Evaluation of Moss Sampling as a Methodology for Evaluating Air Toxics, Using Portland as a Study Area

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DEQ is a leader in restoring, maintaining and enhancing the quality of Oregon's air, land and water.

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Department of Environmental Quality

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1. Background

Mosses have been used as bioindicators of air quality in many studies (e.g., Zhou et al. 2017, Chen et al. 2015, Kłos et al. 2018). Still, little is understood about how concentrations found in moss relate to atmospheric concentrations or how concentrations found in moss vary over time. In December 2013, the U.S. Forest Service (USFS) collected 346 samples of *Orthotrichum lyellii*, a moss that readily grows on hardwood trees, around Portland to determine the concentrations of 22 elements that have important implications in plant biology and are environmentally important trace elements (Gatziolis et al. 2016). Additional moss samples were collected by USFS in October 2015 in areas where 2013 results showed elevated concentrations of cadmium. In 2017 DEQ staff collected samples of *O. lyelii* at selected USFS locations. The purpose of this DEQ sampling effort was to determine how concentrations of metals found in eight *O. lyellii* samples collected by DEQ in March 2017 compared to concentrations found in USFS samples (seven from December 2013, one from October 2015). Additionally, the purpose of this sampling effort was to validate the protocols for field sampling, laboratory sample processing, and analytical techniques necessary to determine the concentrations of metals in moss.

2. Methods

2.1 Field Sampling

DEQ collected samples of *O. lyellii* from eight sites previously sampled by the USFS. Five of those sites were located in a dense urban area and showed elevated concentrations of arsenic, cadmium, chromium, cobalt, lead and nickel based on the USFS distributions of each element. Three sites were located in a light urban area and showed lower concentrations of metals in moss.

Additionally, DEQ collected three moss samples each from two rural areas where it is believed that moss would be minimally exposed to levels of metals via the atmosphere due to the lack of nearby urban and industrial sources. The USFS did not previously sample these two sites (Figure 1).

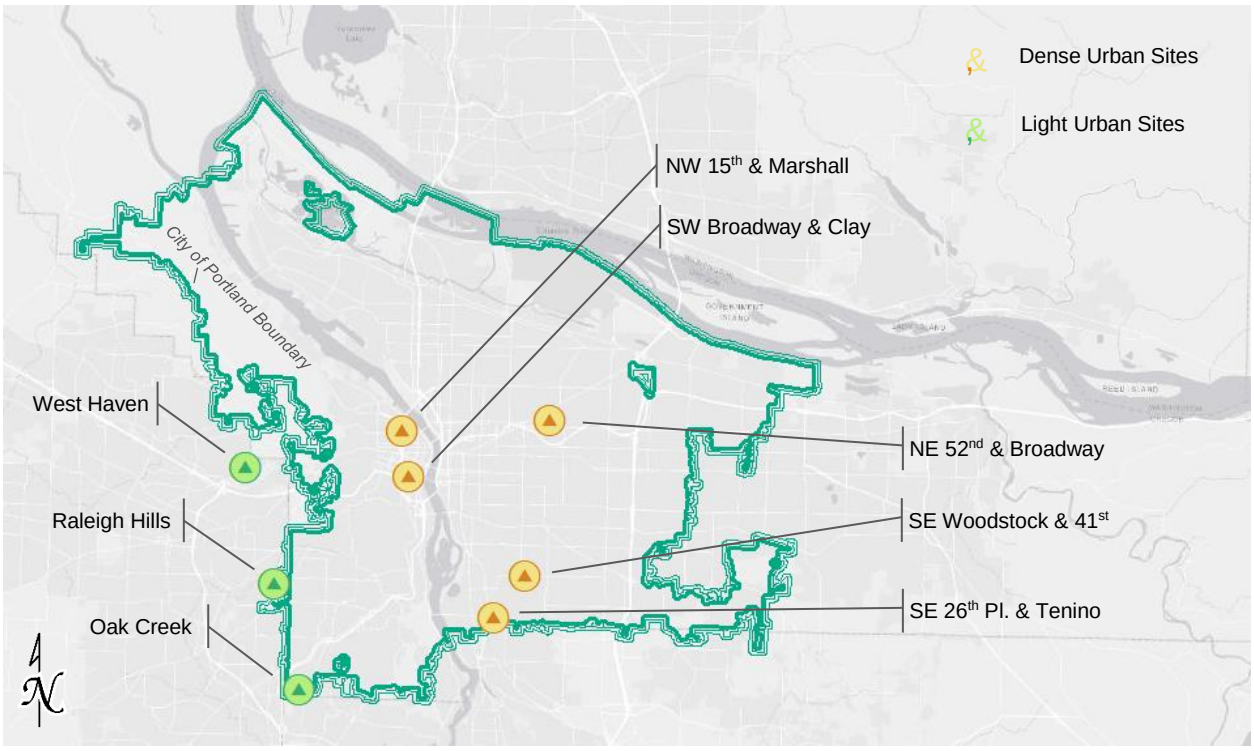

Figure 1a. Location of USFS moss collection sites that were resampled by DEQ.

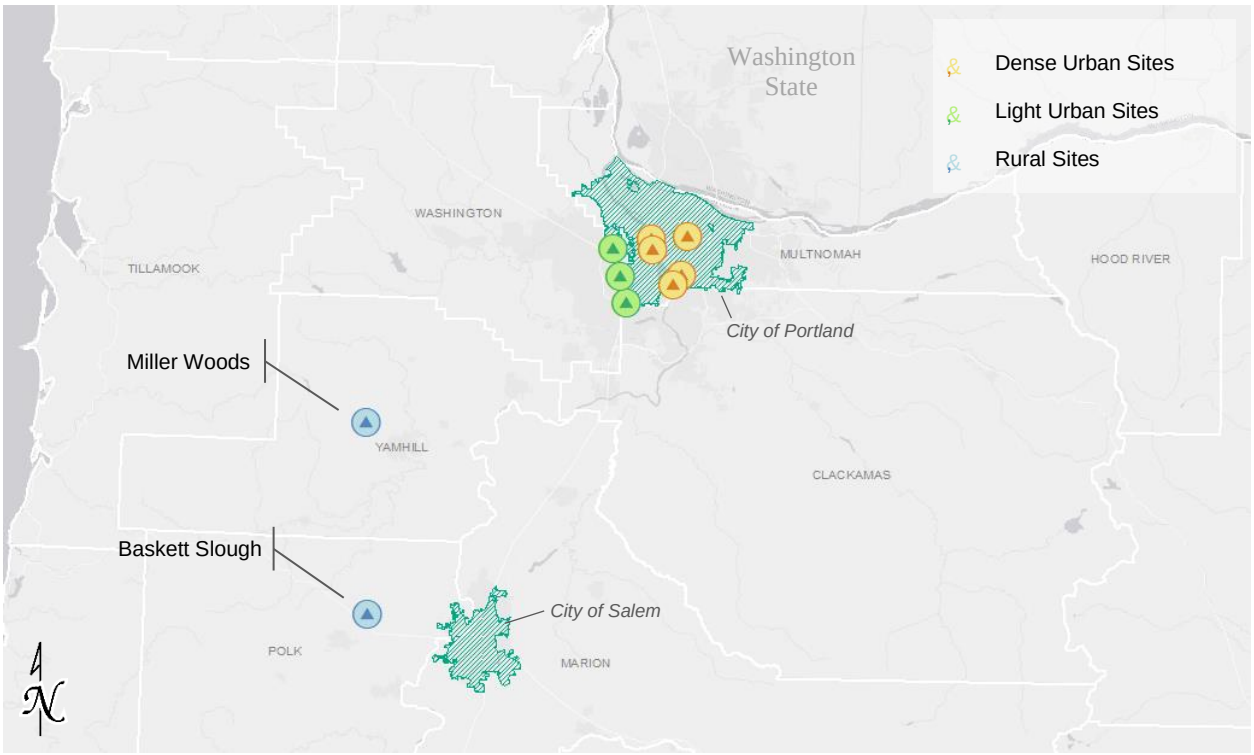

Figure 1b. Location of rural moss collection sites. See Figure 1a for detail of Dense Urban and Light Urban sites.

DEQ collected supplemental moss samples at four sites surrounding each of the original sites sampled by USFS. A sampling grid, consisting of four quadrants with the original sample tree at the center, facilitated the collection of these supplemental samples. The goal was to collect one supplemental sample from each quadrant to compare concentrations over short distances (<1,500ft.). The sampling grid was defined using ESRI ArcGIS and based on the configuration of the streets at the SW Broadway and Clay site. The presence of a dense urban environment at this location dictated that only trees located along the sidewalks were available for sampling and therefore the sampling grid followed the orientation of the streets accordingly.

Moss was collected outside of the desired quadrant in the event that no suitable moss was available to collect within the quadrant, no trees existed within the desired quadrant, or due to access restrictions. Detailed field sampling and preservation methods were documented in Moss Field Collection and Sample Preparation for Atmospheric Pollutant Analysis (ODEQ 2017). In total, DEQ staff collected 45 moss samples.

2.2 Laboratory Analysis

Moss samples were prepared for laboratory analysis by trimming, drying, and grinding the live green portions of the total amount of moss harvested from field sites (Figure 2). A minimum of 1.8g of trimmed and dried material was required per sample. Moss was dried at 40°C for 24 hours and then ground into a homogenous powder. The resulting powder was then dissolved into an aqueous solution consisting of 20% nitric acid (HNO_3) and 5% hydrochloric acid (HCl), and heated by microwave at 200°C for 4.5 minutes. Following the microwave digestion, the solution was diluted by five times and analyzed using inductively coupled plasma mass spectrometry (ICPMS). Samples were analyzed for 21 elements (Table 1).

Figure 2. Photo showing the location of where individual moss fronds were trimmed (yellow line). The live green sections were kept and further prepared for laboratory analysis while unused portions were discarded.

Table 1. List of elements analyzed in moss samples collected by DEQ.

Element	*Element Symbol*	*Element*	*Element Symbol*
Aluminum	Al	Lead	Pb
Arsenic	As	Magnesium	Mg
Barium	Ba	Manganese	Mn
Beryllium	Be	Molybdenum	Mo
Boron	B	Nickel	Ni
Cadmium	Cd	Potassium	K
Calcium	Ca	Selenium	Se
Chromium	Cr	Sodium	Na
Cobalt	Co	Strontium	Sr
Copper	Cu	Zinc	Zn
Iron	Fe		

3. Results

3.2 Moss Samples at USFS Sites

DEQ staff collected moss samples from seven out of the eight trees previously sampled by USFS – one tree where moss was previously collected by USFS did not have suitable moss for collection by DEQ staff. Table 2 shows the relative percent difference in metals concentrations between USFS and DEQ results

Table 2. Relative percent difference between moss collected by USFS and DEQ. Repeat sites were chosen by DEQ based on the range of concentrations seen in previous USFS results as well as based on urban density.

			Element																				
			mg/kg of dry moss																				
			K	**Mg**	**Ca**	**Mo**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**B**	**Na**	**Sr**	**Ba**	**Al**	**Cr**	**Co**	**Cd**	**Pb**	**As**	**Be**	**Se**
Dense Urban	*SITE: NE 52nd & Broadway*																						
	Sampled December 2013	**USFS**	4500	1800	6900	0.63	67.9	1023	1.84	15.2	49.8	26.1	100	41.9	87.3	628	2.98	0.59	0.33	9.5	0.77	**	**
	Sampled March 2017	**DEQ**	4760	2220	8290	ND	75.8	658	1.87	10.6	31.4	32.6	72.6	48.9	60.7	421	3.31	0.34	0.28	3.25	0.22	ND	ND
	Relative Percent Difference		6%	23%	20%	N/A	12%	-36%	2%	-30%	-37%	25%	-27%	17%	-30%	-33%	11%	-42%	-15%	-66%	-71%	N/A	N/A
	SITE: NW 15th & Marshall																						
	Sampled December 2013	**USFS**	4700	1900	7500	3.44	142	3883	6.34	52.2	250	38	166	70.7	97.6	1497	8.8	2.02	0.67	35	0.46	**	**
	Sampled March 2017	**DEQ**	6130	2030	6780	2.46	40	1480	2.95	36.2	107	37.1	125	58.3	62.9	628	5.54	0.57	1.1	7.13	0.23	ND	ND
	Relative Percent Difference		30%	7%	-10%	-28%	-72%	-62%	-53%	-31%	-57%	-2%	-25%	-18%	-36%	-58%	-37%	-72%	64%	-80%	-50%	N/A	N/A
	*SITE: SW Broadway & Clay**																						
	Sampled December 2013	**USFS**	*4800*	*2000*	*6600*	*3.4*	*123*	*3614*	*7.43*	*45.5*	*154*	*24.6*	*186*	*43.5*	*78.2*	*1169*	*8.07*	*1.41*	*0.43*	*34.7*	*0.32*	**	**
	Sampled March 2017	**DEQ**	*5430*	*1690*	*7770*	*3.26*	*86.5*	*2380*	*5.44*	*30.8*	*109*	*33.9*	*120*	*60.4*	*70.3*	*1050*	*10.1*	*0.97*	*0.77*	*7.04*	*0.43*	*ND*	*ND*
	Relative Percent Difference		*13%*	*-16%*	*18%*	*-4%*	*-30%*	*-34%*	*-27%*	*-32%*	*-29%*	*38%*	*-35%*	*39%*	*-10%*	*-10%*	*25%*	*-31%*	*79%*	*-80%*	*34%*	*N/A*	*N/A*
	SITE: SE Woodstock & 41st																						
	Sampled December 2013	**USFS**	3600	1300	4700	1.55	53.9	2013	13.81	18.2	106	5.2	139	30	41.9	901	4.4	1.35	0.26	20	0.3	**	**
	Sampled March 2017	**DEQ**	5170	1600	5230	1.21	57.6	1810	10.2	16.7	107	ND	136	36.7	44.5	996	8.62	1.65	0.23	4.77	0.26	ND	ND
	Relative Percent Difference		44%	23%	11%	-22%	7%	-10%	-26%	-8%	1%	N/A	-2%	22%	6%	11%	96%	22%	-12%	-76%	-13%	N/A	N/A
	SITE: SE 26th Pl. & Tenino																						
	Sampled October 2015	**USFS**	3990	986	7058	**	51.25	1111	61.16	19.86	65.58	**	245	37.29	**	615	**	**	0.75	3.79	ND	**	1.1
	Sampled March 2017	**DEQ**	5290	1310	8280	1.5	35	1240	89.3	20.9	76.5	135	104	49.3	60.5	659	8.61	1.7	3.49	2.45	0.21	ND	2.53
	Relative Percent Difference		33%	33%	17%	N/A	-32%	12%	46%	5%	17%	N/A	-58%	32%	N/A	7%	N/A	N/A	365%	-35%	N/A	N/A	130%
Light Urban	*SITE: Oak Creek*																						
	Sampled December 2013	**USFS**	4000	1700	5300	0.42	54.5	690	0.98	6.7	36.7	9.7	81	40.9	46.8	346	1.33	0.39	0.08	2.1	ND	**	**
	Sampled March 2017	**DEQ**	5160	1860	5250	ND	30.2	566	1.36	6.63	24.4	11.8	92.2	55.5	53.8	338	3.49	0.25	0.07	0.74	0.14	ND	ND
	Relative Percent Difference		29%	9%	-1%	N/A	-45%	-18%	39%	-1%	-34%	22%	14%	36%	15%	-2%	162%	-36%	-13%	-65%	N/A	N/A	N/A
	SITE: West Haven-Sylvan																						
	Sampled December 2013	**USFS**	6100	2300	4400	0.4	21.9	616	0.83	16.7	24.1	20.8	139	31.3	28.7	287	1.23	0.34	0.07	1.4	ND	**	**
	Sampled March 2017	**DEQ**	6460	2750	4340	ND	20.1	659	1.15	23.9	23.3	22.7	117	37.2	33.1	433	2.02	0.31	0.11	0.98	0.13	ND	ND
	Relative Percent Difference		6%	20%	-1%	N/A	-8%	7%	39%	43%	-3%	9%	-16%	19%	15%	51%	64%	-9%	57%	-30%	N/A	N/A	N/A
	SITE: Raleigh Hills																						
	Sampled December 2013	**USFS**	5800	2700	4500	0.49	49.9	540	0.77	6.5	25.2	29.8	159	26.8	21.3	253	1.02	0.3	0.1	1.2	ND	**	**

	Sampled March 2017	**DEQ**	5990	2500	3710	0.42	40.1	491	1.08	6.73	19.2	15.6	176	29.9	19.4	330	1.63	0.23	0.07	0.78	0.12	ND	ND
	Relative Percent Difference		3%	-7%	-18%	N/A	-20%	-9%	40%	4%	-24%	-48%	11%	12%	-9%	30%	60%	-23%	-30%	-35%	N/A	N/A	N/A

* No suitable moss was available for collection by DEQ field staff on the tree previously sampled by USFS in 2013, therefore moss was taken from a nearby tree. Concentrations of elements are shown for informational purposes only.

** Not analyzed by USFS.

3.2 Supplemental Moss Samples

Table 3 shows descriptive statistics for each analyte based on all moss samples collected by DEQ. The Kaplan-Meier method was used to calculate the mean concentration due to its ability to account for any non-detect (ND) results present in the datasets. Figure 3 shows histograms for each analyte along with a vertical chart showing the individual results for each location. No histograms are shown for beryllium and selenium. Beryllium was not detected in any moss samples while selenium was detected only at the SE 26th and Tenino site (2.53 mg/kg).

Table 3. Summary statistics for elements found in DEQ moss samples. The Kaplan-Meier (KM) mean was calculated to account for any non-detect (ND) results. The shaded columns highlight information regarding ND values in each dataset.

Analyte	*# Obs*	*# Detects*	*# NDs*	*% NDs*	*Min. ND*	*Max. ND*	*Maximum*	*KM Mean*
Aluminum	45	45	0	0.00%	--	--	1540	560.6
Arsenic	45	37	8	17.78%	0.02	0.03	0.43	0.187
Barium	45	45	0	0.00%	--	--	106	46
Beryllium	45	0	45	100.00%	0.01	0.02	N/A	N/A
Boron	45	15	30	66.67%	0.99	1.11	135	10.84
Cadmium	45	41	4	8.89%	0.02	0.03	3.49	0.348
Calcium	45	45	0	0.00%	--	--	10400	5340
Chromium	45	45	0	0.00%	--	--	10.3	4.368
Cobalt	45	45	0	0.00%	--	--	2.22	0.629
Copper	45	45	0	0.00%	--	--	69.8	15.4
Iron	45	45	0	0.00%	--	--	2460	1017
Lead	45	45	0	0.00%	--	--	23.9	3.37
Magnesium	45	45	0	0.00%	--	--	3090	1679
Manganese	45	45	0	0.00%	--	--	236	73.54
Molybdenum	45	25	20	44.44%	0.05	0.05	3.72	0.786
Nickel	45	44	1	2.22%	0.1	0.1	89.3	5.237
Potassium	45	45	0	0.00%	--	--	9490	5229
Selenium	45	1	44	97.78%	0.25	0.38	2.53	0.301
Sodium	45	45	0	0.00%	--	--	412	117.9
Strontium	45	45	0	0.00%	--	--	89.2	38.87
Zinc	45	45	0	0.00%	--	--	164	58.85

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.

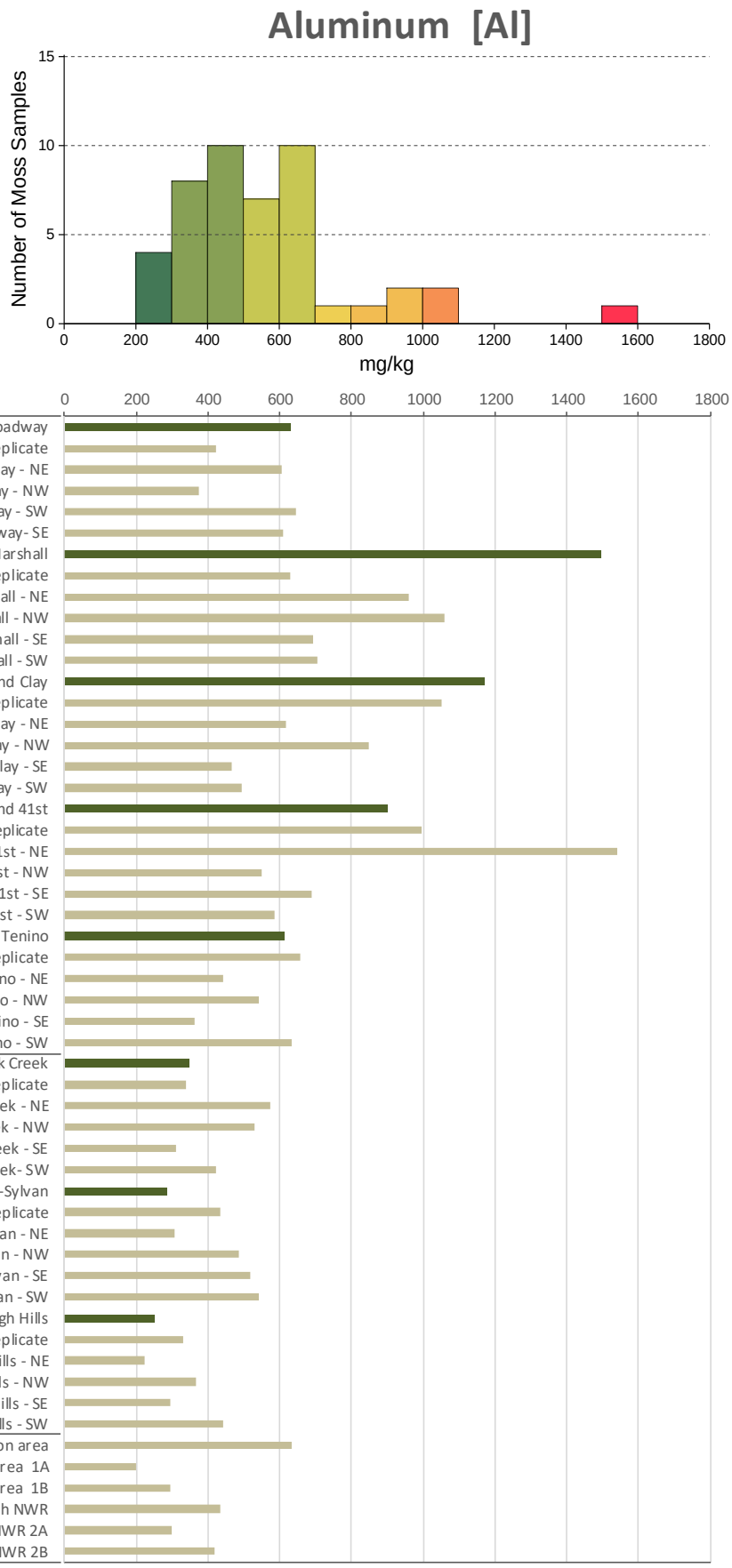

Figure 3a. Aluminum concentrations found in moss samples and corresponding histogram.

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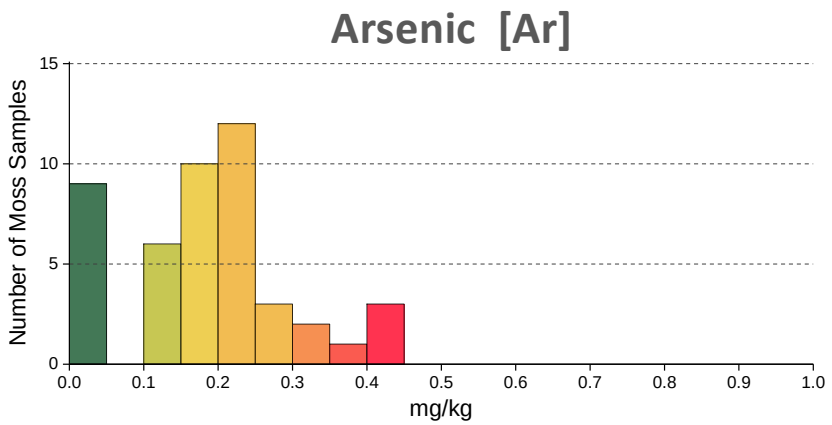


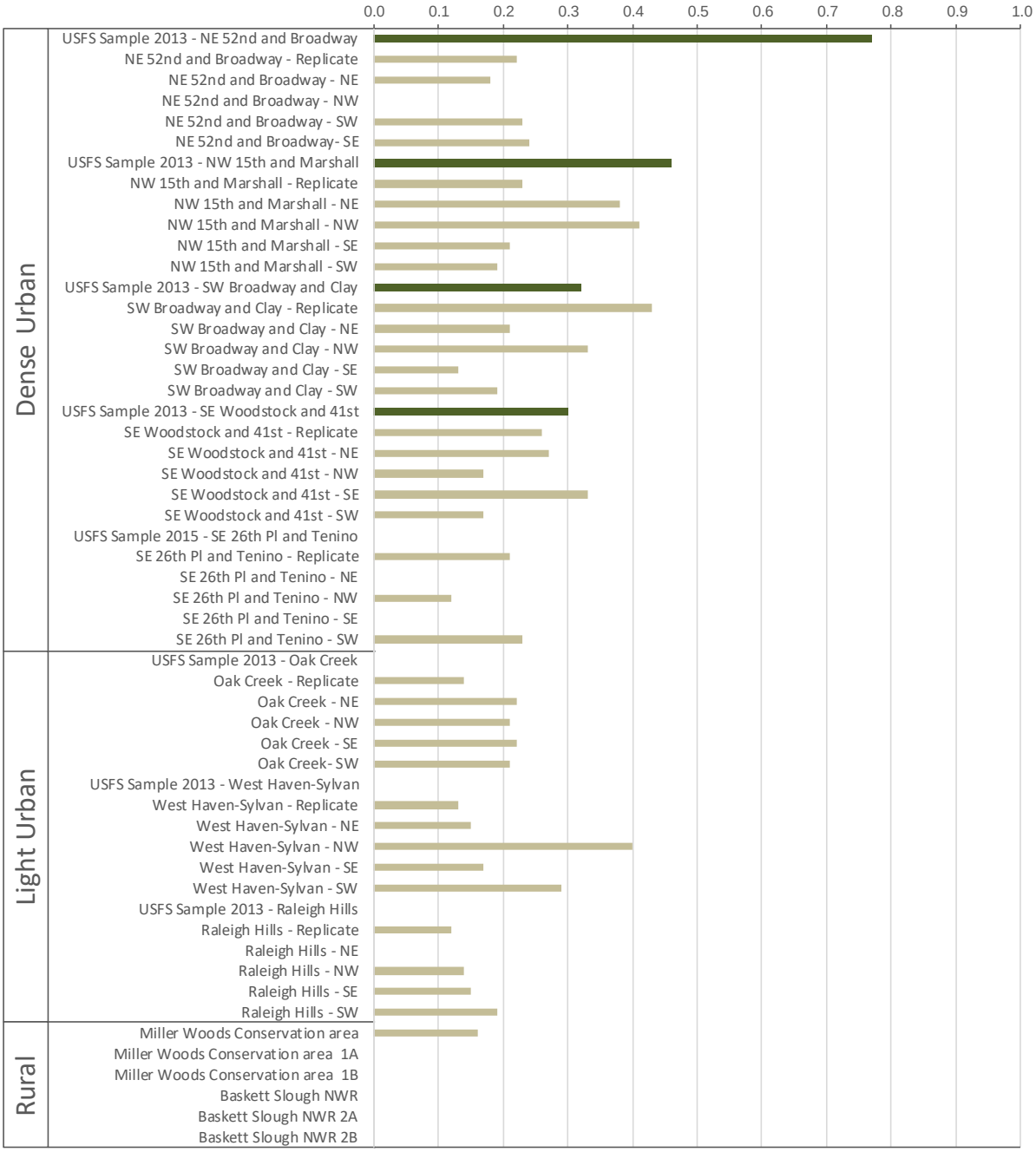
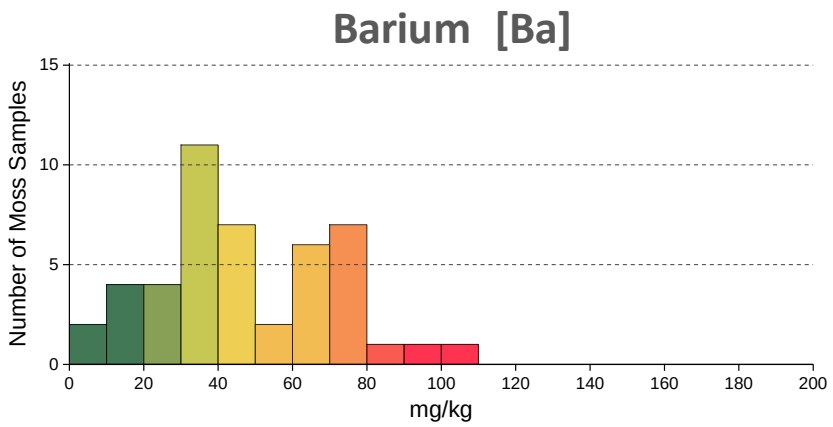


Figure 3b. Arsenic concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



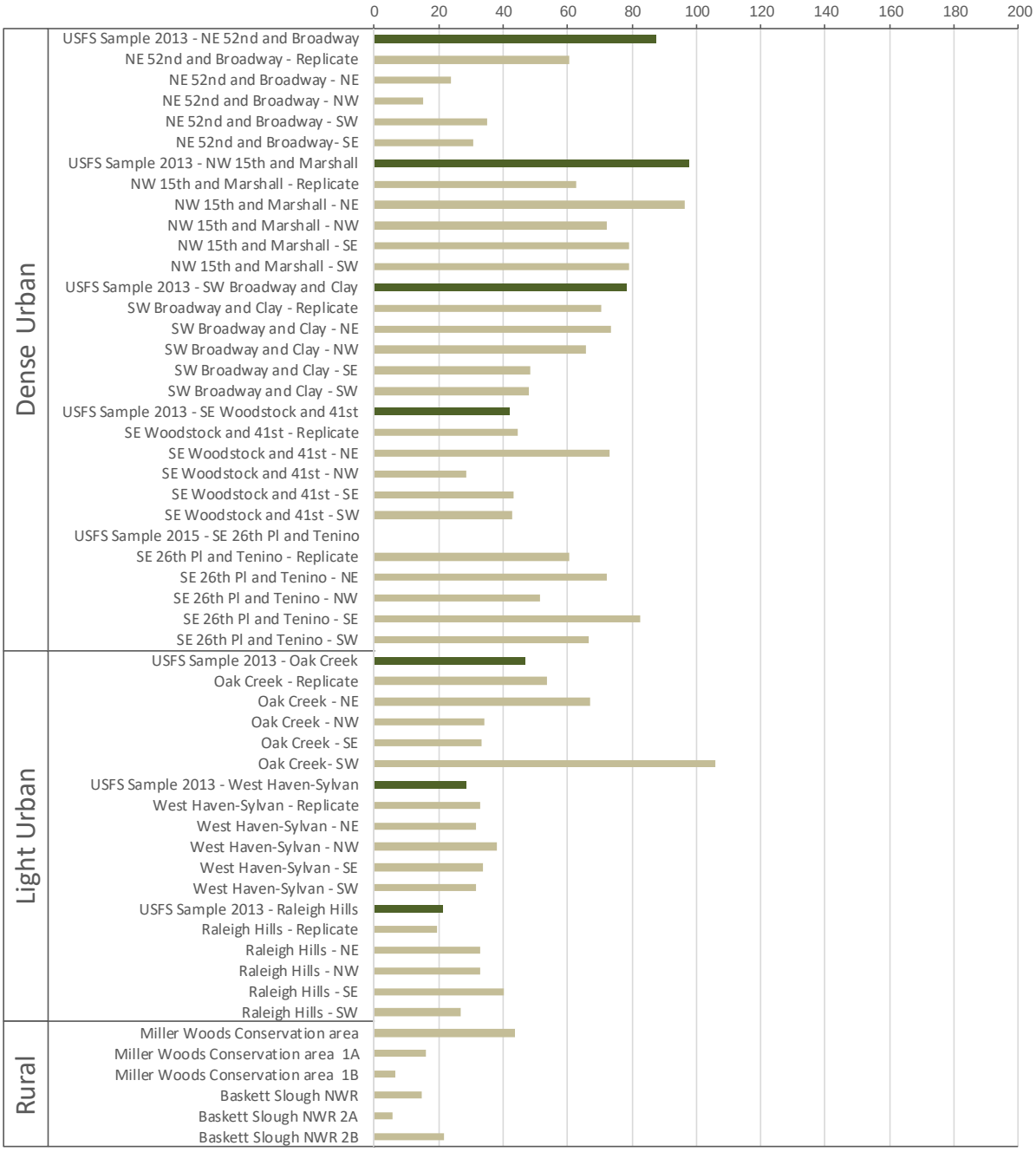
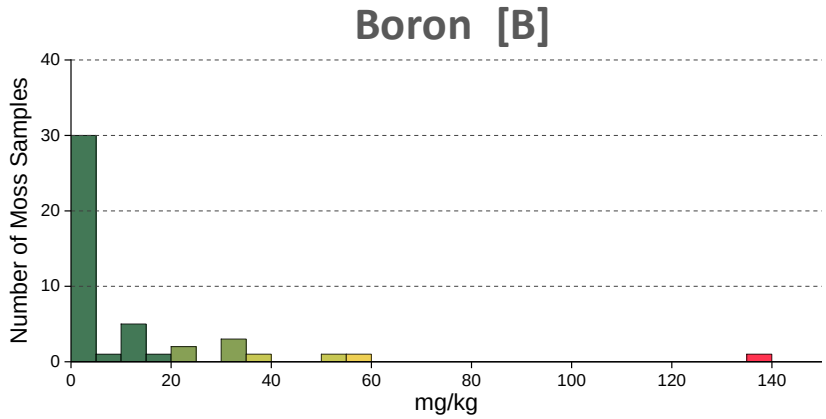


Figure 3c. Barium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



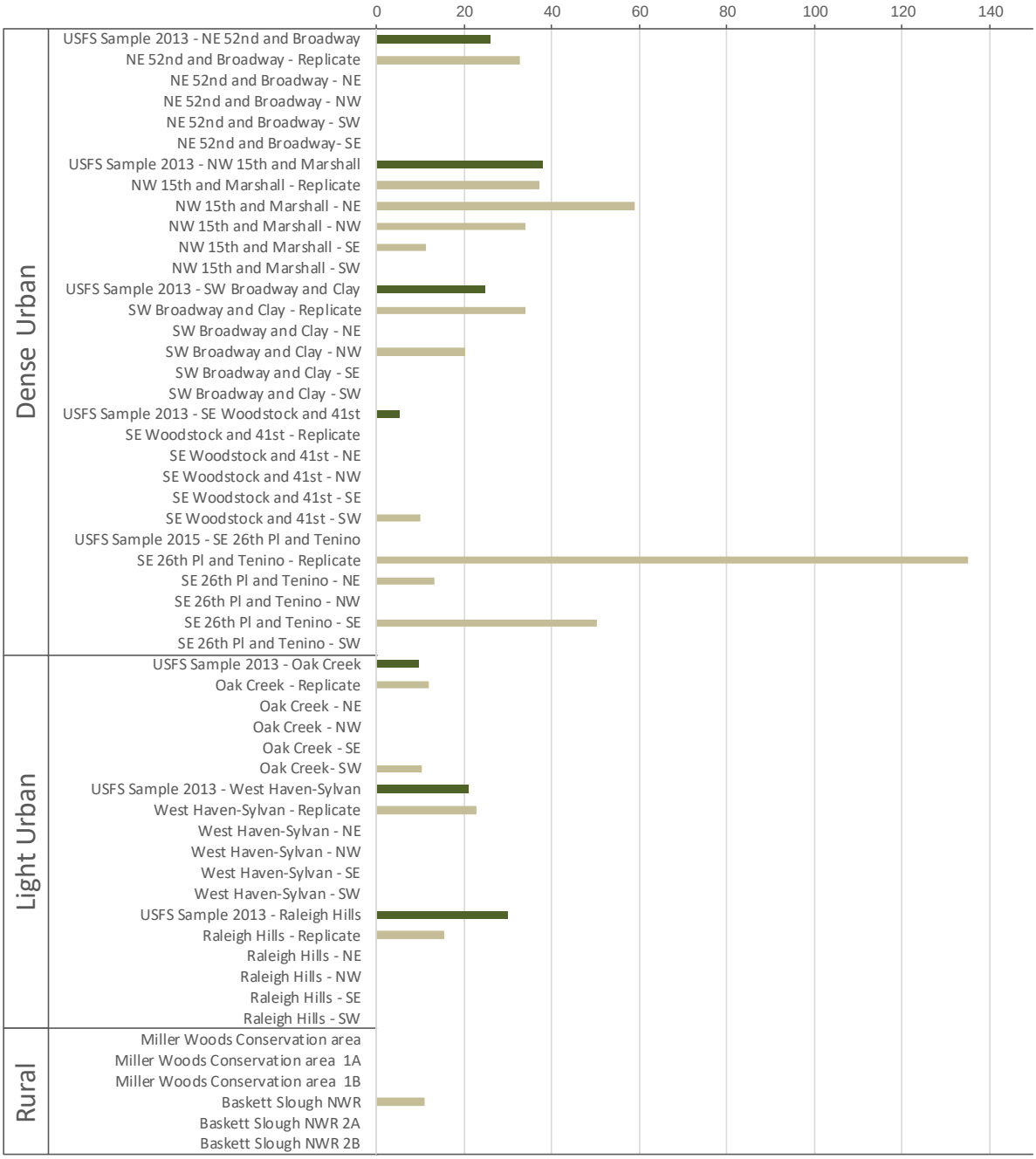
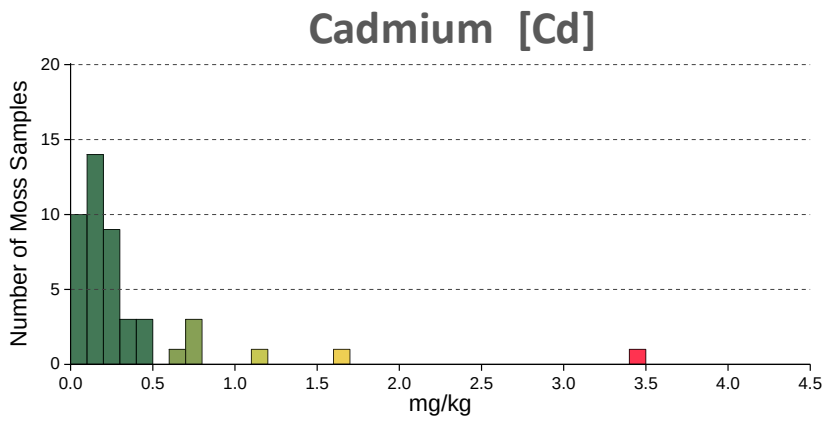


Figure 3d. Boron concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



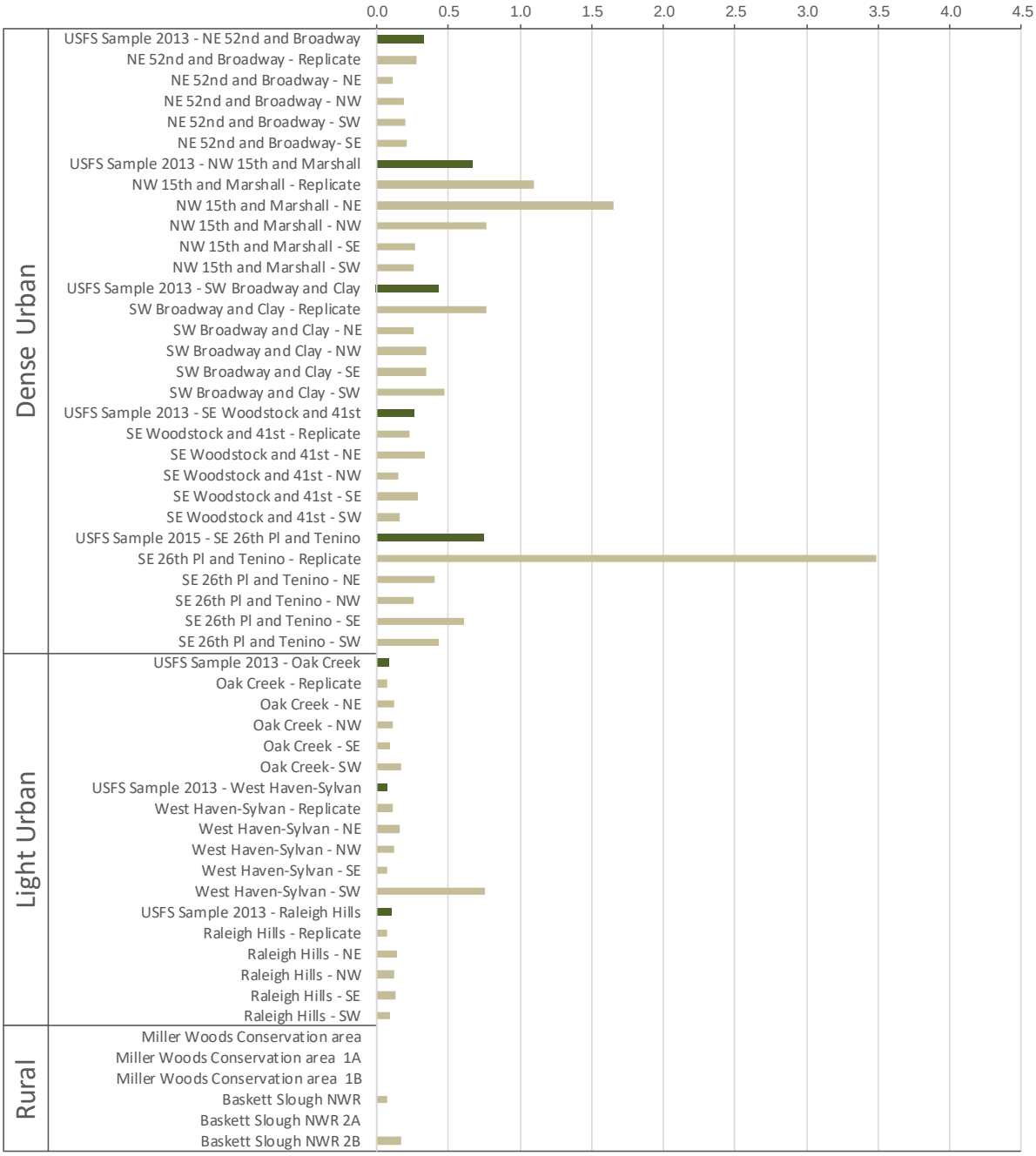
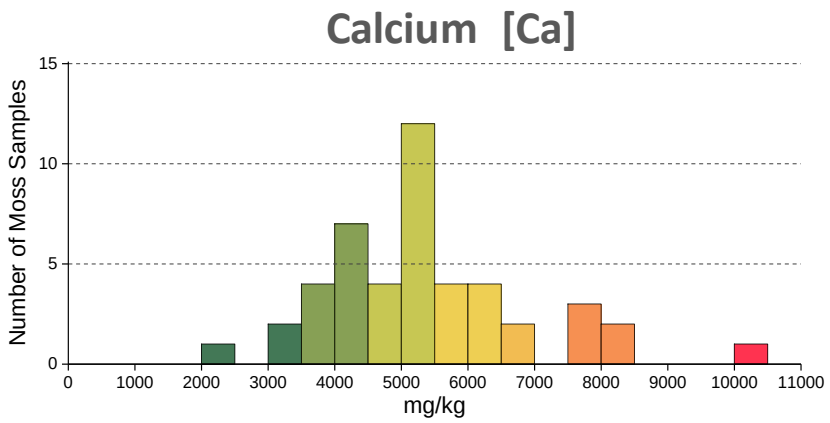


Figure 3e. Cadmium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



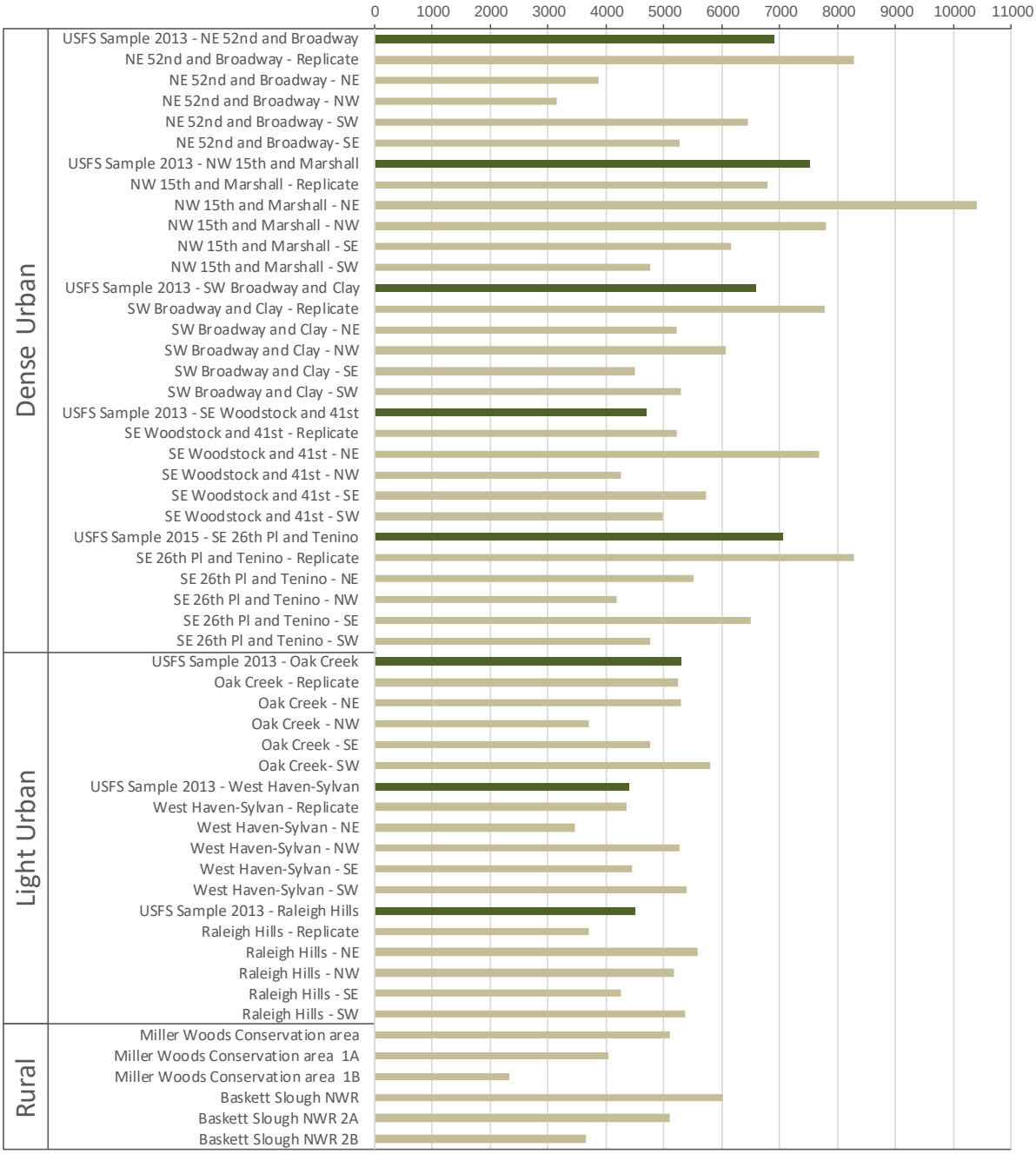
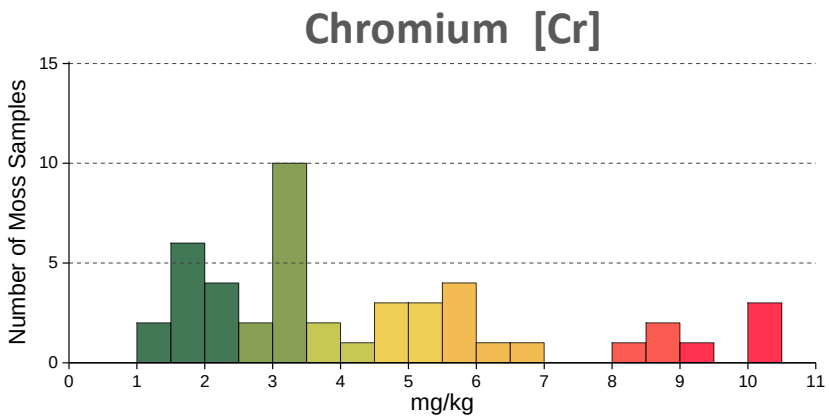


Figure 3f. Calcium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



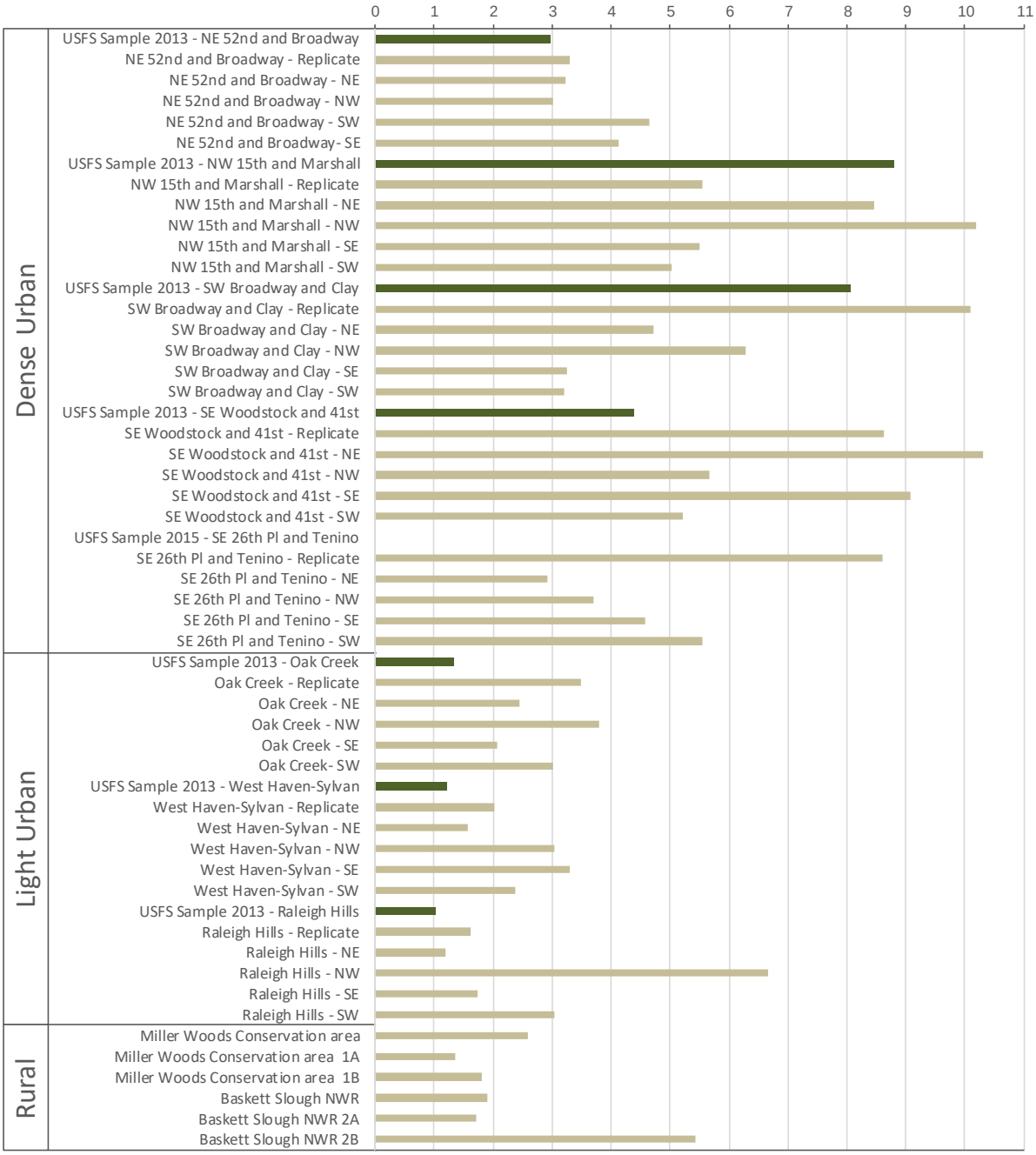
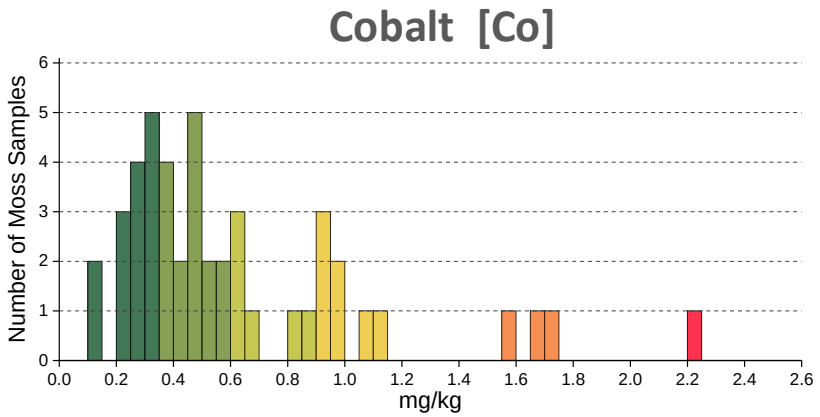


Figure 3g. Chromium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



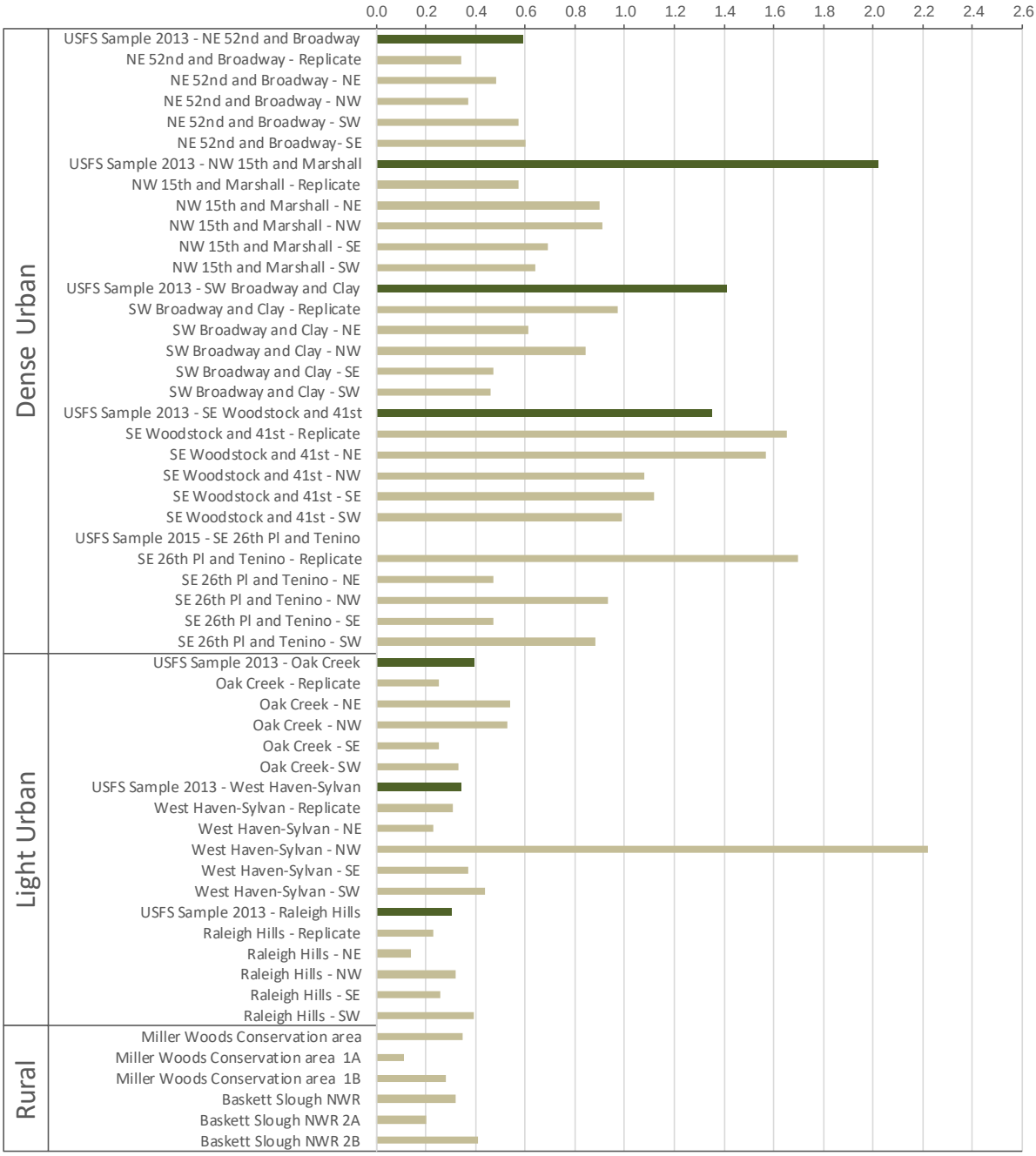
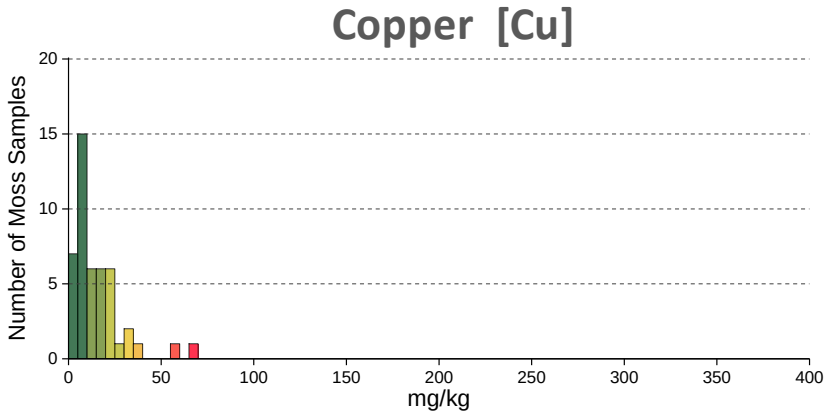


Figure 3h. Cobalt concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



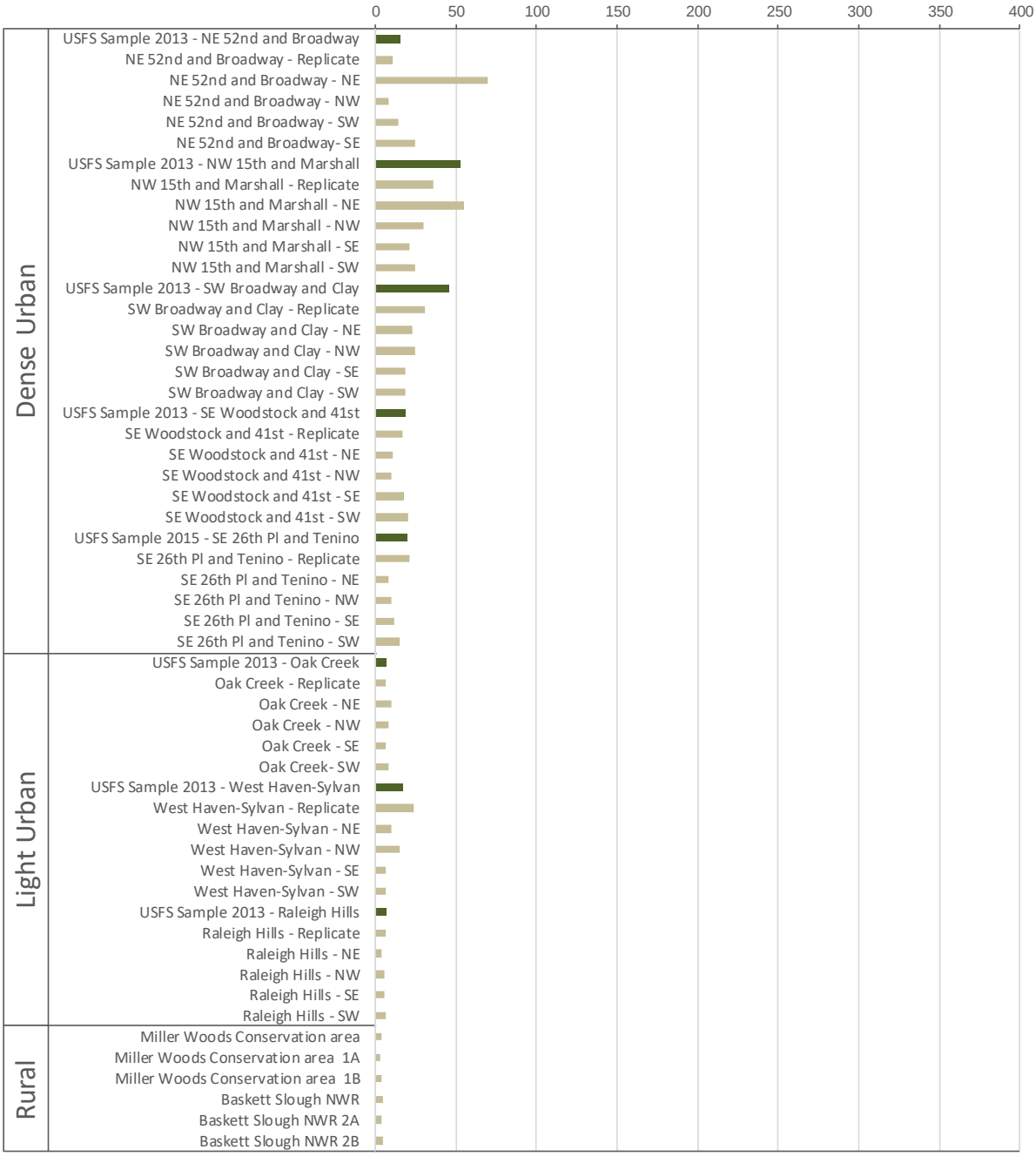
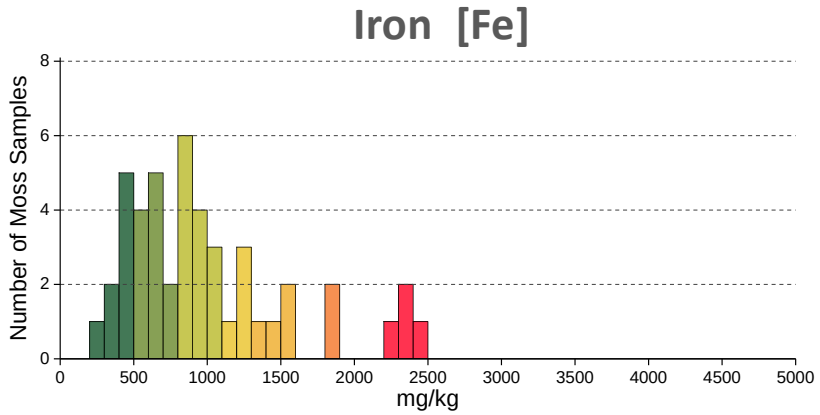


Figure 3i. Copper concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



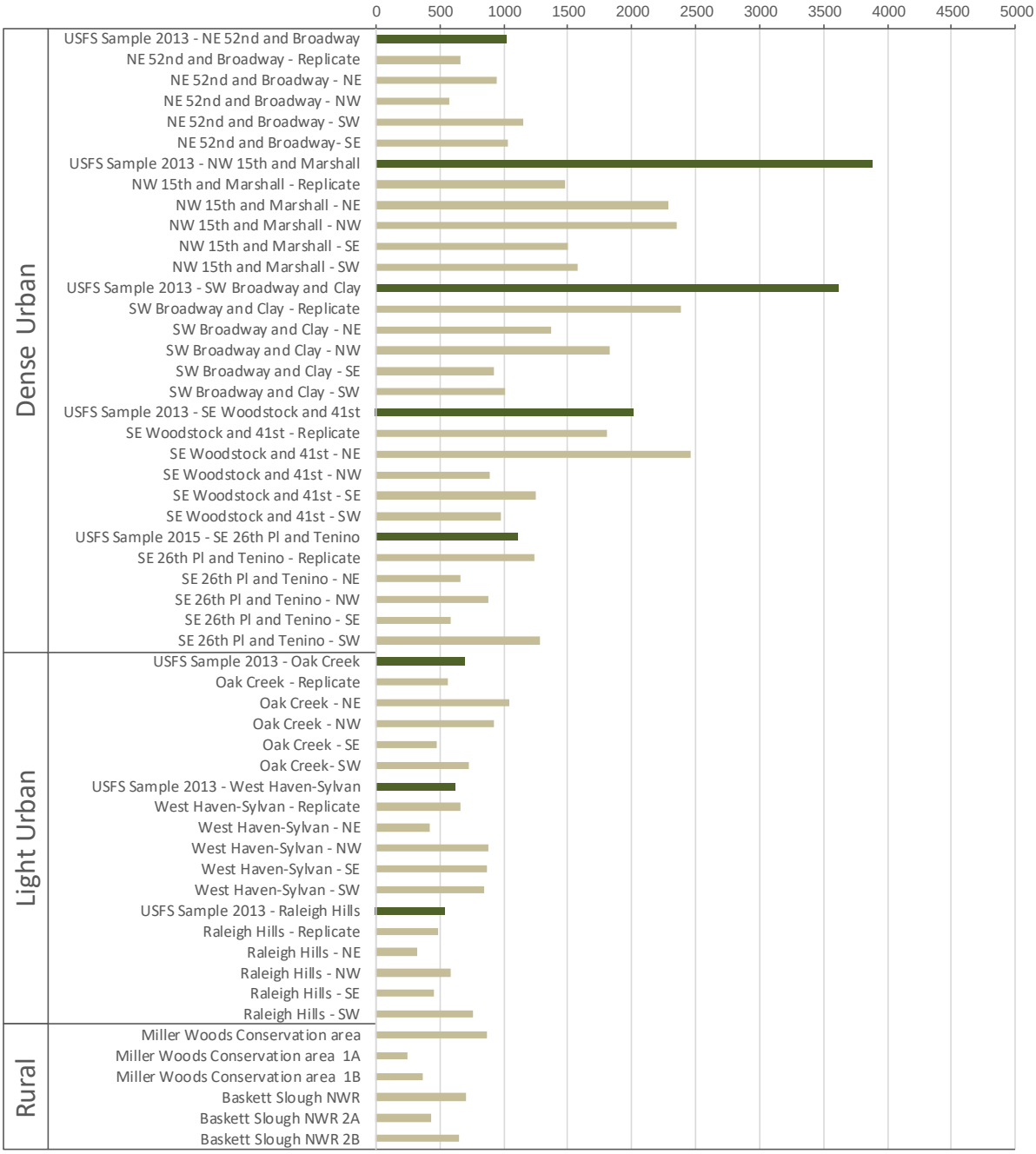
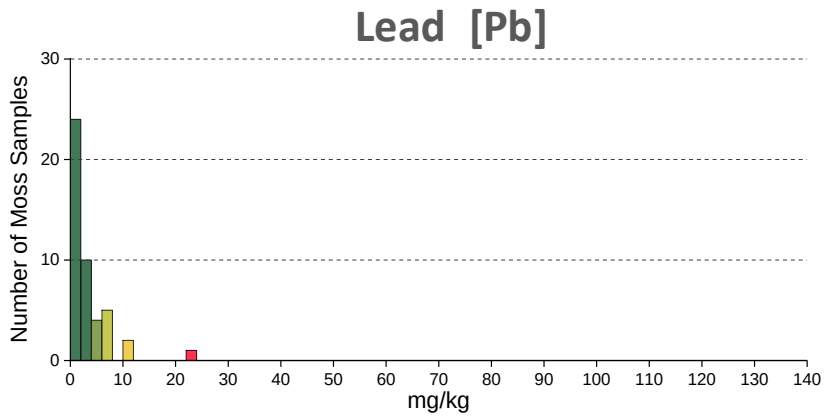


Figure 3j. Iron concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



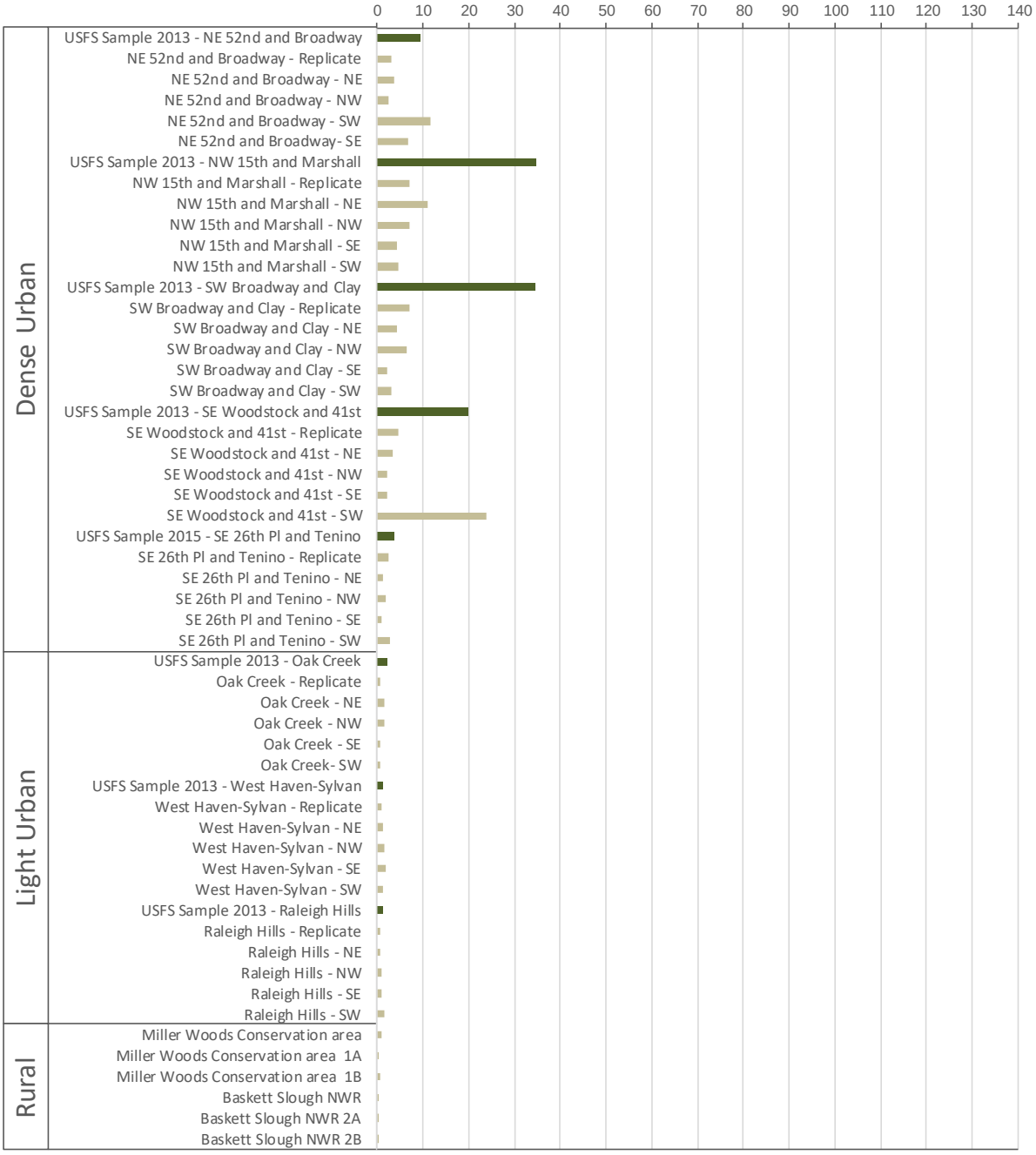
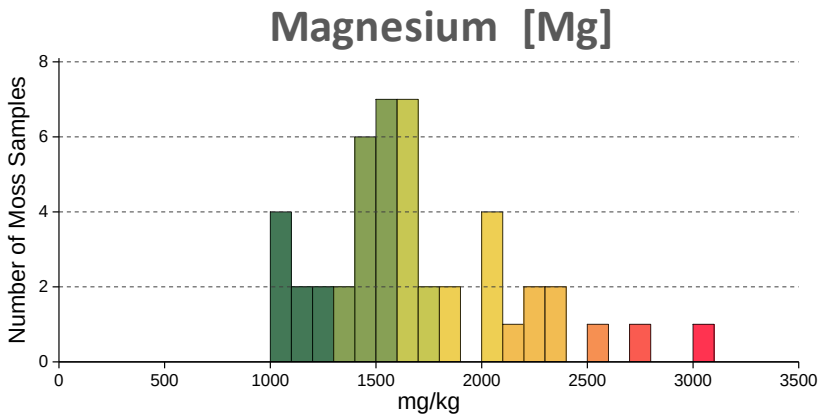


Figure 3k. Lead concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



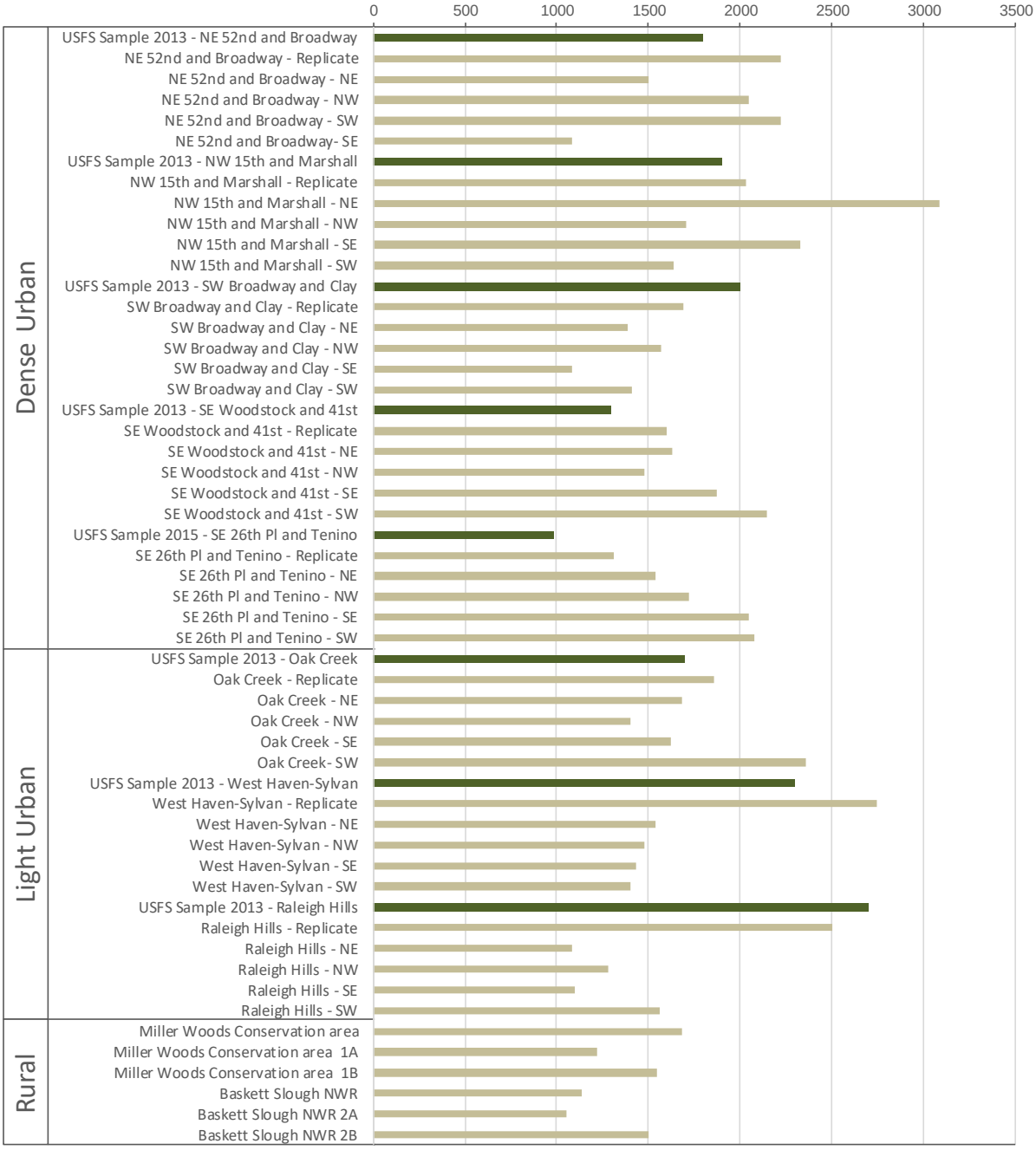
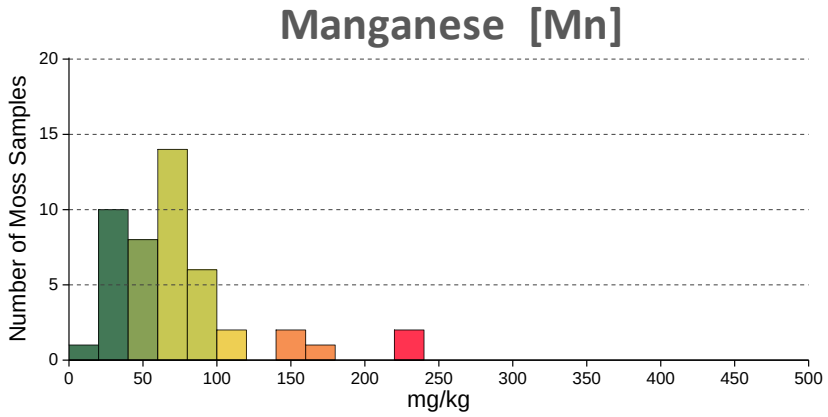


Figure 3l. Magnesium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



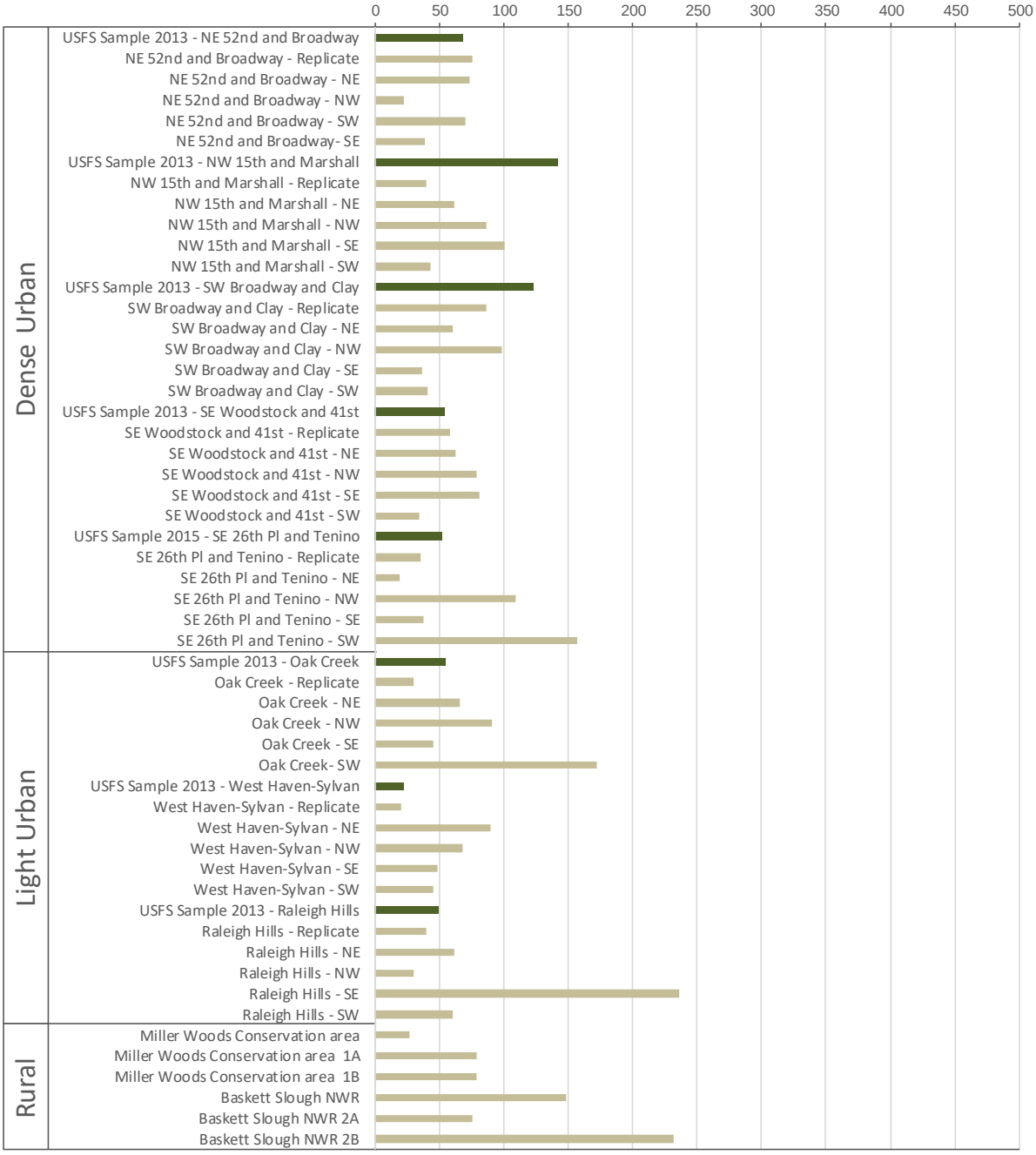
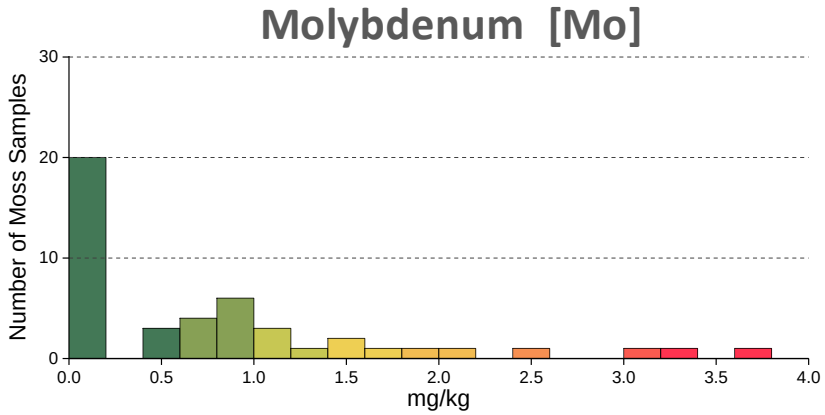


Figure 3m. Manganese concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



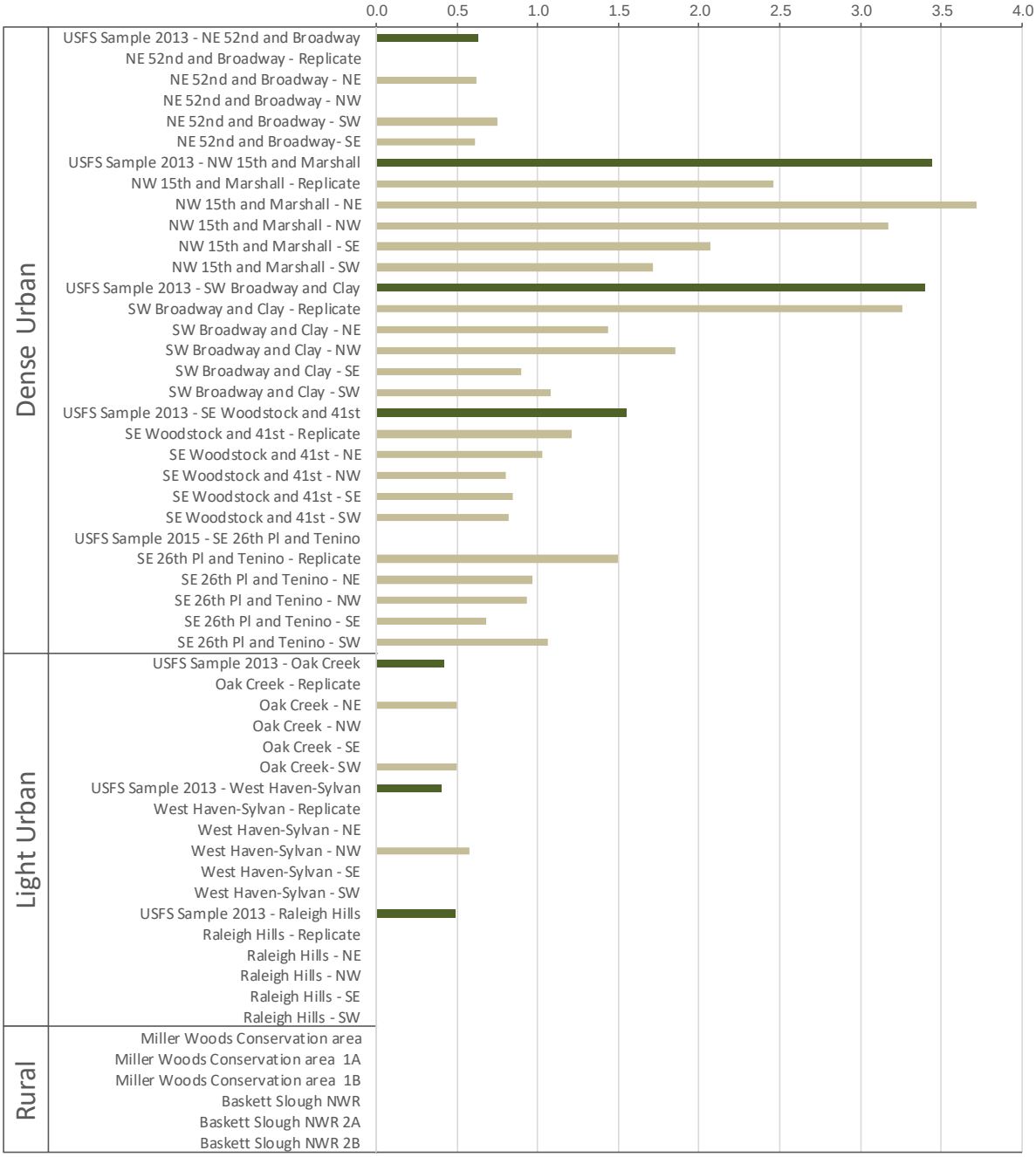
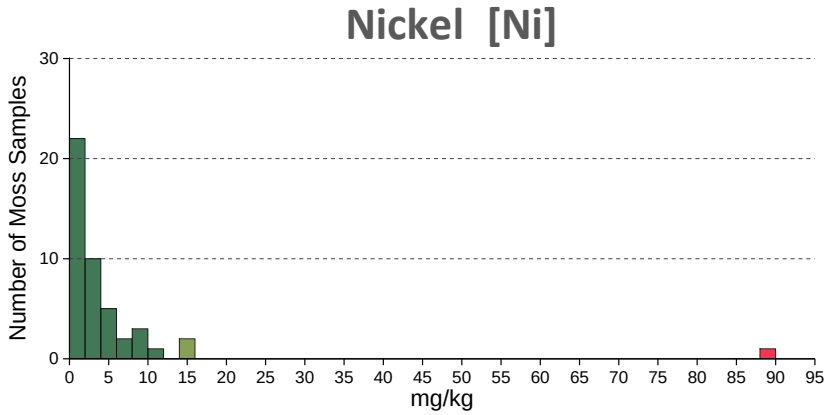


Figure 3n. Molybdenum concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



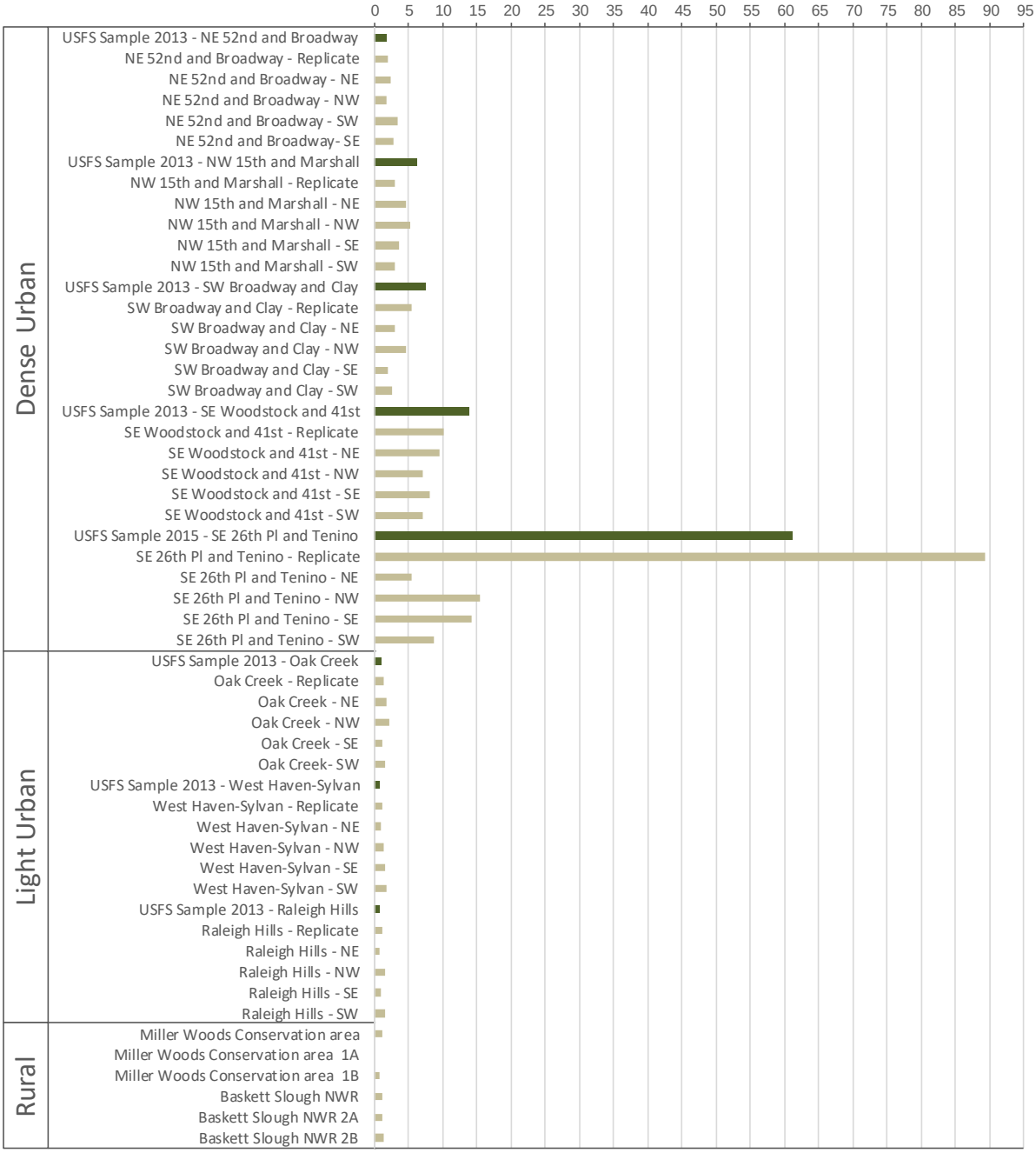
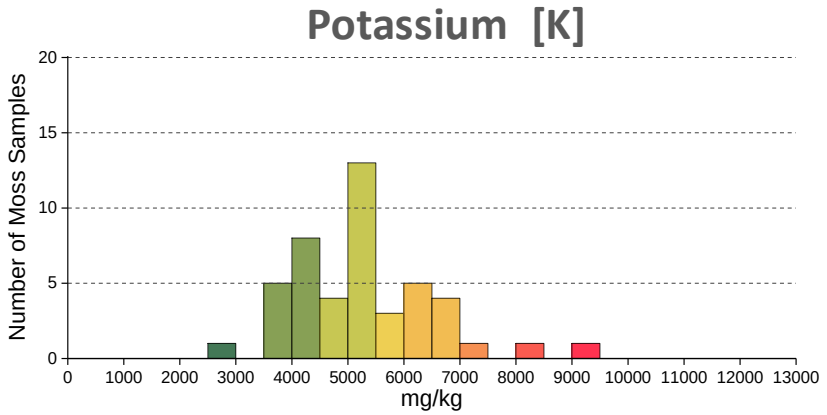


Figure 3o. Nickel concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



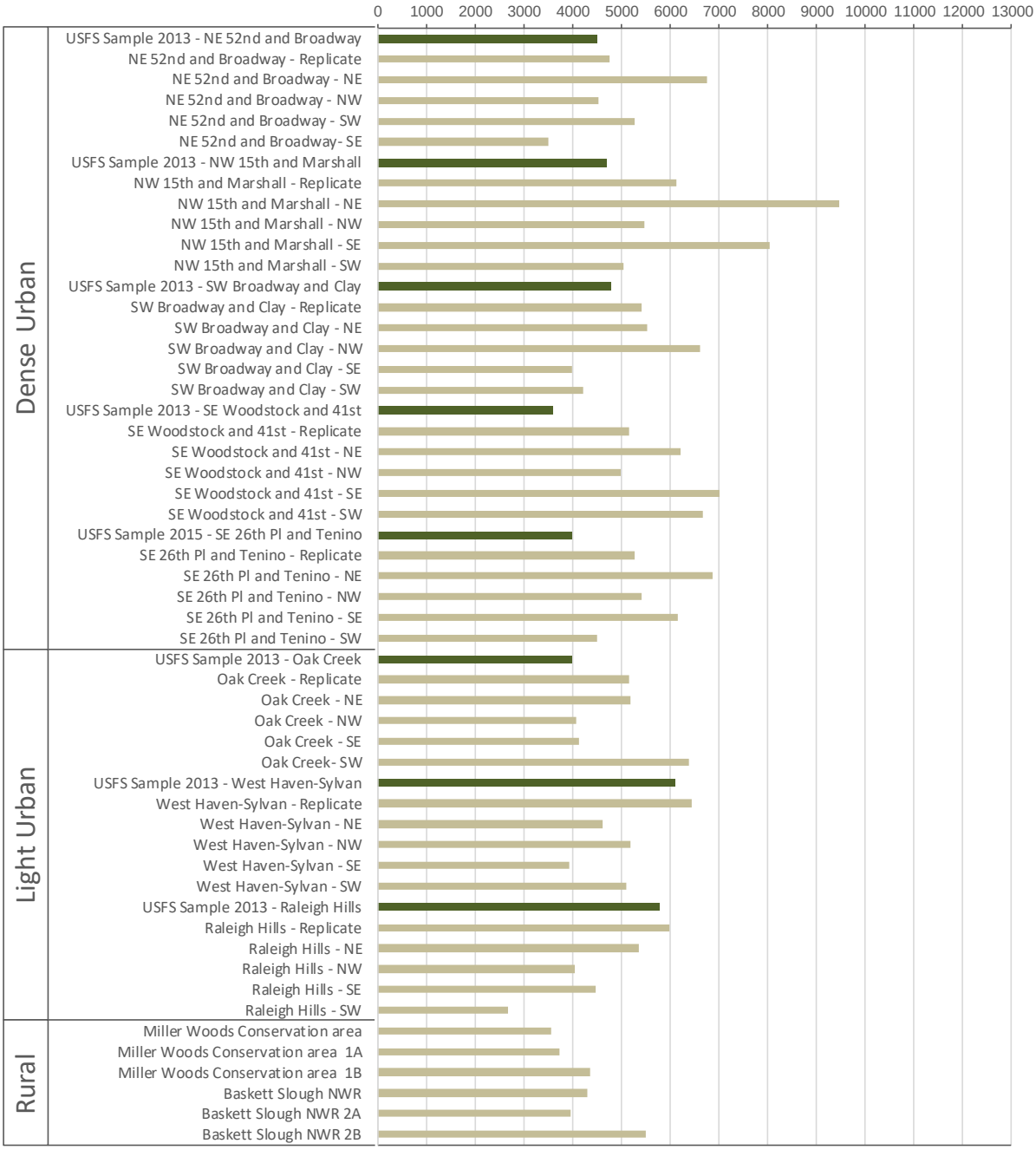
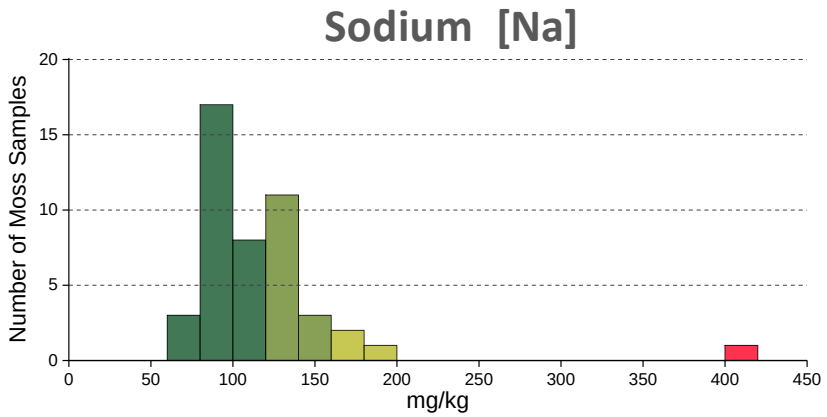


Figure 3p. Potassium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



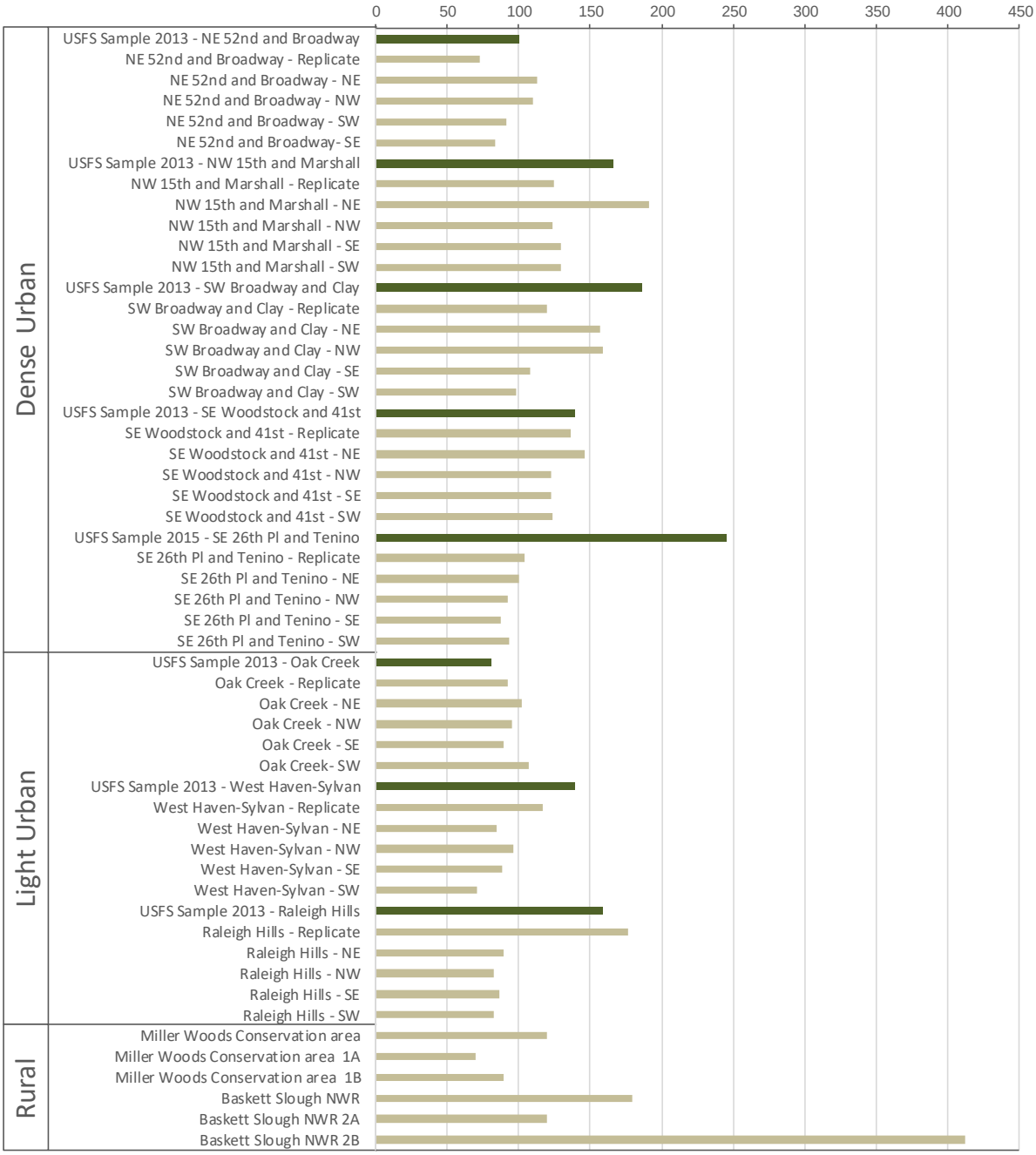
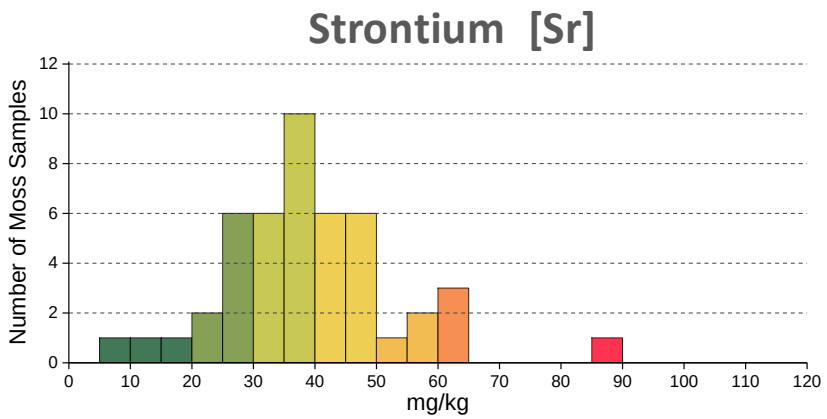


Figure 3q. Sodium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



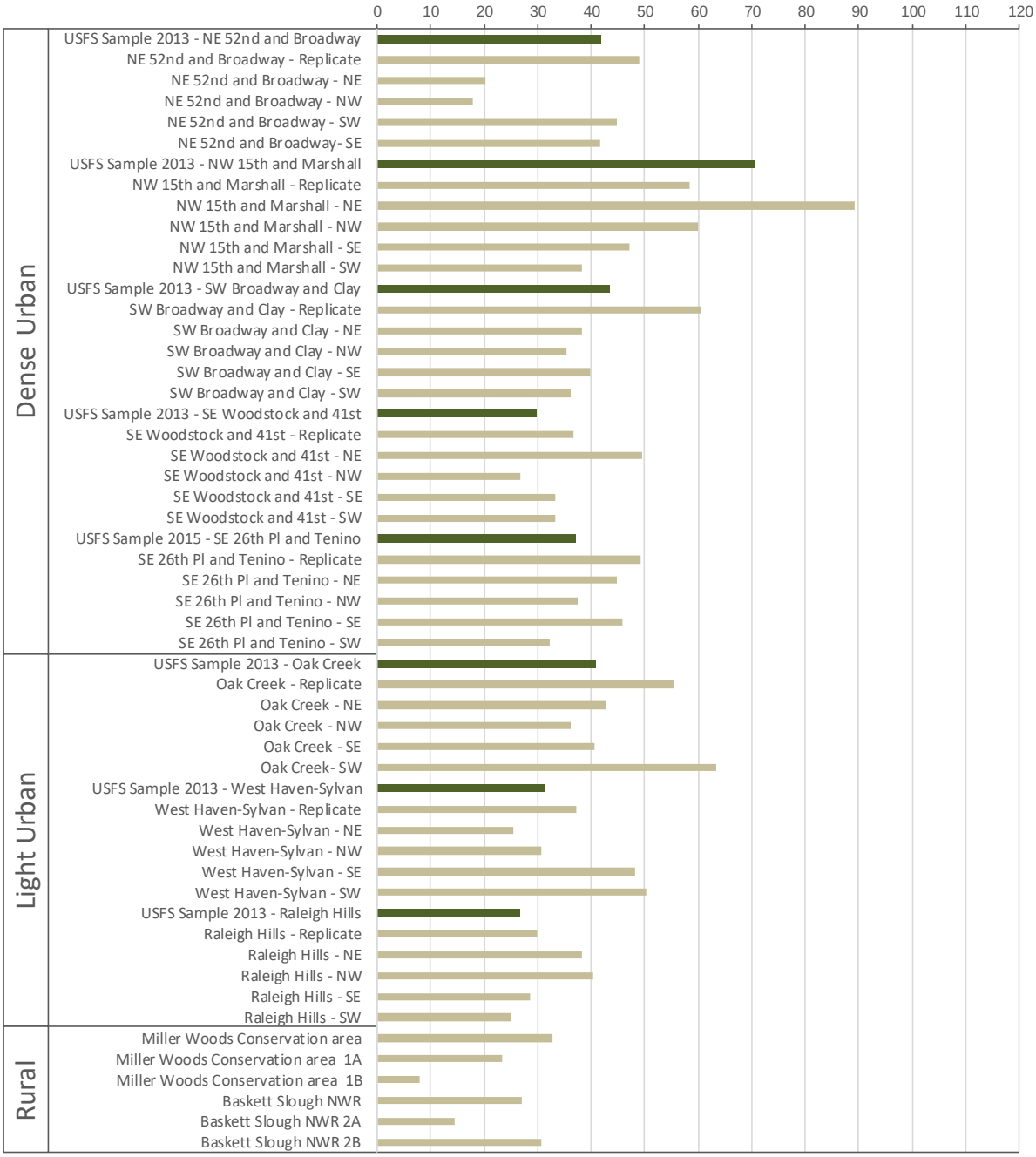
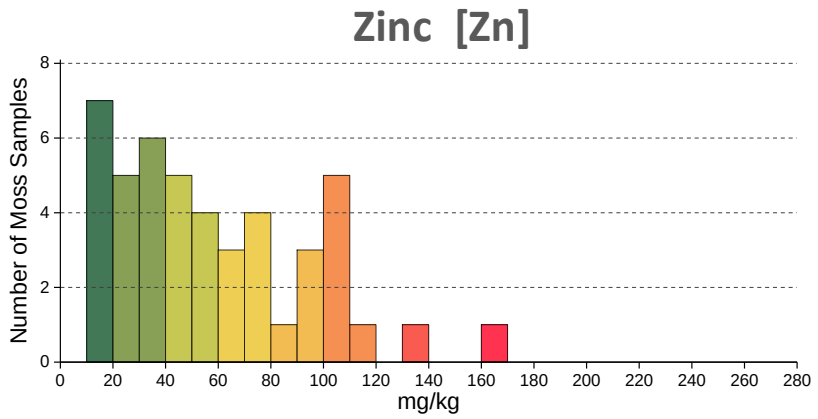


Figure 3r. Strontium concentrations found in moss samples and corresponding histogram.

*USFS Sample results are shown for reference purposes only. Laboratory analytical methods differed and may affect the comparability of results. USFS results were not included in the histogram.



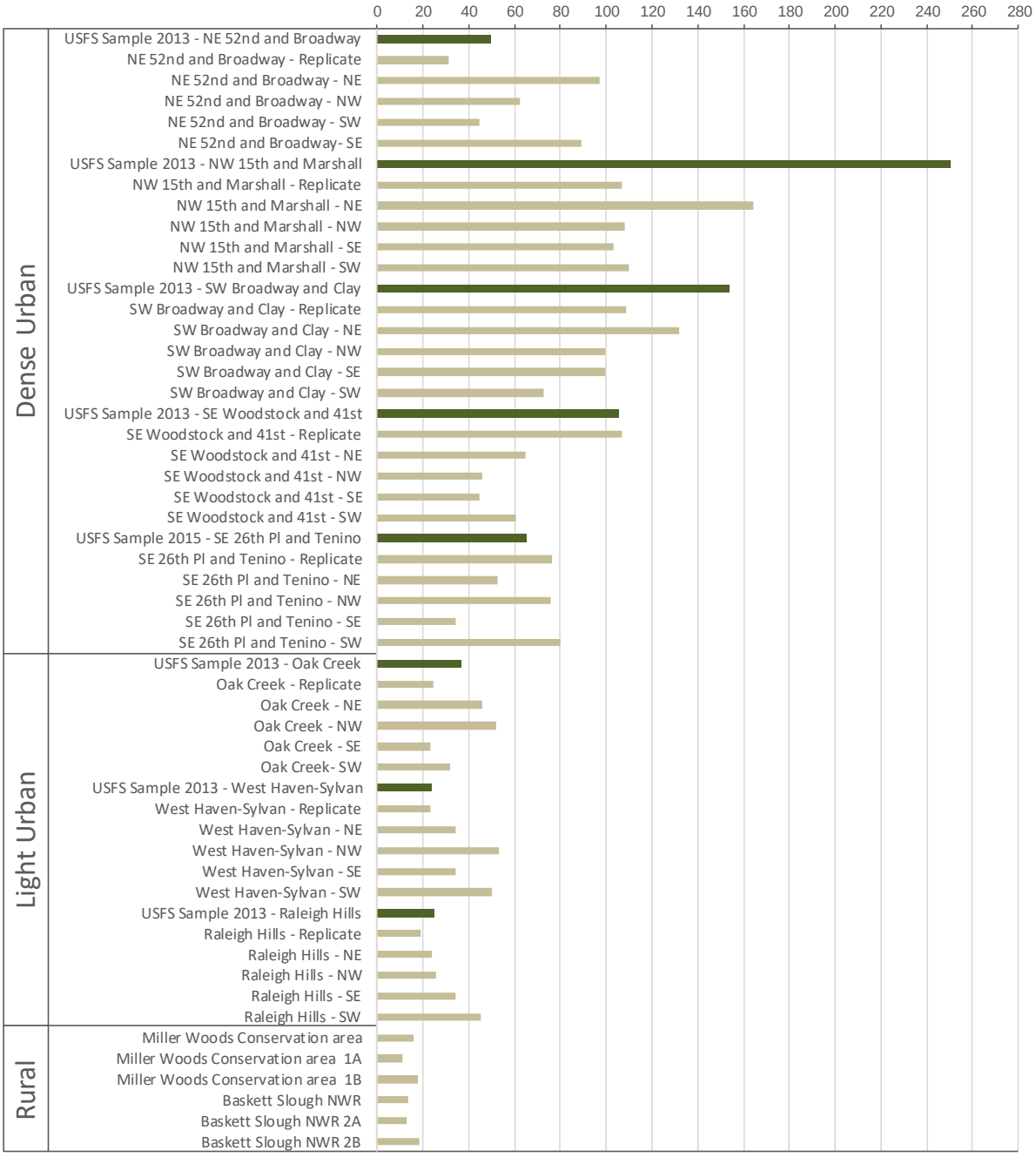


Figure 3s. Zinc concentrations found in moss samples and corresponding histogram.

4.0 Discussion

4.1 Variability at Repeat Sites

High variability was seen between concentrations of metals found in moss samples collected by DEQ and moss previously sampled at the same locations by USFS (Figure 4). The largest differences in concentrations were seen in moss sampled from the SE 26th Pl. and Tenino and Oak Creek sampling locations. Results from the DEQ sample at SE 26th Pl. and Tenino showed a cadmium concentration that was 365% higher and a selenium concentration that was 130% more than what was found in the USFS sample collected in October 2015. Results from the DEQ sample at Oak Creek showed a chromium concentration that was 162% higher than the USFS sample collected in December 2013 (see Table 2).

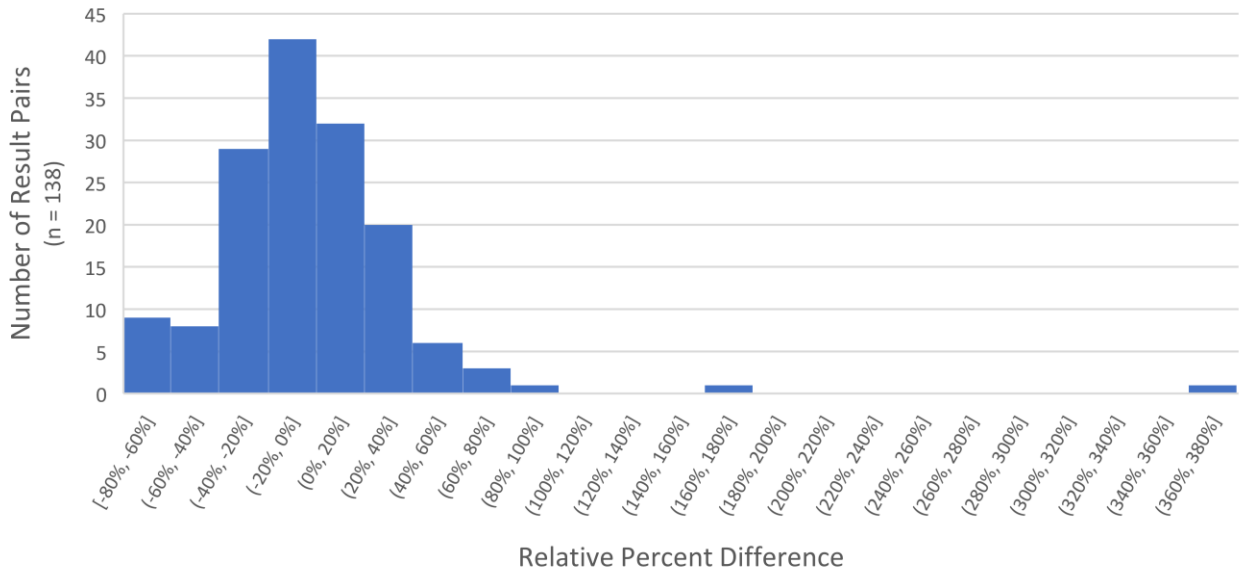


Figure 4. Distribution of relative percent differences (RPDs) between moss collected by DEQ and moss previously sampled at the same locations by USFS. The histogram includes the RPDs for all analytes listed in Table 1 and excludes RPDs that could not be calculated due to concentrations that were below the analytical detection limit.

One unknown factor in this comparison is the time period over which these moss samples have accumulated the metals. Gatziolis et al. (2016) estimate that the time period could range between several months to 3 years. This suggests that concentrations of metals found in moss collected by DEQ in March of 2017 may be indicative of air quality impacts that were not reflected in the USFS moss samples collected in 2013 at the same sites.

4.2 Laboratory Methods

DEQ laboratory analytical techniques used to process moss samples differed from those used by USFS. The method used by DEQ to digest samples yielded a higher percent recovery of the standard reference material used to evaluate the digestion method for several analytes. This difference likely led to concentrations that were higher than would be if samples were digested using the method used by USFS. In order to compare laboratory processes, DEQ analyzed four moss samples which were previously analyzed by USFS. Results were compared by calculating the RPD for each element (Table 4).

Table 4. Comparison of concentrations determined by DEQ and USFS in four moss samples. Results were obtained using different laboratory analytical techniques.

USFS Moss Sample ID --->	USFS 1-22			USFS 1-62			USFS 3-55			USFS 3-40			USFS 2-83		
Analyte	*DEQ*	*USFS*	*RPD (%)*	*DEQ*	*USFS*	*RPD (%)*	*DEQ*	*USFS*	*RPD (%)*	*DEQ*	*USFS*	*RPD (%)*	*DEQ*	*USFS*	*RPD (%)*
*Units: **mg/kg** of dry moss weight*															
Aluminum, Total	488	344.2	29%	1030	688.295	33%	1020	741.88	27%	1400	1168.88	17%	1050	697.44	34%
Arsenic, Total	0.24	ND	N/A	0.43	0.46	-7%	0.39	0.33	15%	0.55	0.32	42%	0.4	ND	N/A
Barium, Total	61.1	58.95	4%	37.1	37.69	-2%	86.5	82.61	4%	75	78.16	-4%	45.3	36.36	20%
Beryllium, Total	ND	Not Analyzed	N/A	ND	Not Analyzed	N/A	ND	Not Analyzed	N/A	ND	Not Analyzed	N/A	ND	Not Analyzed	N/A
Boron, Total	31.1	28.06	10%	29.8	28.72	4%	56.1	46.43	17%	27.8	24.59	12%	28.1	18.67	34%
Cadmium, Total	0.1	0.1	0%	0.43	0.4	7%	0.83	0.835	-1%	0.39	0.43	-10%	0.7	0.56	20%
Chromium, Total	3.68	1.405	62%	8.59	4.86	43%	11.2	5.89	47%	11.9	8.065	32%	8.1	3.21	60%
Cobalt, Total	0.57	0.325	43%	0.87	0.81	7%	1.06	0.98	8%	1.28	1.41	-10%	1.07	0.84	21%
Copper, Total	10.2	8.47	17%	67.3	59.1	12%	29.5	26.055	12%	48.7	45.485	7%	27	19.225	29%
Iron, Total	782	632.115	19%	1880	1564.725	17%	2420	2092.985	14%	3680	3613.985	2%	2400	1840.805	23%
Lead, Total	3.17	4.065	-28%	17.5	19.925	-14%	7.12	8.16	-15%	28	34.745	-24%	14.9	12.995	13%
Manganese, Total	113	102.465	9%	79.4	73.1	8%	128	101.05	21%	129	122.9	5%	134	98.65	26%
Molybdenum, Total	0.57	0.485	15%	1.58	1.35	15%	3.26	2.84	13%	3.71	3.40	8%	2.09	1.44	31%
Nickel, Total	3.64	1.515	58%	5.66	3.725	34%	8.44	5.58	34%	9.2	7.43	19%	6.25	3.14	50%
Selenium, Total	ND	ND	N/A	ND	ND	N/A	ND	ND	N/A	ND	1.755	N/A	ND	ND	N/A
Sodium, Total	205	180.85	12%	161	164.29	-2%	206	187.75	9%	194	185.75	4%	150	113.95	24%
Strontium, Total	43.9	37.67	14%	38.1	32.855	14%	51.4	43.07	16%	47.4	43.46	8%	34.1	22.86	33%
Zinc, Total	26.7	27.365	-2%	105	119.28	-14%	92.7	98.605	-6%	138	154.11	-12%	103	87.25	15%
*Units: **percent** of dry moss weight (DEQ results converted from mg/kg dry wt.)*															
Calcium, Total	0.489	0.491546	-1%	0.683	0.6848885	0%	0.794	0.759608	4%	0.623	0.660608	-6%	0.493	0.380786	23%
Magnesium, Total	0.195	0.1815445	7%	0.173	0.181681	-5%	0.225	0.217192	3%	0.185	0.20	-7%	0.161	0.13335	17%
Potassium, Total	0.751	0.6161575	18%	0.433	0.4430525	-2%	0.68	0.6286265	8%	0.475	0.4824765	-2%	0.405	0.3193415	21%

4.3 Conclusion

The purpose of this DEQ sampling effort was to explore moss sampling as a new methodology for evaluating air toxics, specifically metals, and the method's usefulness as a low cost screening tool to help identify sources of air pollution. This study allowed DEQ to validate protocols for field sampling, laboratory sample processing, analytical techniques and data evaluation. In addition, DEQ was able to determine how concentrations of metals found in O. lyellii moss collected by DEQ in March 2017 compared to concentrations found in seven USFS samples from December 2013 and one USFS sample from October 2015. A comparison of the seven locations that were re-sampled showed large variations in the RPDs across elements.

Despite not knowing the exact relationship between levels of metals found in moss and atmospheric concentrations of metals, moss sampling remains useful for its ability to identify areas where follow-up air quality investigation may be needed and due to its low cost relative to traditional air sampling methods. Sites with levels of elements that are "high" relative to the other moss samples in the area may signal that further investigation is needed. For example, the maximum cadmium level was over 13 times the interquartile range (IQR) for all cadmium levels where typical statistical analysis uses a criterion of 1.5 times the IQR to identify suspected outliers, or high values relative to other values in a dataset. The maximum nickel concentration was almost 20 times the IQR. Both of those values were found in the replicate moss sample taken at the SE 26th Pl. and Tenino site.

This sampling effort also showed a decreasing trend in metals concentrations based on urban density. Levels of metals found in moss were generally highest in the dense urban area and least in the rural area as is expected based on the concentration of pollution sources within the city of Portland.

5.0 Citations

Aboal, J.R., Fernandez, J.A., Boquete, T., Carballeira, A. (2010). Is it possible to estimate atmospheric deposition of heavy metals by analysis of terrestrial mosses? Science of The Total Environment, 408.

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