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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