in Figure 3.4. The calibration curves of As in each medium are shown in Figure 3.5.

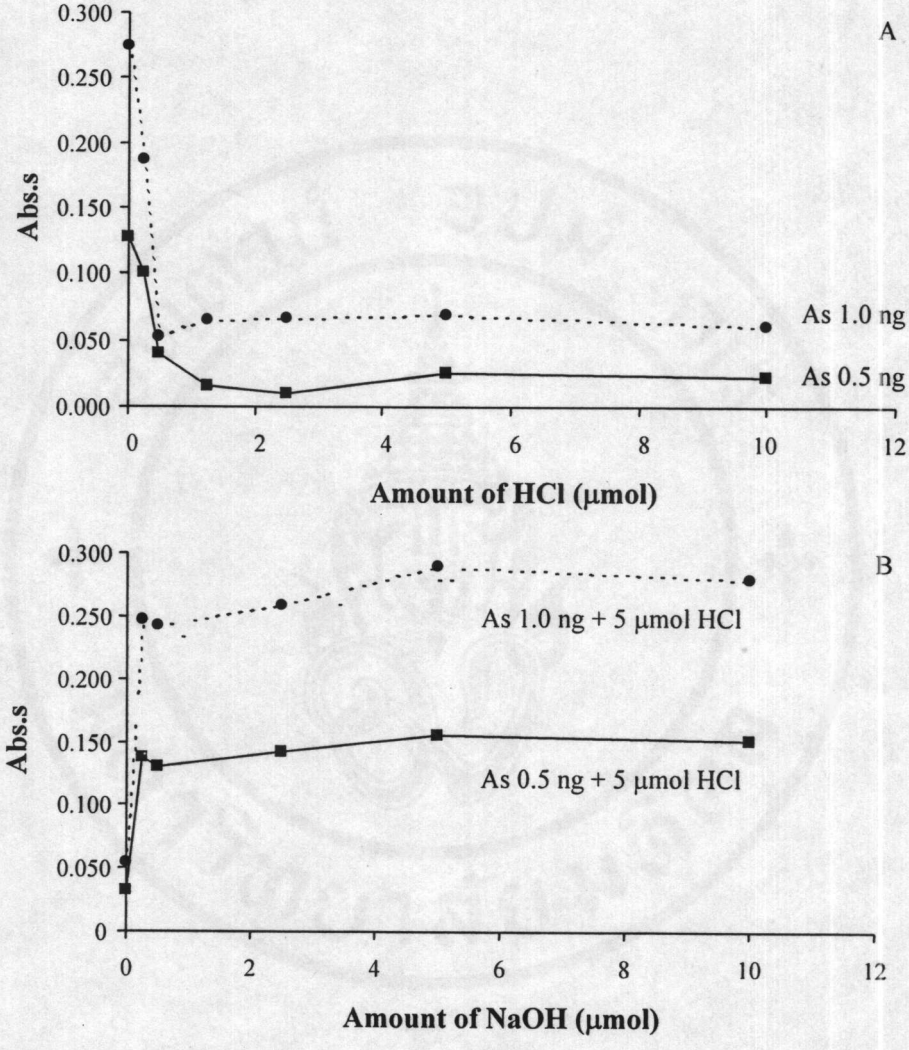


Figure 3.4 Effect of HCl on AA signals of As (A) and recovery upon addition of NaOH (B)

As (0.5 ng) by varying amount of HCl (■) (A)

As (1.0 ng) by varying amount of HCl (●) (A)

As (0.5 ng) in 5 µmol of HCl with varying amount of NaOH addition (■) (B)

As (1.0 ng) in 5 µmol of HCl with varying amount of NaOH addition (●) (B)

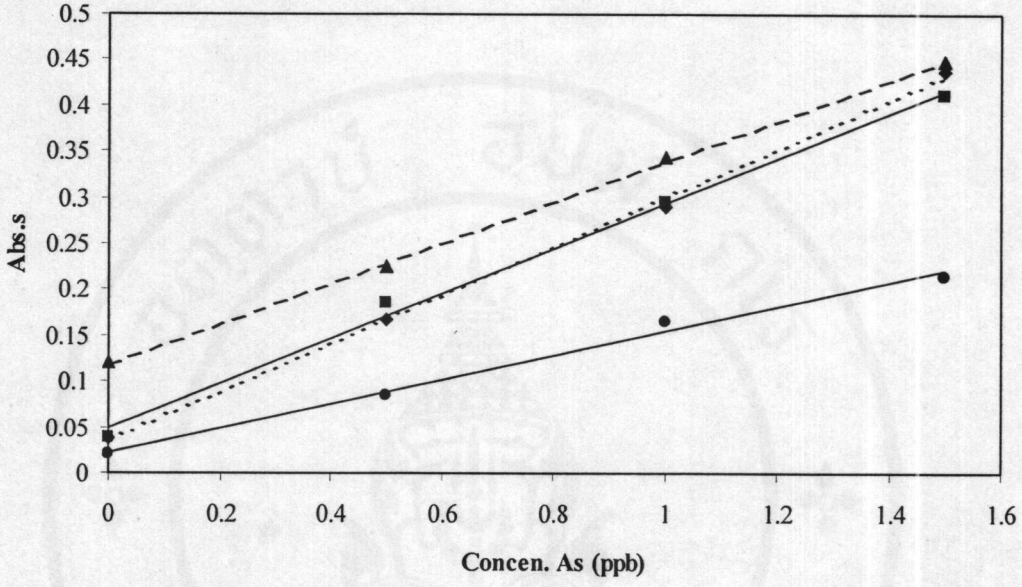


Figure 3.5 Standard calibration of As in aqueous (●), 0.5 *M* $NaHCO_3$ (▲), 0.1 *M* NaOH (x) and 1.0 *M* HCl (◆). (5 μg Pd was added in all cases and 0.25 μmol NaOH in the last cases)

3.2 Solid Sampling ETAAS Determination of Arsenic

The solid sampling ETAAS was chosen for determination of both total and residual As because this technique offers many advantages over conventional digestion such as reduced sample preparation time, simplicity, decreased analyte loss through volatilization during sample digestion, and elimination of hazardous acid use.

The slurry sample introduction is one of method to introduce directly solid sample into the ETAAS atomizer by using 75% glycerol to maintain a stable slurry until the time of sample introduction [55]. The precision of analysis of As by slurry sample introduction method is dependent on the presicion of pipetting slurry. The studies on effect of pipetting speed and injection temperature are presented in the next section.

3.2.1 Effect of injection temperature and pipetting speed on analytical precision

The pipetting speed and injection temperature can influence the precision and speed of ETAAS measurement. So the optimum condition of the pipetting speed and injection temperature was investigated. The results are shown in Figure 3.6.

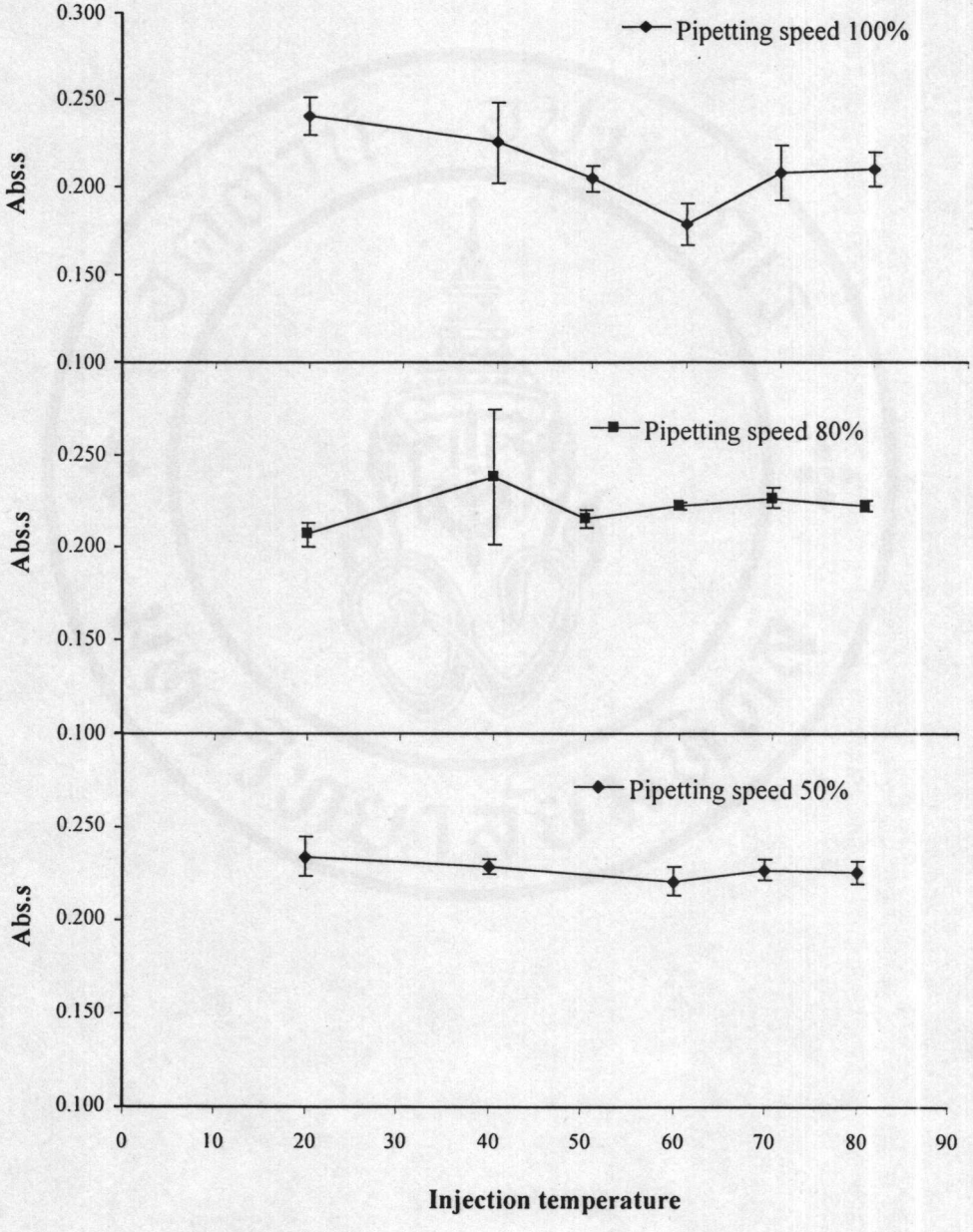


Figure 3.6 Effect of injection temperature and pipetting speed on analytical sensitivity and precision

The 50% pipetting speed showed the best performance in term of precision. At higher pipetting speed, poor precision was obtained due to incomplete transfer of sample slurry to the tube. The optimal injection temperature was found to be 40 °C. At too low temperature, the slurry may percolate into graphite tube. At too high temperature, the slurry may be splashed to the wall tube as a result of vigorous boiling.

3.2.2 Pyrolysis and atomization temperature

The pyrolysis and atomization curves of As using 5 μg Pd as modifier are presented in Figure 3.7, The optimum temperature of pyrolysis and atomization was obtained at 1200, 2400 °C respectively. Therefore, the graphite furnace operating condition for solid sample introduction as given in Table 3.2 was used throughout

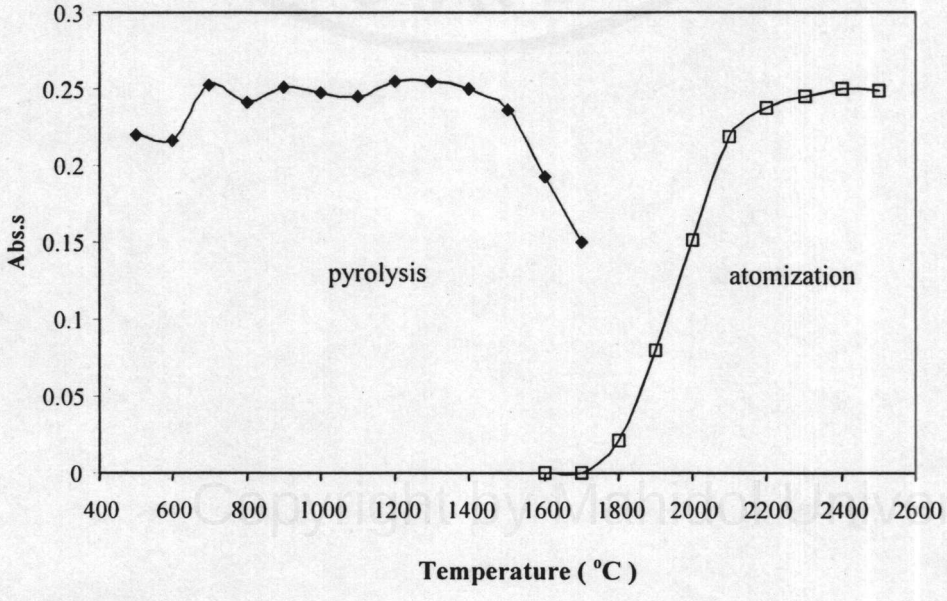


Figure 3.7 Effect of pyrolysis and atomization temperature on AA signals

3.2.3 Effect of internal Ar flow and absorption wavelength

The absorption wavelengths used in this study were at 193.7 and 197.2 nm. The former line is 2 times more sensitive than the latter line. Flowing argon gas at 50 and 250 ml/min during the atomization stage can reduce analytical sensitivity to approximately 1/2 and 1/8 times that at 0 ml/min respectively (Figure 3.8).

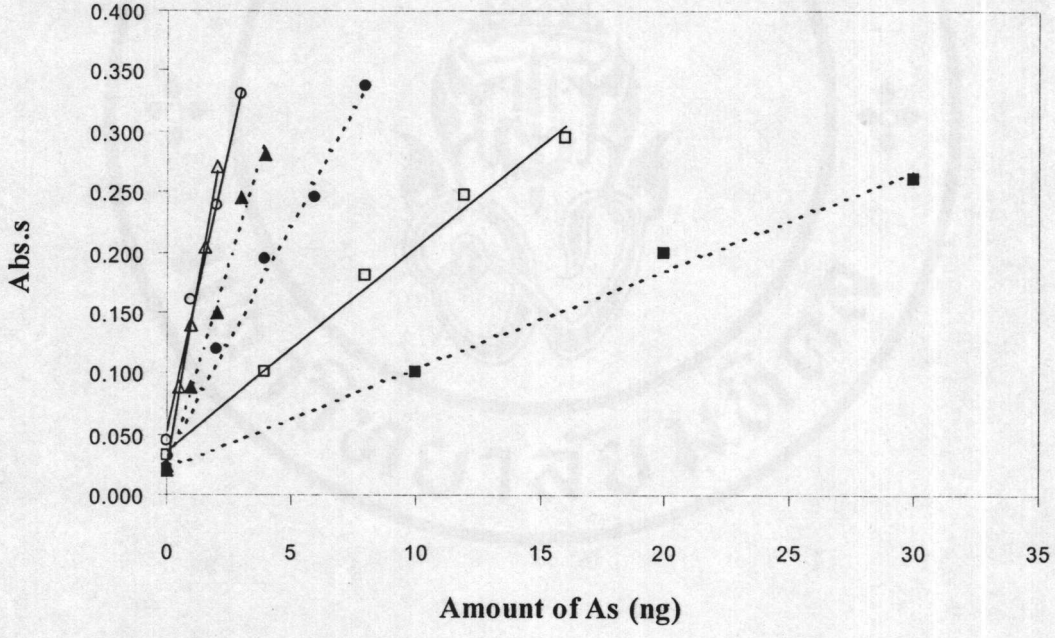


Figure 3.8 Effect of internal Ar gas flow and absorption wavelength

▲ 1. 197.2 nm stop Ar flow ● 2. 197.2 nm Ar flow 50 ■3. 197.2 nm Ar flow 250 ml/min

△ 4. 193.7 nm stop Ar flow ○ 5. 193.7 nm Ar flow 50 □6. 193.7 nm Ar flow 250 ml/min

y1 = 0.0677x + 0.0216 R^2 = 0.9872 y4 = 0.1218x + 0.0240 R^2 = 0.9982

y2 = 0.0378x + 0.0322 R^2 = 0.9894 y5 = 0.0937x + 0.0528 R^2 = 0.9939

y3 = 0.0082x + 0.0221 R^2 = 0.9926 y6 = 0.0168x + 0.0364 R^2 = 0.9929

3.2.4 Evaluation of solid sample introduction ETAAS

To evaluate the solid sample introduction technique, certified reference materials (SRM 2704, SRM 2710 and SRM 2711) were analysed.

Table 3.2 The concentration of total As in SRMs by solid sample introduction ETAAS

Sample	%slurry (w/w)	Total As concen.±SD.[a] (μg/g)	Certified values (μg/g)	% Recovery
SRM 2704	1.4206	25.9±2.3	23.4±0.8	110.7
SRM 2710	0.6104	523.6±35.2	626±28	84.1
SRM 2711	1.5010	110.3±5.3	105±8	105.0

[a] : (n=5)

The results from Table 3.2 show that sediment SRM 2704 and soil SRM 2711 gave As concentrations close to the certified values. The soil SRM 2710 that contains high level of As showed lower concentration than the certified value. The low percentage of slurry concentration (only 0.6104%) for sample with high arsenic concentration may have attributed to inhomogeneity of slurry and consequent inaccuracy owing to the small sample weight taken. The certified reference materials have been guaranteed for homogeneity for minimum sample weight of 250 mg, which is higher than the amount taken of 60 mg. The previous study [55] indicated the optimum slurry concentration as 1-5 %.

3.3 A Continuous-Flow Sequential Extraction System

Our research group has successfully developed a continuous flow sequential extraction system [35] for solving many problems encountered in the batch extraction. In this section, the continuous flow system was applied to the fractionation of As in soils. The extraction scheme was performed following the scheme shown in Section 2.5. Parameters affecting the extraction efficiency such as sample weight/chamber volume ratio and the evaluation of this system have been investigated.

3.3.1 Extractogram of arsenic from a continuous flow sequential extraction system

A continuous flow sequential extraction system was developed and the 4-step sequential extraction procedure was used for determination of As species in soils. The extractograms of As fractionation by the continuous flow system are shown in Figure 3.9.

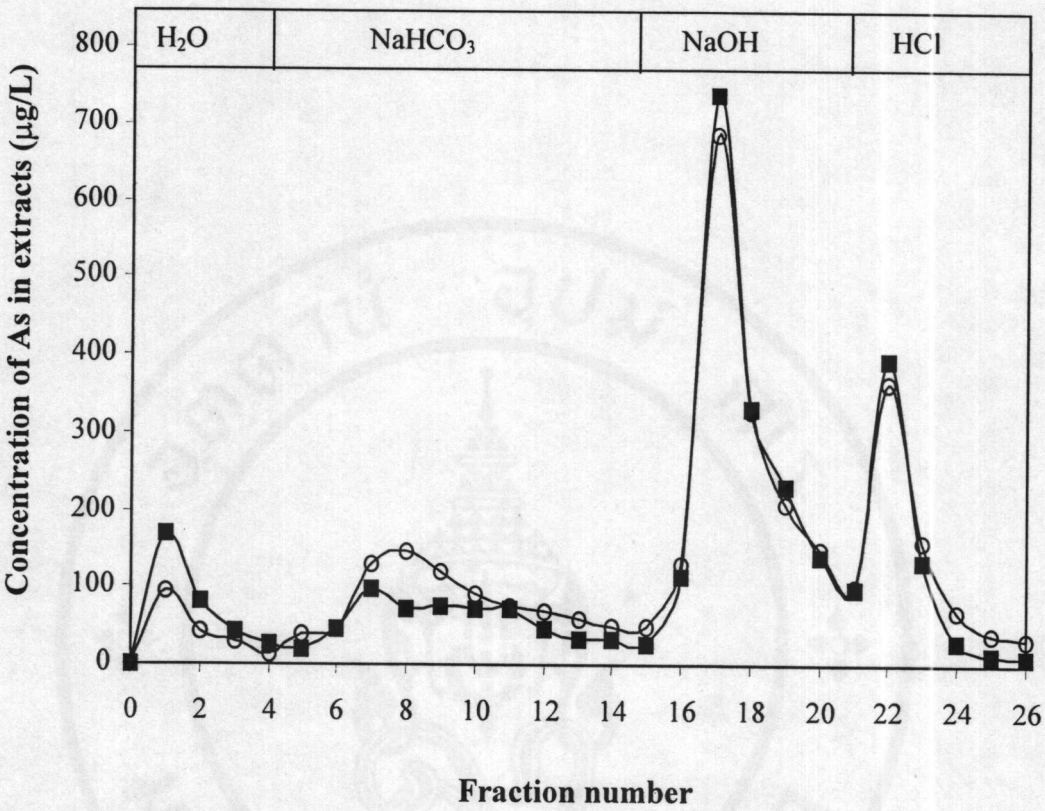


Figure 3.9 The overlay of two extractograms showed the reproducibility of As fractionation in SRM 2710

3.3.2 Effect of sample weight/chamber volume ratio

The effect of sample weight/chamber volume [S/V] ratio was investigated. The ratio used varies from 1/15 to 1/40 by adding the various sample weights (0.2000, 0.1500, 0.1000 and 0.0700 g) into the 3-ml of chamber size. The results are shown in Figure 3.10 and Table 3.3

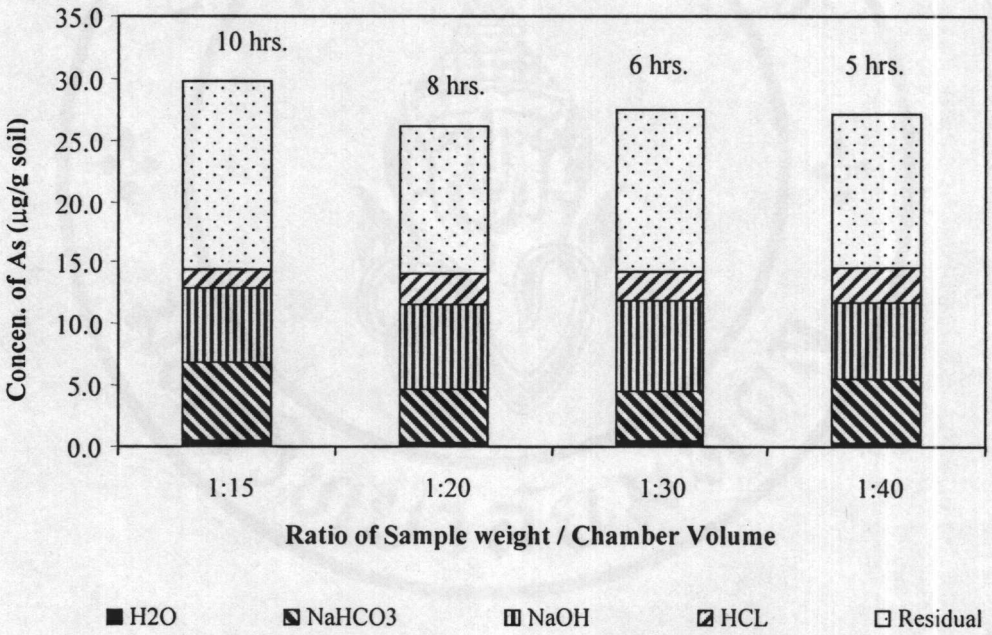


Figure 3.10 Distribution of As fractions determined at varying sample weight/chamber volume ratio. Sample : SRM 2704, Chamber volume : 3 ml. The time at the top of each column indicate total extraction time. Certified value for total concentration of As 23.4 µg/g.

Table 3.3 The As concentration in each fractions at varying S/V ratio (SRM2704)

Fraction	As concentration at varying S/V ratio (μg/g) (Mean ±SD.)			
	1/15	1/20	1/30	1/40
H_2O	0.6±0.1	0.4±0.04	0.5±0.02	0.3±0.1
$NaHCO_3$	6.4±0.6	4.3±0.4	4.0±0.7	5.3±0.7
NaOH	5.9±1.3	6.9±1.3	7.3±0.7	6.0±0.7
HCl	1.6±0.4	2.5±0.7	2.4±0.6	3.0±0.4
Residual	15.4±4.6	12.1±0.3	13.2±1.4	12.5±0.7
Total	29.9±4.6	26.2±1.6	27.4±1.8	27.1±1.3

(n = 2)

The results in Figure 3.10 and data in Table 3.3 indicate that the ratios of sample weight/volume chamber volume did not affect the total amount of As extracted. However, use of higher ratios can affect the flow rate of extractants in the flow system and the extractions takes longer time to complete. The extraction time required for 4 steps of sequential extraction were shown at the top of each column in Figure 3.10.

Decreasing the ratio or sample weight can reduce the extraction time. However, if the sample weight used is too low and weighing error can affect analytical performance. Therefore, a larger chamber volume of 10 ml is used hereafter instead of the 3 ml of chamber volume in further investigation.

3.3.3 Evaluation of arsenic speciation by a batch and a continuous flow sequential extraction system

The evaluation of the continuous flow sequential extraction system for soils and sediments were performed by analyzing certified reference materials (CRMs). The batch method was also performed for comparison. Both systems were evaluated by comparing the total As concentration from the certified values with the sum of As found in each fraction. The results of the continuous flow system were found to be in good agreement with the certified value as shown in Table 3.4

Table 3.4 Total and extractable soil As concentrations by a batch and a continuous flow system.

Sample	System	As fraction concentration (μg As g^{-1} soil) (Mean ± SD)					Total As concentration (μg As/g soil)	Certified value (μg As/g soil)	% recovery
		H_2O	$NaHCO_3$	NaOH	HCl	Residual			
SRM 2704	Batch (n=3)	0.4±0.03	3.3±0.2	4.7±0.06	1.6±0.3	20.7±2.6	30.8±2.6	23.4±0.8	131.6
	Flow (n=5)	0.5±0.5	5.2±1.1	6.6±0.6	2.2±0.6	12.4±2.5	26.8±2.8		114.5
SRM 2710	Batch (n=3)	2.6±0.3	31.6±3.6	247.6±15.1	226.1±5.9	100.1±2.6	607.6±16.8	626±28	97.1
	Flow (n=5)	21.7±12.2	110.5±40.0	225.2±34.1	93.1±25.6	151.5±27.8	601.8±65.9		96.1
SRM 2711	Batch (n=3)	7.5±1.4	23.7±2.2	27.7±2.4	28.3±0.3	42.6±2.5	129.9±3.6	105±8	123.7
	Flow (n=5)	9.2±3.0	19.3±2.7	20.6±2.9	20.3±0.3	40.0±5.5	109.4±7.4		104.2

* The continuous flow was carried out at 1/100 sample extraction chamber volume and at 10-20 ml fraction collection interval.

The %recoveries of all CRMs for the flow method were acceptable with the value of 114.5, 96.1 and 104.2% for SRM 2704, SRM 2710 and SRM 2711 respectively. The results of batch system were found to be larger than the continuous flow system, the value were 131.6, 97.1 and 123.7%. The As fraction distribution in SRMs as determined using the batch and flow system were also compared in Figure 3.11and 3.12.

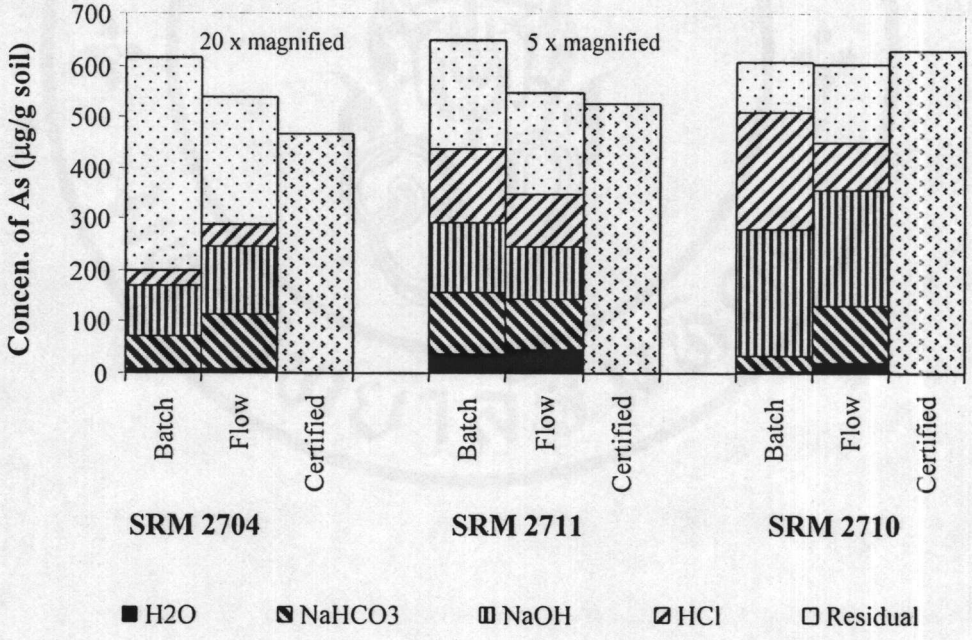


Figure 3.11 Comparison of results of sequential extraction As species in SRMs between batch system and flow system

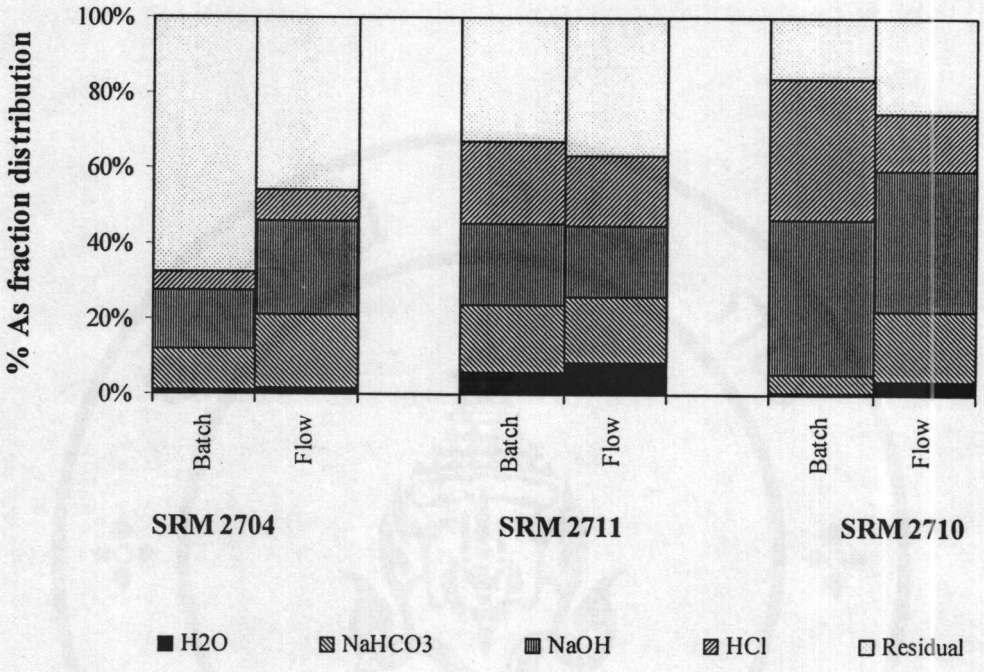


Figure 3.12 Comparison of results of % As species in soils between the continuous flow system, the batch system from SRMs

3.3.4 Arsenic fractionation for soil from different origins

The continuous flow sequential extraction was applied to fractionate As species in soil samples. The soil samples are from two sources, one is from Thailand and the other is from the cattle dip sites, Australia. The results of As fractionation of soil samples from the cattle dip site performing by a batch system have been taken from an earlier report of McLaren et al. [28]. The results of arsenic fraction distribution from the 2 sites using both methods are shown in Figure 3.13-3.16

Ronphiboon, Thailand

The soil samples from Ronphiboon, Thailand came from 2 different spots; one was from residential area and the other from mining factory nearby. The soils were separated into 3 sizes at <106, 106-150 and >150 μm for determining As fraction distribution in each particle sizes. The As concentrations in the tin mining factory was found to be approximately 25 times higher than those of the residential area (Figure 3.13 upper graph).

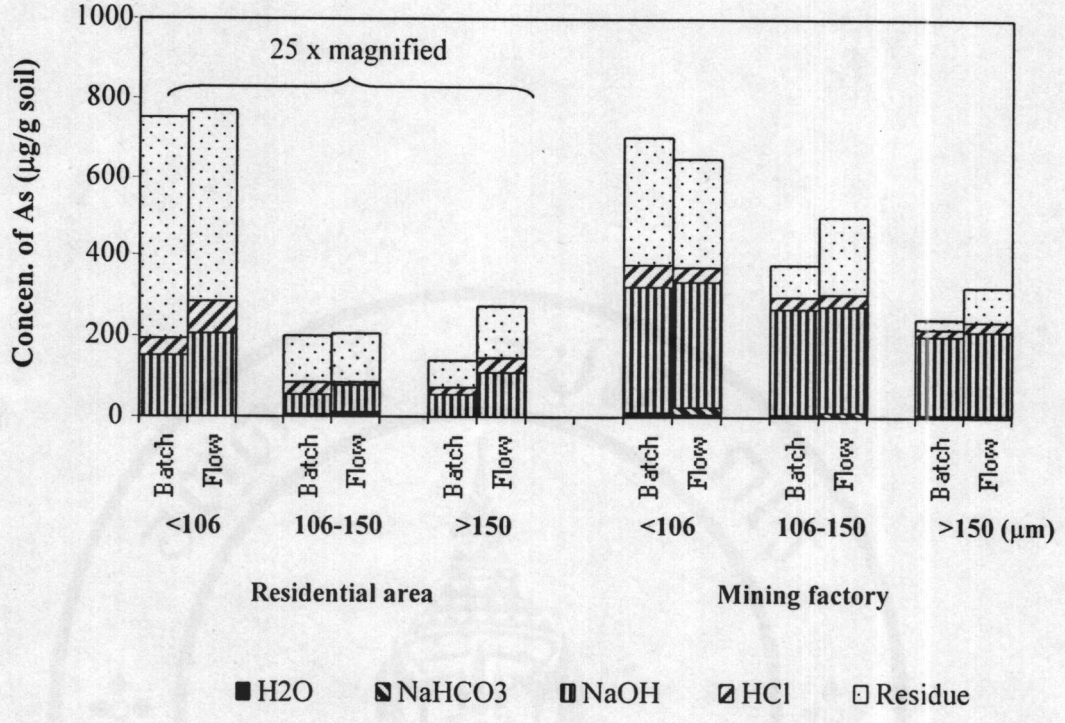


Figure 3.13 Results of As fractionation by using the continuous flow system and the batch system for soil from Ronphiboon, Thailand (Data in Appendix)

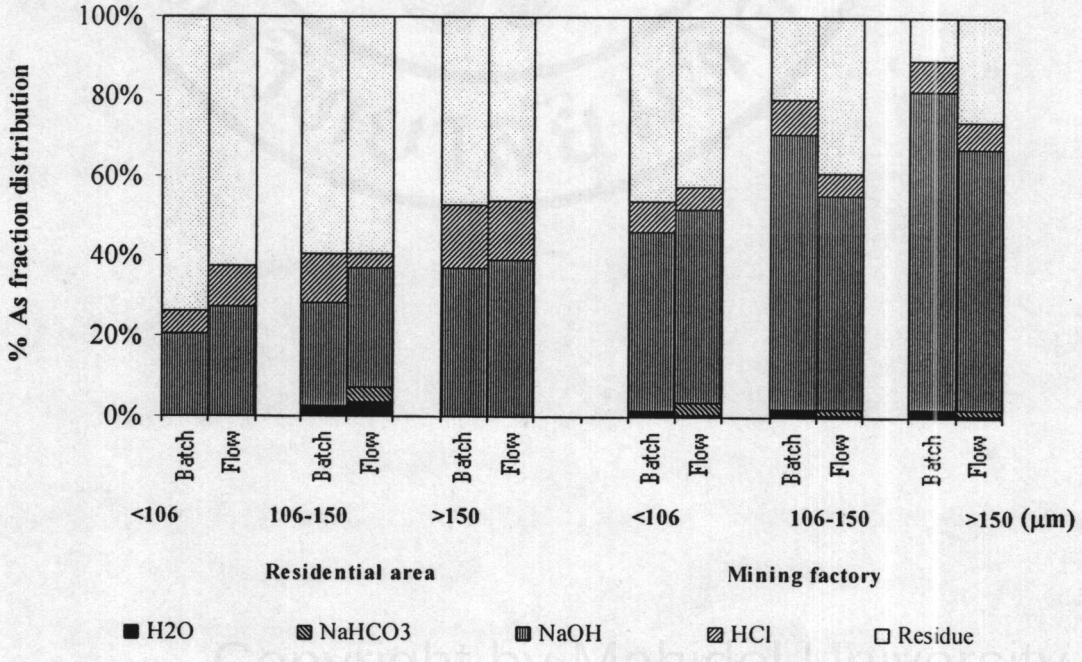


Figure 3.14 Arsenic fraction distribution by using the continuous flow system and the batch system for soil from Ronphiboon, Thailand (Data in Appendix)

The fraction distribution shown in the lower part of Figure 3.14 indicates that only small amount of arsenic is present in the bioavailable forms (water soluble+$NaHCO_3$ soluble), i.e. less than 5%. The results from both batch and flow system are close, with the exception of soil sample of residential area particle size > 150 μm. The reason is not known at this stage. Larger particle size can suffer more from sample inhomogeneity may be one of the reasons.

Cattle dip sites, Australia

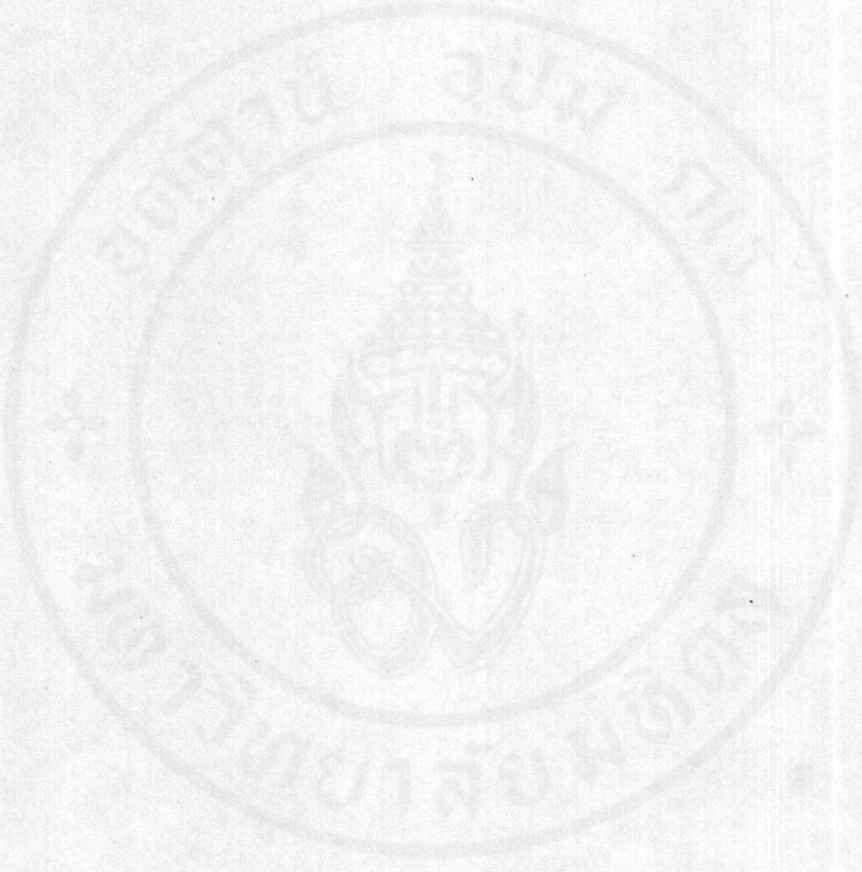
The results of the batch method for soil samples from cattle dip sites in Australia were taken from Mclaren et al. [28]. Although the extraction scheme was slightly different from the flow method, the results can be approximately compared by matching fractions from the two methods as followed.

Batch		**Flow**
Resin exchangeable	compared with	Water extractable
$NaHCO_3$ extractable	compared with	$NaHCO_3$ extractable
NaOH extractable + Sonication/NaOH extractable	compared with	NaOH extractable
HCl extractable	compared with	HCl extractable
Residual	compared with	Residual

The comparison of As concentrations by batch and flow system in each step is shown in Figure 3.15 (Data in Appendix Table 3A). By so doing, it was found that most of the results were well close eventhough the detection techniques as well as the extraction schemes were different. Higher arsenic concentration was observed only in the residual fraction of the flow method probably owing to the error from the solid sampling ETAAS method used. The method can be erroneous for sample with wide range of particle sizes.

It can be observed from Figure 3.13 and 3.15 that the concentrations of As obtained in the continuous-flow system can give different fraction distribution although the summation of all fractions is well close. The slight differences observed between both methods in some samples could be explained by different extraction conditions between the two systems and/or the errors from analytical measurements. The other probable explanation of the different results between the batch and the flow system is the carry-over effect in the batch system. In the batch system, the As extracted in one has to be washed away before the next step is carried out to avoid the

carry-over. Incomplete washing will increase the amount of As extracted in the next step. This is the reason of the higher As concentrations in some step of the batch system. (Data a in Appendix Table 1A, 3A).



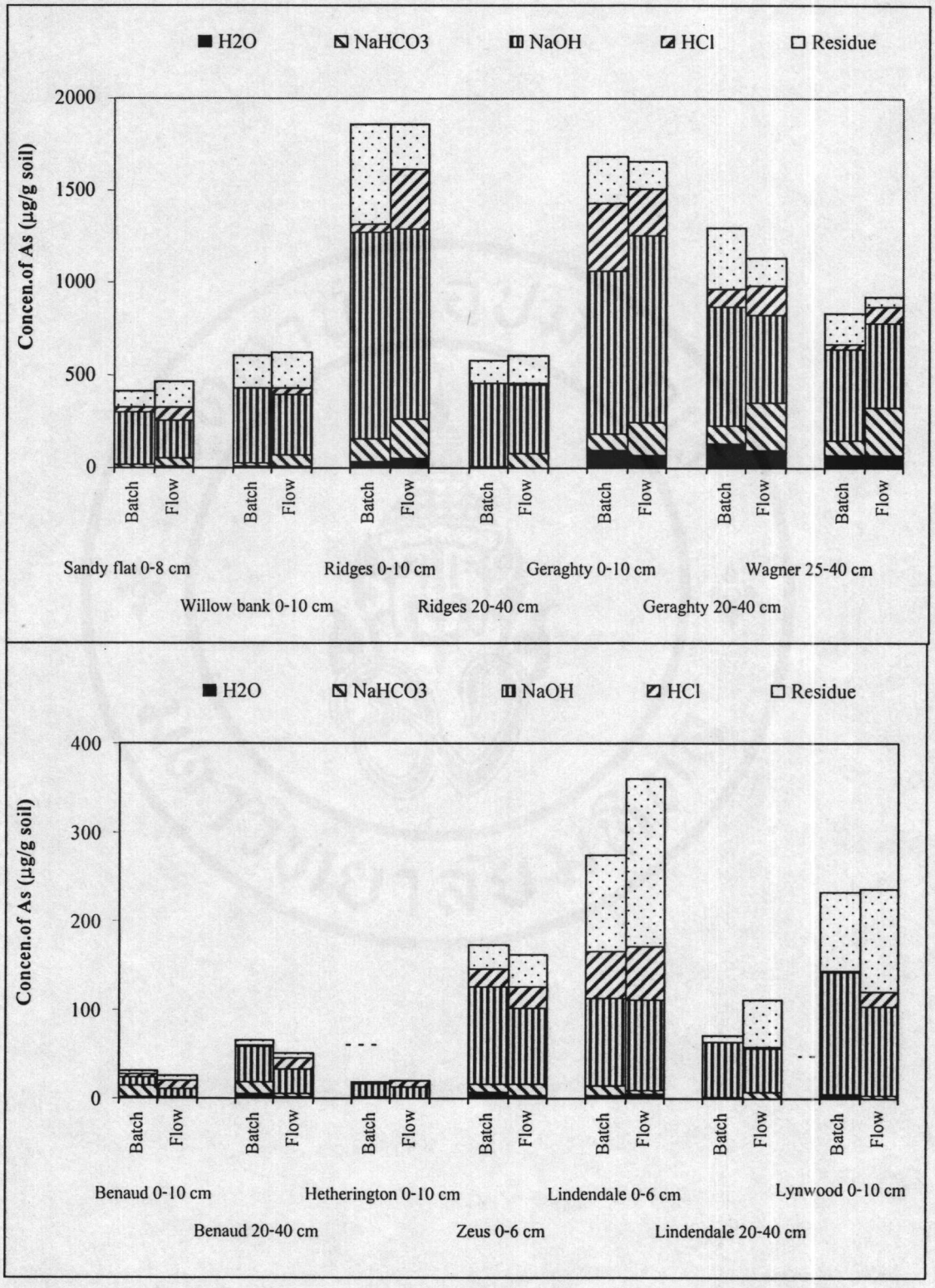


Figure 3.15 Results of As fractionation using the continuous flow system and the batch system for soils from cattle dip sites in Australia (Data of Batch system from McLaren [28])

3.3.5 Association of elements in soil phases

The development of continuous flow sequential extraction system does not only give the quantity of As in soils, but also give the information of As association with many elements in soils. The extractograms of As, Fe, Al and Ca fractionation in soils are shown in Figure 3.16

A. SRM 2704 and SRM 2710

In Figure 3.16 the sediment and soil, As was found to distribute quite evenly in $NaHCO_3$, NaOH and HCl fractions. The overlayed extractograms (A1, A2) indicated association of As with Al in the NaOH fraction in sediment and with Al and Fe in soil. As in HCl fraction were associated with Ca, Al and Fe.

B. Cattle dip sites, Australia

For soils from Cattle dip sites Australia, As were found to be mostly in the NaOH and HCl fractions. The extractograms (B3 ,B4, B5) showed the association of As with Al and Fe in the NaOH fraction in soils. As in HCl fraction were associated with Ca, Al and Fe.

C. Soil from contaminated sites, Thailand

The soils from Yala province (C6) and Ronphiboon, Nakornsrithammarach province (C7, C8), As were found to be mostly in the NaOH fraction and moderately in HCl. The Overlayed extractograms (C6, C7, C8) indicated that the dominant As in this site associated with Al, and Fe oxides in soils obtained in the NaOH fraction. As in HCl indicated the association of As with Ca, Fe and Al.

The extractograms in Figure 3.16 showed the dominant As species were present in NaOH solution (step III), the lower As fraction was dissolved in HCl, $NaHCO_3$, and H_2O, respectively. The Fe traces showed the soluble Fe concentrations strongly obtained in HCl fraction (step IV), while dissolved Al obtain in both NaOH and HCl solutions. The Ca lines were presented in HCl fraction owing to Ca-compounds was unstable at low pH.

All samples seemed to show the highest As concentration in the NaOH fractions. Many workers (Sadiq [9], Woolson [32], Jacobs [16], McLaren [28]) have suggested that As is strongly sorbed by many materials such as the amorphous Fe and Al oxides which are the dominant composition of soils. Sadiq [9] have reported that the high pH enhances net negative charge on the soil surface (Figure 1.2) and there As oxyanions can be released from the negative charge of soil surfaces to the extractant because of the repulse of the same charge. Tiessen [34]and McLaren [28] have reported that hydroxide-extractable As was associated with amorphous Fe and Al oxide and some crystalline Fe and Al arsenate. The Al traces obtained in the

extractograms showed the high Al concentration in NaOH, from the dissolution of Al oxide/hydroxide at high pH. The Fe concentrations in NaOH fraction are lower than Al concentration because Fe oxide/hydroxide is more stable at high pH. Furthermore, Sadiq [20] suggests that $FeAsO_4$ (crystalline, (c)) and $AlAsO_4$ (c), may form in As contaminated soils and dissolve in alkaline solution as NaOH.

In most cases, the soluble As presented in the HCl fraction was lower than in the NaOH fraction. Tiessen [34], Mclaren [28] reported that As that obtained in this extracts was from Ca compounds in soils. Sadiq [9] have reported that the Ca compounds in soil such as $Ca_3(AsO_4)_2$ (c) is stable in alkaline condition and unstable in acidic solution (Figure 1.3). The extractograms showed Ca traces in the final step (HCl fraction). Fe was also found at high concentration in HCl fraction than in NaOH fraction. The results indicated that some other forms of Fe which are not associated with As in soil may be present and dissolve at low pH.

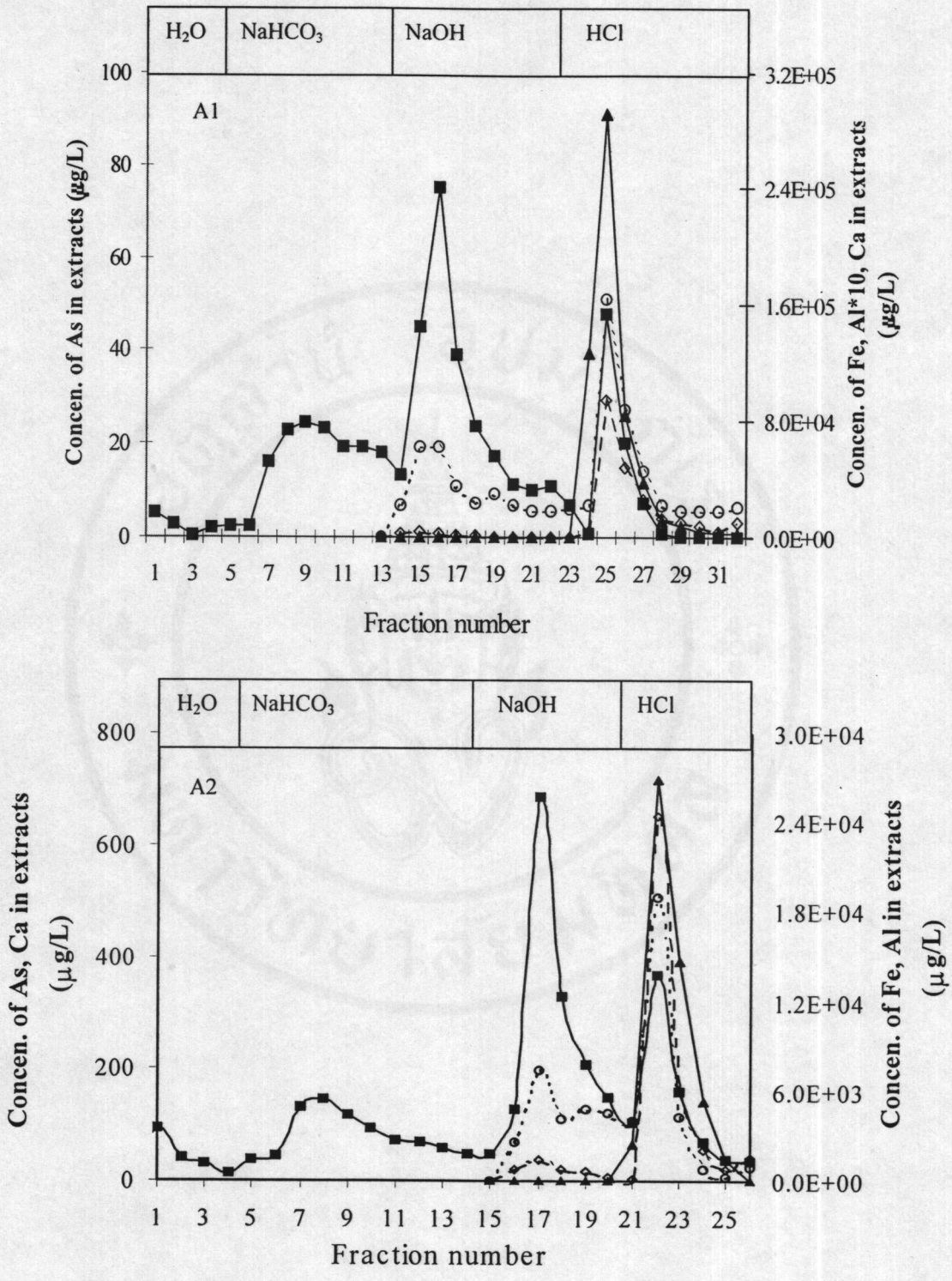


Figure 3.16 Overlayed extractograms showing association of As (■) with Fe (◇), Al (○) and Ca (△). A1) SRM 2704, A2) SRM 2710, B3) Lindendale 20-40 cm, B4) Zeus 0-6 cm, B5) Benaud 20-40 cm, C6) Tin Mining Yala province, 7C) Residential area and C8) Mining factory

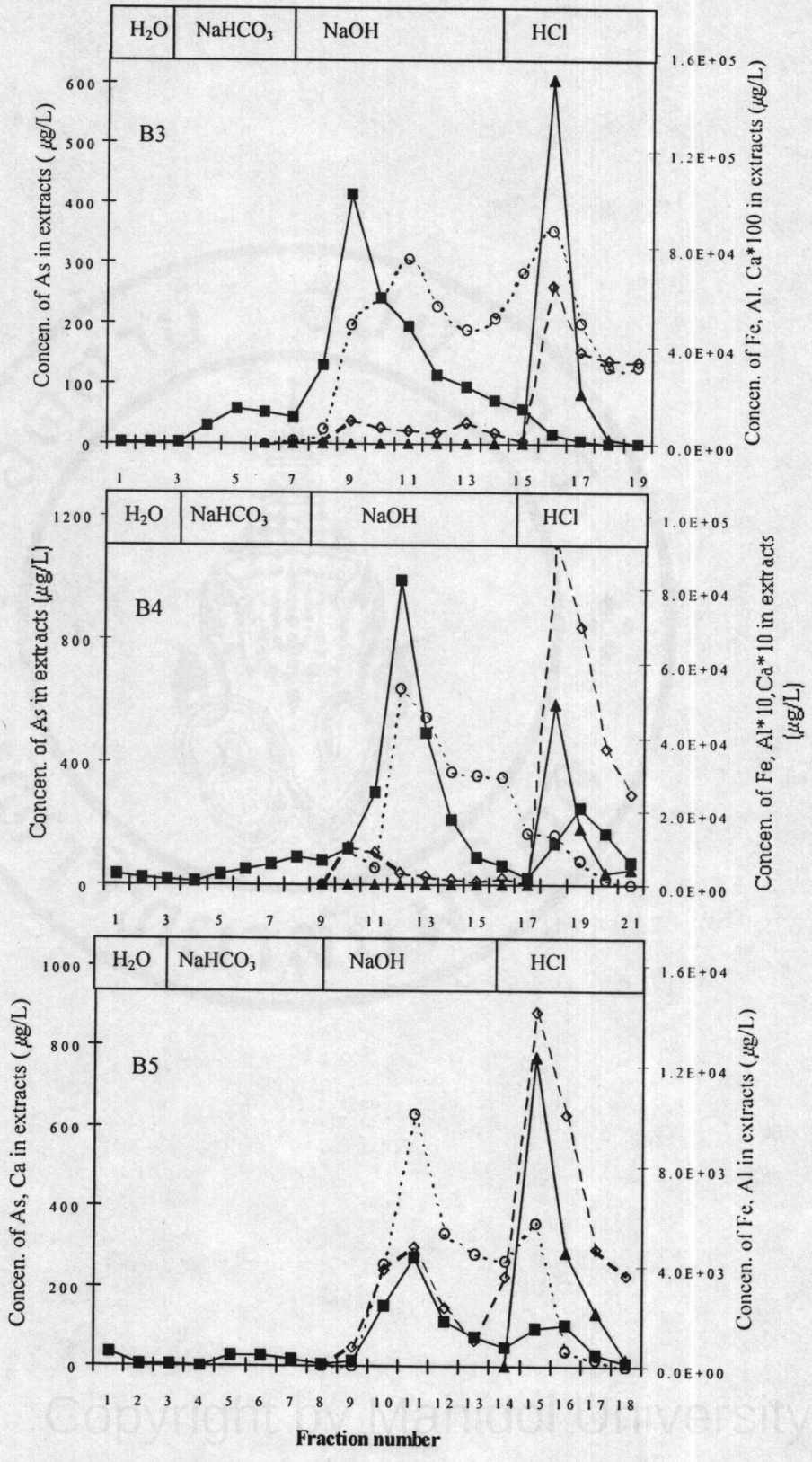


Figure 3.16 (Continued). (B3) Lindendale 20-40 cm, (B4) Zeus 0-6 cm, (B5) Benaud 20-40 cm

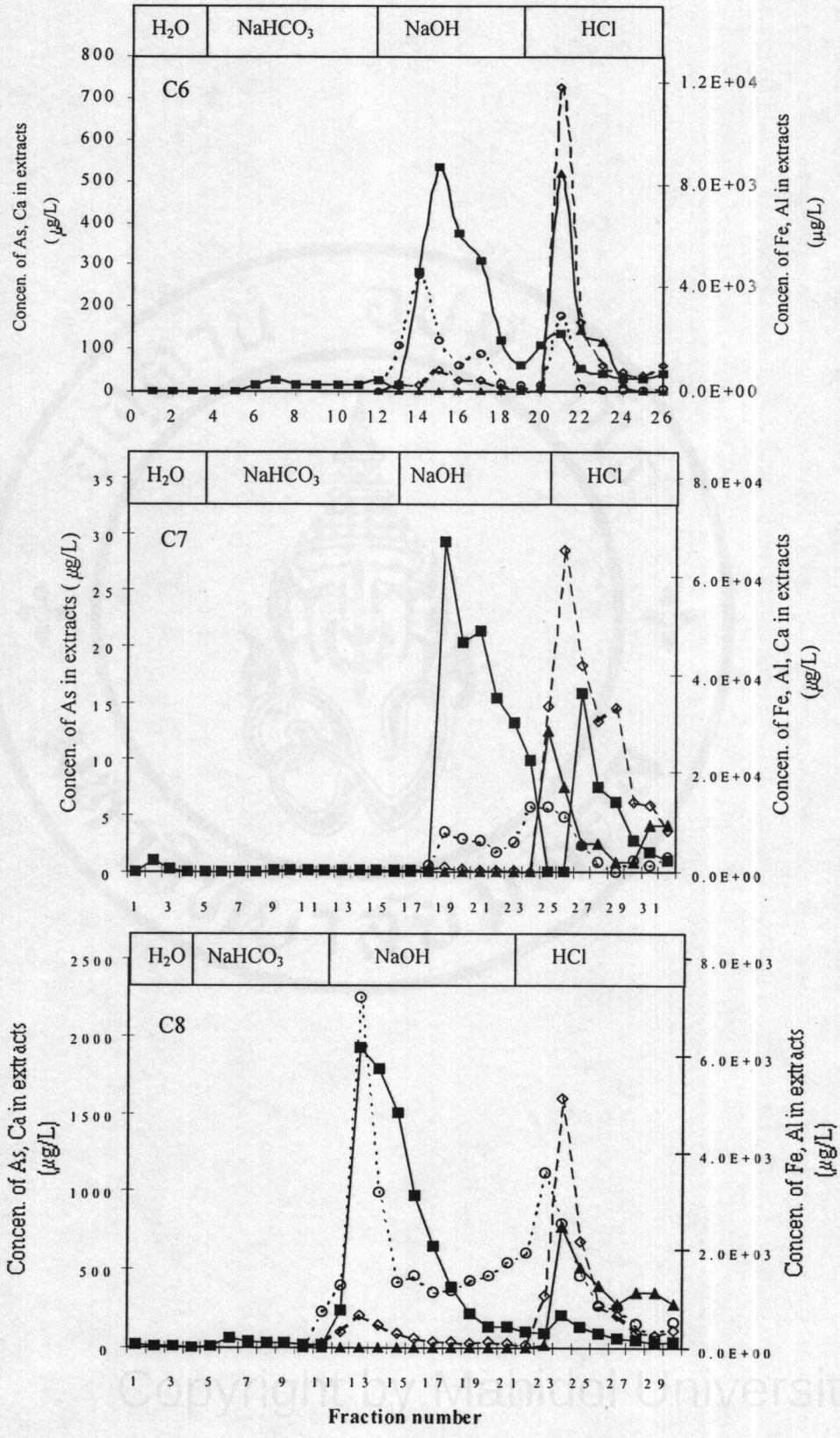


Figure 3.16 (Continued). C6) Tin Mining Yala province, 7C) Residential area and C8) Mining factory

CHAPTER IV

CONCLUSION

The existence of a multitude of As emission sources threatened to contaminate large areas of surrounding agricultural soils, industrial sites and mining factory. Arsenic is phytotoxic and hazardous to human health. It is generally recognized that total concentration of toxic elements in soil gives a good indication of extent of soil contamination, but it is not necessarily a reliable indicator of potential bioavailability or mobility. Sequential extraction of the element of interest in soil helps to differentiate between the forms of element that is readily labile and that is bound strongly by the soil.

The sequential extraction scheme for As was performed based on the P fractionation scheme reported earlier to investigated the chemical nature of As contaminated soils. The scheme used in this work consists of 5 extraction steps namely water-soluble (Step:1), $NaHCO_3$-extractable (Step:2), NaOH-extractable (Step:3), HCl-extractable (Step:4) and residual fraction.

The As content obtained from step 1and step 2 fractions provided a good index of potential As biavailability and mobility. The As in step 3 estimated the As in associated with the amorphous Fe and Al oxide in soil. The As in step 4 is the forms associated with Ca and the As final fraction is the recalcitrant from. The As obtained in step 3, step 4 and step 5 are considered to be slowly mobile (or immobile) fractions.

The batch sequential extraction is widely used for determination of As species in soil. In this work a continuous flow sequential extraction system for separating As species with subsequent ETAAS determination was used.

The different extractants can affect As signals by ETAAS. It was found that As signals increased at the presence of $NaHCO_3$ and NaOH. In contrast, the signals of As decreased in the presence of HCl. The decreasing signals of As in HCl can be overcome by adding NaOH. Pd 5 μg was added as modifier for stabilizing As to obtained high sensitivity of detection.

The slurry sampling ETAAS was used to determine As in residues and total As in soil, by using 75% glycerol as a suspension medium. The optimum condition of ashing and atomization are 1200 and 2400 °C, respectively.

Optimum conditions of extraction were investigated. The varying of sample/chamber volume ratio did not affect the extractable amounts of As. However, the sample/chamber volume ratio can affect the flow rate of extractant in the flow system. The flow rate of extractant increased with decreasing sample/chamber volume ratio. Therefore, the 10-ml of chamber is used for As fractionation by decreasing the error of weighing.

The evaluation of the continuous flow sequential extraction system was performed by comparing the sum of As species in each fraction with the total As concentration from certified values of CRMs. The results were in good agreement.

The continuous flow system was found to benefit the extraction by less extraction time and simple extraction procedure.

This system of extraction was applied for As fractionation of contaminated soils in Ronphiboon, Thailand and a cattle dip sites, Australia.

The soils from Ronphiboon, Thailand were taken form 2 sites. The first site contained 5-30 ppm As was the residential area. The other site, Mining factory contained 300-700 ppm. The important As concentration are appearance in fraction of NaOH. It indicated that the most As in soil associated with Fe and Al oxide/hydroxide that dominant composition of soils.

The As in soil from cattle dip sites, Australia as determined by the batch system and the continuous flow system were found to be comparable. The third step (NaOH), the As concentrations are strong appearance. The As in this fraction is from As associated with Fe and Al oxide/hydroxide in soils.

For all the soil samples, As was mostly found in the NaOH fraction. Low proportion of As in step 1 and step 2 does not mean that As toxicity is unlikely to be a problem. The extremely high total As concentrations means that the labile forms of As are often well above the levels likely to cause phytotoxicity.

The continuous flow sequential extraction system gives additional information of As association with the other elements soil. The extractograms of As, Fe, Al and Ca showed that low concentration of As in the first two fractions. The other elements can not be observed in this fraction. The third step (NaOH), the important As concentration are appearance because the high pH can change the charge of soil surface, that dominant composition of As adsorption, from positive charge to negative charge. Since As oxyanions is released to NaOH solution owing to As oxyanions and soil surface, are Fe and Al oxides/hydroxides, have the same charge. The strongly dissolved Al are present in NaOH solution because Al oxides can dissolved at high pH. The final fraction, As associated with Ca are extracted because Ca compounds are unstable in HCl.

APPENDIX

Data of arsenic fractionation in soils

Table 1A Arsenic fractionation by the batch and the continuous flow sequential extraction system for soils from Ronphiboon, Thailand.

Sample	System	As concentration in sample (μg As g^{-1} soil)					Sum
		H_2O	$NaHCO_3$	NaOH	HCl	Residual	
Residential area <106 μm	Batch	n.d.	n.d.	6.1	1.8	22.1	33.1
	Flow	n.d.	n.d.	8.4	3.1	19.3	30.8
Residential area 106-150 μm	Batch	0.2	n.d.	2.1	1.0	4.8	8.1
	Flow	0.3	0.2	2.5	0.3	5.0	8.2
Residential area >150 μm	Batch	n.d.	n.d.	2.1	0.9	2.7	5.7
	Flow	n.d.	n.d.	4.3	1.6	5.1	11.0
Mining factory <106 μm	Batch	7.9	4.4	311.0	54.3	325.7	703.2
	Flow	4.0	20.2	312.6	35.8	275.0	647.6
Mining factory 106-150 μm	Batch	6.3	2.2	258.0	33.7	77.3	377.5
	Flow	1.9	7.8	267.5	26.7	195.2	469.1
Mining factory >150 μm	Batch	3.4	1.1	196.6	19.3	25.9	246.3
	Flow	1.3	5.6	208.7	21.3	83.8	320.7

Table 2A Arsenic fraction distribution by the batch and the continuous flow sequential extraction system for soils from Ronphiboon, Thailand.

Sample	System	As fraction distribution (%)					Sum
		H_2O	$NaHCO_3$	NaOH	HCl	Residual	
Residential area <106 μm	Batch	0	0	18.4	5.4	66.8	100
	Flow	0	0	27.2	10.0	62.8	100
Residential area 106-150 μm	Batch	2.5	0	25.9	12.3	59.3	100
	Flow	3.9	1.7	30.9	3.9	61.1	100
Residential area >150 μm	Batch	0	0	36.8	15.8	47.4	100
	Flow	0	0	38.8	14.2	46.9	100
Mining factory <106 μm	Batch	1.1	0.7	44.2	7.7	46.3	100
	Flow	0.6	3.1	48.3	5.5	42.5	100
Mining factory 106-150 μm	Batch	1.5	0.6	62.8	8.2	26.9	100
	Flow	0.4	1.6	53.6	5.3	39.1	100
Mining factory >150 μm	Batch	1.4	0.4	77.4	7.6	13.2	100
	Flow	0.4	1.7	65.1	6.7	26.1	100

Table 3A Arsenic fractionation by the batch and the continuous flow sequential extraction system for soils from Cattle dip sites, Australia.

Sample	System	As concentration in fractions (μg As g^{-1} soil)					Sum
		H_2O[a]	$NaHCO_3$	NaOH[b]	HCl	Residual	
Sandy flat 0-8 cm	Batch	4	15	279	26	88	412
	Flow	6.5	42.1	209.0	64.5	140.9	463
Willow bank 0-10 cm	Batch	5	22	401	0	177	605
	Flow	12.6	61.6	322.7	33.4	192.6	622.9
|Ridges 0-10 cm	Batch	33	127	1116	38	548	1860
	Flow	55.7	208.5	1027.9	317.6	249.4	1859.1
Ridges 20-40 cm	Batch	3	5	445	0	130	583
	Flow	2.0	73.7	374.8	8.1	146.2	604.8
Geraghty 0-10 cm	Batch	95	87	876	371	259	1688
	Flow	68.3	177.9	1007.0	256.8	50.7	1660.7
Ge raghty 10-40 cm	Batch	133	92	644	95	335	1300
	Flow	95.4	259.8	465.6	158.2	149.2	1128.2
Wagner 25-40cm	Batch	73	80	487	28	167	835
	Flow	73.5	247.0	463.0	81.5	56.8	921.8

[a] resin-extractable As used in the batch system

[b] NaOH-extractable+sonication/NaOH-extractable As in a batch system (Data from RG. McLaren [28])

Table 3A Arsenic fractionation by the batch and the continuous flow sequential extraction system for soils from Cattle dip sites, Australia (Continued).

Sample	System	As concentration in sample (μg As g^{-1} soil)					Sum
		H_2O[a]	$NaHCO_3$	NaOH[b]	HCl	Residual	
Benaud 0-10 cm	Batch	2	13	8	5	3	31
	Flow	0.9	1.1	8.8	8.7	5.6	25.1
Benaud 20-40 cm	Batch	5	13	40	2	6	66
	Flow	2.2	3.5	27.2	12.4	6.3	51.6
Hetheringt on 0-10 cm	Batch	1	0	15	2	0	18
	Flow	0.5	0	13	5.7	1.3	20.5
Zeus 0-10 cm	Batch	7	9	109	21	27	173
	Flow	2.9	14.2	84.6	24.6	35.3	161.6
Lindendale 0-6 cm	Batch	4	10	98	54	109	275
	Flow	5.7	4.3	100.3	59.7	190.1	360.1
Lindendale 20-40 cm	Batch	1	0	62	0	8	71
	Flow	0.4	7.0	48.6	3.0	51.4	110.4
Lynwood 0-10 cm	Batch	3	3	135	2	90	233
	Flow	0.1	3.4	100.2	16.0	116.1	235.8

[a] resin-extractable As used in the batch system

[b] NaOH-extractable+sonication/NaOH-extractable As in a batch system (Data from RG. McLaren [28])

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