

Appendix F
In Vitro Bioassay Report

**Laboratory Report: Results and QA/QC
For In Vitro Bioassay Results using the
Relative Bioavailability Leaching Procedure
(RBLP)**

Omaha Lead Site

**For
USEPA Region 7**

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1.0 Introduction

In February, 2004 Black and Vetch, Inc., on behalf of U.S. Environmental Protection Agency (USEPA) shipped the Laboratory for Environmental and Geological Studies (LEGS), at the University of Colorado soils from the Omaha Lead Site to undertake a bioavailability study. In response to this an in vitro bioassay was conducted to determine relative lead bioavailability. Samples were acquired from the community by representatives of Black and Vetch on behalf of the USEPA. Results of that study are summarized in Table 1.1 and all raw data are supplied.

In addition, a set of 61 samples from the EPA speciation report entitled "THE SOURCE OF ANOMALOUS LEAD CONCENTRATIONS IN SOILS FROM THE OMAHA COMMUNITY--- OMAHA, NEBRASKA, September 22, 2002 are included in Table 1. 2 to provide a more comprehensive estimate of relative bioavailability across the site.

TABLE 1. Preliminary Summary Of In Vitro Bioassay Results

Sample	ID	Pb in bulk soil mg/kg	mass soil (g)	calc Pb #1	ICP Pb (mg/l)	solution amt (l)	% Relative Pb Bioavailability
13271		1303.286	1.00518	1.31	12.12818	0.1	93
18343		664.6751	1.00613	0.67	5.17	0.1	77
	22258	858.0319	1.00652	0.86	6.421	0.1	74
	22412	1172.359	1.0059	1.18	11.343	0.1	96
	29478	259.2703	1.00657	0.26	2.008	0.1	77
	33449	1114.666	1.00037	1.12	9.888775	0.1	89
	34544	1432.589	1.0035	1.44	12.07975	0.1	84
	36276	206.2425	1.00811	0.21	1.510855	0.1	73
	37666	697.0887	1.00763	0.70	5.618096	0.1	80
	38573	1362.08	1.00085	1.36	10.53043	0.1	77
	38775	860.9752	1.01228	0.87	6.82711	0.1	78
	40182	498.2988	1.00679	0.50	3.50987	0.1	70
	40299	929.7609	1.00629	0.94	7.451902	0.1	80
	41449	964.4801	1.00741	0.97	8.420272	0.1	87
	41488	465.823	1.00255	0.47	3.844448	0.1	82
	44481	85.14883	1.00933	0.09	0.808408	0.1	94
	44837	509.5865	1.00546	0.51	4.312079	0.1	84
	47483	605.1829	0.9607	0.58	4.615424	0.1	79
	47618	384.7143	1.524	0.59	2.91381	0.1	50
QA/QC							
blank-proc					0.003887		
blank-spk(10 ppm)					9.6669		
blank-proc					0.003456		
blank-spk(10 ppm)					9.7445		
47618-spk(10ppm)			1.00611		12.57789		
47483-AD		605.1829	0.9607	0.58	4.58444	0.1	79
44481 spk(10ppm)			1.00276		10.40966		
29478-dup		259.2703	1.01052	0.26	2.015063	0.1	77
22258-DUP		858.0319	1.00435	0.86	6.548	0.1	76
NIST2710		5508.907	1.00944	5.56	43.16877	0.1	78

Table 1.2. Relative Bioavailability Estimates for Omaha Samples.

Run at 1.5pH for 1 hr @ 39°C

Lab ID	Sample ID	Pb in bulk soil (ppb)	max sol (%)	calc Pb (%)	ICP Pb (ppb)	solubility ratio	1.5pH BSA
OM-1	AZZSGPZA12565	1287	1.00513	1.29	9.747	0.1	75
OM-2	AZZSGPZA12566	144079	1.0046	144.74	1100.09	0.1	76
OM-3	AZZSGPZA12568	4273	1.00384	4.29	31.829	0.1	74
OM-4	AZASGPZZ12563	45337	1.00731	45.67	367.6	0.1	80
OM-5	AZASGPZZ12544	50036	1.00747	50.41	430.68	0.1	85
OM-6	AZCESGPZZ12552	13970	1.00634	14.06	97.67	0.1	69
OM-7	AZZSGPZA12569	4733	0.98423	4.66	35.664	0.1	77
OM-8	AZASGPZZ12567	103509	1.00971	104.51	844.12	0.1	81
OM-9	AZZSGPZA12563	38260	0.99834	37.89	321.42	0.1	85
OM-10	AZCESGPZZ12561	7129	1.00357	7.15	66.409	0.1	73
OM-11	AZCESGPZZ12550	81268	1.00783	81.75	464.42	0.1	74
OM-12	AZZSGPZA12582	105666	1.00207	106.89	874.02	0.1	82
OM-13	AZZSGPZA12564	891	1.00543	0.89	5.455	0.1	79
OM-14	AZCESGPZZ12548	14407	1.00486	14.48	106.40	0.1	73
OM-15	AZDSGPZZ12547	22088	1.00364	22.17	164.18	0.1	74
OM-16	AZZSGPZA12581	39190	1.00333	39.32	296.36	0.1	75
OM-17	AZZSGPZA12574	197206	1.0062	198.43	1043.7	0.1	53
OM-18	AZZSGPZA12573	96181	1.00632	96.98	789.88	0.1	79
OM-19	AZZSGPZA12571	6109	1.00265	6.12	46.78	0.1	91
OM-20	AZCSGPZZ12544	7958	1.00558	8.00	56.38	0.1	70
OM-21	AZZSGPZA12576	4201	1.00644	4.23	37.24	0.1	88
OM-22	AZZSGPZA12675	42220	1.00738	42.53	356.84	0.1	84
OM-23	AZCSGPZZ12543	8601	1.00889	8.68	58.245	0.1	87
OM-24	AZOSGPZZ12541	528	1.00782	0.53	5.647	0.1	106
OM-25	AZCNSGPZZ12542	52861	1.00557	53.26	416.267	0.1	78
Community soils							
5003DZ		2389	1.00242	2.39	23.159	0.1	97
5063 B1		2220	1.00089	2.22	21.358	0.1	96
5062 B1		808	1.00187	0.81	7.251	0.1	90
5060 B2		567	1.00576	0.57	4.65	0.1	82
5048 B2		514	1.00657	0.52	3.921	0.1	76
5079 B2		2010	1.0033	2.02	15.403	0.1	78
5081 F2		693	1.0002	0.69	6.024	0.1	87
5046 B2		1973.744	1.00357	1.98	17.71	0.1	83
5046 B1		394.23062	1.0022	0.40	3.124	0.1	79
5063 F2		1184.4449	1.0014	1.19	10.44	0.1	88
5081 F2		569.39369	1.0058	0.57	4.89	0.1	82
5058 F2		586.06163	1.0012	0.59	4.90	0.1	84
5044 B1		432.9642	1.0048	0.44	3.62	0.1	83
5055 F1		830.97308	1.0037	0.83	5.17	0.1	82
5088 F2		836.87574	1.0011	0.84	5.18	0.1	81
5034 B1		450.73448	1.0036	0.49	4.60	0.1	91
5017 F1		944.56151	1.0002	0.94	8.10	0.1	86
5006 B1		986	1.0017	0.99	8.80	0.1	89
5087 G		978.31268	1.0065	0.98	7.91	0.1	80
5080 B2		1699.696	1.0002	1.94	17.38	0.1	89
5020 B2		805.85158	1.0073	0.81	6.44	0.1	79
5098 B2		52.707758	1.0082	0.68	0.41	0.1	84
5008 B1		547.10847	1.0040	0.66	5.69	0.1	88
5056 F2		878.71722	0.9989	0.86	7.98	0.1	91
5030 B1		131.19291	1.0065	0.13	1.01	0.1	76
5007 F2		520.6223	1.0007	0.52	4.54	0.1	87
5059 B1		793.9715	1.0064	0.80	7.4	0.1	93
5041 B1		124.74159	1.0068	0.13	1.1	0.1	87
Goold "Park"							
Loc#1		9546	1.0000	9.55	42.6	0.1	77
Loc#2		8219	1.0028	8.24	62.8	0.1	78
Loc#3 6-9"		3545	1.0051	3.66	26.7	0.1	73
Loc#4 10-15"		399	1.0038	0.60	3.0	0.1	51
Loc#4		2508	1.0018	2.51	19.1	0.1	76
Loc#5 10-12"		16636	1.0057	16.73	144.7	0.1	86
Loc#6 5-8"		4368	1.00	4.38	32.0	0.1	73
Loc#7 9-11"		1353	1.00157	1.36	9.2	0.1	68
QA/QC							
BLANK					0.082		
BLANK					0.029		
BLANK					0.022		
BLANK					0.006		
BLANK					0.048		
BLANK					0.038		
BLANK					0.014		
OM-4-AD		45337	1.00731	45.67	377.12	0.1	83
5007 F2-AD		520.6221	1.0007	0.53	4.51	0.1	87
OM-6-DUP		13970	1.00457	14.06	105.112	0.1	73
OM-24-DUP		528	1.00392	0.53	5.522	0.1	84
5020 B2-DUP		805.85158	1.0013	0.81	6.59	0.1	82
5020 B2-DUP		805.85158	1.0013	0.81	6.44	0.1	83
5046 B1-DUP		394.23062	1.00501	0.40	3.154	0.1	83
5046 B1-DUP		394.23062	1.00501	0.40	3.25	0.1	83
5020 B2-DUP-spike-20ppm					27.30		
5046 B1-DUP-spike-10ppm					13.78		
BLANK-spike-10ppm					11.301		

AD= Analytical duplicate

2.0 In Vitro Standard Operating Procedure

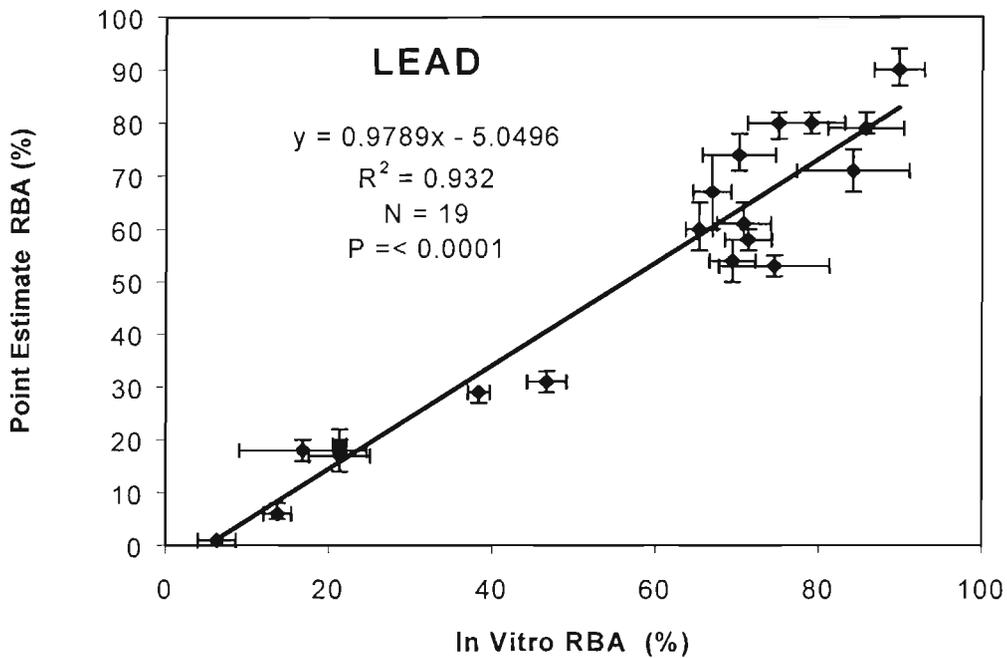
2.1 Background

An increasingly important property of contaminated media found at environmental sites is the bioavailability of individual contaminants. Bioavailability is the fraction of a contaminant that is absorbed by an organism via a specific exposure route. Many animal studies have been conducted to experimentally determine oral bioavailability of individual metals, particularly lead and arsenic. During the period 1989-97, a juvenile swine model developed by USEPA Region VIII was used to predict the relative bioavailability of lead and arsenic in approximately 20 substrates (Weis and LaVelle 1991; Weis et al. 1994). The bioavailability determined was relative to that of a soluble salt (i.e. lead acetate trihydrate). The tested media had a wide range of mineralogy, and produced a range of lead and arsenic bioavailability values. In addition to the swine studies, other animal models (e.g. rats and monkeys) have been used for measuring the bioavailability of lead and arsenic from soils and paint.

Several researchers have developed in vitro tests to measure the fraction of a chemical solubilized from a soil sample under simulated gastrointestinal conditions. The in vitro tests consist of an aqueous fluid, into which the contaminant is introduced. The solution then solubilizes the media under simulated gastric conditions. Once this procedure is complete, the solution is analyzed for lead concentrations. The mass of the lead found in the filtered extract is compared to the mass introduced into the test. The fraction liberated into the aqueous phase is defined as the bioavailable fraction of lead or arsenic in that media. To date, for lead-bearing materials tested in the USEPA swine studies, this in vitro assay has correlated well ($R^2 = 0.93$, $p = .0001$), Figure 2.1.1, with relative bioavailability. Arsenic results are still in review and data should be considered for screening purposes only at this time.

Further background on the development and validation of in vitro test systems for estimating lead and arsenic bioaccessibility can be found in; Ruby et al. (1993, 1996); Medlin (1972); Medlin and Drexler, 1997; Drexler, 1998; and Drexler et al., 2004. Background information for the USEPA swine studies may be found in (Weis and LaVelle, 1991; Weis et al. 1994; and Casteel et al., 1997) and in the USEPA Region VIII Center in Denver, Colorado.

Figure 2.1.1



2.2 Sample Preparation

All media were prepared for the in vitro assay by first drying (<40° C) all samples and then sieving to < 250 m. The <250 micron size fraction was used because this particle size is representative of that which adheres to children's hands. Samples were thoroughly mixed prior to use to ensure homogenization. Samples were archived after the study completion and retained for further analysis for a period of six months unless otherwise requested. Prior to obtaining a subsample for testing in this procedure, each sample was homogenized in its sample container by end-over-end mixing.

2.3 Apparatus and Materials

2.3.1 Equipment

The main piece of equipment required for this procedure is the extraction device. The device holds ten; 125 ml, wide-mouth, high-density polyethylene (HDPE) bottles. These were rotated within a Plexiglas tank by a TCLP extractor motor with a modified flywheel. The water bath must be filled such that the extraction bottles remained immersed. Temperature in the water bath was maintained at 37 +/- 2° C using an immersion circulator heater (Fisher Scientific Model 730). The 125-ml HDPE bottles had an airtight screw-cap seal (Fisher Scientific #02-893-5C), and care was taken to ensure that the bottles did not leak during the extraction procedure.

2.3.2 Standards and Reagents

The leaching procedure for this method used an aqueous extraction fluid at a pH value of 1.5. The pH 1.5 fluid was prepared as follows:

Two liters of aqueous extraction fluid were prepared using ASTM Type II deionized (DI) water. The buffer was made up in the following manner. To 1.9 L of DI water, 60.06 g glycine (free base, reagent grade), were added bringing the solution volume to 2 L (0.4M glycine). The mixture was placed in the water bath at 37° C until the extraction fluid reached 37 °C. The pH meter (using both a 2.0 and a 4.0 pH buffer for standardization) was standardized using temperature compensation at 37° C or buffers maintained at 37° C in the water bath. Trace metal grade, concentrated hydrochloric acid (12.1N) was added until the solution pH reached a value of 1.50 +/- 0.05 (approximately 60 mL).

All reagents were free of lead and arsenic, and the final fluid was tested to confirm that lead and arsenic concentrations were less than one-fourth the project required detection limit (PRDL) of 100 (less than 25 $\mu\text{g/L}$ lead 5 $\mu\text{g/L}$ arsenic) in the final fluid.

Cleanliness of all materials used to prepare and/or store the extraction fluid and buffer is essential. All glassware and equipment used to prepare standards and reagents were properly cleaned, acid washed, and finally, triple-rinsed with deionized water prior to use. When possible, disposable “poly” tubes were used.

2.4 Leaching Procedure

100 +/- 0.5 mL of the extraction fluid was measured, using a graduated cylinder, and transferred to a 125 mL wide-mouth HPDE bottle. 1.00 +/- 0.5 g of test substrate (<250 μm) was added to the bottle, ensuring that static electricity did not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, an antistatic brush was used to eliminate static electricity prior to adding the media. The mass of substrate added to the bottle was recorded. Each bottle top was hand tightened and shaken/inverted to ensure that no leakage occurred, and that no media was caked on the bottom of the bottle.

The bottle was placed into the modified TCLP extractor, making sure each bottle was secure and the lid(s) were tightly fastened. The extractor was filled with 125 mL bottles containing test material or QA samples.

The temperature of the water bath was 37 +/- 2° C.

The extractor was turned on and rotated end-over-end at 30 +/- 2 rpm for 1 hour. The start time of rotation was recorded.

When extraction (rotation) was complete, the extractor rotation was immediately stopped and the bottles were removed. They were then wiped dry and placed upright on the bench top.

Extract was removed directly from the reaction vessel into a disposable 20 cc syringe with a Luer-Lok attachment. A 0.45 μm cellulose acetate disk filter (25 mm diameter) was attached to the syringe, and the extract was filtered into a clean 15 mL polypropylene centrifuge tube (labeled with sample ID) or other appropriate sample vial for analysis.

The time that the extract was filtered was recorded (i.e. extraction was stopped). If the total time elapsed was greater than 1 hour 30 minutes, the test was repeated.

The pH of the remaining fluid was measured in the extraction bottle. If the fluid pH was not within +/- 0.5 pH units of the starting pH, the test was discarded and the sample reanalyzed as follows:

If the pH had changed more than 0.5 units, the test was re-run in an identical fashion. If the second test also resulted in a decrease in pH of greater than 0.5 s.u. this was recorded, and the extract filtered for analysis. If the pH had increased by 0.5 s.u. or more, the test was repeated, but the extractor stopped at specific intervals and the pH manually adjusted down to pH of 1.5 with dropwise addition of HCl (adjustments at 5, 10, 15, and 30 minutes into the extraction, and upon final removal from the water bath { 60 min}). Samples with rising pH values might better be run following the method of Medlin, 1997.

Filtered samples were stored in a refrigerator at 4° C until analyzed. Analysis for lead and arsenic concentrations occurred within 1 week of extraction for each sample.

In general, extracts were analyzed for lead and arsenic, following EPA methods 6010B, 6020, or 7061A.

2.5 Quality Control/Quality Assurance

Quality Assurance for the extraction procedure consisted of a series of quality control samples. Controls, control limits and corrective actions are listed in Table 2.5.1 .

Table 2.5.1.

	Analysis Frequency	Control Limits	Corrective Actions
Reagent Blank	once per batch	< 25 $\mu\text{g/L}$ lead	Make new fluid and re-run all analyses.
Bottle blank	1 in 10	<50 $\mu\text{g/L}$ lead	Check calibration and re-analyze as necessary.
Blank spike*	1 in 10	85-115% recovery	Check calibration and/or source of contamination and re-analyze.
Matrix spike*	1 in 20	75-125% recovery	Flag
Duplicate sample	1 in 20	+/- 20% RPD**	Flag
Control soil***	1 in 25	+/- 10% RPD	Flag

- Spikes contained 10 mg/L lead . ** RPD= relative percent difference.
- *** The National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) RPD is based upon mean RBA-lead values of 84% and 75% for MS2711 and MS2710, respectively.

3.0 Bulk Soil Analyses

Analysis of the <250 μ soil sample was carried out using Good Laboratory Practice (GLP) protocols. Samples were digested following EPA Method 3050B. Analysis of the digest was similar to EPA Methods 6020 A or B, but with somewhat reduced QA/QC. Controls, control limits and corrective actions are listed in Table 3.0.1. Initial calibration was based on a 4- point calibration curve with a minimum 0.999 R^2 value.

	Analysis Frequency	Control Limits	Corrective Actions
Method Blank	once per run	25 μ g/L lead	Check calibration and/or source of contamination and re-analyze all samples.
IVC Initial Calibration Verification	once per run	90-110% recovery	Check calibration and start run over.
Interference Check	once per run	90-110% recovery	Flag
Matrix spike*	1 in 20	75-125% recovery	Flag
CCV Continuing Calibration Verification	1 in 10	90-110% recovery	Check calibration and re-analyze preceding samples.
Duplicate sample	1 in 20	+/- 20% RPD	Flag

* Spikes contained 500 mg/L lead

4.0 Chain-of-Custody Procedures

All media once received by the Laboratory were maintained under standard chain-of-custody. Samples were maintained within secured facilities, with limited access, for 60 days post laboratories final report.

5.0 Data Handling and Verification

All sample and fluid preparation calculations and operations were recorded on data sheets. Finally, all key data were entered into EXCEL spreadsheets for final delivery and calculation of percent relative bioavailability.

6.0 Data Evaluation

Data evaluation is based on in vitro analyses from the current set of samples, not historical samples that have been added to this report for comprehensiveness. Bulk soil analyses met or exceeded all required QA/QC and therefore no corrective action was necessary. Results are summarized below.

3050B	Analysis Frequency	Control Limits	Corrective Actions
Method Blank	1 Method blank run	<3 $\mu\text{g/L}$ lead no values above limit.	None
IVC Initial Calibration Verification	once per run	99% recovery	None
Interference Check	once per run	99% recovery	None
CCV Continuing Calibration Verification	2 CCV run	98-99% recovery	None
Matrix spike	2 matrix spike run	98-99% recovery-Pb	None
Duplicate sample	2 duplicates run	3-9% RPD-Pb	None

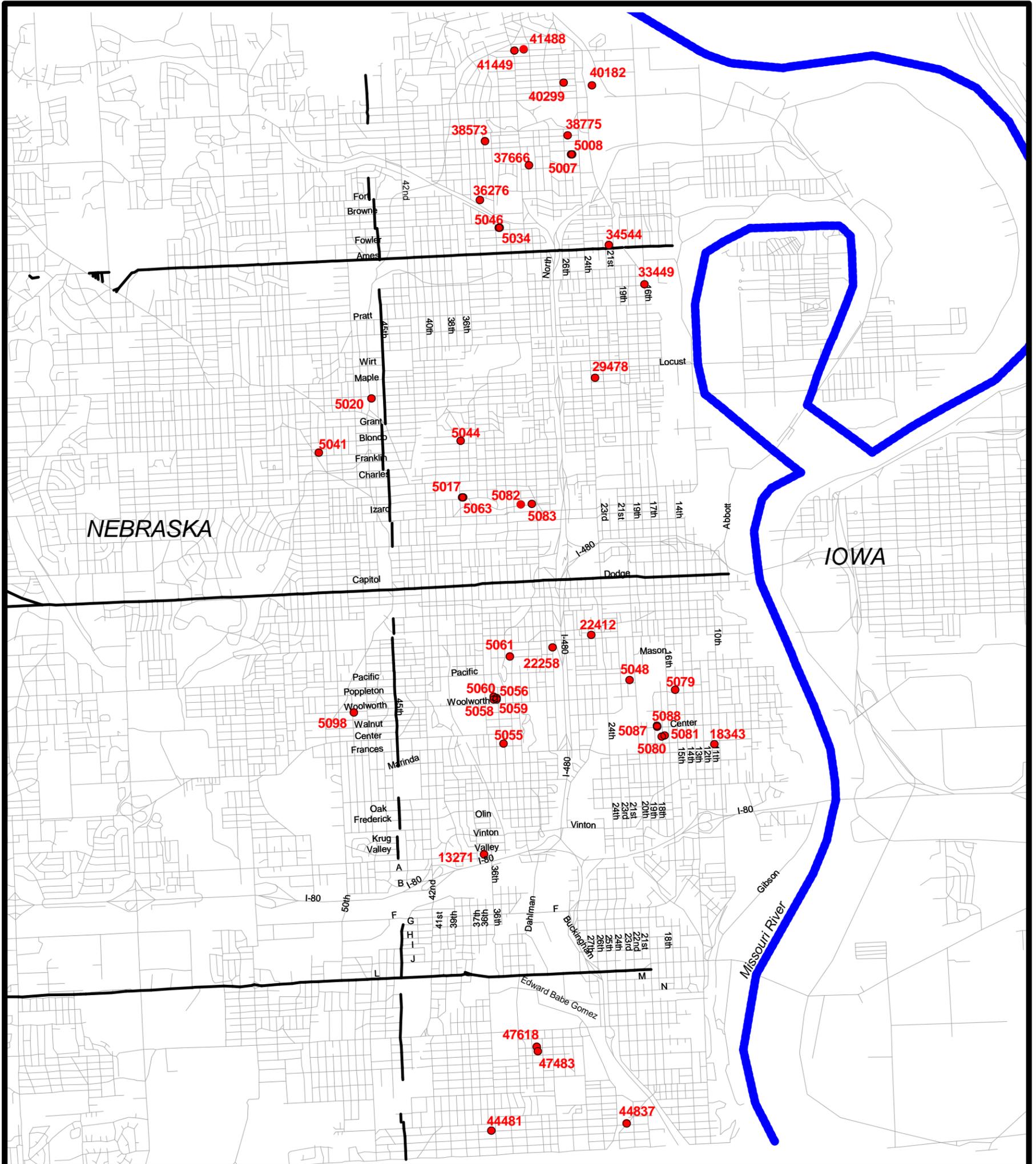
RBLP analyses did meet all QA/QC and no corrective action is suggested.

RBLP	Analysis Frequency	Control Limits	Corrective Actions
Reagent Blank	One Reagent Blank	<1 $\mu\text{g/L}$ lead	None
Bottle blank	1 blank run	<1 $\mu\text{g/L}$ lead	None
Blank spike	2 blank spikes run	98-99% recovery	None
Matrix spike	2 matrix spikes run	96-97% recovery	None
Duplicate sample	2 duplicates run	1-2% RPD	None
Control soil	1 control run	4% RPD	None

7.0 References

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Figure 1 Omaha Lead Site In Vitro Sample Locations



LEGEND

- In Vitro Sample
- Roads
- State Line

Resident properties for which access has been granted have been sampled.



1000 0 1000 2000 3000 Feet