APPENDIX A

PRESERVATION BY FREEZING AS AN EFFECTIVE METHOD FOR STABILIZATION OF DISSOLVED NITRATE PLUS NITRITE IN AQUEOUS SOLUTIONS

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ABSTRACT

A review of the literature was conducted to substantiate freezing as a scientifically valid method for preserving leachate for NO_3-N analysis. Due to an extremely large number of samples collected from three locations and the time consuming ortho-P analyses conducted on the same samples, a large backlog of samples quickly accumulated, necessitating long term frozen storage of the samples. The literature and our own test indicate that there were not statistically significant differences in concentrations of samples analyzed for NO_2-N + NO_3-N utilizing EPA method 353.2, regardless of whether analyzed at 0-28 days per FDEP SOP's or analyzed after extended frozen periods without preservation.

Situation:

Between May 2005 and August 2011 the University of Florida conducted contract work for FDEP on nutrient leaching from turfgrass grown at three UF research facilities – Jay (northwest Florida), Citra (central Florida), and Ft. Lauderdale (south Florida). In 2007, an FDEP audit raised concerns about the freezing preservation and the delay in time required to analyze the samples. For example, at the Jay research site, 40,550 groundwater leachate samples were collected; 20,750 utilized freezing as the sole preservation method. Holding to analysis periods for the frozen samples ranged from 2 to 439 days as a result of laboratory backlogs and delays; of these only 250 were greater than 365 days. Similar samples numbers were obtained from the other two locations. From the audit outcome, the following was stated:

"Freezing is not mentioned as an acceptable practice in the DEP SOPs. It is mentioned in the QAPP, but the implication was that it would be used only if the samples could not meet the holding time. This was interpreted by the DEP QA reviewers to be the exception, not the routine procedure. If freezing continue to be the practice, a holding time study that compares results of properly preserved samples with frozen samples must be submitted to DEP for review and approval. If freezing is determined to be an acceptable practice, a conventional freezer should be used. A frost-free freezer may be acceptable if it can be demonstrated through continuous monitoring that the temperature of the freezer compartment does not exceed -10°C at any time during the freezing cycle."

Holding Time Study Findings:

Water samples were collected from the Winter Fertilization Study (Gainesville site) and sampled with one of four preservation methods:

1. Acidified and analyzed within 28 days
2. Frozen and analyzed within 48 days
3. Frozen and analyzed within 111 days
4. Frozen and analyzed within 179 days

Data were analyzed for effects of timing, N rate and their interaction.

Table 1. Analysis of preservation methods on stability of NO_3-N in water samples taken from turfgrass research plots.

ANOVA	NO_3-N (ppm)
Preservation Method	NS
Nitrogen Rate	**
Preservation Method X Nitrogen Rate	NS
MEANS	
Preservation Method	
1. Acidified and analyzed within 28 days	0.01667 a
2. Frozen and analyzed within 48 days	0.00083 a
3. Frozen and analyzed within 111 days	0.02125 a
4. Frozen and analyzed within 179 days	0.00792 a
Nitrogen Rate (kg ha^{-1})	
98.0	0.00063 b
49.0	-0.02875 b
24.5	-0.02938 b
12.3	0.06188 a
6.1	0.00750 b
0.0	-0.03188 b

† Within columns, means followed by different letters differ significantly at $P = 0.05$.
** Significant at the 0.01 probability level.
NS Not significant at the 0.05 probability level.

The ANOVA reveals that no differences in NO_3-N were detected from water samples regardless of preservation method. A difference was detected in the NO_3-N concentration related to N application rates but it is likely a data anomaly. The second lowest application rate yielded the highest NO_3-N rate; no differences were detected among the other application rates, regardless of N application rate. No interaction between N Rate and preservation method was observed.

Freezing as a suitable preservation method has been demonstrated by Proctor, who compared unpreserved seawater fortified with $NaNO_2$ to a concentration of 1.0µg/L of NO^-_2-N. All the frozen samples out to 220 plus days when analyzed matched baseline analysis within the precision limits of the analytical method (Proctor, 1962 Vol 4). Avanzino and Kennedy found freezing to be an effective preservation method for NO_2-N + NO_3-N out to 8 years with a 95% confidence level (Avanzino & Kennedy, 1993). While studying effects of frozen storage out to 1 year on N in saline and low saline water samples, McDonald and McLaughlin found freezing to decrease NO_3-N concentrations for saline samples by approximately 1.6%, while having no effect on the low saline samples (McDonald & McLaughlin, 1982). Investigating preservation methods to preserve inorganic N in unfiltered; surface, tile drain, and stream water samples, out to 12 weeks, Klingaman and Nelson found that storage at

subzero temperature was as effective as 4°C samples preserved with PMA or $HgCl_2$ in lieu of H_2SO_4 (to prevent any N loss from oxide formation) (Klingaman & Nelson, 1976). While determining proper storage techniques for leachate collected from low volume ceramic cup samplers, Park (unpublished data) reported freezing as the most suitable practice for preserving sample integrity when immediate analysis of NH_3 and NO_2-N + NO_3-N was not possible. This conclusion was arrived at by applying the paired sample *t*-test to samples; immediately analyzed, analyzed after 20 hours, refrigerated at 4°C and analyzed at 24 hours, frozen at -20°C and analyzed at 11 days, and refrigerated at 4°C , acidified to a pH of 2 with H_2SO_4 and analyzed at 11 days (Park, 2007).

Establishment and justification of freezing to preserve NO_3-N + NO_2-N concentrations in samples of aqueous solutions when time to analysis would result in altered NO_2-N + NO_3-N composition due to biotic and physical chemistry changes has been addressed in the literature and supports our own findings, which indicate there are no differences found between leachate samples that were immediately acidified, placed on wet ice until refrigerated at 4°C or less with analysis for NO_2-N + NO_3-N utilizing EPA method 353.2 performed at 28 days and samples that were immediately placed on wet ice, frozen and subsequently analyzed with the same method at; 48 , 111, and 179 days.

Sample Storage at the Jay Site:

Stored samples preserved for later analysis need to be consistently maintained at temperatures less than -10 °C. This can be adequately demonstrated by utilizing temperature data loggers and the temperature data they collect. The freezer utilized for holding the Jay (FL) research site samples was electronically logged from October 25, 2007 through November 8th 2011 at 15 minute intervals following a post audit discussion with FDEP that was later formalized by email on January 8th of 2008 stating, "A frost-free freezer may be acceptable if it can be demonstrated through continuous monitoring that the temperature of the freezer compartment does not exceed -10 °C at any time during the freezing cycle." Temperature logging indicated seven significant power failures with only one of those resulting in a temperature logged above 0 °C and then only for two consecutive 15 minute logging periods. Otherwise, no more than one 15 minute logging period in any one day on average would exceed the -10 °C temperature requested. Since compartment air temperature and not sample temperature was being logged it is reasonable to assume that once frozen all stored samples remained in that state until analyzed.

Conclusions:

Though not approved as a preservation method under FDEP and EPA guidelines, research supports freezing as an effective preservation method for stabilization of dissolved NO_2-N + NO_3-N in aqueous solutions when circumstances do not allow immediate analysis,

Literature Cited

Avanzino, R. J., & Kennedy, V. C. (1993). Long-term frozen storage of stream water samples for dissolved orthophosphate, nitrate plus nitrite, and ammonia analysis. *Water Resources Research*, 3357-3362.

Klingaman, E., & Nelson, D. (1976). Evaluation of methods for preserving the levels of soluable inorganic phosphorus and nitrogen in unfiltered water samples. *Journal Environmental Quality*, 42-46.

McDonald, R., & McLaughlin, F. (1982). The effect of storage by freezing on dissolved inorganic phosphate, nitrate and reactive silicate for samples from coastal and estuarine waters. *Water Research*, 95-104.

Park, D. (2007). *Influence of sample storage on stability of nitrogen from low volume leachate.* Gainesville: Unpublished.

Proctor, R. (1962 Vol 4). Stabilization of the nitrite content of sea water by freezing. *Limnology and Oceanograph*, 479-480.