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Additional analysis of dendrochemical data of Fallon, Nevada

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ABSTRACT

Previously reported dendrochemical data showed temporal variability in concentration of tungsten (W) and cobalt (Co) in tree rings of Fallon, Nevada, US. Criticism of this work questioned the use of the Mann–Whitney test for determining change in element concentrations. Here, we demonstrate that Mann–Whitney is appropriate for comparing background element concentrations to possibly elevated concentrations in environmental media. Given that Mann–Whitney tests for differences in shapes of distributions, inter-tree variability (e.g., "coefficient of median variation") was calculated for each measured element across trees within subsites and time periods. For W and Co, the metals of highest interest in Fallon, inter-tree variability was always higher within versus outside of Fallon. For calibration purposes, this entire analysis was repeated at a different town, Sweet Home, Oregon, which has a known tungsten-powder facility, and inter-tree variability of W in tree rings confirmed the establishment date of that facility. Mann–Whitney testing of simulated data also confirmed its appropriateness for analysis of data affected by point-source contamination. This research adds important new dimensions to dendrochemistry of point-source contamination by adding analysis of inter-tree variability to analysis of central tendency. Fallon remains distinctive by a temporal increase in W beginning by the mid 1990s and by elevated Co since at least the early 1990s, as well as by high inter-tree variability for W and Co relative to comparison towns.

1. Introduction

Previously reported dendrochemical data showed temporal variability in concentration of tungsten (W) and cobalt (Co) in tree rings of Fallon, Nevada, US [1]. Criticism of this work questioned the use of Mann–Whitney for determining change in element concentrations was questioned. Here, we demonstrate that Mann–Whitney is appropriate for comparing background element concentrations to possibly elevated concentrations in environmental media. The Mann–Whitney test, a nonparametric test of differences in the cumulative distribution functions of two groups, is sensitive to and will detect differences in medians and other measures of elevated concentrations.

Fallon (39° 28′ 25″ N, 118° 46′ 35″ W) experienced a cluster of childhood leukemia beginning in 1997. Extensive research was conducted in Fallon to determine what might have caused this childhood leukemia cluster. Multiple lines of evidence indicated that the heavy metals W and Co are elevated in airborne particulates of Fallon. See Supplemental Material and the rest of this special issue of Chemico–Biological Interactions for details of the Fallon cluster, of general research done in Fallon, and of specific research on airborne particulates of Fallon.

Temporal change in airborne W and Co in Fallon was not discernable from the spatial environmental monitoring techniques employed in Fallon because they are not resolvable in multiple increments of time [2]. Dendrochemistry, the measurement and interpretation of element concentrations in tree rings [3], can reflect temporal variability of elements with resolution as fine as individual years. Dendrochemical measurements are typically used to evaluate relative change in concentrations through time in environmental availability of elements as well as to compare absolute concentrations across different trees or different sites [4].

Accordingly, dendrochemistry was applied in Fallon to assess temporal change in W and Co since the late 1980's, that is, since before the onset of the cluster of childhood leukemia [1]. Dendrochemical data in the form of medians of concentrations of elements in aggregated tree rings showed that W increased in Fallon trees relative to nearby comparison towns beginning by the mid-1990s, slightly before the onset of the cluster, and that Co has been generally high in Fallon trees relative to other communities since the late 1980's. From this analysis, a coarse temporal correspondence was noted between the onset of the childhood leukemia cluster and rising or elevated levels of airborne W and Co. It was

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also acknowledged that environmental data alone cannot directly link childhood leukemia with exposure to metals, but biomedical research was called for to directly test for such linkage.

We used the nonparametric Mann–Whitney test to evaluate differences in median concentrations in trees within Fallon versus trees outside of Fallon. It was subsequently asserted that Mann–Whitney is not valid for testing samples with unequal variances, in spite of its being a nonparametric test for which equal variances is not assumed [5–7]. This assertion is supported in some applied literature:

- "Two-sample tests, whether parametric or not, make one important assumption: the two distributions that are compared are assumed to have the same shape and variance, [i.e.,] homoscedasticity" [8].
- "The performance of the Wilcoxon–Mann–Whitney test depends on the performance of the T test. More specifically, the shortcomings of the T test are inherited by the Wilcoxon–Mann–Whitney test whenever the ranked data are subject to violations of the assumptions of normality and equal variances" [9].

Samples tested in our original dendrochemical analysis certainly had unequal variances. If equal variances really were required for Mann–Whitney to be valid, then Mann–Whitney would not have been appropriate in our original dendrochemical analysis of Fallon. Thus, a central question arose: Is the assertion correct that Mann–Whitney requires the two data groups to have the same shapes and variances?

Answer: No, this assertion is not correct. Mann–Whitney tests for differences in shapes of distributions (i.e., cumulative distribution functions) in addition to central tendencies (usually given as medians). Mann–Whitney is a test of the null hypothesis H_0 that two independent samples of observations X and Y come from the same distribution, against one of the following alternatives [10]:

H_1: Y observations tend to be larger than X observations

H_2: Y observations tend to be smaller than X observations

H_3: Y observations tend to be either larger or smaller than X observations

Mann–Whitney may be used when samples are from two distributions with identical cumulative distribution functions under H_0, but under H_1, one cumulative distribution curve lies beneath the other apart from some points where the curves touch. Under H_1, low or high ranks should dominate in one sample, as opposed to a fairly even distribution of ranks under H_0. Such H_1 are referred to as dominance alternatives [11]. Thus, the statistics literature does not support the previous two assertions made by authors in applied fields.

Mann–Whitney does not take the approach of the traditional *t*-test in attempting to hold the standard deviation of groups constant so that any measured difference is attributed only to a shift in the means. Instead, it determines whether one group "dominates" the other, i.e., whether one group results in generally higher values than the other, regardless of whether this is due to a shift in the median or to a different pattern in the cumulative distribution, such as an increase in the highest third of the data set. Because Mann–Whitney is computed on the data ranks, it does not require a constant standard deviation of the two groups in order to compute its *p*-value, as does the normal-theory *t*-test. Mann–Whitney *p*-values are determined by comparing the observed pattern of data to all results possible when no difference occurs between the two groups. More recently, this method of determining *p*-values has been adopted by permutation tests and applied to measures of difference other than those used by Mann–Whitney.

Accordingly, we argue that Mann–Whitney was appropriate in our original dendrochemical analysis of Fallon. However, our description of Mann–Whitney was incomplete. We described it as the "Mann–Whitney test of medians" [1], but an indication of testing shapes of distributions should have been included. Therefore, it should have been described as the "Mann–Whitney test of differences in cumulative distribution functions." This nuance is important enough that we hope it is understood by the wider community of the applied environmental sciences. We made this correction, a minor one of expression, in the journal that published our original dendrochemical analysis of Fallon [12].

In considering whether Mann–Whitney was appropriate in our original dendrochemical analysis of Fallon, an additional question emerged: Given that Mann–Whitney tests for differences in shapes of distributions, in what ways were shapes of distributions of element concentrations of tree rings of our Fallon study different or similar to one another? Shape characteristics of distributions include spread (variance), having a long tail on one side (skewness), and/or being humped or peaked (kurtosis) [13]. These characteristics of distributional shape are quantifiable, so we now analyze our original dendrochemical data for these characteristics, focusing here on inter-tree variability. The second objective of this article is to report this additional statistical analysis of temporal change in W and Co in Fallon.

2. Methods

2.1. Fallon, Nevada

Details of the field and laboratory methods employed in this Fallon dendrochemical research are described in our original publication [1]. In short, cottonwoods (*Populus* sp.) were sampled in Fallon (the "treatment" sample) and cottonwoods and elms (*Ulmus* sp.) were sampled in the comparison towns of Lovelock, Fernley, and Yerington (the "control" sample). See Supplemental Material for maps of west-central Nevada. Four time periods of rings were selected for measurement for concentrations of multiple elements. Two periods predate the 1997 onset of excessive childhood leukemia in Fallon (1989–1992 and 1993–1996) and two periods postdate it (1997–2000 and 2001–2003 or –2004, depending on the last ring available for measurement).

2.2. Sweet Home, Oregon: an independent test case

To independently test the accuracy of dendrochemistry specifically for W, the Fallon research design was repeated in a different town that has a known source of airborne W. Sweet Home, Oregon (44° 23′ 51″ N, 122° 44′ 10″ W, see Supplemental Material for maps of west-central Oregon), has a tungsten-powder facility that was established in November, 2000. Spatial environmental techniques had confirmed that airborne W is elevated in the area immediately surrounding this known industrial source compared to the rest of Sweet Home, to other nearby towns, and to outlying open areas [14]. Douglas-firs (*Pseudotsuga menziesii*) and cottonwoods were sampled near the W facility (the "treatment" sample), and Douglas-firs were sampled outside Sweet Home at a rural location near Crawfordsville (the "control" sample). Approximately the same four time periods of rings that were measured in the Nevada trees were selected in the Oregon trees for measurement of concentrations of multiple elements.

2.3. Additional statistical analysis

Going beyond the statistical analysis done earlier, other moment statistics of distributions [13] were calculated for each measured element across trees within subsites and time periods. In particular, the second moment statistic, the variance, was converted to standard deviation and then relativized to central tendency, for which we continued using the median as a conservative estimate. This is a variant on the typical calculation for coefficient of variation, referred to here as "coefficient of median variation" (CMV).

2.4. Data simulation

To illustrate the performance of the Mann–Whitney test, two distributions of data were simulated. The first distribution ("background") was lognormal with mean log of 0 and standard deviation of logarithms of 1. The second distribution ("treatment") was a mix of 60% background with 40% from a lognormal distribution with mean log of 1.5 and standard deviation of logarithms equal to 1. This simulated groups with different standard deviations in the original units where a portion of the contaminated group concentrations are elevated above background, though the median may not be dramatically affected. From the two distributions, 1000 subsamples of $n = 5$, 10, 20, and 50 samples were randomly chosen and tested for differences using the Mann–Whitney test and the *t*-test (one-tailed in both cases, with H_a being that the background sample is less than the contaminated sample). Given that data were simulated to show a difference due to the addition of the 40% high data in the treatment distribution, the proportion of cases where a difference was found is the statistical power. See Supplemental Material for details and figures of these simulations.

3. Results

3.1. Inter-tree variability

3.1.1. Fallon, Nevada

For W and Co, the metals of highest interest in Fallon, inter-tree CMV (i.e., inter-tree variability) was always higher within versus outside of Fallon (Fig. 1). By comparison, no other measured metal consistently showed higher inter-tree variability within versus outside of Fallon. Inter-tree CMV of most other metals fluctuated through time without any apparent pattern within versus outside of Fallon. For aluminum and copper, inter-tree CMVs were always higher outside of Fallon than within. Also, lead showed anomalously high inter-tree variability both within and outside of Fallon.

For copper, high inter-tree variability outside of Fallon accurately reflects the fact that one of the comparison towns, Yerington, is located at the base of a tailings pile of an open-pit copper mine. Even though that mine is no longer active, copper is elevated in airborne particulates of Yerington [15], indicating high environmental availability of copper in that town. The other comparison towns, Fernley and Lovelock, have no copper mining nearby, hence the high inter-tree variability of copper across trees of these three comparison towns.

When viewed across all four time periods of this research, median CMVs of W and Co within Fallon were about double those of outside of Fallon (Table 1). No other measured metal showed ratios of CMVs within versus outside of Fallon even close to 2.0. Only two of the other measured metals had ratios as high as 1.0 (Mn and Sr). All other measured metals exhibited ratios below 1.0 (Al, Cd, Cu, and Pb), indicating higher inter-tree variability outside of Fallon than within.

3.1.2. Sweet Home, Oregon

For W, the only metal of interest in Sweet Home (Co is not processed at the W facility in Sweet Home), inter-tree CMV was low in the cottonwoods near the W facility for the first two time periods, but then it increased dramatically in the third period (just before the W facility was established) and stayed relatively high in the fourth period (just after the W facility was established) (Fig. 2). By contrast, inter-tree CMV for W was low (below 1.0) throughout all four time periods of this research for trees outside of Sweet Home, which were Douglas-firs. This accurately confirms the timing of establishment of the W facility in Sweet Home.

The high inter-tree variability of W in Sweet Home cottonwoods during the 1997–2000 period, which predates the establishment of the W facility in Sweet Home, is possible evidence of movement of W taken up after 2000 into pre-2000 rings, perhaps due to endogenous factors within trees [16,17]. Incorporation of elements into already-formed growth rings is of general concern in dendrochemistry [18]. However, W probably did not migrate across more than four rings because inter-tree variability of W in the Sweet Home cottonwoods for the first and second periods (1989–1992 and 1993–1996) was low.

Inter-tree CMV for W was also low throughout all four time periods of this research for the Douglas-firs that were sampled

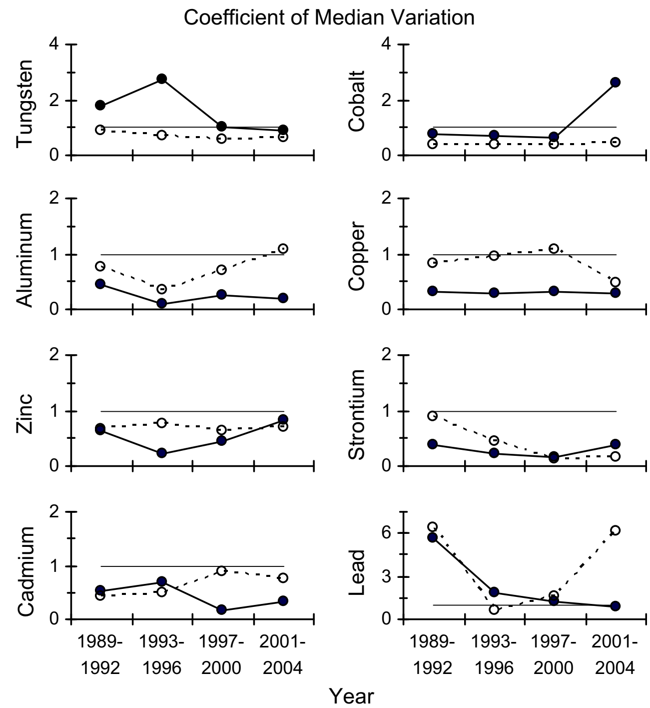

Fig. 1. Inter-tree coefficient of median variation for metals measured in trees sampled within Fallon (solid lines, filled circles, $n = 5$ trees) and outside of Fallon (dotted lines, open circles, $n = 6$ trees). Reference lines are at 1.0, indicating standard deviation equal to median.

Table 1
Summary results for coefficient of median variation[a] for trees within versus outside of fallon. In all cases, $n = 4$ time periods.

Absolute value			Ratio	
Element	Within Fallon	Outside Fallon	Element	Within ÷ outside
Lead	1.55	3.86	Tungsten	2.04
Tungsten	1.40	0.68	Cobalt	1.97
Cobalt	0.72	0.37	Manganese	1.18
Manganese	0.71	0.60	Strontium	1.00
Zinc	0.53	0.69	Zinc	0.77
Cadmium	0.43	0.61	Cadmium	0.70
Copper	0.30	0.88	Lead	0.40
Strontium	0.29	0.29	Copper	0.34
Aluminum	0.23	0.73	Aluminum	0.32

[a] Coefficient of median variation = standard deviation ÷ median.

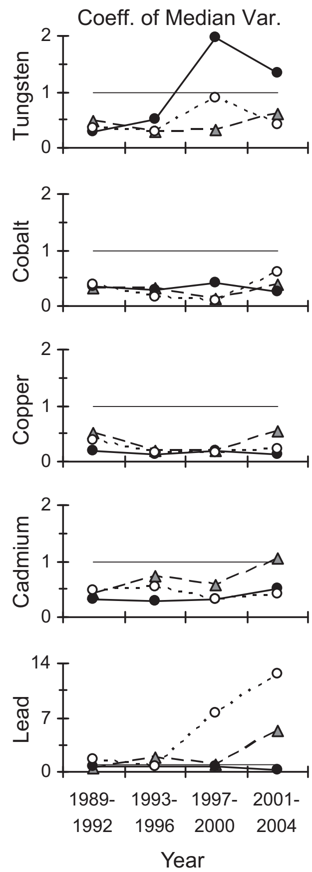

Fig. 2. Inter-tree coefficient of median variation for metals measured in cottonwoods sampled within Sweet Home (solid lines, filled circles, $n = 4$ trees), Douglas-firs sampled within Sweet Home (dashed lines, gray triangles, $n = 4$ trees), and Douglas-firs sampled outside of Sweet Home (dotted lines, open circles, $n = 4$ trees). Reference lines are at 1.0, indicating standard deviation equal to median.

within Sweet Home near the W facility. This result, in conjunction with the Douglas-firs outside of Sweet Home also showing low inter-tree variability, might be interpreted as poor ability of the species Douglas-fir to reflect variability in environmental availability of W, which would contradict the ranking of Douglas-fir as a "highly recommended" species for dendrochemistry [19].

On the other hand, this Douglas-fir result could be indicating something environmental. The Douglas-firs sampled within Sweet Home are growing nearly due south of the facility (see Supplemental Material for map of Sweet Home). Prevailing winds stronger than $2\ m\ s^{-1}$ ($4.5\ mile\ hr^{-1}$) of west-central Oregon are from the west, southwest, and/or south (Fig. 3), and because of this it could very well be that the Douglas-firs sampled within Sweet Home are not receiving much deposition of airborne W from the industrial facility in spite of their growing within about 100 m of the facility. Almost certainly, the sampled Douglas-firs are receiving less W deposition than the sampled cottonwoods, which are growing in a more downwind direction from the W facility. In this sense, low inter-tree variability of W in the Douglas-firs sampled within Sweet Home might be accurately reflecting environmental conditions in Sweet Home.

Most other measured metals consistently showed low inter-tree variability, with CMVs mostly well below 1.0 for all three sites, within and outside of Sweet Home (Fig. 2). Inter-tree CMVs of these other metals also did not fluctuate much through time.

Lead was the lone exception to this lack of inter-tree variability. Lead showed anomalously high inter-tree CMVs during the last two time periods (Fig. 2). These high inter-tree CMVs for lead were due essentially to one value at each subsite and time period being substantially higher than other values, which defies explanation environmentally, physiologically, or even technically (e.g., measurement error). Dendrochemistry of lead has been notably problematic [20–22], and these lead results here, both in Sweet Home and in Fallon, confirm that dendrochemistry of lead continues to need development.

3.2. Simulations

For random samples of $n = 5$ (i.e., five "trees" sampled from each distribution, which was the approximate size of samples of Sheppard et al. [1], rejection rates were generally low (below 20%) (Table 2), indicating low power, presumably because of the small sample size. The fact that significant differences were found at all in Sheppard et al. [1], surmounting the handicap of low sample size, indicates that true differences do exist in those data sets.

Additionally, with samples of $n = 5$, the rejection rate of Mann–Whitney was twice that of the *t*-test (Table 2). Thus, the Mann–Whitney test committed less type II error, i.e., had more power to find a difference when one really does exist [23], than the *t*-test at this low sample size.

As sample size increased, rejection rates increased and became approximately equal for both tests (Table 2). This is the expected behavior of the *t*-test based on the Central Limit Theorem, that it is able to equal the power of the Mann–Whitney when sample sizes are sufficiently large. How large depends on the skewness of the data, and is often larger than 30 observations for the considerable skewness found in environmental concentrations. It should be noted that samples of $n = 10$ trees per subsite would be considered high in dendrochemistry due to the high expense of chemical measurement of samples. Sample sizes of $n = 20$ and $n = 50$ trees per subsite would be extraordinary in dendrochemistry.

4. Discussion

4.1. Dendrochemistry

This research adds a new dimension to dendrochemistry of point-source contamination. Dendrochemistry usually focuses on concentrations of elements or other chemical substances in tree rings. Isotopic ratios of elements are also commonly analyzed. Given that more than one tree is sampled at any given site, results are reducible to an estimate of central tendency of measurements across trees within a time period. Central tendencies are then plotted as time series to assess temporal change in tree-ring chemistry that might reflect temporal change in environmental conditions. This is a straight-forward, obvious approach to analyzing dendrochemical data.

By plotting inter-tree variability, shape of distributions is emphasized in addition to central tendency. It is logical to extend dendrochemical analysis in this way, as environmental change, especially contamination from a point source, can affect shapes of sample distributions as much as, if not more than, central tendencies. For example, point-source contamination can affect some, but not all, trees, which would increase the variance of a treatment distribution over that of a control distribution. This response to point-source contamination might actually not affect the median of a treatment distribution, but changes to the shape of a treatment distribution could, if not should, be considered real treatment effects independent of changes in the medians.

This additional statistical analysis of dendrochemical data of Fallon and Sweet Home puts this concept into practice. In Sweet Home, inter-tree variability in W increased at or about the time of

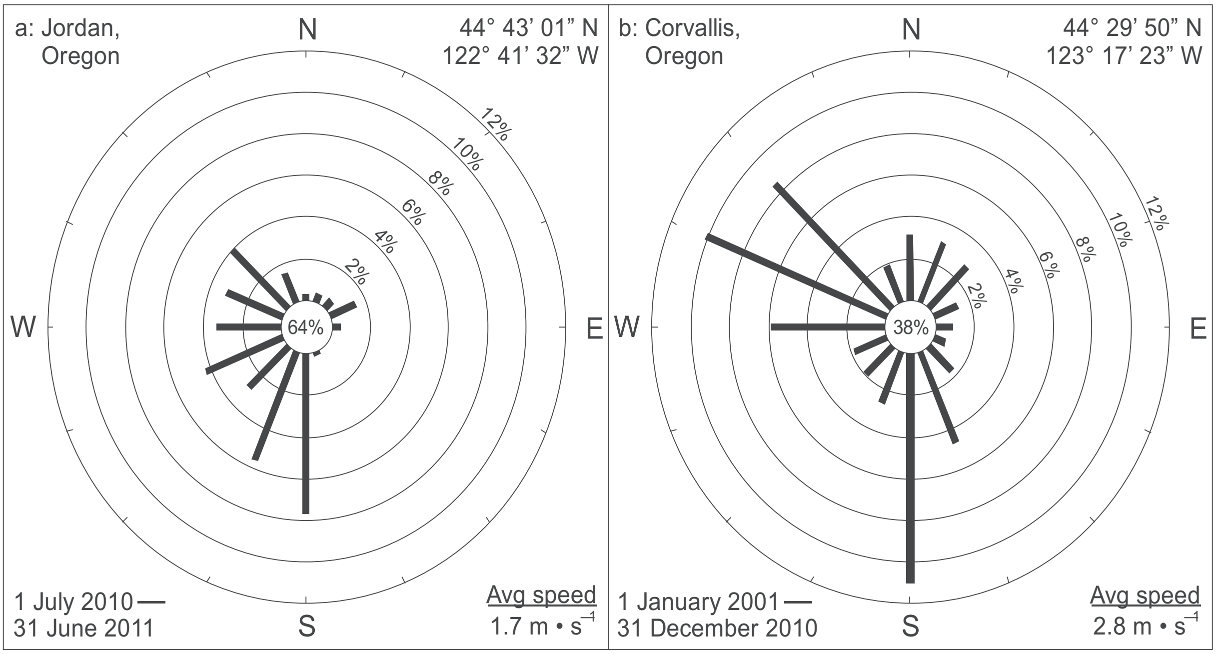

Fig. 3. Wind rose diagrams for two locations near Sweet Home: (a) Jordan (one year of data) and (b) Corvallis (11 years of data) (see Supplemental Material for map of west-central Oregon). Numbers inside center circles are percent calm winds, including winds up to 2 m s^{-1}. For Jordan, hourly wind data are from a remote automatic weather station; for Corvallis, hourly wind data are from the municipal airport. Wind rose diagrams were generated using software of the Western Regional Climate Center.

establishment of a point source of airborne W in that town, accurately reflecting that known change in environmental chemistry. By extension, high inter-tree variability in W and Co in Fallon can be viewed as evidence of a point source of those two metals within Fallon.

This additional statistical research has another potential ramification for dendrochemistry. Because of inherent differences in suitability of tree species for dendrochemistry [19], it has been logical to restrict any given tree-ring chemical study to comparing data within one tree species, e.g., cottonwoods versus cottonwoods or Douglas-firs versus Douglas firs. Now, with analysis of shapes of distributions in addition to central tendencies, it might be acceptable to compare data from different subsites using different species, e.g., cottonwoods within Sweet Home versus Douglas-firs within or outside of Sweet Home. Whatever the ability of a species might be to take up and retain any given element or chemical substance in its growth rings, changes in inter-tree variability within a subsite could indicate point-source contamination more or less the same across species. This would allow more flexibility in research of temporal change of environmental chemistry. This concept needs more replication before it is proven useful, but this additional statistical analysis at least provides rationale for pursuing this concept further.

4.2. To transform data, or not

An alternative approach to the Mann–Whitney test of raw data could be to log transform data sets in order to achieve homoscedasticity (equal variances) and then apply the parametric *t*-test [24]. However, transforming data for this purpose has drawbacks. With small sample sizes, it is inherently difficult to assess distributional shape and thereby choose the appropriate transformation [25,26]. Log transformation can have the arbitrary effect of reducing the importance of high observations and inflating the importance of small observations [27]. Even if log transformation reduced differences in variances of two samples, it still might not necessarily succeed to the extent that parametric testing would be appropriate [28]. Interpreting test results from transformed data back into original units also can be difficult [6,29].

Table 2
Rates of rejection of null hypotheses (i.e., statistical power) for Mann–Whitney and *t*-tests of 1000 subsamples of simulated distributions.

Sample size[a]	% Rejection of H_0	
	Mann–Whitney	*t*-test
5	19	9
10	29	27
20	56	57
50	94	98

[a] Number of "trees" sampled from each simulated distribution.

5. Conclusion

The general approach of the Mann–Whitney test, to determine whether "one group produces larger values than a second group," is often exactly what is needed in environmental investigations. Its insensitivity to distributional shape requirements renders data transformation unnecessary. Its sensitivity to not just a shift in the median, but also to an increase in the top 40% of data as exemplified here, mirrors types of contamination effects often seen in the real world.

This additional statistical analysis of our Fallon dendrochemical data strengthens our original conclusion: Fallon is distinctive by a temporal increase in W beginning by the mid 1990s as well as by elevated Co since at least the early 1990s [1], and also by high inter-tree variability and skewness for W and Co (this study). Accordingly, we continue to encourage environmental research in Fallon to confirm current and past airborne loadings of W and Co.

As usual, linkage between a disease and an environmental condition cannot be made from environmental data alone in an ecologic study [30], [31 (this issue)]. Nonetheless, merely the co-occurrence in Fallon of elevated W and Co in airborne particulates and a cluster of childhood leukemia logically should prompt biomedical research to evaluate the potential linkage between the combined exposure to both W and Co and leukemia.

Conflict of interest statement

Paul R. Sheppard and Mark L. Witten have provided documents, data, and declarations in Cases CV03-03482, Richard Jernee et al. vs Kinder Morgan Energy et al., and CV03-05326, Floyd Sands et al. vs Kinder Morgan Energy et al., Second Judicial District Court of Nevada, Washoe County, which are related to the childhood leukemia cluster of Fallon. In these cases, the law firm of Dunlap and Laxalt, representing the plaintiffs, with full disclosure to all defendants and their counsels, made an unsolicited donation of $15,000 to assist Witten and Sheppard in furthering their research, with a request that defendants provide similar donations. Neither Witten nor Sheppard have profited personally as a result of doing their research in Fallon or from providing material in these cases. Robert J. Speakman was also listed by plaintiffs as a potential consultant/expert in these cases, but he has not testified in these cases. Dennis R. Helsel and Gary Ridenour declare no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cbi.2011.12.009.

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