

Using Lichen Chemistry to Assess Airborne Tungsten and Cobalt in Fallon, Nevada

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Abstract This paper describes the use of lichen chemistry to assess airborne tungsten and cobalt in Fallon, Nevada, where a cluster of childhood leukemia has been on going since 1997. Lichens and their rock substrates were collected from Rattlesnake Hill within Fallon as well as from four different rock outcrops located north, east, south, and west of Fallon and at least 20 km away from the town center. In the lichens themselves, W and Co are significantly higher within Fallon than in the combined control site outside of Fallon. In the rock substrates of the lichens, no differences exist in W and Co. The W and Co differences in lichens cannot be attributed to substrate geochemistry. Fallon is distinctive in west central Nevada for high airborne W and Co, and given its cluster of childhood leukemia, it stands to reason that

additional biomedical research is in order to test directly the leukogenicity of combined airborne W and Co.

Keywords Fallon, Nevada · Childhood leukemia · Tungsten · Cobalt · Lichen chemistry

1 Introduction

This paper describes the use of lichen chemistry to assess heavy metals in Fallon, Nevada, where a cluster of childhood leukemia has been on going since 1997. Officially, 16 cases of childhood leukemia were diagnosed from 1997 to 2002 inclusive (Expert Panel, 2004), and one additional case was reported in December, 2004 (Nevada State Health Division, 2004). Given Fallon's pediatric population of ~2,500 children up to 19 years in age (US Census, 2000) and a national expected rate of childhood leukemia of 4.1 cases per 100,000 children up to 19 years in age per year (US NCI, 2003), the expected rate of childhood leukemia for Fallon should be only one case every 10 years.

This cluster, deemed “one of the most unique ever reported” (Steinmaus, Lu, Todd, & Smith, 2004), has prompted extensive research in an effort to determine if an environmental cause might be responsible. This research has included drinking water (Moore, Lu, & Smith, 2002), jet fuel (US ATSDR, 2002), pesticides

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(US CDC, 2003a), surface water (US ATSDR, 2003a), outdoor air (US ATSDR, 2003b), surface soil and indoor dust (US ATSDR, 2003c), potential lingering effects of underground nuclear bomb testing in the area (Seiler, 2004), and groundwater (Seiler, Stollenwerk, & Garbarino, 2005). Although few definitive conclusions have been made, significantly elevated airborne tungsten and cobalt has been identified within Fallon (Sheppard, Ridenour, Speakman, & Witten, 2006a). Toxicological research on effects of these combined metals on leukemia has been recommended, as has additional environmental monitoring in Fallon to confirm its airborne W and Co (Sheppard et al., 2006a). In the spirit of applying multiple lines of evidence to complex environmental issues (Reid & Thompson, 1996; Reimann & de Caritat, 2005), the objective of this study is to continue assessing airborne W and Co in Fallon using lichen chemistry as an additional source of environmental data that records atmospheric chemistry.

2 Materials and Methods

Fallon is a small, rural farming community (Greater Fallon Chamber of Commerce, 2005) located in west central Nevada (Figure 1a). Its climate is cool to mild and dry, with a mean annual temperature and

precipitation of 10.7°C and 127 mm, respectively, as typified from meteorological data from the Fallon Experiment Station (monthly data from 1931 to 2004 obtained on-line from the National Climatic Data Center, NOAA). Along with service industries and small businesses, Fallon has an industrial facility that specializes in hard-metal metallurgy, which includes tungsten carbide and cobalt powders (Harris & Humphreys, 1983). This hard-metal facility has been in Fallon for over 50 years (Reno Gazette-Journal, 2006), and the facility has been suggested to be a candidate source of W within Fallon generally (Mullen, 2003) and of elevated airborne W and Co found in total suspended particulates of Fallon specifically (Sheppard et al., 2006a).

2.1 Lichen chemistry background

Lichen chemistry is the measurement and interpretation of element concentrations in lichens, which are symbiotic associations of fungi and algae (Baron, 1999). Lichens are ideal biomonitors of atmospheric chemical composition, including heavy metals. Lichens colonize tree bark and rock surfaces (Wolterbeek & Bode, 1995), and specifically they are common on rock surfaces throughout the Great Basin Desert of Nevada. Lichens lack true roots, thereby diminishing the influence of growth substrates on their chemical composition (Szczeplaniak & Biziuk, 2003; Wolterbeek, 2002). They also lack a cuticle, allowing easy incorporation of atmospheric constituents directly into their tissues (Conti & Cecchetti, 2001; Falla, Laval-Gilly, Henryon, Morlot, & Ferard, 2000). Lichens are widespread geographically (Richardson, 1992, 1995), and they can live for decades or even beyond 100 years (Bull, King, Kong, Moutoux, & Phillips, 1994), thereby integrating their chemical environment over an extended period of time (Sensen & Richardson, 2002). Many case studies exist worldwide of using lichen chemistry to monitor or assess airborne elements (e.g., Bennett & Wetmore, 2003; Chiarenzelli et al., 2001; Conti, Tudino, Stripeikis, & Cecchetti, 2004; Cuny, Davranche, Thomas, Kempa, & Van Haluwyn, 2004; Freitas & Pacheco, 2004; Helena, Franc, & Cvetka, 2004; Juran, Jaćimović Batič, Smodiš, & Wolterbeek, 1996; Loppi & Pirintsos, 2003; Nash et al., 2003; Scerbo et al., 1999; Yeniso-y-Karakas & Tuncel, 2004; Yun, Longerich, & Wadleigh, 2003; Zhang, Chai,

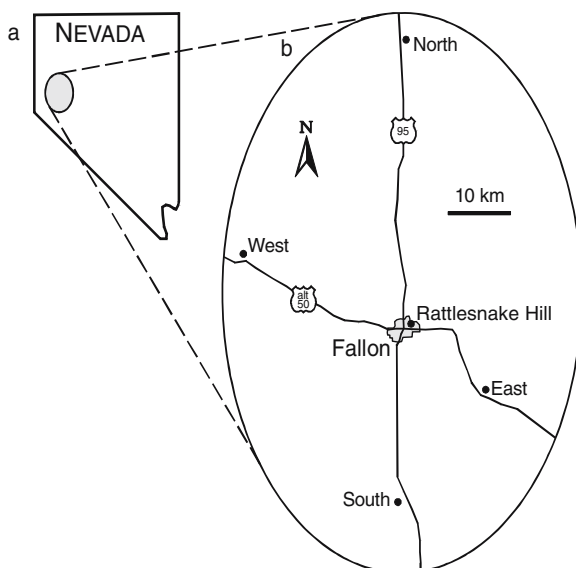


Figure 1 Maps of (a) Nevada, and (b) lichen sampling sites in and around Fallon (filled circles).

Mao, & Chen, 2002; Zschau et al., 2003). Lichen chemistry was used in and around Fallon to continue assessing airborne W and Co, which are elevated in total suspended particulates within Fallon (Sheppard et al., 2006a).

2.2 Lichen chemistry methods

The strategy for lichen chemistry was to compare levels of metals in lichens within Fallon versus outside of Fallon. Ten lichens were collected from Rattlesnake Hill, a Quaternary outcrop of basalt near the center of town (Willden & Speed, 1974) and the only reasonable location within Fallon for abundant sampling of epilithic lichens (Figure 1b). Sampled lichens were on their own distinct rocks located meters apart from one another. For comparison control (Ernst, 1995), lichens were also collected from four different late Tertiary basalt or rhyolite outcrops located north, east, south, and west of Fallon and at least 20 km away from the town center (Figure 1b). Five lichens, again from distinct rocks located meters apart from one another, were collected from each site outside of Fallon, making for 20 lichens sampled outside of Fallon and a total of 30 lichens sampled for the entire study. All collection locations were at least a quarter-kilometer from principal roads or highways, minimizing effects of automobile traffic on lichen chemistry.

Lichen type was kept constant across all sites to minimize differential uptake of metals by different species (MacNaeidhe, 1995). The lichen type chosen is epilithic, crustose, lobulate, and yellow-green with a whitish pruina and blackish lobes. This type belongs to the *Lecanora garovaglii* group (sagebrush rim-lichen), which is common and widespread throughout semiarid deserts of the West (Brodo, S. D. Sharnoff, & S. S. Sharnoff, 2001). Several grams of tissue were collected for each lichen using a ceramic, non-metallic scraper. Samples were placed in clean, air-permeable paper envelopes and stored in a dust-free box. Prior to chemical measurement, lichen samples were rinsed lightly for just a few seconds in de-ionized water to remove surface dust that might be present on the samples (MacNaeidhe, 1995; Markert, 1995), and non-lichen material was removed manually. After air-drying, lichens were ground to a size class of ~0.1 mm using a ceramic, non-metallic mortar and pestle.

For each lichen, approximately 100 mg of tissue were placed into solution and measured for metals using inductively coupled plasma mass spectroscopy (ICP-MS), which has very low detection limits. Freeze-dried lichens were digested using a laboratory microwave system and a combination of high purity HNO₃ and HF acids. A 10% H₃BO₃ solution was used to reduce formation of insoluble fluorides that are known to precipitate some elements out of solution (Kingston & Haswell, 1997). Digestates were spiked with internal standards and analyzed on a VG Axiom high-resolution ICP-MS system. Blank and lichen quality control samples (CRM-482; Quevauviller, Herzig, & Muntau, 1996) were processed similarly. The median recovery from the lichen CRM for all measured elements with a certified value was 92%, indicating excellent performance of ICP-MS on lichen tissue. Twenty-four elements were measured in a broad search for metals that might differ within versus outside of Fallon and for suites of metals that might serve as environmental fingerprints (Wolterbeek & Bode, 1995). Element concentrations were compared between Rattlesnake Hill within Fallon and the composite site outside of Fallon made up of lichens from the four subsites. The two-tailed Mann-Whitney test of median values was used as a conservative analysis (Sokal & Rohlf, 1981).

2.3 Rock substrate chemistry methods

To assess the role of rock substrate in causing differences in metal concentrations of the lichens, rock samples from each lichen site were also analyzed chemically. Three basalt samples were collected from distinct rocks at each lichen site, making for a total of 15 rock samples. All rock samples were washed with deionized water to remove surface dirt and other loose particles. Samples were wrapped in clean paper towels, placed between two steel plates, and crushed with a Carver Press to obtain 50- to 100-mg fragments. Interior fragments (those exhibiting no cortex) were examined with a magnifier to eliminate those with metallic streaks or crush fractures that could indicate contamination. Several grams of clean fragments were obtained from each sample and stored in plastic bags.

Rock fragments were then prepared and measured for tungsten and cobalt by instrumental neutron activation analysis (INAA). INAA of rocks, which consists of two irradiations and a total of three gamma counts, constitutes a superset of procedures used at most INAA

Table 1 Element concentrations and standard errors in lichens and rock substrates

Element	Median (ppm) Lichens	Standard error	Median (ppm)	Standard error	Ratio within:outside
	Within Fallon (<i>n</i> =10)		Outside Fallon (<i>n</i> =20)		
Lithium	8.02	0.82	5.37	3.61	1.49
Beryllium	0.32	0.06	0.19	0.07	1.71
Vanadium	21.65	3.88	13.40	5.52	1.62
Chromium	8.52	1.56	6.21	2.26	1.37
Manganese	137	52	118	43	1.16
Iron	8,122	1,403	6,290	1,852	1.29
*Cobalt	3.59	0.73	1.79	0.65	2.01
Nickel	4.38	0.94	4.40	0.99	0.99
Copper	8.99	0.72	7.73	1.43	1.16
Zinc	43.55	2.31	34.60	4.58	1.26
Arsenic	4.83	0.72	3.07	0.99	1.57
Strontium	251	20	179	20	1.40
Molybdenum	0.63	0.06	0.60	0.12	1.05
Silver	0.17	0.01	0.14	0.01	1.26
Cadmium	0.30	0.01	0.32	0.02	0.94
Tin	0.61	0.07	0.44	0.08	1.40
Antimony	0.77	0.08	0.71	0.13	1.09
Cesium	1.44	0.14	0.93	0.44	1.54
Tantalum	0.09	0.02	0.06	0.02	1.47
***Tungsten	24.95	1.91	1.99	0.30	12.54
Thallium	0.12	0.01	0.08	0.03	1.55
Lead	13.70	1.15	17.45	5.13	0.79
Thorium	2.28	0.33	1.35	0.31	1.69
Uranium	0.96	0.11	0.52	0.19	1.85
	Rock substrates				
	Within Fallon (<i>n</i> =3)		Outside Fallon (<i>n</i> =12)		
Cobalt	24.00	0.58	23.00	0.47	1.04
Tungsten	2.40	0.17	2.45	0.06	0.98

Differences in medians were tested using the two-tailed Mann-Whitney test of medians (Sokal & Rohlf, 1981).

*Significance at the 0.06 level.

***Significance at the <0.001 level.

laboratories (Glascok, 1992; Neff, 1992, 2000). A short irradiation was carried out through a pneumatic tube irradiation system (Glascok, 1992). Samples in polyvials were sequentially irradiated, two at a time, for 5 s by a neutron flux of $8 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$. The 720-s count yielded gamma spectra containing peaks for nine short-lived elements: Al, Ba, Ca, Dy, K, Mn, Na, Ti, and V. For the second irradiation, samples were encapsulated in high-purity quartz vials and were subjected to a 12-h irradiation at a neutron flux of $5 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$. This long irradiation is analogous to the single irradiation utilized at most other laboratories. After the long irradiation, samples decayed for

five days and then were counted for 1,800 s (the “middle count”) on a high-resolution germanium detector coupled to an automatic sample changer. The middle count yielded determinations of eight medium half-life elements: As, La, Lu, Nd, Sm, W, U, and Yb. After an additional three-week decay, a final count of 10,000 s was carried out on each sample. The latter measurement yielded 17 long half-life elements: Ce, Co, Cr, Cs, Eu, Fe, Hf, Ni, Rb, Sb, Sc, Sr, Ta, Tb, Th, Zn, and Zr. Element concentrations were determined using the standard-comparator method in which the unknown samples (i.e., rock samples) were determined by ratioing the measured activities per unit

weight of the unknown sample to those for a reference standard (SRM-1633a, SRM-1648, and SRM-688) with known concentrations. The median recovery from a rock quality control standard (SRM-288) for all measured elements with a certified value was 102%, indicating excellent performance of INAA on rock samples.

3 Results

3.1 Lichens

From lichen samples, all but two of the measured elements show no significant differences within versus outside of Fallon (Table I). For these elements, ratios of median concentrations within Fallon relative to outside of Fallon average 1.35, i.e., about one-third higher inside Fallon compared to outside of Fallon. This establishes a typical background pattern of airborne elements being only slightly elevated within Fallon compared to outside of Fallon in undeveloped desert. This background conforms to the typical pattern of urban areas having slightly higher airborne particulates than undeveloped areas due to automobile and/or industrial activity in towns (e.g., Monn et al., 1995).

In sharp contrast to these slight differences, tungsten and cobalt are significantly higher in lichens within Fallon compared to outside of Fallon, with W being very significantly higher (Table I). Median values of W and Co within Fallon are $12.50\times$ and $2.00\times$ higher, respectively, than median values outside of Fallon. Thus, Fallon is distinctive environmentally by very high levels of W and slightly high levels of Co in lichens of Rattlesnake Hill.

Looking more closely at W, it was compared with all other measured elements combined. Values for each element were standardized to their mean value, yielding data sets of dimensionless indices with an average of 1.0. All standardized elements except W were averaged together. Compared to this composite average, W is clearly elevated in lichens of Rattlesnake Hill inside Fallon (Figure 2). The 10 Fallon lichens have W indices well above the mean value, with indices up to $3.5\times$ the mean. No other element shows such a clear difference as W between lichens within Fallon versus outside of Fallon. The North control site lichens are slightly elevated in other elements, but only by a factor of $\sim 2\times$ the mean at most.

3.2 Rock substrates

From the basalt samples, median concentrations of tungsten and cobalt do not differ significantly between inside versus outside of Fallon (Table I). For both metals, ratios of medians within versus outside of Fallon are near 1.0. Furthermore, rock concentrations of both W and Co match their typical crustal abundances of 1.2 ppm for W and 22 ppm for Co (Krauskopf, 1995), indicating that these particular rock outcrops in and around Fallon are normal with respect to W and Co in crust. As a specific rock type, basalt typically has even higher levels of Co (48 ppm, Krauskopf, 1995), so Co in basalt in and around Fallon is actually lower than expected by half. Consequently, rock substrate does not play a discernible role in elevating W and Co in lichens within Fallon compared to outside of Fallon, countering the hypothesis that Fallon's elevated airborne W and Co is attributable to natural geology of Nevada (Expert Panel, 2004).

4 Discussion

Previous environmental research related to the cluster of childhood leukemia in Fallon, Nevada, has noted

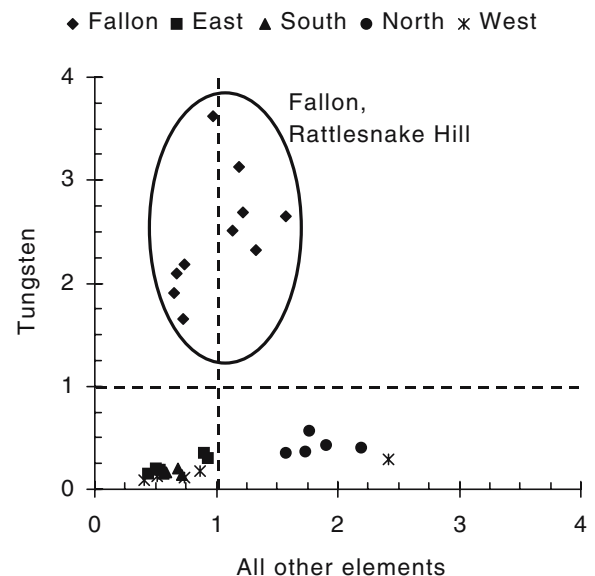


Figure 2 Tungsten concentration in lichens compared to concentrations of all other elements measured. All elements were indexed to their respective means, after which all elements other than tungsten were averaged together. Dashed lines indicate the reference value of 1.0, the expected value of each indexed element series.

elevated tungsten in and around Fallon. Tungsten has been shown to be high in drinking water (US CDC, 2003b), in groundwater (Seiler et al., 2005), and in blood and urine samples of residents (US CDC, 2003b). However, a consensus has been that W is not related to the childhood leukemia of Fallon because W is a natural environmental feature of the region (Expert Panel, 2004; Pardus, Sueker, & Gass, 2005). For example, W is found throughout northern Nevada (Stager & Tingley, 1988), in groundwater near Fallon (Seiler et al., 2005), and in desert varnish outside of Fallon (Wayne, Diaz, Fairhurst, Orndorff, & Pete, 2006).

Nonetheless, Fallon has been shown to be distinctive from nearby comparison towns in Nevada by significantly elevated airborne W as well as Co in total suspended particulates (Sheppard et al., 2006a). This present study corroborates the total suspended particulate findings with the environmental monitoring and assessment method of lichen chemistry. The spatial resolution of this lichen data set is not fine enough to conclusively isolate the source of airborne W and Co in Fallon. Other environmental assessment techniques that are more finely resolvable spatially should be employed in Fallon for that purpose.

The Fallon cluster of childhood leukemia began in 1997 (Expert Panel, 2004), but data types tested in Fallon so far do not indicate temporal variability of airborne metals at the temporal scale of several years. Lichens integrate their environment throughout their entire lives, so long-lived lichens can reflect atmospheric chemistry and deposition at the decadal to multi-decadal scale (Garty, 1993). Total suspended particulates reflect atmospheric chemistry at the hourly to daily scale. It remains to be shown that airborne W and Co has varied in Fallon at the annual to sub-decadal scale. Dendrochemistry, the analysis of element concentrations in dated tree rings (Amato, 1988; Lewis, 1995), can speak to environmental chemical changes on the scale of several years, and that research is being pursued as a logical next step in environmental monitoring and assessment of Fallon related to its cluster of childhood leukemia.

5 Conclusions

Based on multiple lines of evidence that confirm one another, Fallon is unique environmentally by high

airborne tungsten and cobalt. Comparison towns of west central Nevada do not have elevated airborne W and Co (Sheppard et al., 2006a), nor are these metals elevated in lichens of non-developed desert lands outside of Fallon (this study).

It cannot be concluded from environmental data alone that elevated airborne tungsten and/or cobalt causes childhood leukemia. Such a connection requires biomedical research. Given that Fallon's incidence of childhood leukemia is the "most unique cluster ever reported" (Steinmaus et al., 2004) and that Fallon is distinctive by elevated airborne W and Co, it stands to reason that additional biomedical research is in order to test directly the leukogenicity of airborne W and Co (Sheppard, Speakman, Ridenour, & Witten, 2006b, 2006c).

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