CHAPTER 5 - DEVELOPMENT OF A SEQUENTIAL EXTRACTION SCHEME TO FRACTIONATION SOIL ARSENIC AND ANTIMONY

5.1. INTRODUCTION

The current project aims to examine the fate and behaviour of Sb with comparisons to As in the study site as defined in Chapter 3, and in particular its interactions with acid sulfate processes in some of the agricultural and aquatic ecosystems of the floodplain. In order to better understand Sb behaviour and mobility in such environments, there is a need to characterise the chemical forms and phase associations of Sb (and As) in these soils. One approach, reviewed in some detail in Chapter 2, is to extract the soil with a sequence of reagents, each designed to dissolve the element in question from a particular soil pool or phase (Hirner 1991). This approach can be used to infer soil phase distributions of contaminants and nutrients, and may be used to indicate bioavailability (Gummuluru et al. 2002; Krishnamurti and Naidu 2000). In contrast to more direct methods of contaminant speciation such as HPLC-ICP-MS, sequential soil chemical extractions also have the advantage of being relatively cheap and widely available to most, if not all laboratories.

As identified in Chapter 2 as a requirement for future research, any sequential extraction scheme for Sb (and As) must be applicable to the soils under investigation, and must also consider the properties and behaviour of the elements or metalloids being examined. As described in Chapter 2, many aspects of Sb chemistry are similar to those of As and P. Even with the differences in ionic radii, structure and protonation effects (Chapter 2, section 2.4), it is to be expected that sequential extraction schemes that have been used for P and As (Filella et al. 2002a; Matera and Le Hecho 2001) should be more appropriate for Sb than those designed for cationic metal contaminants (Tessier et al. 1979). Single and sequential extractions for P have been in development for several decades, driven by its importance as a plant nutrient, with much of the work from the 1980s onwards being based on the original scheme of Hedley et al. (1982a; 1982b). Basically, Hedley's scheme used 0.5 M $NaHCO_3$ to extract exchangeable and labile P, 0.1 M NaOH to extract organic and chemisorbed P, 1 M HCl to target more resistant phases (e.g. apatite), and a strong acid digest to

dissolve a residual P fraction associated with silicate minerals. A notable omission from this sequence was a specific extractant for the amorphous Al and Fe hydroxide phase, which strongly adsorbs phosphate. Guo and Yost (1999) have demonstrated the importance of non-crystalline phases for binding P in some acid soils (Chapter 2, section 2.7.2).

Some extensions of P extraction methods for As in soil have occurred. Woolson et al. (1971) showed significant correlations between plant growth reductions and mixed acid or bicarbonate based extracts for As in soils. As with P, a strong association between As and non-crystalline hydroxides has been repeatedly demonstrated via oxalate or hydroxylamine hydrochloride based extractions (e.g. McLaren et al. 1998; Chapter 2, section 2.5.3).

Research into the use of sequential extractions to characterise Sb associations in soil has been more limited (Lintschinger et al. 1998; Wilson et al. 2004). The role of organic fractions, labile concentrations, non-crystalline hydroxide and residual phases has not been determined for acidic soils with moderate levels of Sb enrichment (< 40mg kg^{-1}) such as those occurring on the Macleay Floodplain. In this chapter the sequential extraction scheme of Hedley et al. (1982a) was compared with a modified scheme which was applied to two soils from the study site. The aim was to develop the necessary analytical methods for ICP-OES analysis of the extracts at the University of New England, Armidale NSW Australia (UNE), and to compare the original and modified extraction schemes as they were applied to 2 test soils from the study area, in preparation for routine use of the extract sequence in later experiments (Chapter 7). Extractant efficiencies for Sb removal from these soils are compared to those for As and P and inferences are made in terms of elemental soil phase distribution.

5.2. METHODS

5.2.1. General analysis

Excluding the $NaHCO_3$ extract, extracts were analysed for Al, Fe, P, As and Sb via a Varian Vista MPX CCD ICP-OES equipped with a Vista SPS-5 autosampler at the University of New England (UNE), NSW, Australia. All standard solutions and

extracts were prepared with analytical grade reagents and Milli-Q water. Sodium bicarbonate extracts were analysed commercially via ICP-MS at ALS Environmental, Brisbane, Queensland, Australia, for As and Sb. Commercial relative standard deviations (RSDs) were < 12.2 % for duplicate analyses of both elements, with spiked sample recoveries of 94 - 122 %.

For the detailed comparisons between soil 1 and 2 (section 5.2.3), P concentrations in the 0.5 M $NaHCO_3$ extracts were analysed via ICP-OES at UNE. Phosphorus concentrations in $NaHCO_3$ and NaOH extracts had only limited quality assurance and quality control (QA/QC), specifically the calculation of extract RSDs and the inclusion of the $NaHCO_3$-P and NaOH-P in the summation of soil P fractions (section 5.2.2). Discussion and interpretation of P concentrations in these extracts when applied to soils 1 and 2 were limited to comparisons with published P data and extracted As and Sb concentrations, and are not included in further extraction based studies (Chapter 7). Due to the verification of acceptable extract summations of each element (Table 5.7), all elemental data are presented as the concentration within a phase compared to the soil total concentration:

$$\% = (E_i / \Sigma^5_{i=1} E_i) \times 100$$

Where % = elemental concentration in phase i as a percent of the total soil concentration.

E = elemental concentration in phase i (e.g. μg ml^{-1}).

i = phase of interest (e.g. soil extract concentration).

Aluminium and Fe concentrations in the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ extract have been presented as mg kg^{-1} values (Figure 5.3).

5.2.2. Method development

For the extracts detailed in Table 5.3, several complementary approaches were undertaken for QA/QC and method development. These were:

Optimisation of ICP-OES parameters

Viewing height (mm), nebulizer pressure (kPa) and power (kW) were varied sequentially while signal intensity was recorded for P, As and Sb for the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ and 0.1 M NaOH extracts. Other parameters were not altered and are presented in Table 5.1. The signal to background ratio (SBR) was taken as an indicator of optimisation by maximising the raw counts per second at which SBR ≤ 2 (Varian 2000), using the lowest standard solution as a constant (Table 5.1). Aluminium and Fe were omitted, as preliminary investigations showed the SBR for any setting was never less than 2 (due to the high concentrations of each element in the standard solutions).

Independent laboratory verification

Aliquots of the 0.1 M NaOH and 1 M HCl extracts (n = 9) from a range of soils were analysed via ICP-MS at ALS Environmental for As and Sb, and also via ICP-OES at UNE. Metalloid concentrations detected were compared to assess any bias in analysis.

Method detection limits

Method detection limits for ICP-OES analysis of Al, As, Fe, P and Sb in the 0.1 M NaOH and the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ extracts were calculated, using the method of the US EPA (1986a), which is based on the variability inherent in repeated analyses of the standard solution with the lowest elemental concentrations. Initially detection limits for the 1 M HCl extract were estimated from continuous calibration variability, based on the more rigorous methods of the US EPA (EPA 1986a). However, actual detection limits were not calculated, as the majority of extracts showed non-detectable concentrations of metalloids, and the use of this extract was discontinued (section 5.2.3). Detection limits for the aqua regia digestion used to determine residual elemental fractions are presented in Chapter 4 and Tighe *et al.* (2004), and are restated in Table 5.6. Detection limits of the NaHCO₃ extract were determined commercially. These limits are not given, as no results from this extract were below detection.

Table 5.1. Conditions for ICP-OES parameter optimisation for 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ and 0.1 M NaOH extract analysis.

Operating conditions	
Polychromator	Echelle
Grating	94.74 lines mm^{-1}
Power	Varied
Frequency	40 MHz
Plasma flow	15.0 L min^{-1}
Auxiliary flow	1.5 L min^{-1}
Nebulizer flow	Varied
Viewing height	Varied
Replicate read time	45 s
Instrument stabilisation delay	30 s

Sample introduction settings	
Sample uptake delay	30 s
Pump rate	15 rpm
Rinse time	30 s

Element	Wavelength (nm)	Standard concentration (μg ml^{-1})	
		0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O$	0.1 M NaOH
Al	396.152	37.5	0.5
As	188.980	0.25	0.25
Fe	238.204	200	1.0
P	213.618	8.75	0.5
Sb	217.582	0.25	0.25

Analysis of spiked samples

As independent laboratory validation of the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ extracts was not possible (due to funding constraints), extract aliquots spiked with known concentrations of Al, As, Fe, P and Sb (standard concentrations given in Table 5.1) were measured via ICP-OES at UNE during routine analysis. Element recoveries (percent of known additions) were calculated to determine suppression or amplification effects during ICP-OES analysis (Table 5.7).

Summation analysis of extracted soil fractions

For a subset (n = 18) of soil samples with varying elemental concentrations and properties, the summation of the metalloid and co-occurring element concentrations across the individual extracts were compared with the total aqua regia extractable element values (Table 5.7).

5.2.3. Comparison of extractions

Characteristics of the soils used for the extraction comparisons

The location, land uses and environmental details of the Macleay catchment coastal floodplain, northeastern NSW, Australia, are described in Chapter 3. For the detailed extraction comparisons two soils were used from the study area (Table 5.2). Soils were sampled to a depth of 100 mm with a stainless steel spade, with a composite of 4 – 5 sub samples in each location to provide a sample of 1 – 1.5 kg. Visible plant material and surface organic matter were physically removed prior to sampling. Soils were air-dried, ground and sieved to < 150 μm. Air-drying was undertaken as this approach was used in the subsequent glasshouse trial (Chapter 7). The justification for this is given in Chapter 7.

Electrical conductivity (EC) (μS cm^{-1}) and pH (1:5 soil:water) were measured using a TPS-81 combination meter (TPS Pty. Ltd. Brisbane, Australia). Loss on ignition (LOI) organic matter was determined at 400°C. Determining loss on ignition at < 500°C minimises decomposition of hydrated minerals (Schulte and Hopkins 1996), and is taken here as an indicator of soil organic matter content. Particle size distribution was determined using the pipette method (Day 1979). Results are detailed in Table 5.2. Relative to soil 1, soil 2 was more acidic (an actual acid sulfate soil), more saline and had higher proportions of organic matter and clay. Both soils had elevated concentrations of As and Sb compared to the catchment calculated background values as described in Chapter 3. Soil 1 (n = 3) was used in the comparison of the original extraction scheme with the modified (+ the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ step) scheme. Soil 2 (n = 10) was used to compare the extraction efficiencies (or relative extraction strength) of the extracts used in the modified scheme.

Table 5.2. Properties of soils selected for the extraction comparisons. Elemental values are given in mg kg^{-1}.

	Soil 1	Soil 2
pH $_{1:5 \text{ Water}}$	5.40	3.55
EC$_{1:5 \text{ Water}}$ (µS/cm)	152	1726
Loss on ignition 400°C (%)	5.9	22
Texture[†]	Silty clay loam	Silty clay
P	846	696
As	30.5	22.5
Sb	26.9	23.0
Fe	31750	35400
Al	89520	44670

[†] McDonald *et al.* (1990).

The modified sequential extraction scheme

The original scheme of Hedley *et al.* (1982a) was not used in its entirety, due to the moderate (22.5 – 30.5 mg kg^{-1}) total soil concentrations of As and Sb in the floodplain soils. Anion resin extraction and aliquot inorganic/organic partitioning as described in the original scheme were omitted as it was expected that the further partitioning would result in concentrations below method detection limits. The basic original extraction scheme of Hedley *et al.* (1982a) without these steps was adapted for the modified scheme by the insertion of a 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ extraction after the initial bicarbonate extraction, as presented in Table 5.3. The extraction scheme shown in Table 5.3, without the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ step, was taken as the original scheme of Hedley *et al.* (1982a) and is referred to as such in the remainder of this chapter.

Preliminary analysis of soil 1 showed non-detectable levels of As and Sb in the 1 M HCl extract, and this extract was not applied to soil 2.

Table 5.3. The extraction sequence, modified from that of Hedley *et al.* (1982a).

Extractant	Method	Target association	Reference
0.5 M NaHCO$_3$	To 1.5 g soil, add 30 ml, shake for 16 hrs. Centrifuge. Filter (Whatman no. 1) and dilute to 50 ml	Freely exchangeable and readily labile fractions	Hedley *et al.* (1982a)
0.2 M C$_2$H$_2$O$_4$ - (NH$_4$)$_2$C$_2$O$_4$	To residue add 30 ml, shake for 4 hours in the dark. Centrifuge. Filter (Whatman no. 1) and dilute to 50 ml	Moderately reducible phase (primarily amorphous and poorly crystalline Al and Fe oxyhydroxides)	Guo and Yost (1999); Lintschinger *et al.* (1998)
0.1 M NaOH	To residue add 30 ml, shake for 16 hrs. Centrifuge. Filter (Whatman no. 1) and dilute to 50 ml	Elements sorbed by Fe and Al components	Hedley *et al.* (1982a)
1 M HCl	To residue add 30 ml, shake for 16 hours. Centrifuge. Filter (Whatman no. 1) and dilute to 50 ml	Elements in partially resistant phases (e.g. apatite type minerals and strongly occluded elements)	Hedley *et al.* (1982a)
Aqua regia	To residue add 9 ml conc. HCl and 3 ml conc. HNO$_3$, digest, filter (Whatman no. 1) and make to volume	'Residual' such as primary minerals including partial dissolution of silicate minerals	

Oxalic acid/ammonium oxalate extractable Fe and Al

To determine whether the 0.5 M NaHCO$_3$ extract step in the sequential scheme altered the amount of Al or Fe in the following oxalate extract, the concentrations of these elements in the oxalate step of the sequential program were compared to a single oxalate extract of soil 1 and 2 (n = 3).

Relative extraction strengths for P, As and Sb

Applying the modified sequence to soil 2, the relative strength of each extract (taken as the proportion of each element extracted in each step relative to the total soil elemental concentration extraction) was determined for P, As and Sb. This was also investigated using the values obtained from the comparisons of the two extraction schemes as applied to soil 1.

Statistical analysis and further quality control

Arsenic and Sb concentrations extracted with NaOH and with HCl and quantified by independent ICP-MS and 'in-house' ICP-OES were compared using linear regression, with R^2 values denoting the extent of the linear relationship. Comparisons between Fe and Al extracted with the single oxalate extract and the oxalate extract following the 0.5 M $NaHCO_3$ extract were made using a 2-sample t-test. All other statistical tests were performed using linear and general linear modelling, with appropriate contrasts and pairwise comparisons specified. All statistical analyses were performed using R version 1.6.2 (Ihaka and Gentleman 1996). Precisions of analyses are given both as standard errors within tables and figures, and stated as relative standard deviations (RSDs) where applicable.

5.3. RESULTS

5.3.1. Method development

Parameter optimisation

Table 5.4 shows the optimal parameter settings for elemental analysis of the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ extract, based on the standard solution concentrations of As, P and Sb as given in Table 5.1, and are; viewing height = 4 mm, nebulizer flow = 0.45 L min^{-1}, power = 1.30 kW, with other parameters as given in Table 5.1.

While the 3 mm viewing height gave slightly higher counts per second for As, P and Sb (Table 5.4), there was a pronounced decline in intensities for Fe and Al (data not shown), due to plasma fluctuations at this height. The selected parameter settings were used during all further use of this extract.

Table 5.5 shows the optimal combination of parameters for analysis of the 0.1 M NaOH extract, based on the standard solution concentrations given in Table 5.1. These were; viewing height = 4 mm, nebulizer flow = 0.30 L min^{-1}, power = 1.40 kW, with other parameters set as presented in Table 5.1.

Table 5.4. Sequential optimisation of viewing height (mm), power (kW) and nebulizer flow ($L\ min^{-1}$) settings for ICP-OES analysis of the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ standard solution. Elemental units are in raw counts per second (intensities). Selected parameter combinations are given in bold.

Settings	Varied parameter	P	As	Sb
Viewing height = varied,	9	609	122	225
power = 1.4,	8	424	87	166
nebulizer flow = 0.5	7	489	99	191
	6	496	101	197
	5	621	123	227
	4	**759**	**147**	**260**
	3	832	156	277
Viewing height = 4,	0.40	776	148	269
power = 1.4,	**0.45**	**817**	**155**	**277**
nebulizer flow = varied	0.55	723	142	258
	0.60	807	155	266
Viewing height = 4,	1.35	737	143	248
power = varied,	**1.30**	**947**	**183**	**305**
nebulizer flow = 0.45	1.25	837	163	276

Similar to the oxalate extract, the 3 mm viewing height gave slightly higher counts per second for P and Sb (Table 5.5), but there was a pronounced decline in intensities for Fe and Al (data not shown). These parameter settings were used during all further use of this extract.

Detection limits

The calculated detection limits are presented in Table 5.6. The high detection limits for Al and Fe in the oxalate extract were a direct result of the concentrations of these elements in the standard solution used (Table 5.1), which were chosen to provide a wide linear calibration curve for the ranges of Al and Fe expected in samples. The method detection limits for Sb in both the oxalate and NaOH extracts were also elevated compared to those for 40 % aqua regia.

Table 5.5. Sequential optimisation of viewing height (mm), power (kW) and nebulizer flow (L min^{-1}) settings for ICP-OES analysis of the 0.1 M NaOH standard solution. Elemental units are in raw counts per second (intensities). Selected parameter combinations are given in bold.

Settings	Varied parameter	P	As	Sb
Viewing height = varied,	9	352	77	152
power = 1.4,	8	415	87	179
nebulizer flow = 0.5	7	428	90	178
	6	440	92	181
	5	453	94	187
	4	**461**	**95**	**191**
	3	468	94	192
Viewing height = 4,	0.45	486	99	203
power = 1.4,	0.40	535	109	226
nebulizer flow = varied	0.35	598	121	262
	0.30	**633**	**129**	**298**
Viewing height = 4,	1.35	583	121	287
power = varied,	1.30	560	116	278
nebulizer flow = 0.30				

Table 5.6. Elemental method detection limits (µg ml^{-1}) in the $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ and NaOH soil extracts and the aqua regia digestion.

Element (nm)	0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$	0.1 M NaOH	Aqua regia (40 %)
Al (396.152)	10.8	0.26	6.28
As (188.980)	0.06	0.05	0.05
Fe (238.204)	34.4	0.08	6.89
P (213.618)	1.22	0.05	0.22
Sb (217.582)	0.12	0.16	0.04

Independent laboratory verification

Figure 5.1 shows the linear relationships and the theoretical 1:1 relationship between the metalloid concentrations extracted with NaOH (a) and HCl (b) and analysed by both independent ICP-MS and ICP-OES at UNE.
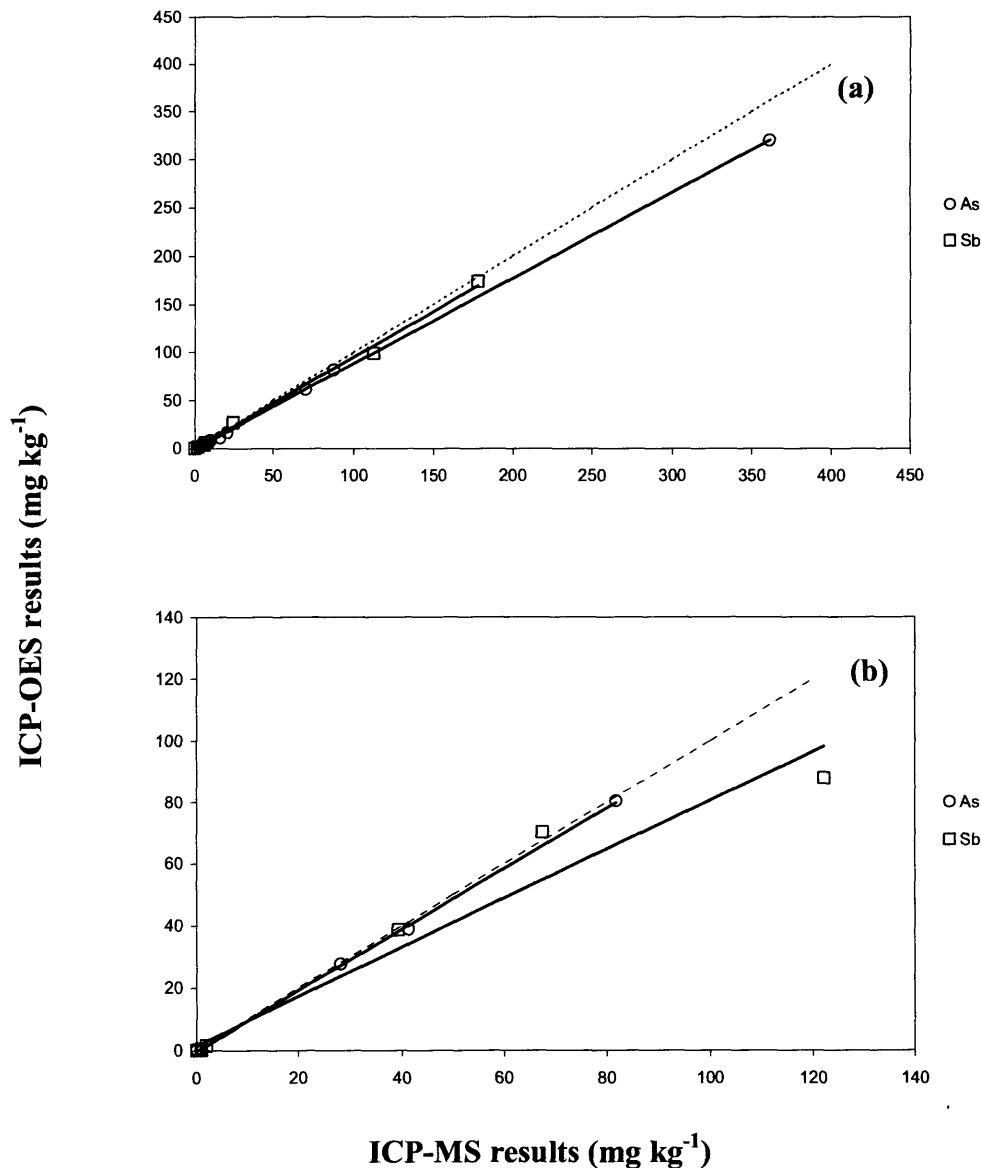
Figure 5.1a, b. The relationship between independent ICP-MS and 'in-house' ICP-OES analysis of As and Sb in 0.1 M NaOH (a) and 1 M HCl (b) extracts. Solid lines = linear regressions with $R^2 > 0.96$ for As and Sb. Dashed line = theoretical 1:1 relationship between ICP-MS and ICP-OES data.

There is a high level of agreement between the independent ICP-MS and UNE ICP-OES analysis of As and Sb in the 0.1 M NaOH extract. There is a noticeably lower amount of As detected by ICP-OES at high (> 200 mg kg^{-1}) concentrations of this metalloid, but at As extract concentrations < 100 mg kg^{-1} the relationship is very close to 1:1. Similarly, Figure 5.2b shows linear relationships between the ICP-MS and ICP-OES analysis for both As and Sb in the 1 M HCl extract over a wide range of concentrations. There is a decline in detection of Sb concentrations by ICP-OES at

higher levels (> 80 mg kg⁻¹) compared to the ICP-MS measurements, but, similar to the As concentrations in the NaOH extract, at lower HCl Sb concentrations (< 60 mg kg⁻¹) the ICP-OES:ICP-MS ratio is close to 1:1.

Spiked sample analysis and summations

The results of spiked sample analysis for the 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ extracts during routine analysis are shown in Table 5.7, as are the summation of elemental fractions.

Table 5.7. Percent recovery (± standard error) for spiked extracts of 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ (n = 4) and summation (percent ± standard error) of extract concentrations relative to aqua regia total elemental values (n = 18).

Element	Extract spike recovery	Summation of fractions
Al	107 ± 2.74	98.9 ± 2.05
As	122 ± 9.02	97.4 ± 2.56
Fe	94.5 ± 10.3	114 ± 1.97
P	93.1 ± 15.1	98.9 ± 2.23
Sb	106 ± 1.78	92.0 ± 4.53

Generally, Table 5.7 shows acceptable recoveries (as percent values) of elements in spiked samples. The average recovery of As was slightly higher than 100 % (121.9 %), while the spike recoveries of both Fe and P had relatively high standard errors. Table 5.7 also shows that the summation of extract concentrations is consistently close to 100 % of the total (aqua regia) soil element concentrations, including As and Sb. As both the spike recoveries and summation of fractions were within ± 20 % of the expected values, no corrections were made for subsequent analytical values.

While percent summations of Al and Fe in all soil extracts were close to 100 %, there was a lack of QA/QC data for Al and Fe measurements in the NaOH and HCl extracts. This negated their use in further analysis and interpretation (these elements in these extracts were not subject to independent laboratory verification or recovery calculation by sample spiking due to the additional cost of analysis). Acceptable spike recoveries for these elements in the oxalate extract (Table 5.7) did, however, prompt

the further use of oxalate extractable Al and Fe in the analysis and interpretation during the detailed soil comparisons of Chapter 7.

5.3.2. Comparisons of extractions

The fractionation of P, As and Sb

There were large differences in the fractionation of P, As and Sb in soil 1 when the oxalic acid – ammonium oxalate extraction step was included (Figure 3 a-c).
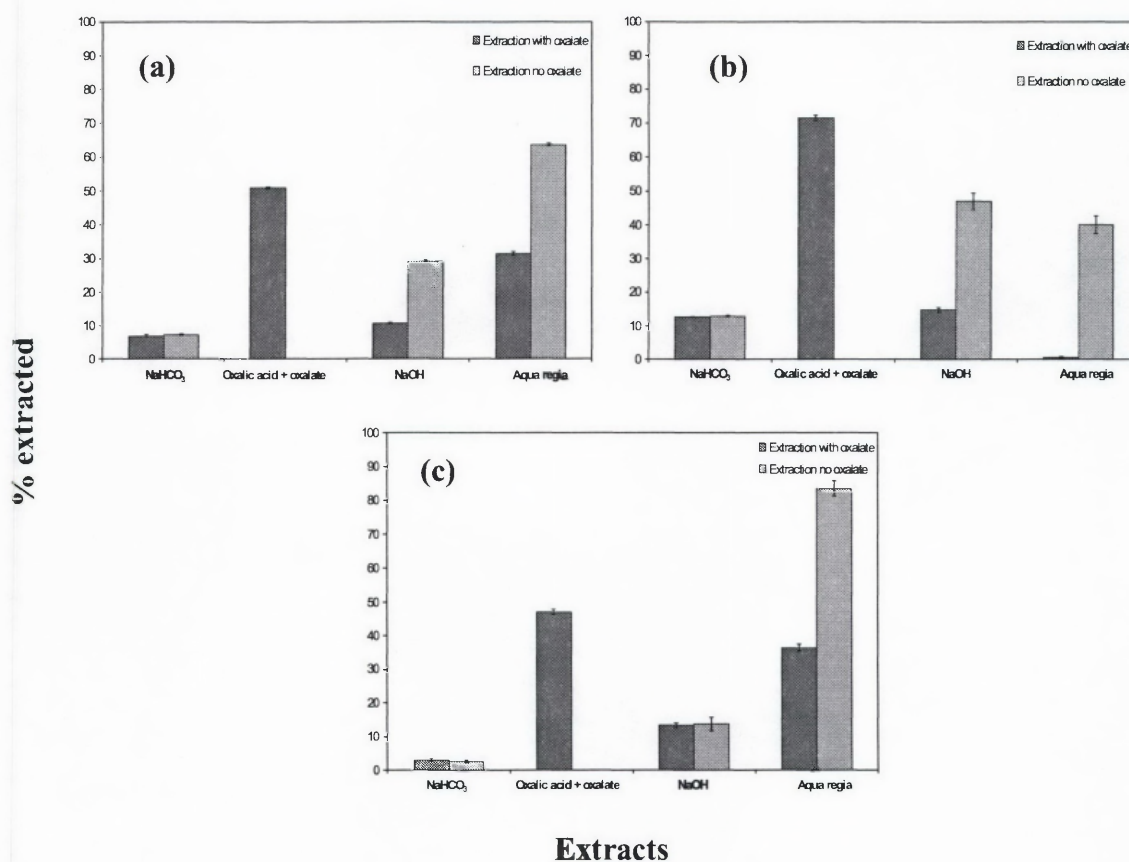

Figure 5.2a-c. Proportions of P (a), As (b) and Sb (c) extracted from soil 1 (n = 3) by the modified and original extraction schemes. Error bars are standard errors of the means.

The oxalate step removed 51, 71 and 47 % of P, As and Sb respectively. In the original scheme (i.e. in the absence of the oxalate step), these proportions of P and As were extracted in part by both the NaOH (P < 0.0001) and the aqua regia steps (P < 0.0001) (Figures 5.2a and b). For Sb in soil 1, the 47 % extracted by the oxalate step was extracted exclusively in the aqua regia fraction in the original scheme (Figure 5.2c) (P < 0.0001). In the absence of oxalate, the aqua regia fractions of P, As and Sb

were 32, 40 and 47 % higher than with the oxalate included (P < 0.0001). Analytical precisions varied. For P all RSDs were < 6.8 %. The aqua regia As fraction in the modified (+ oxalate scheme) had a high RSD (38 %) due to the residual As fraction being below the method detection limit. All other As fractions had RSDs < 13.9 %. The RSDs for Sb were highest (25.6 %) for the NaOH extract (in the absence of the oxalate step) and the $NaHCO_3$ extracts (19.4 – 21.9 %). All other extracts gave RSDs of < 8.6 % for Sb.

Oxalic acid/ammonium oxalate extractable Fe and Al

In both soil 1 and soil 2, the amount of Al and Fe extracted with 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ was not significantly different when the extract was used singly or following the 0.5 M $NaHCO_3$ extract (all P > 0.28) (Figures 5.3a and b).
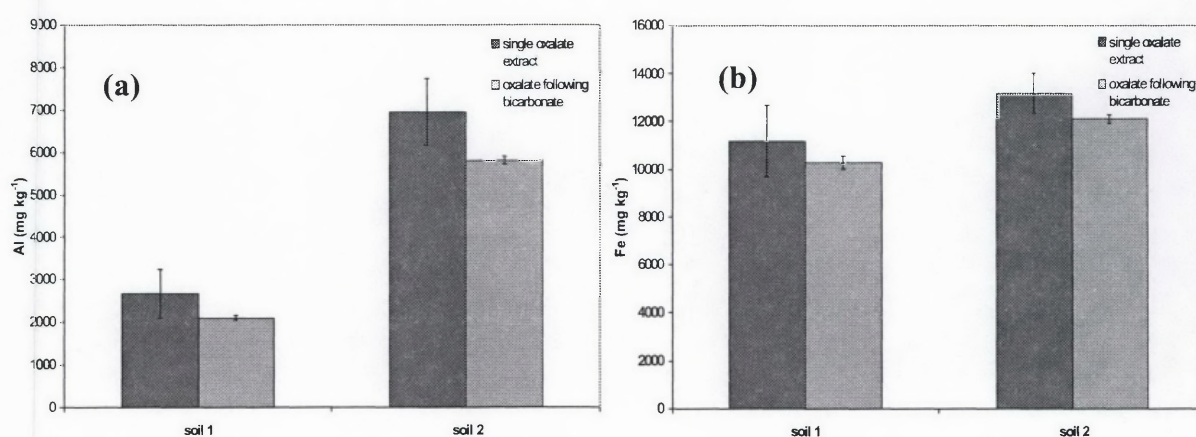

Figure 5.3a, b. Aluminium (a) and Fe (b) (mg kg^{-1}) extracted with 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ as a single extract and following the 0.5 M $NaHCO_3$ extract as part of the sequential extraction scheme in soils 1 and 2 (n = 3). Error bars are standard errors of the mean.

In soil 1, the oxalate extractable Fe and Al equated to 32 and 2.3 % of the soil totals of the respective elements. In soil 2, the oxalate extractable Fe and Al equated to 34 and 13 % of the respective soil total values. Across both soils, using 0.2 M $C_2H_2O_4$ - $(NH_4)_2C_2O_4$ as a single extract reduced the analytical precision. The average RSDs for Al and Fe in the single use oxalate extract were 28 and 17 % respectively. Following the $NaHCO_3$ extract, the RSDs for oxalate extractable Al and Fe were 3.47 and 3.48 % respectively.

The relative strengths of P, As and Sb extraction in the modified scheme were compared within each soil. For soil 2, differences are shown in Figure 5.4. For soil 1, the relative extraction strengths for P, As and Sb are depicted in Figure 5.2 and are also noted below. For soil 2, as with soil 1, the oxalate step extracted substantial amounts of all 3 elements; 41, 50 and 30 % for P, As and Sb respectively. The relative importance of the NaOH fraction decreased from P to As to Sb (Figures 5.4 a-c) in soil 2, which contained a higher proportion of organic matter than soil 1 (22.0 versus 5.9 % LOI, Table 5.2). Compared to P and As, there was a high proportion of Sb in the residual phase in both soils (Figures 5.2c and 5.4c). Conversely, there was a low proportion of residual As in both soils (Figures 5.2b and 5.4b).

In soil 1;

Extraction strengths for P, As and Sb were $NaHCO_3$ < NaOH < Aqua regia < Oxalic acid + oxalate (all $P < 0.0001$).

For soil 2;

Extraction strengths for P were in the order $NaHCO_3$ < Aqua regia < NaOH ≈ Oxalic acid + oxalate.

Extraction strengths for As were in the order $NaHCO_3$ < Aqua regia < NaOH < Oxalic acid + oxalate.

Extraction strengths for Sb were in the order $NaHCO_3$ < NaOH < Oxalic acid + oxalate < Aqua regia.

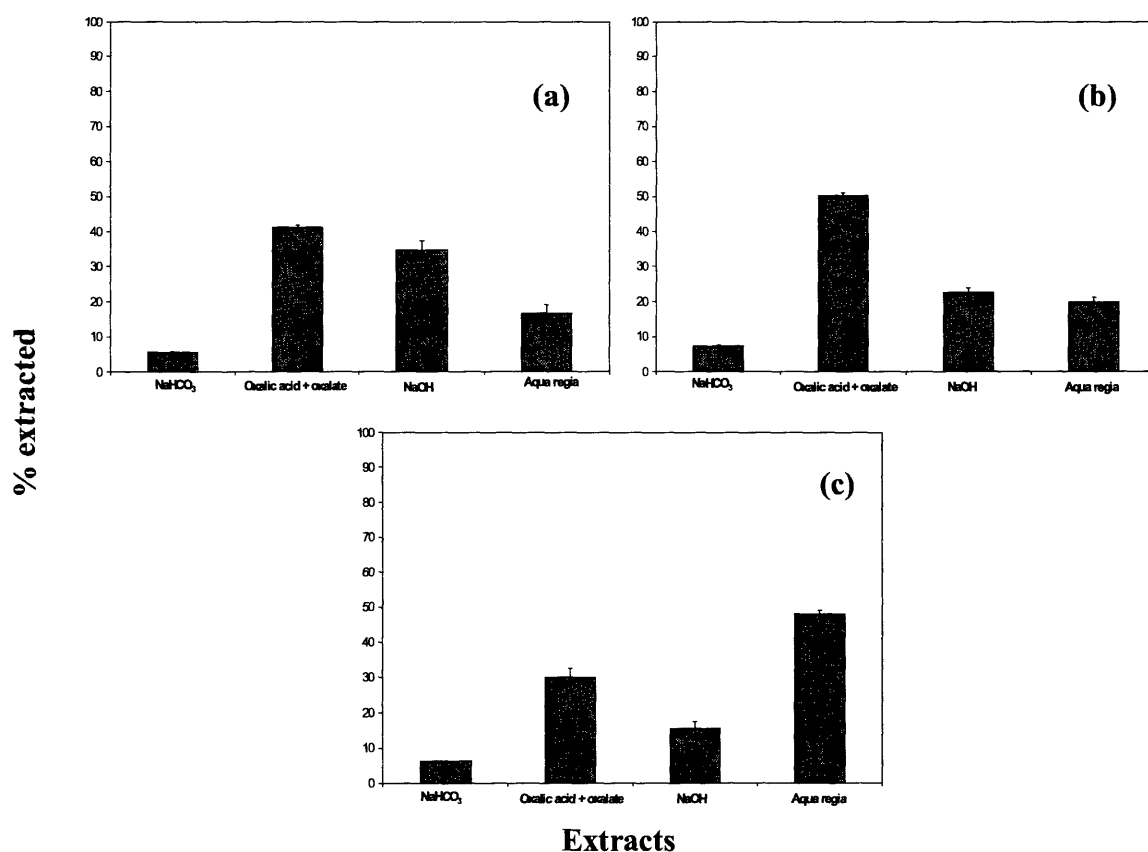All of the differences in extraction strengths given above are significant ($P < 0.05$).

Figure 5.4a-c. Proportions of P (a), As (b) and Sb (c) extracted from soil 2 using the modified extraction scheme. Error bars are standard errors of the means.

As with soil 1, the RSDs for all 3 elements in the fractions of soil 2 varied. For P, RSDs for $NaHCO_3$, oxalate and aqua regia fractions were < 13 %. For the NaOH-P and the HCl-P fractions the RSDs were 28 and 22 % respectively. The P contents of these fractions had limited quality assurance data (i.e. summation comparisons only).

For As and Sb, the highest RSD was given by the NaOH extractable fraction (19 and 34 %, respectively). This corresponded to values that were occasionally below the elemental method detection limits for this fraction. All other RSDs were below 14.3 and 11.3 % for As and Sb respectively.

5.4. DISCUSSION

5.4.1. Method development

Parameter optimisation and detection limits

The optimisation of ICP-OES parameters and the calculation of method detection limits were dependent upon the standard solution concentrations used (Table 5.1), which in turn were predetermined depending upon the expected typical soil concentrations of the elements. High Al and Fe in the oxalate standard solution resulted in the relatively high method detection limits for these elements (10.8 and 34.4 μg ml^{-1} respectively, Table 5.6). The higher concentrations of these elements expected in this extract (for soils of similar origin to that of soils 1 and 2) means most, if not all, of the floodplain soils should still have quantifiable Al and Fe concentrations despite these high detection limits. Conversely, and despite parameter optimisation, the relatively high detection limits for Sb in the oxalate and NaOH extracts were due to the high background intensities of Sb in these matrices, which resulted in less precise determination of Sb in both the oxalate and NaOH extracts. This has been previously recognised for ICP-OES measurements of Sb (Nash *et al.* 2000) and is an inherent limitation of this type of analysis. Use of an ICP-MS, with or without a hydride generation system, would lower the method detection limits by at least an order of magnitude (Soltanpour *et al.* 1998; Thompson *et al.* 1978) and increase the use of various extracts for soils with moderate As and Sb (e.g. < 40 mg kg^{-1}). This is currently not an available option for routine analysis at UNE.

Spikes and recoveries

The slightly higher average recovery of As (121.9 %) shown in Table 5.7 was attributed to the marginally elevated recoveries in spiked samples where the original (unspiked) sample concentration was close to the detection limit for As in one or more of the samples. It is known that the accuracy of trace element analyses decreases as the method detection limit is approached (Cline 2001). When this was taken into account, the recovery of As was deemed to be acceptable, and no adjustments were made to any subsequent oxalate extractable As concentrations based on spike recoveries. Similarly, the higher standard errors associated with the Fe and P spike recoveries were attributed to the wide concentrations ranges of calibration solutions (0 - 800 μg ml^{-1} for Fe, and 0 - 35 μg ml^{-1} for P). These ranges were chosen based on

preliminary investigations of the soil concentration ranges of these elements, and resulted in a loss of precision for finer scale determinations.

The summations of soil elemental fractions were acceptable for all of the elements analysed (Table 5.7). While this does not rule out matrix specific or elemental interferences, it does indicate that there were no gross extract specific errors (such as amplification interferences causing raw intensities of orders of magnitude higher than actually possible).

Independent validation

It was evident that the ICP-OES detectable As and Sb concentrations in the NaOH and HCl extracts were commensurate with the concentrations determined via independent ICP-MS at metalloid concentrations of less than $60 - 80$ mg kg^{-1} in the extracts. While there appeared to be some deviation from this 1:1 relationship at higher concentrations of NaOH extractable As and HCl extractable Sb (Figures 5.1 a and b), within the concentration ranges expected in soils specific to this study (such as soils 1 and 2) there was no noticeable bias between the ICP-OES and ICP-MS analysis for either metalloid. During preliminary investigations, the majority of 1 M HCl extractable As and Sb in soils 1 and 2 were found to be below the detection limits. Subsequently this extract was omitted from the detailed examinations of both soils 1 and 2.

Summary

It was determined in the method development section of this chapter that ICP-OES analysis of As and Sb concentrations in the oxalate and NaOH (and aqua regia) extracts were not subject to any suppression, amplification, or matrix specific interference that negated the application of these extracts to the soils examined. There is a possibility that method detection limits may be too high to accurately measure metalloid concentrations in the oxalate and NaOH extracts in all of the floodplain soils sampled. Similarly, there will be a loss of analytical precision at concentrations approaching the MDL. At this stage, there are no alternatives for routine analysis at UNE, and both analysis and interpretation must consider these difficulties. Some possible interpretations using the sequential soil extractions are discussed below.

5.4.2. Comparisons of extracts and sequences

NaHCO₃

Wenzel *et al.* (2001) state that coefficients of variation (or RSDs) less than 20 % imply acceptable analytical precision. The RSDs for the NaHCO$_3$ extractable P and As in both test soils did not exceed 13 %. For NaHCO$_3$ extractable Sb in soil 1 the RSDs were $\leq$ 21.9 %. This was similar to the RSDs for a dilute salt found by Lintschinger *et al.* (1998), which was attributed to the low proportion of Sb detectable in that fraction. As the proportion of total soil Sb extracted by NaHCO$_3$ in the current work was low, a relatively small change in the concentration detected in the extract would account for this large change in precision.

The proportions of P, As and Sb extracted in both soils were all < 12.7 % in soil 1 and < 7.40 % in soil 2. Johnston and Barnard (1979) extracted 1.2 – 7.4 % of soil As with NaHCO$_3$ in soils containing up to 51 mg kg^{-1} As. Other measures of 'labile' As fractions have given similar results across a range of soils. McLaren *et al.* (1998) found a mean value of 9.3 % of As extracted with NaHCO$_3$ in soils with total As ranging from 15 to 4310 mg kg^{-1}, while Hamon *et al.* (2004) found 2.6 and 12 % of total soil As (4770 and 725 mg kg^{-1}) in a labile fraction as determined by isotopic labelling and an NH$_4$H$_2$PO$_4$ based extract.

The use of NaHCO$_3$ to extract soil Sb is not common. In the few existing studies, dilute salts and acids have been used to estimate mobile or labile fractions (see Chapter 2, section 2.5.3). For example, Baroni *et al.* (2000) found < 8.6 % of total Sb was extracted with acetic acid in highly contaminated soils, while Lintschinger *et al.* (1998) found < 1% of soil Sb was extracted by NH$_4$NO$_3$ in soils with $\leq$ 196 mg kg^{-1} Sb. While the specificity of dilute salts in detecting 'labile' fractions needs case-by-case validation, it can be concluded that similar proportions of As and Sb are 'weakly' bound to soils 1 and 2, as have been found in previous studies (and as discussed in section 2.5.3).

Bicarbonate extracts of P have had considerable success as agricultural soil P availability indicators (Coventry *et al.* 2001). Furthermore, as early as the 1970s, the use of NaHCO$_3$ extractable – As was suggested as a predictor of phytotoxicity based

on P studies (Walsh and Keeney 1975). The similarity in proportions extracted, coupled with the success of $NaHCO_3$ extractable P as a measure of plant availability indicates that investigations into the predictive capability of $NaHCO_3$ extractable As and Sb for plant uptake of these metalloids is needed.

Oxalic acid/ ammonium oxalate

Investigations into the specificity of oxalate based soil extractions have been undertaken previously. For example, Wenzel *et al.* (2001) tested an oxalate extract on synthetic hydroxides, and determined the extract was specific for amorphous Fe and Al oxyhydroxides. This extract is often used in isolation to quantify these soil phases and associated elements (Chao and Zhou 1983; McLaren *et al.* 1998). As the preceding $NaHCO_3$ step did not affect the Fe or Al extracted by the oxalate step (Figures 5.3a and b), it is a reasonable assumption that this extract in the modified sequence is targeting the non-crystalline Al and Fe hydroxide phases in the soil, although there may also be displacement of specifically adsorbed metalloids from other sites, such as on the crystalline oxyhydroxides phases.

Relative standard deviations for the oxalate extracts in the sequential scheme in both soils were < 13.0, 14.3 and 11.3 % for P, As and Sb respectively. These were well within the 'acceptable' ranges as stated by Wenzel *et al.* (2001). The high proportion of each element extracted by the oxalic acid/ammonium oxalate step (30 - 71 %, Figures 5.2 and 5.4) demonstrates the importance of the non-crystalline oxyhydroxide phase in these soils, and is consistent with the known scavenging potential of these phases for P, As and Sb (Guo and Yost 1999; Pierce and Moore 1982; Thanabalasingam and Pickering 1990), as discussed in Chapter 2. For example, Lintschinger *et al.* (1998) extracted over 50 % of total Sb from contaminated soils with an oxalate extract, while Wilson *et al.* (2004) found non-crystalline hydroxide phases to be the dominant component of the residual or strongly bound fraction of Sb in grossly contaminated soils. In the original scheme (i.e. in the absence of the oxalate step), Sb associated with the non-crystalline hydroxide phase was measured as part of the residual (or immobile) fraction by the aqua regia digestion (Figure 5.2c). This was also partly true for P and As (Figures 5.2a and 5.2b), and shows the general need for adapting extraction sequences for specific soils. This also suggests that for

Sb at least, release from other phases (such as the crystalline oxyhydroxides) is not occurring to any noticeable degree.

Soils 1 and 2 occur in areas affected by acid sulfate processes. In these types of soils, dissolution and re-formation of non-crystalline oxyhydroxides is known to occur, which implies intermittent mobility of elements associated with this fraction (Dent 1986; Willet and Bowman 1990). This may have management implications for As and Sb as contaminants. In the study area, there is interest in resubmergence of pastures underlain by ASS as a possible means of controlling and perhaps reversing oxidation of sulfides and hence sulfuric acid production (Smith and Yerbury 1996). However if resubmergence results in reduction and dissolution of hydroxide phases, such as the Fe (III) hydroxide phase, metalloid mobility and possibly bioavailability may increase.

NaOH

In general, the NaOH extract showed the worst precision for P, As and Sb. For both As and Sb, the high RSDs (26 % for Sb in soil 1, 19 and 34 % for As and Sb in soil 2 respectively) appeared to be the result of metalloid concentrations approaching the MDLs as given in Table 5.6. The high RSDs for P were not attributable to low P concentrations in the extracts. A possible cause could not be determined due to the lack of QA/QC data for this extract.

Opinions differ on the pool targeted by a NaOH extract. The conflicting mechanisms were outlined in Chapter 2, section 2.5.3. To reiterate, Hedley *et al.* (1982a) emphasised the role of the added hydroxide in causing desorption from pH dependent sites. Others attribute elements released in NaOH extracts to deprotonation of organic acids leading to dissolution of the organic phase (Sposito *et al.* 1982). In soils, both mechanisms must operate to some extent. In soils 1 and 2 there was a decline in the amount of each element extracted with NaOH from P to As to Sb. This is in accordance with the known general decreasing associations of these elements with organic matter (Thanabalasingam and Pickering 1986), although this trend is based on very limited data for Sb (Pilarski *et al.* 1995).

For P and As, inclusion of the oxalic acid - ammonium oxalate step decreased the amounts extracted by the subsequent NaOH step, which may have been due to the removal of pH dependent adsorption sites associated with the non-crystalline oxyhydroxides. Conversely, the application of the oxalate extract did not change the amount of NaOH-Sb subsequently extracted in soil 1 (Figure 5.2c). This suggests that the Sb associated with non-crystalline oxyhydroxides (as determined by the oxalate based extract) is bound in this soil in such a way as to not be affected by pH dependent desorption, or pH based dissolution of organics. Thus, Sb may be co-precipitated with the hydroxide soil phase, rendering it inaccessible to the NaOH extract. This is a very preliminary indication that the oxalate and NaOH extracts may be targeting independent soil fractions of Sb. Detailed investigations, such as batch adsorption trials, may aid in determining the relative importance of Sb binding to different soil phases.

Aqua regia

The RSDs for P, As and Sb in the aqua regia analyses were deemed to be acceptable, and commensurate with the precisions given in Chapter 4, excluding As in the modified scheme as it was applied to soil 1. The high RSD for As extracted with aqua regia in soil 1 (in the modified scheme) was due to residual As concentrations less than the aqua regia method detection limit as given in Chapter 4 and Table 5.6.

The substantial proportion of residual, or highly immobile, Sb in the test soils support the findings of Ashley and Graham (2001) that Sb in the Macleay floodplain results predominantly from flood-related sediment deposition, sourced from historic mining related activities upstream, and thus occurs at least partly in primary mineral form. This contrasts with the lower proportions of residual As, which is probably sourced partly from flood deposition, and partly from *in situ* development (Ashley and Graham 2001; Tighe *et al.* 2005). The longer period of time that *in situ* As would have been exposed to local weathering and ASS processes has presumably resulted in a smaller portion present in the highly immobile phase.

5.5. CONCLUSIONS

Analysis of soil extracts for P, As and Sb via ICP-OES, in terms of the measured method parameters, was generally acceptable. There were difficulties in analysing the HCl extract for metalloids due to the low concentrations of the metalloids in the extract, being routinely below the MDLs of the ICP-OES. Following parameter optimisation, there were no obvious matrix specific or elemental interferences that affected the summation of the extracted fractions, spiked sample recoveries or the independent cross validation of the various extracts. The analysis of P concentrations in the $NaHCO_3$, NaOH and HCl extracts had very limited QA/QC, and requires more verification before routine use.

The proportion of Sb removed from the test soils by each extractant was broadly similar to that of both P and As. Analytical precisions for all 3 elements were generally acceptable, with the exceptions directly attributable to the ICP-OES detection limits, standard solution concentrations and calibration ranges. Some interpretation of metalloid fractionation in the soils was possible, including:

There were similarities in the proportions of P, As and Sb 'weakly' bound to the soil, as extracted by $NaHCO_3$. These proportions were generally less than 10 % of the total soil element concentration.

Large proportions of all three elements were bound to the non-crystalline hydroxide soil phases (30 - 71 %).

Less Sb than As or P was extracted by NaOH, possibly indicating less Sb was associated with organic matter.

The proportion of residual Sb was high compared to P and As. This supported the existing hypotheses of partial differences in elemental sourcing of As and Sb (Ashley and Graham 2001).

The importance of an extractant step in these soils targeting the non-crystalline oxyhydroxide phase was shown for As, P and Sb. Excluding this step led to an overestimation of the immobile soil P, As and Sb by 30-71 %. Dissolution of this phase by chemical reduction or pH changes associated with seasonal flooding or ASS processes in the floodplain may liberate relatively large quantities of these elements from the soil.

Finally, there has been some success in using single extracts to measure the bioavailability of P and As (Guo and Yost 1999; Schoenau and Huang 1991; Woolson *et al.* 1971; Woolson *et al.* 1973) and as discussed in Chapter 2, section 2.7.2. This suggests that an extract, or combination of extracts and measured environmental variables, may show some relationship with the bioavailability of As and/or Sb in the soils of the study area. This is investigated in conjunction with the effect of flooding these soils in Chapter 7.

CHAPTER 6 - SOIL, WATER AND PASTURE ENRICHMENT OF ARSENIC AND ANTIMONY WITHIN A COASTAL FLOODPLAIN SYSTEM

This chapter has been published as:

The results presented in Table 6.3, and Figures 6.2 and 6.3 and the discussions of these results are an addition to the previously published data.