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Summary of a Preliminary Study on the use of 0.1M HNO_3 for Preserving the Stomach and Intestine Solutions from the Unified BARGE Method

Chemical & Biological Hazards Programme

Open Report OR/07/037

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Mark Cave and Joanna Wragg

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British Geological Survey offices

BGS Central Enquiries Desk

Tel 0115 936 3143 Fax 0115 936 3276

email enquires@bgs.ac.uk

Kingsley Dunham Centre, Keyworth, Nottingham NG12 5GG

Tel 0115 936 3241 Fax 0115 936 3488

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Forde House, Park Five Business Centre, Harrier Way, Sowton EX2 7HU

Tel 01392 445271 Fax 01392 445371

Maclean Building, Crowmarsh Gifford, Wallingford OX10 8BB

Tel 01491 838800 Fax 01491 692345

Geological Survey of Northern Ireland, Colby House, Stranmillis Court, Belfast BT9 5BF

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Natural Environment Research Council, Polaris House, North Star Avenue, Swindon SN2 1EU

Tel 01793 411500 Fax 01793 411501

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Foreword

This report is the published product of a preliminary trial for the BioAccessibility Research Group of Europe (BARGE) Unified Bioaccessibility Method (UBM) carried out by the Medical Geology project team. The trial describes a study investigating the usefulness of employing an acid solution as a preservative for bioaccessibility extracts prior to analysis, with the long term aim of utilizing the methodology in a follow-on international inter-laboratory trial.

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Summary

This report describes a preliminary study undertaken in advance of the BARGE UBM international inter-laboratory trial to investigate the use of 0.1M nitric acid as an effective preservative for the 'stomach' and 'stomach & intestinal' extracts produced in the *in vitro* methodology. Results for preserved and unpreserved extracts are described for measurements collected over a period of c. 1 month, with an evaluation of the extract stability and recommendations for the full BARGE inter-laboratory trial.

1 Introduction

The Bioaccessibility Research Group of Europe (BARGE) made a joint decision to progress research in the field of *in vitro* bioaccessibility methods in 2005 and carry forward a single harmonised methodology for the determination of soil associated contaminant bioaccessibility applicable to human health risk assessment. The chosen method was a modified version of that previously published by researchers at the Dutch Institute of Public Health, the RIVM (Oomen, 2000; Oomen et al., 2002) because this method was considered to be the most physiologically representative of the *in vitro* methods available to the group. Modifications to the method, described by Wragg *et al.,* (2008), were undertaken to ensure conservatism and robustness across the local geological conditions and regulatory requirements associated with each member country. Evaluation of the modified RIVM method, know as the BARGE Unified Bioaccessibility Method (UBM) was undertaken by carrying out an international round robin exercise, led by the Environment and Health project of the BGS.

Because of the international nature of the inter-laboratory trial, resulting in sample transportation over long distances, concerns were raised over the stability and the potential degradation of the extraction fluids. To address such concerns, an investigation was undertaken by the BGS Environment and Health project with associated analysis by the BGS Analytical Geochemistry Laboratories, prior to undertaking the full UBM trial, to evaluate the preservation of samples for transport by means of addition of a 0.1M HNO_3 matrix and the application of a HNO_3/H_2O_2 digestion to the bioaccessibility extracts in order to stop sample degradation and ensure matrix uniformity prior to analysis.

1.1 METHOD

In order to assess the need and the suitability of using 0.1M HNO_3 as a preservation agent for the UBM bioaccessibility extracts, the UBM protocol as described by Wragg et al., (2008) was carried out in duplicate on the following samples:

- NIST 2710;
- NIST 2711;
- Spiked solution of As, Cd and Pb, taken through all extractions as if it were a soil.

Two samples for analysis were taken from each phase of the UBM *in vitro* test (the 'stomach' and the 'stomach & intestine', and were prepared as follows:

- Preservation 1 - diluted 1 ml sample (stomach or intestine) + 9 ml 0.1 M HNO_3;
- Preservation 2 - diluted 1 ml sample (stomach or intestine) + 9 ml deionised water.

The preservations were analysed the next day after extraction, after seven days and then again after 37 days, by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Details of how the ICP-AES analysis was undertaken have been reported in the corresponding report on the full UBM international inter-laboratory study (Wragg *et al.*, 2008).

The ICP-AES results for As, Cd and Pb measured in preservations 1 and 2 over the 1st seven days of the trial are summarised as bar charts estimating an analytical uncertainty of ± 5%. For the analysis of the longer term stability of the preserved and unpreserved NIST 2710 and 2711 samples, the mean relative change in the measured As, Cd and Pb concentration of the 7 and 37 day analysis was compared to the initial analysis. The uncertainty on the long term experiments was calculated using a non-parametric resampling approach (Blank *et al.*, 2001).

1.1.1 Spike Tests

Figure 1 shows the results for the As spike taken through both phases of the UBM. There is some evidence that overall there is higher recovery in the 'stomach & intestine' phase solution than in the 'stomach' phase, although the differences are small. There are no significant effects, however, between acid and non-acid preservation or whether the samples were analysed immediately or after 7 days.

Figure 2 shows that, for Cd, again there is possibly a slightly higher recovery in the 'stomach & intestine' phase solution compared to the 'stomach' phase solution for the acid preserved samples. For the 'stomach' solution, there is no significant difference between acid and non-acid preservation or between immediate and 7 day analysis. For the 'stomach & intestine' solution, however, the recovery for the non-acid preserved samples is c. 50% lower than for acid preservation, which decreases a further c. 10 –15 % after 7 days. There is no significant loss (i.e. within the repeatability error) of Cd in the acid preserved 'stomach & intestine' solutions after 7 days.

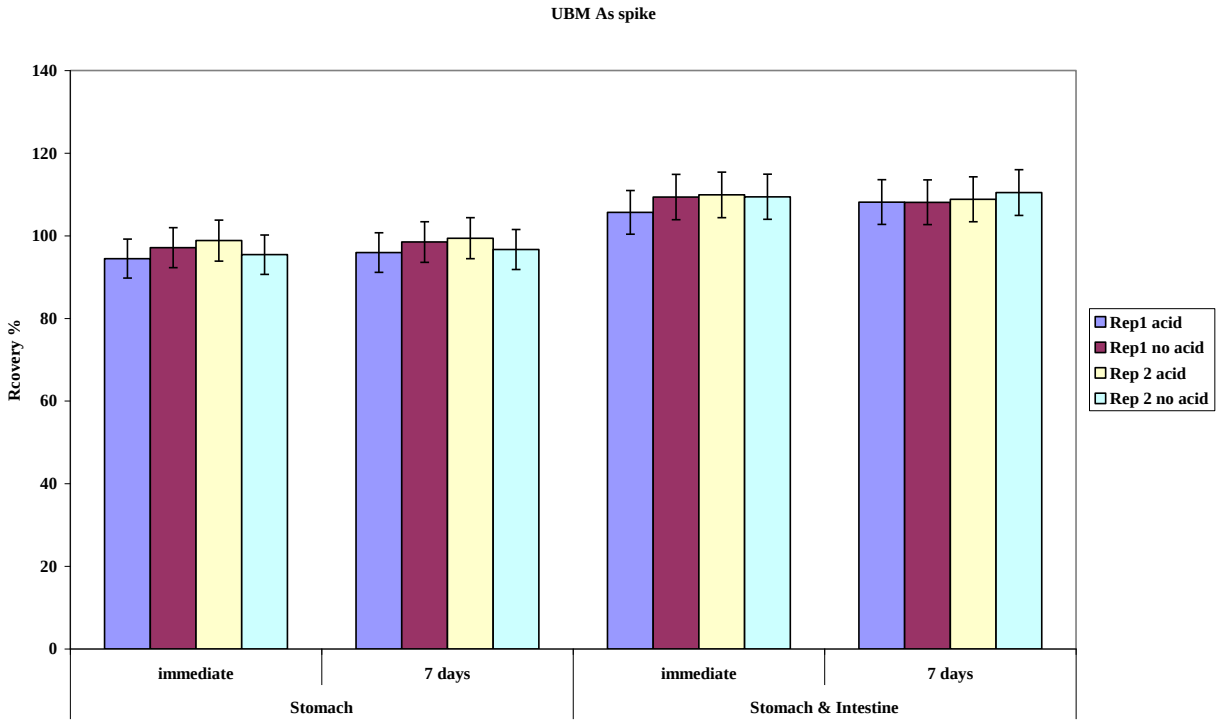

Figure 1 Recovery data for the As spike after Extraction by the UBM

For the Pb spike recovery test, Figure 3 shows that there is c. 35-40% decrease in the recovery between the 'stomach' and 'stomach & intestine' phase solutions. For the 'stomach' phase solution, there is no significant differences between acid and non-acid preservation or between immediate and 7 day analysis. For the 'stomach & intestine' phase solution there is a decrease in recovery of c.10% in the non-acidified solution compared to the acidified preservation that decreases by a further c. 40% after 7 days. There is no significant loss (i.e. within the repeatability error) of Pb in the acid preserved 'stomach & intestine' phase after 7 days.

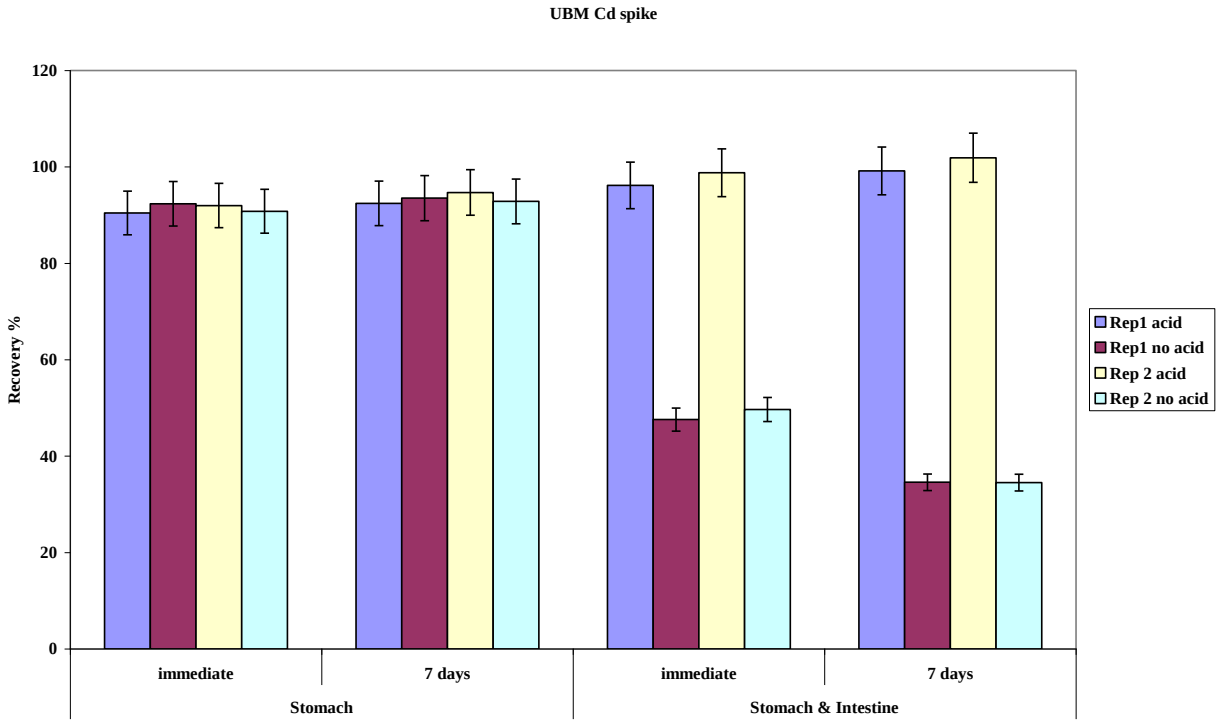

Figure 2 Recovery data for the Cd spike after Extraction by the UBM

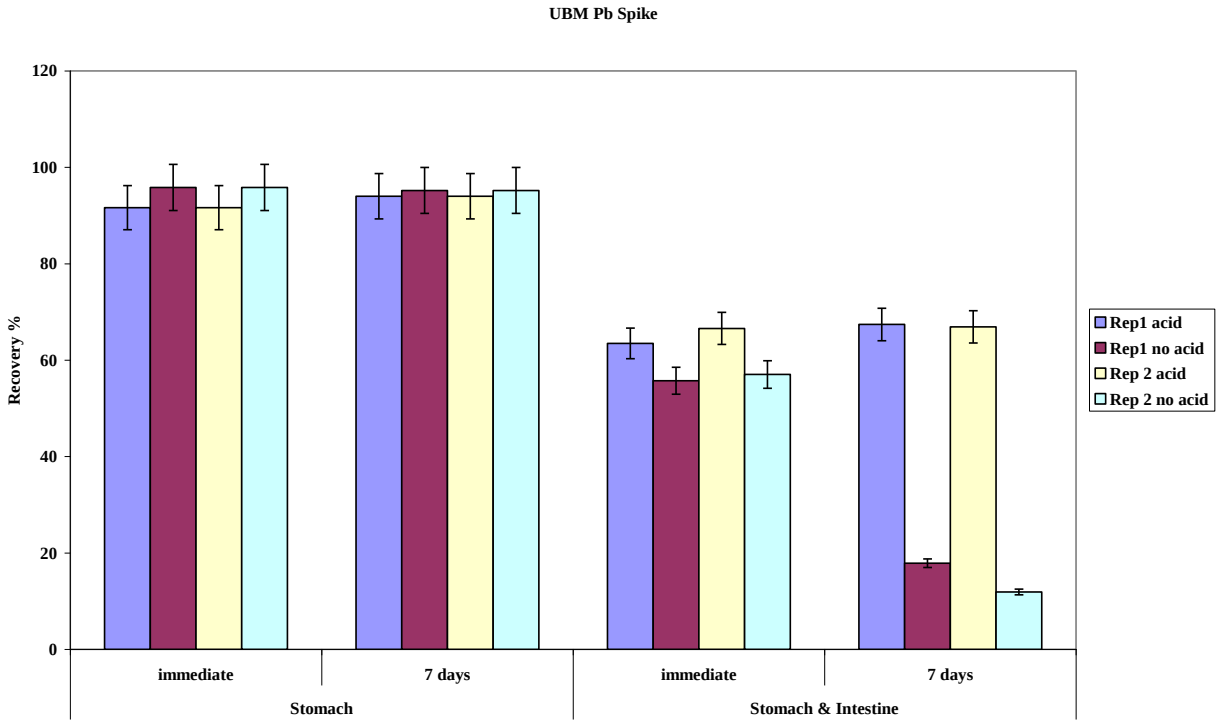

Figure 3 Recovery data for the Pb spike after Extraction by the UBM

1.1.2 NIST 2710 and 2711

Figure 4 shows the As extraction results for the duplicate samples of NIST 2710 and 2711. The results for both soils are very similar to the spike test in that they show that there are no

significant differences (i.e. within the repeatability error) between acid and non-acid preservation and between immediate and analysis after 7 days.

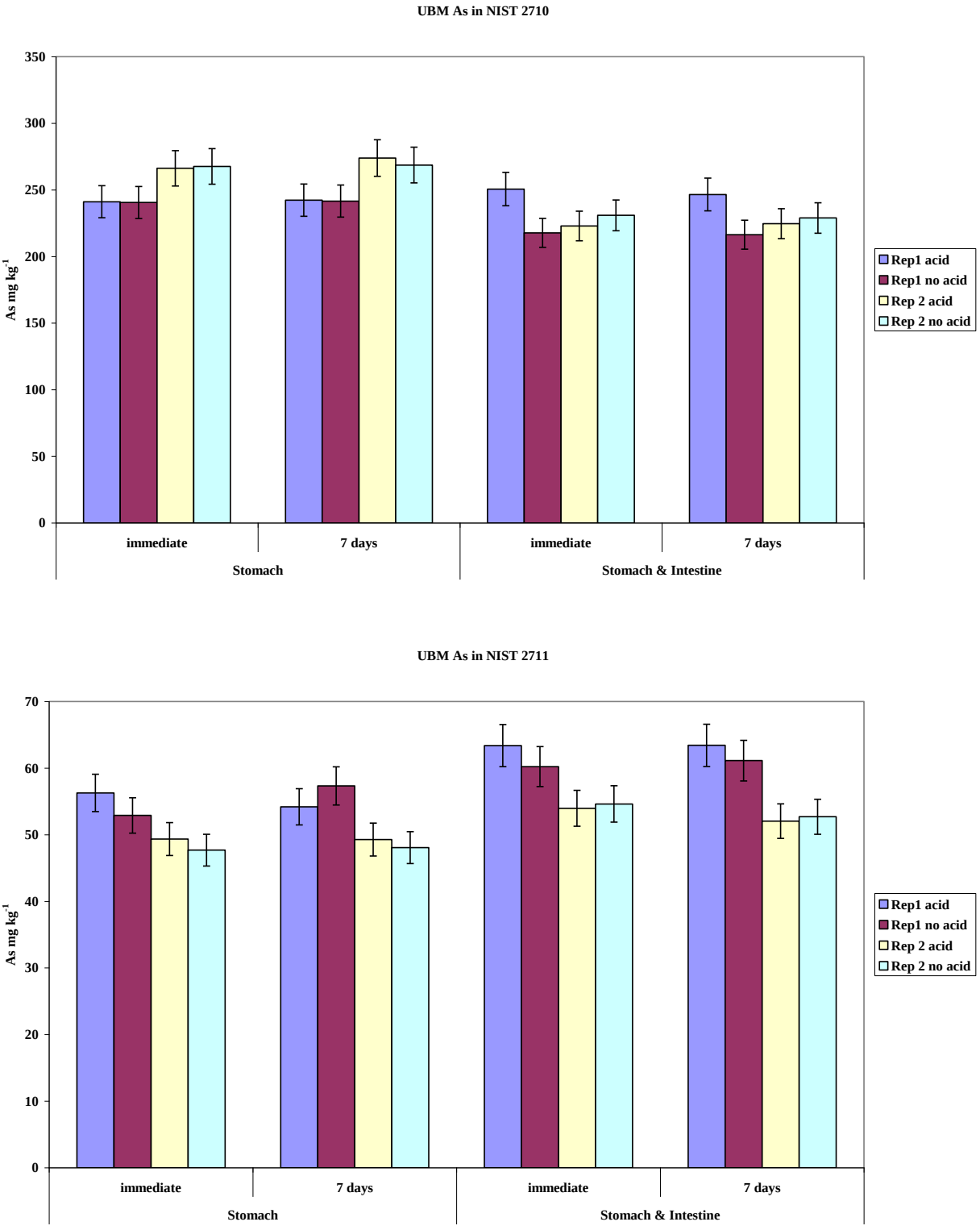

Figure 4 UBM As extraction data for NIST 2710 and 2711

Figure 5 summarises the long term stability of As in the 'stomach' and 'stomach & intestine' phases of the UBM extracts. This figures shows that there is no significant deterioration in the 'stomach' or 'stomach & intestine' phases over the 37 day trial period.

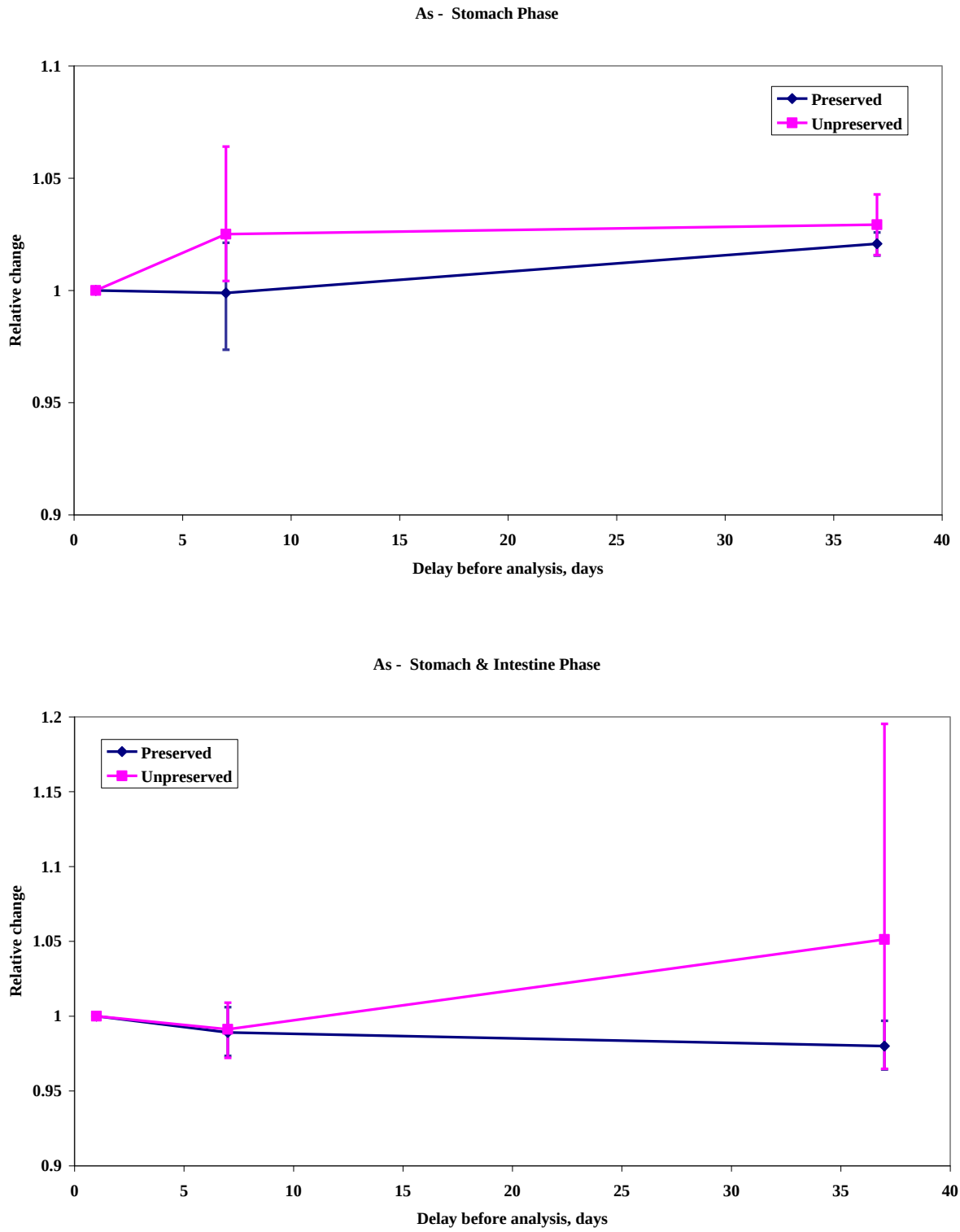

Figure 5 As stability plot for preserved and unpreserved extracts

Figure 6 shows the Cd extraction results for the duplicate samples of NIST 2710 and 2711. Both soils show very similar patterns of extraction. The 'stomach' phase solutions show no significant

differences (i.e. within the repeatability error) due to acid and non-acid preservation and immediate or 7 day analysis. The 'stomach & intestine' phase solutions show a lower Cd content of c. 5 mg kg^{-1} in the non-acidified samples (apart from one duplicate value in the 7 day analysis that increased) and no significant difference between immediate and 7 day analysis.

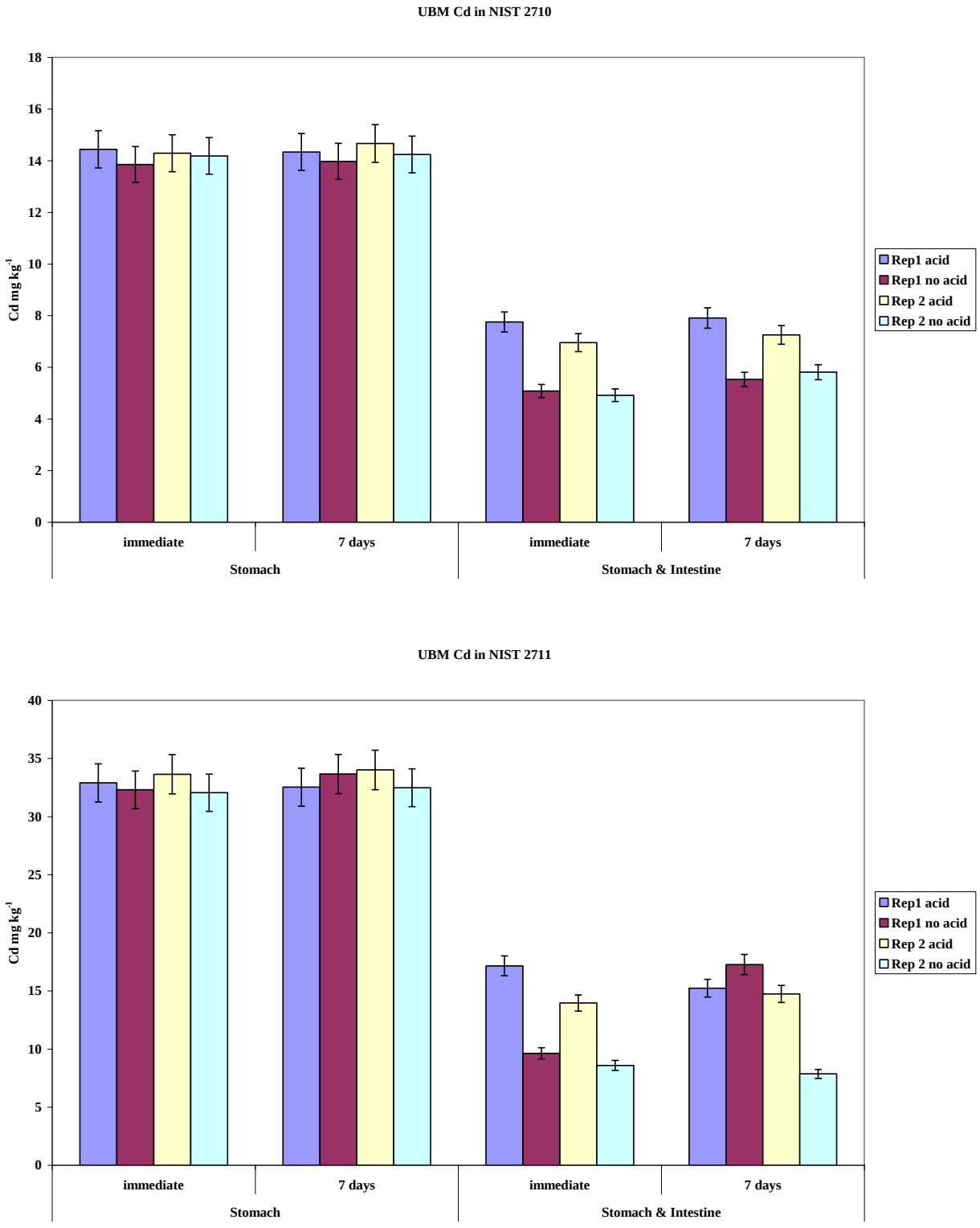

Figure 6 UBM Cd extraction data for NIST 2710 and 2711

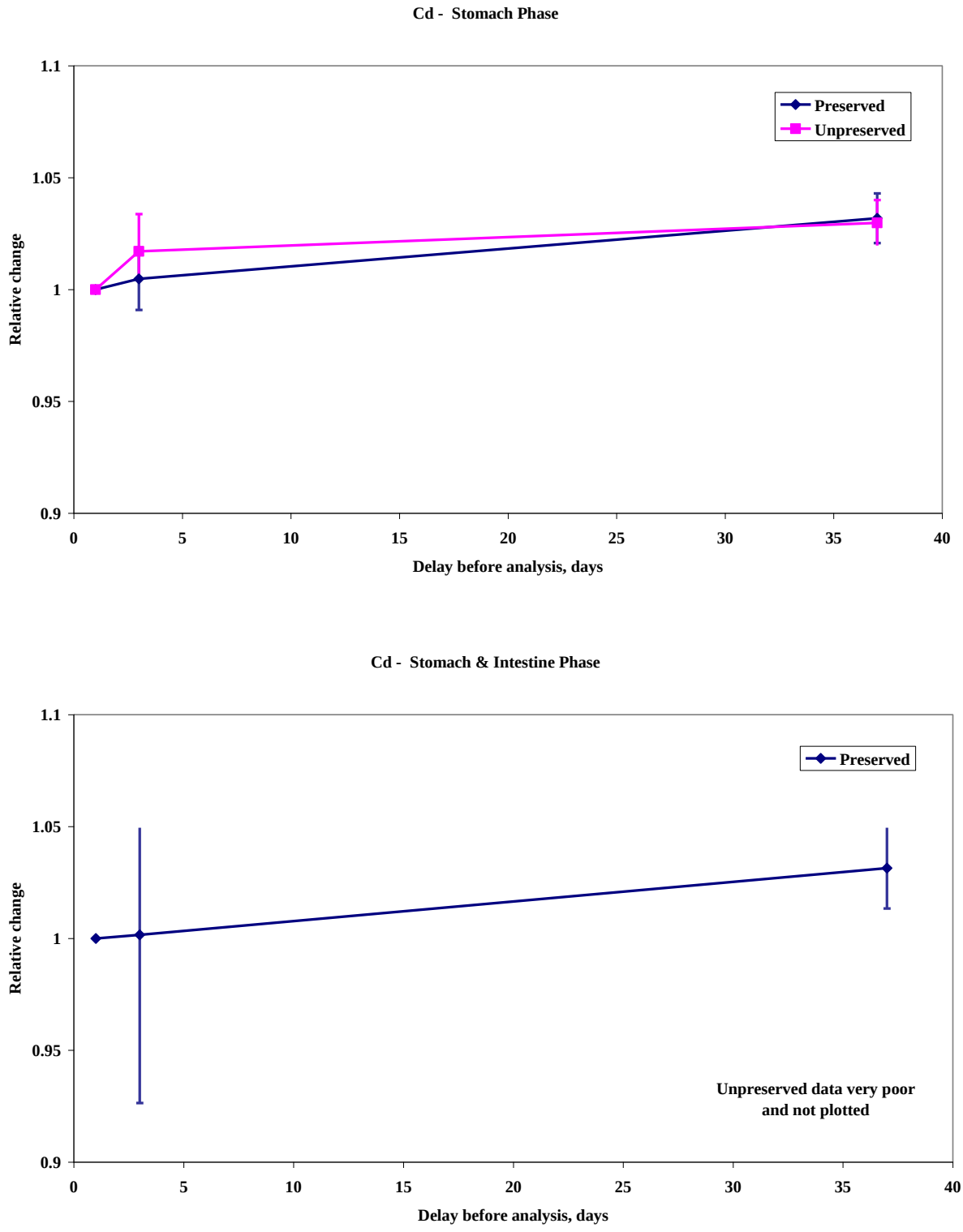

Figure 7 Cd stability plot for preserved and unpreserved extracts

Figure 7 shows no appreciable deterioration of the measured Cd in the unpreserved 'stomach' phase samples compared to the preserved over the trial period. However, the unpreserved 'stomach & intestine' phase extractions returned poor results for Cd, and have not been shown, indicating either sample deterioration through effects such as precipitation in the neutral matrix.

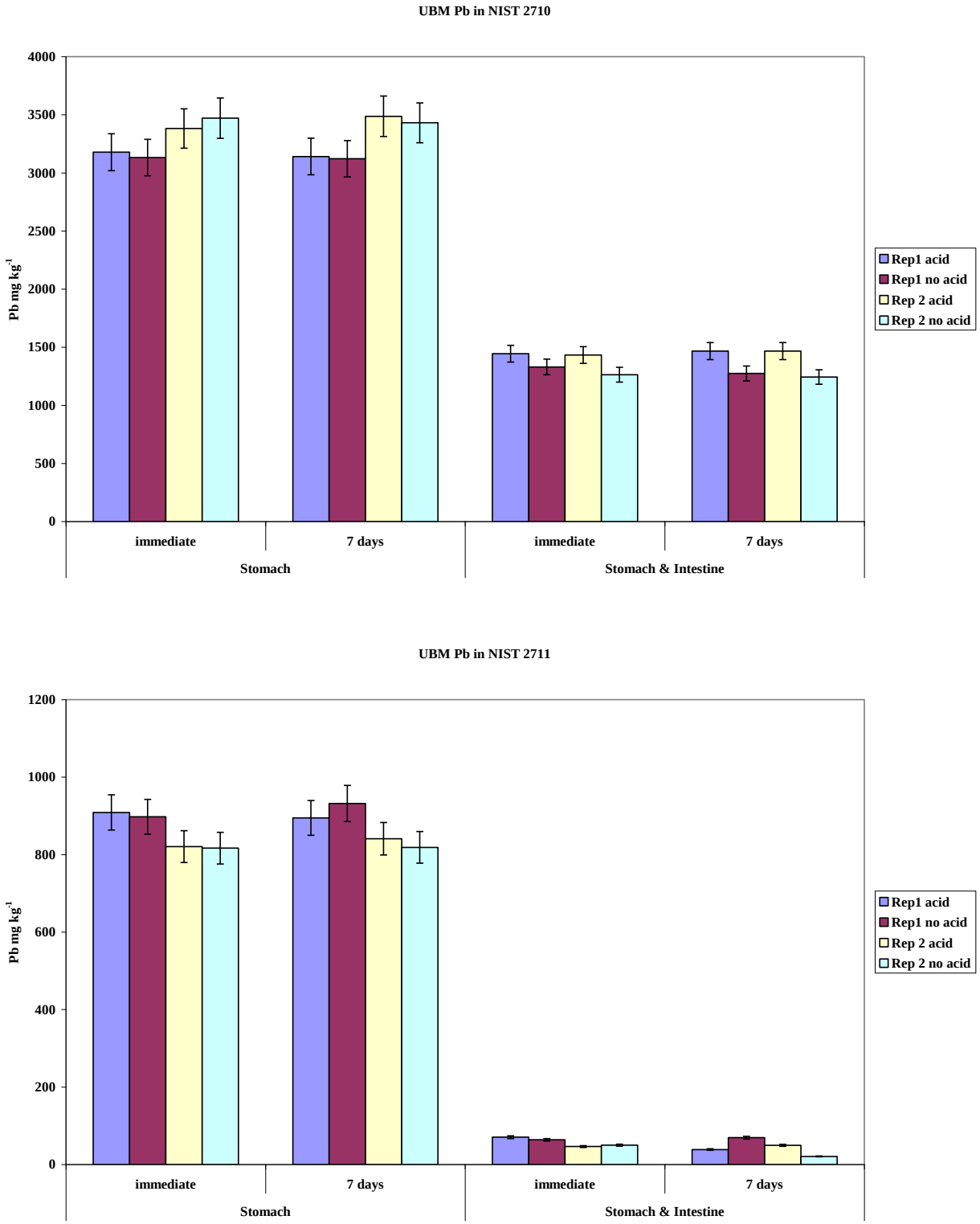

Figure 8 UBM Pb extraction data for NIST 2710 and 2711

Figure 8 shows the Pb extraction results for the duplicate samples of NIST 2710 and 2711. Both soils show very similar patterns of extraction. The 'stomach' phase solutions show no significant differences due to acid and non-acid preservation or immediate and 7 day analysis. The 'stomach & intestine' phase solutions suggest a lower Pb content of c. 5 mg kg^{-1} in the non-acidified samples (apart from one duplicate value in the seven day analysis that increased) although this is

within analytical uncertainty. There is no significant difference between immediate and 7 day analysis in the 'stomach & intestine' phase solutions. In a similar manner to the stability plots presented for Cd in the 'stomach' and 'stomach & intestine' phases of the UBM (figure 7), figure 8 indicates that preservation of the extraction solutions prevents loss of Pb in both extracted phases. Figure 9 indicates that, for Pb, lack of preservation increases the risk of analyte loss in the 'stomach & intestine' phase. In this instance the data was too poor to be presented.

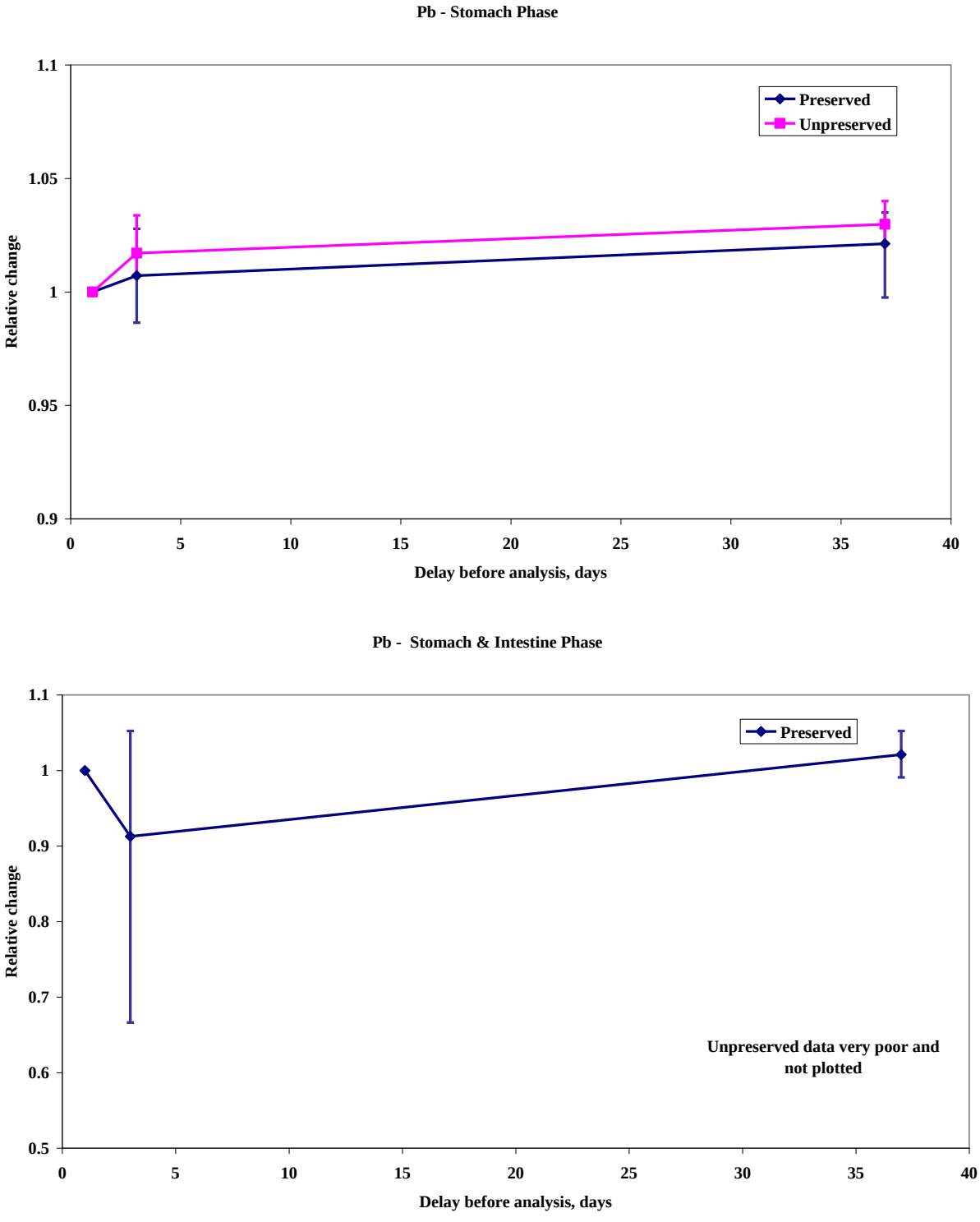

Figure 9 Pb stability plot for preserved and unpreserved extracts

1.2 CONCLUSIONS

For the spike test and the NIST 2710 and 2711 reference soils the use of acid preservation shows no evidence of reduced As, Cd and Pb concentrations due to precipitation of metals from solution. For Cd and Pb there is very clear evidence that if acid preservation is not used then both of these metals can drop out of solution in the 'stomach & intestine' phase extracts.

1.3 RECOMMENDATIONS

It is recommended that the preservation methodology of dilution of the 'stomach' or 'stomach & intestine' phase solutions using 1 ml of sample with 9 ml 0.1M HNO_3 should be adopted in the BARGE UBM. The added benefits of this method of preservation are: the reduction/removal of the extraction matrix used in the UBM, which has a high total dissolved solids (TDS) content and may cause problems for the chosen method of analysis; and the removal of the need to matrix match calibration and quality control standards to the sample matrix.

Appendix 1

		As						Cd						Pb					
Sample	Aliquot	Stomach			Stomach & Intestine			Stomach			Stomach & Intestine			Stomach			Stomach & Intestine		
		Immediate	7 days	37 days	Immediate	7 days	37 days	Immediate	7 days	37 days	Immediate	7 days	37 days	Immediate	7 days	37 days	Immediate	7 days	37 days
2710 (1)	Acid	241	242	244	218	216	216	13.9	14.0	14.3	5.08	5.53	5.53	3131	3121	3126	1330	1274	1274
2710 (1)	No acid	241	242	244	251	247	247	14.4	14.3	14.7	7.75	7.91	7.91	3178	3141	3136	1444	1466	1466
2710 (2)	Acid	268	269	272	231	229	224	14.2	14.2	14.4	4.92	5.81	15.2	3471	3431	3433	1263	1244	38.6
2710 (2)	No acid	266	274	273	223	225	224	14.3	14.7	15.0	6.96	7.26	7.26	3381	3487	3483	1432	1466	1466
2711 (1)	Acid	52.9	57.3	55	60.2	61.1	61.1	32.3	33.7	33.8	9.62	17.3	21.3	898	932	909	63.9	69.2	79.1
2711 (2)	No acid	56.3	54.2	57	63.4	63.4	72.0	32.9	32.5	33.7	17.2	15.2	17.3	909	895	932	70.6	38.6	69.2
2711 (2)	Acid	47.7	48.1	50	54.6	52.7	52.7	32.1	32.5	32.8	8.59	7.86	7.86	817	819	804	49.9	20.9	20.9
2711 (2)	No acid	49.4	49.3	51	54.0	52.0	52.0	33.6	34.0	35.0	14.0	14.7	14.7	821	841	852	46.6	49.6	49.6
mix spike (1)	Acid	97.1	98.5	97.9	109	108	108	92.4	93.5	95.3	47.6	34.6	34.6	95.8	95.2	94.2	55.7	17.9	17.9
mix spike (1)	No acid	94.5	95.9	101	106	108	108	90.4	92.4	97.2	96.2	99.2	99.2	91.6	94.0	96.0	63.5	67.4	67.4
mix spike (2)	Acid	95.4	96.7	98.9	109	110	110	90.8	92.8	96.6	49.7	34.5	34.5	95.8	95.2	94.3	57.0	11.9	11.9
mix spike (2)	No acid	98.8	99.4	99.4	110	109	108	91.9	94.7	94.7	98.8	102	102	91.6	94.0	94.7	66.6	66.91	66.9

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