Evaluation of Chemical Data from Samples Collected February 2008 at the Cactus Flat Main Lake Depression and Surrounding Area, Nevada Test and Training Range

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prepared by

Desert Research Institute, Nevada System of Higher Education

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ABSTRACT

The U.S. Bureau of Land Management contracted with Desert Research Institute in January 2008 to investigate water and sediment chemistry at the Nevada Test and Training Range Cactus Flat Main Lake depression. The focus of the investigation was the source of nitrogen compounds that apparently led to the death of 71 wild horses in July 2007. Although conditions at the depression had changed significantly in the period between the wild horse deaths and the initiation of the contract, the managing agencies wanted to proceed with sampling and analysis, as any potential insight on what led to the wild horse deaths could help protect wild horses in the future. Consequently, this report is an attempt to determine the possible sources of the nitrogen compounds in spite of the seasonal and water-level changes that had taken place between the time of the wild horse deaths and the time of sampling. Samples were also collected to screen for possible anthropogenic compounds.

The primary objective of this study was to answer four questions:

- Do data indicate whether nitrogen compounds in the Main Lake depression are man-made or naturally occurring?
- Can later time data be modeled to replicate initial water sample results?
- Are water nitrogen concentrations a possible cause of death?
- Can similar conditions that could result in another wild horse kill be predicted?

Water in the Cactus Flat Main Lake depression was significantly less saline in February 2008 than in summer 2007, likely because of low evaporation and dilution by recent precipitation. Chloride concentrations suggest that the water in the Main Lake depression in July 2007 had been concentrated approximately 38-fold by evaporation, as compared to the water present in February 2008. Although total dissolved solids concentrations were not measured on either water, summation of known dissolved solids concentrations in the two samples shows that total dissolved solids were less than 1,000 mg/L in February 2008, but over 30,000 mg/L in July 2007.

Based on a simple model of evaporation and insight from the nitrogen isotope data, it appears that the dilute water observed in the Main Lake depression in February 2008 could be altered by purely natural processes to yield the high dissolved solids concentrations (including nitrate and nitrite) observed in July 2007. Analysis of stable isotopes of nitrogen suggests that the most likely cause of the high nitrate in the Main Lake depression in July 2007 was a combination of two natural processes: evaporative concentration of natural nitrate, and addition of nitrate via nitrification of natural materials, including animal waste and natural soil nitrogen.

It appears unlikely that human influence, such as contamination from urea or glycol-based deicing fluids played a significant role in the high nitrogen concentrations. However, because of the large time difference between the wild horse deaths and the sampling conducted by DRI, anthropogenic contamination cannot be definitively discounted since glycol deicers and other organic compounds can quickly degrade with time.

Because there is little information regarding nitrate/nitrite toxicity to horses, it is not possible to predict the exact conditions that would lead to future wild horse deaths. Nitrogen and oxygen isotopes suggest that increases in nitrogen compounds in the depression from

nitrification may have occurred at the very latest stages of evaporation, although evaporative concentration of nitrogen compounds also contributed to the high nitrate and nitrite concentrations. Additionally, assuming the wild horses have used the depression over many years, it would appear that the deaths from high nitrate concentrations in the depression are a rare occurrence. Monitoring of the chemical evolution of the water chemistry in the Main Lake depression as a function of water depth, in conjunction with reliable estimates of nitrogen toxicity in horses, would produce information that could be used to develop a strategy for managing herd access to the Main Lake depression at Cactus Flat.

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INTRODUCTION

The U.S. Bureau of Land Management (BLM) contracted with the Desert Research Institute (DRI) in January 2008 to investigate water and sediment chemistry at the Nevada Test and Training Range (NTTR) Cactus Flat Main Lake depression. The focus of the investigation was the source of nitrogen compounds that apparently led to the death of 71 wild horses in July 2007. Although conditions at the NTTR had changed significantly in the period between the wild horse deaths and the initiation of the contract, the managing agencies (BLM, U.S. Department of Defense, and U.S. Department of Energy) wanted to proceed with sampling and analysis, as any potential insight on what led to the wild horse deaths could help protect wild horses in the future. Consequently, this report is an attempt to determine the possible sources of the nitrogen compounds in spite of the seasonal and water-level changes that had taken place between the time of the wild horse deaths and the time of sampling. Samples were also collected to screen for possible anthropogenic compounds including glycol-based deicers and petroleum hydrocarbons.

The NTTR is located in southern Nevada 130 km north of Las Vegas, and occupies 11,700 km^2. The BLM Nevada Wild Horse Range Herd Management Area comprises nearly 1,900 km^2 (1.3 million acres) within the northern portion of the NTTR; it is occupied by approximately 1,400 wild horses. Between July 20 and 25, 2007, 71 wild horses were found dead in the northern part of the NTTR near the Main Lake depression located in Cactus Flat at a dry lake bed approximately 5 km northeast of an airstrip managed by the NTTR (Figure 1a).

The Main Lake depression was excavated approximately 20 years ago for a U.S. Department of Energy/Sandia National Laboratories project. The Main Lake depression has been used by wildlife (including wild horses) as a consistent source of drinking water under normal precipitation conditions. However, because of below normal precipitation in 2006 and 2007 (www.wrcc.dri.edu/summary/Climsmnv.html [stations Tonopah Ap (268170) and Las Vegas WSO Airport (264436)]), water in the Main Lake depression was approximately 0.3 m deep when the dead wild horses were found (Ronald Lowndes, Sandia National Laboratories, personal communication, 2008).

Toxicology reports prepared by the California Animal Health and Food Safety Laboratory System (CAHFS) indicated that high levels of nitrate (NO_3^-) and nitrite (NO_2^-) were the most probable cause of the wild horse deaths. The primary reason for this attribution was high concentrations of nitrate in serum and ocular fluid (CAHFS, 2007a). In addition, tests for botulin (the toxin that causes botulism), anatoxin-a, and microcystins, and gas chromatograph/mass spectrometry screening for organic compounds were all negative. In July 2007, shortly after the wild horse deaths, two water samples were collected from the Main Lake depression at different depths, and "scum" was collected from the water surface; these samples were analyzed for nitrate and nitrite (in addition to other constituents). The nitrate concentration of the water samples from the different water depths were 5 and 3,670 parts per million (ppm), whereas samples of scum from the surface of the Main Lake depression had 3,440 and 3,940 ppm (CAHFS, 2007b). [Note that concentrations reported in ppm are essentially equivalent to the concentrations reported in mg/L.] An excerpt of the CAHFS is provided in Appendix 1, see page "6 of 8" of this report for the nitrate data. Nitrite levels in the Main Lake depression water were below detection in one sample and 50 ppm in

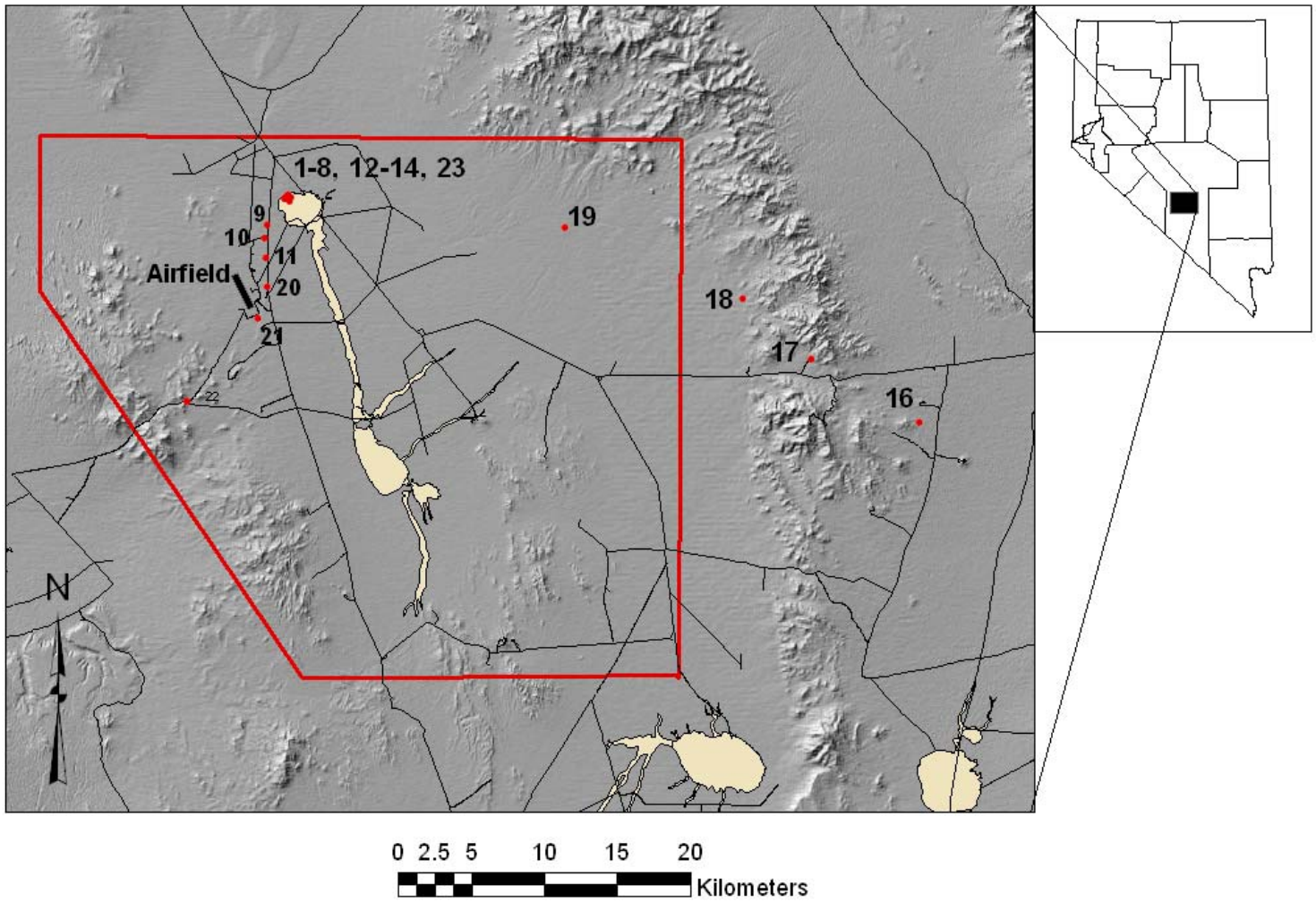

Figure 1a. Overview map showing the location of samples collected by the Desert Research Institute on the NTTR in February 2008. Except for the airfield (labeled), black lines indicate locations of roads. The red polygon represents the boundary of the Tonopah Test Range (located within the NTTR).

another sample; surface scum had nitrite concentrations as high as 848 ppm (CAHFS, 2007)[1]. In samples collected by the U.S. Air Force, other ions were also present in markedly high concentration, with 2,100 mg/L of chloride, and 2,100 mg/L of sulfate (see Appendix 2). Evaporative concentration of waters is the most likely explanation for the high concentrations of dissolved solids in the Main Lake depression in July 2007, but precipitation between September 2007 and the sampling for this report in February 2008 increased the water-level in the Main Lake depression, altering the conditions from those when the wild horses died.

In 1988, 61 wild horses were found dead at a construction pond at the southern end of the airfield runway, which is approximately 5 km from the Main Lake depression. Those deaths were attributed to "ammonia toxicity due to excessive consumption of urea" (Stager and Ruegamer, 1988). The deaths were linked to disposal of a urea-based roadway/runway deicer by contractors working on the NTTR (Stager and Ruegamer, 1988). In soils, urea is degraded to ammonia (NH_3) via hydrolysis, a reaction that is primarily mediated by the enzyme urease (Sills and Blakeslee, 1992). Nitrifying soil organisms convert ammonia from urea to nitrogen by hydrolysis, but both the urea-ammonia and ammonia-nitrogen conversions are much slower during the winter than the summer (Sills and Blakeslee, 1992). Urea deicers have not been used at the NTTR since the "early to mid [19]90s" (R. Schofield, U. S. Air Force, personal communication, 2008), consistent with a 1996 Air Force letter recommending discontinuing the use of urea deicers (U.S. Air Force Center for Environmental Excellence, 1998). In the U.S., urea was commonly used as a runway deicer in the past, because it is much less corrosive than deicers such as sodium chloride; however, its use in recent years has been extremely rare, because other deicers are cheaper, more effective, and have lower environmental impact (Ireland, 1992; Moran *et al.*, 1992).

Nitrogen and the Nitrogen Cycle

Nitrates are commonly found in desert soils at parts-per-hundred levels (Böhlke *et al*., 1997a; Walvoord *et al*., 2003; Graham *et al*., 2008) and arise from natural inputs directly from the atmosphere and via nitrification. Atmospheric inputs are from both wet deposition (nitrate contained in rain water) and dry deposition (the settling of nitrate dust or gaseous HNO_3 on soil surfaces). Atmospheric nitrate originates from the photochemical oxidation of NO_x ($NO + NO_2$) emitted naturally, by lightening and biomass burning, and by humans, as a result of combustion processes such as those in automobiles and power plants. Nitrification is the oxidation of the ammonium ion (NH_4^+) by bacteria, which use the reaction to derive energy for metabolism. Soil ammonium usually occurs naturally, mainly from the breakdown of plant and animal wastes (urine, manure) with small amounts originating from wet and dry deposition of atmospheric ammonia (NH_3/NH_4^+). However, anthropogenic ammonia is a common agricultural fertilizer and is the primary source of soil nitrate in farming regions. The relative importance of atmospheric deposition versus nitrification in the nitrate budget of desert soils is controlled by water. Whereas atmospheric inputs are relatively constant averaged over several years, desert nitrification is usually limited by water availability (Belnap *et al*., 2004; Housman *et al*., 2006; Miller *et al*., 2006). Thus, wetter deserts have a

[1] Note that nitrate concentrations can be converted to nitrate-as-nitrogen (NO_3-N) concentrations by multiplying by 0.226; nitrate-as-nitrogen concentrations can be converted to nitrate concentrations by multiplying by 4.43. Similarly, nitrite concentrations can be converted to nitrite-as-nitrogen (NO_2-N) values by multiplying by 0.305, and nitrite-as-nitrogen concentrations can be converted to nitrite concentrations by multiplying by 3.28.

larger nitrate input from nitrification, whereas in hyperarid deserts, the nitrate is predominately atmospheric (Michalski *et al*., 2004a; 2005; Eqing *et al.*, 2007).

Nitrate accumulates in desert soils because of the general lack of water, which minimizes leaching of nitrate (and other salts) and restricts biologic utilization such as plant assimilation and denitrification. This is in contrast to low nitrate levels in most temperate soils, usually measured at ppb to ppm levels. In desert soils, water flux beneath the root zone further concentrates salts, including nitrate (Tyler *et al*., 1996; Hartsough *et al*., 2001). Nitrate salts are highly soluble and are not removed by precipitation of carbonate or sulfate minerals. Therefore, in closed basins, ground-water discharge and overland flow can transport soil nitrate to terminal lakes and/or playa lake beds where the nitrate can undergo evaporative concentration and reach high concentrations (Tyler *et al.*, 1997; Blank *et al.*, 1999). An examination of nitrate in playa soils in Nevada found concentrations ranging from 0.9 to 6,860 mg/kg (values were reported as nitrate. The reported range is equivalent to 0.2 to 1,550 mg/kg as N; mg/kg units are equivalent to ppm), with approximately half the sites examined having concentrations above 100 mg/kg (Leathem *et al*., 1983). One important anthropogenic source of nitrate in many arid environments is agriculture return flow (McMahon *et al.*, 2006); however, the nearest agricultural operation is over 8 km away, and is separated from the Main Lake depression by a surface drainage flowing to the west, making it unlikely that significant nitrate could migrate to the Main Lake depression. Other possible sources of nitrate include explosives, explosives residue, sewage, and animal waste.

Study Objectives

The primary objective of this study was to answer four questions:

- Do data indicate whether nitrogen compounds in the Main Lake depression are man-made or naturally occurring?
- Can later-time data be modeled to replicate initial water sample results?
- Are water nitrogen concentrations a possible cause of death?
- Can similar conditions that could result in another wild horse kill be predicted?

SAMPLING

Samples were collected at 22 sites (Figure 1a, b, and c). Of the 22 sampling sites, waters were collected at seven sites (five spring waters were sampled, and samples were collected from two depths in the Main Lake depression). At the remaining 15 sites, sediments were collected, with four sites at the bottom of the Main Lake depression, and the remaining 11 sites being natural or engineered drainages. Of the 11 drainage samples, nine were collected from locations between the airfield and the Main Lake depression. Although an analysis of surface-water flow directions was not performed, the topographic relationship suggests flow from the airfield toward the Main Lake. The remaining two samples were collected northeast of the Main Lake depression, upgradient of the Main Lake depression, but downgradient of an old testing target. At each site, multiple sample aliquots were collected for major ion, trace element, and organic constituent determinations. When water samples were collected, electrical conductivity (EC), pH, and temperature were measured on-site at the time of collection.

A list of the samples collected by DRI at each site is given in Appendix 3, along with analytical results. A list of results from U.S. Air Force sampling concurrent with DRI's sampling is provided in Appendix 4. Because sediment sample analysis involved processing an aliquot of a large sediment sample, some sediment samples had two aliquots selected, processed, and analyzed to indicate the variability within each bulk sediment sample. A description of the sample collection, storage, preparation, and analysis procedures for each type of sample is given below. All samples were tracked with chain-of-custody forms and a custody seal was placed on the sample container at the time of sampling, such that the sample container could not be opened without breaking the seal. Sample collection and analysis followed standard procedures appropriate for each analyte, which are described in the following sections.

Water and sediment samples were collected on Wednesday, February 6, through Friday, February 8, and then transported to the DRI Reno facility Friday, February 8. All samples were stored in insulated coolers with ice to maintain a temperature as close to 4 °C as possible until they were transferred to refrigerators for storage as close to 4 °C as possible. Water samples were analyzed for nitrate and nitrite by the DRI Analytical Chemistry Laboratory on February 13. Nitrate and nitrite analysis of sediment extract samples was conducted less than 48 hours after completion of extract process, but water samples from the Main Lake depression and surrounding springs were analyzed after being held (cooled) for five to seven days. U.S. Environmental Protection Agency recommended holding times for aqueous nitrate and nitrite samples are 48 hours for nonpreserved samples and 28 days for samples preserved with sulfuric acid. Although the water and sediment samples were processed and analyzed after more than 48 hours, this should not have affected measured nitrate and nitrite concentrations, nor the conclusions drawn in this report. Research has shown that adding sulfuric acid to water samples causes a rapid conversion of nitrite to nitrate (Roman *et al*., 1991), so this form of preservation is not appropriate if nitrite is an analyte of interest. In addition, nitrate and nitrite concentrations in unacidified water and soil samples stored at 4 °C are stable from one week to more than 30 days (e.g., Roman *et al*., 1991; Khakural and Alva, 1996; Yorks and McHale, 2000; Clough *et al*., 2001)

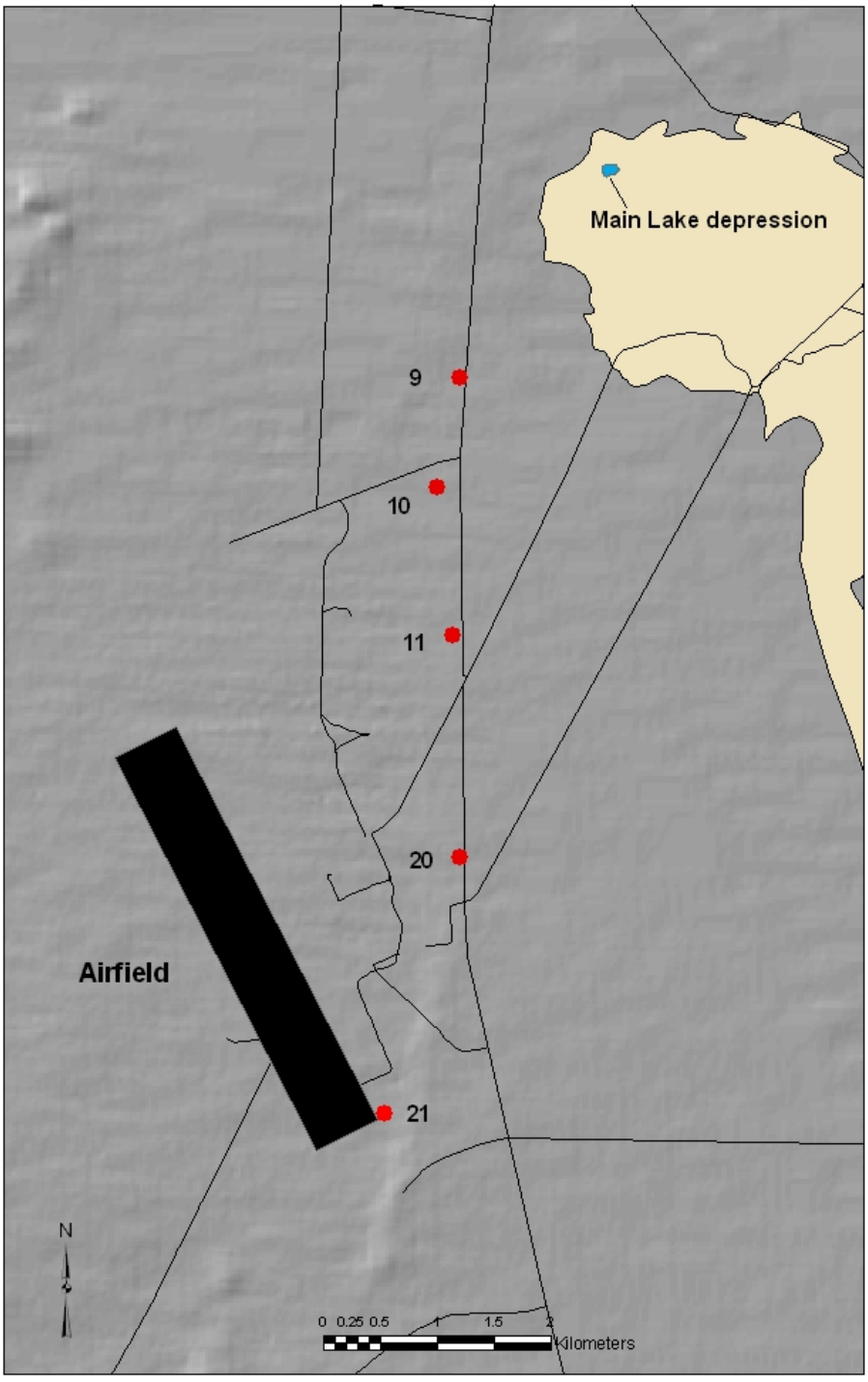

Figure 1b. Close-up view of a portion of the area shown in Figure 1a, focusing on the NTTR airfield and the Main Lake to the northeast, with locations of samples collected by DRI in February 2008.

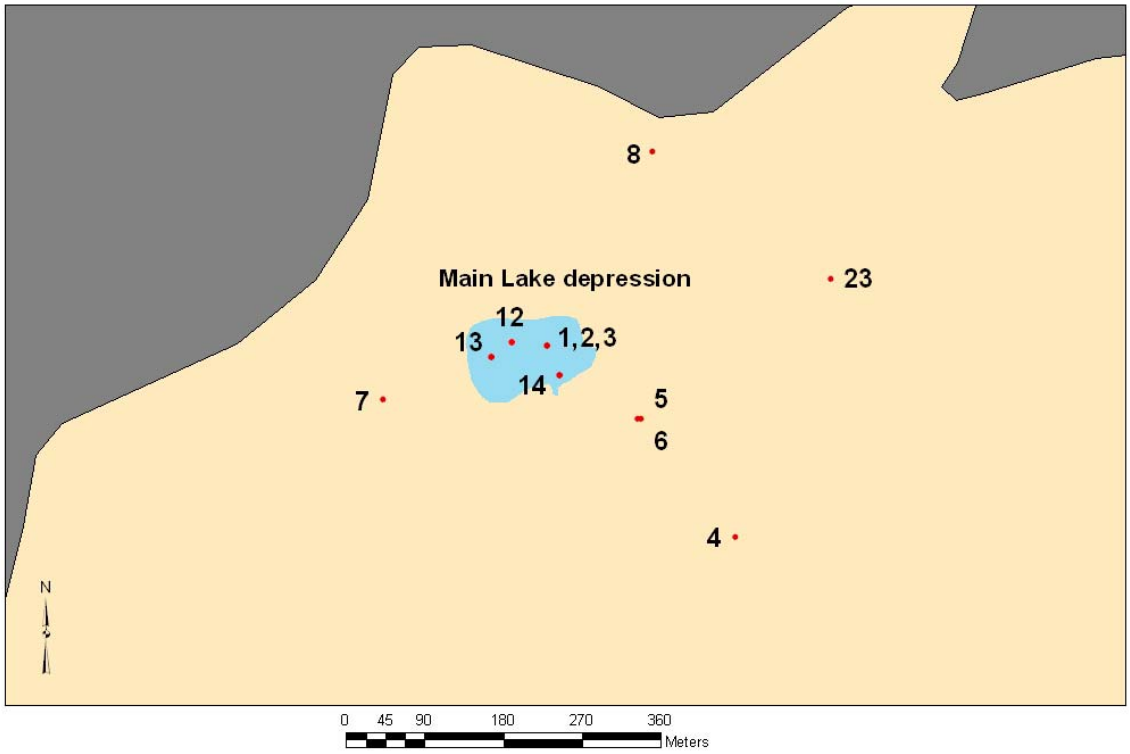

Figure 1c. Close-up view of a portion of the area shown in Figure 1b, focusing on the Main Lake depression and immediate vicinity, with locations of samples collected in and around the Main Lake depression collected by DRI in February 2008.

Water Samples

Main Lake depression water samples were analyzed for major-ion chemistry, trace element content, isotopic composition of dissolved nitrogen and oxygen in nitrate, glycols, petroleum hydrocarbons, and screened for semi-volatile organic compounds that are additives of glycol-based deicers. Spring water samples were analyzed for major-ion chemistry, trace-element content, and the isotopic composition of dissolved nitrogen and oxygen in nitrate.

Samples for major-ion chemistry analysis were collected in two 500-mL poly bottles. The water placed in one of the two bottles was filtered through a 0.45-µm polyethersulfone (PES) filter and then acidified with 10 drops of reagent-grade nitric acid. The water in the second bottle was unfiltered and unacidified (a portion of this sample was also used for trace-element analysis, as described in the following paragraph). In the field, samples were stored on ice in insulated coolers to maintain a temperature as close to 4 °C as possible. After transport to Reno, samples were stored in a refrigerator until transferred to the DRI Analytical Chemistry Laboratory for analysis.

Samples for trace element analysis were collected in pre-cleaned, acid-washed, 500 mL poly bottles after being filtered through a pre-cleaned 0.45-µm PES filter. Each sample had 5 mL of Seastar Baseline trace-metal-grade nitric acid added after collection. In the field, samples were stored in insulated coolers to maintain a temperature as close to 4 °C as possible. After transport to Reno, samples were stored in a refrigerator until transferred to the

DRI Ultra-Trace Chemistry Laboratory for analysis. Water samples from the Main Lake depression contained significant amounts of fine (<0.45 μm) sediment. Because addition of acid (standard metals sample preservation technique) to samples in the field could dissolve some of the suspended material, or release metals adsorbed on the suspended material into solution, aliquots of unfiltered, unacidified water were filtered through 0.1-μm polycarbonate membranes in the laboratory prior to acidification. The trace-element concentrations measured in the laboratory filtered samples are more representative of actual dissolved trace-element concentrations; the concentrations measured in the field-filtered and acidified samples are more representative of the dissolved trace-element concentrations that might result from raw, unfiltered Main Lake depression water encountering the low-pH environment of a horse's stomach (see Merritt, 2003).

Samples for nitrogen isotope analysis were collected in 7.6-L poly containers to obtain sufficient nitrogen (N) to allow isotopic analysis. Most water was filtered through a 0.1-μm cartridge filter prior to collection; the two Main Lake depression water samples contained too much fine sediment for field filtration, so they were collected after filtration through a 0.45-μm cartridge filter. In the field, samples were stored in insulated coolers to maintain a temperature as close to 4 °C as possible. After transport to Reno, samples were stored in a refrigerator until transferred to the Purdue Stable Isotope (PSI) facility at Purdue University for analysis. Samples were conveyed to PSI in insulated coolers packed with ice, and shipped via overnight delivery service. The PSI facility was notified of the fact that the two Main Lake depression water samples had not been filtered through 0.1-μm filters, and the decision was made for PSI to perform the filtration in the laboratory as part of their sample processing.

Water samples from the Main Lake depression were analyzed for organic compounds; spring waters were not sampled for these compounds. Samples for semi-volatile organic screening were collected in 1-L amber glass bottles. The remaining samples for organics analysis were collected in 40-mL volatile organic analysis (VOA) vials. Samples for total petroleum hydrocarbons extractable (TPH-E) and total petroleum hydrocarbons purgeable (TPH-P) were collected in individual VOA vials that had been pre-filled with hydrochloric acid. Vials were filled so as to eliminate headspace without overfilling (which could have caused some of the preservative acid to be lost). Samples for glycol analysis were collected in a VOA vial with no acidification. In the field, samples were stored in insulated coolers to maintain a temperature as close to 4 °C as possible. After transport to Reno, samples were stored in a refrigerator until transferred to Alpha Analytical in Reno for analysis (glycol analyses were performed by Zalco Laboratories in Bakersfield, CA, under subcontract to Alpha Analytical; all other organics analyses were performed in-house at Alpha Analytical).

Sediment Samples

Collection

Most samples of sediment were analyzed for major-ion chemistry, trace element content, the isotopic composition of dissolved nitrogen compounds, glycols, petroleum hydrocarbons, and screened for semi-volatile organic compounds.

Two 0.95-L glass jars were filled with sediment for major-ion chemistry analysis. In the field, samples were stored in insulated coolers to maintain a temperature as close to 4 °C

as possible. After transport to Reno, samples were stored in a refrigerator until transfer to the DRI Soils Laboratory for preparation of sediment extracts.

For trace-element analysis, approximately 1 L of sediment was placed in plastic bags; sampling was conducted to avoid contamination from metal implements. The Main Lake depression sediment samples were collected in a PVC sampler. In all other cases, the upper ground surface was frozen solid, so a rotary hammer was used to break up the frozen crust (approximately 15 cm thick). Once the upper layer was broken apart, the exposed material was soft, and plastic implements were used to scrape away several inches of the surface material in an effort to remove any sediment that might have been in contact with the metal of the rotary hammer. In the field, samples were stored in insulated coolers to maintain a temperature as close to 4 °C as possible. After transport to Reno, samples were stored in a refrigerator until transfer to the DRI Ultra-Trace Chemistry Laboratory for preparation of sediment extracts.

For nitrogen isotope analysis, four 1-L glass jars were filled with sediment. In the field, samples were stored in insulated coolers to maintain a temperature as close to 4 °C as possible. After transport to Reno, samples were stored in a refrigerator until transferred to the PSI facility at Purdue University for analysis. Samples were conveyed to PSI in insulated coolers packed with ice, and shipped via overnight delivery service.

For organics analyses, two sediment samples were collected in glass jars. Sediment from one jar (collection volume 0.24 L) was used for the TPH-E and TPH-P analyses, as well as the semi-volatile screening. Sediment from the second jar (collection volume 0.12 L) was used for glycol analysis. In the field, samples were stored in insulated coolers to maintain a temperature as close to 4 °C as possible. After transport to Reno, samples were stored in a refrigerator until transferred to Alpha Analytical in Reno for analysis (glycol analyses were performed by Zalco Laboratories in Bakersfield, CA, under subcontract to Alpha Analytical; all other organics analyses were performed in-house at Alpha Analytical).

Processing

Analysis of major-ion chemistry and trace elements were performed on sediment extracts prepared at DRI; all other sample processing was carried out by the laboratory to which the samples were submitted. All sediment extracts were made using a 1:10 sediment:liquid ratio by weight.

For major-ion analyses, two types of extracts were prepared; one extract was prepared using deionized (DI) water, the other using a 0.5 M KCl solution. The DI water extract was used for the determination of all values except NH_4^+, $O\text{-}PO_4$, and total dissolved P, which were determined from the KCl extract. These analyses were conducted by the DRI Analytical Chemistry Laboratory after preparation of the extracts. Sediment was passed through a 2-mm sieve to integrate the sample; approximately 4 g of the sieved sediment was collected and placed in a poly centrifuge tube. Forty milliliters of liquid (either deionized water or 0.5 M KCl solution, as appropriate) were added to the tube, at which point the tube was capped and placed flat on a shaker table and agitated for 15 hr. Samples were centrifuged for 30 min at 3,500 rpm, and then filtered through a 0.45-μm filter. Low-nitrogen filters were used for samples destined for nitrogen analysis. The filtrate was transferred in a poly bottle to the DRI Analytical Chemistry Laboratory for analysis.

For trace element analyses, extracts were prepared using deionized (DI) water. All extract preparation was performed wearing gloves and using nonmetallic laboratory equipment. Approximately 4 g of sediment were removed from each sample container. This material was placed in a pre-cleaned, acid-washed, poly centrifuge tube. Forty milliliters of ultra-pure DI water were added to the tube, at which point the tube was capped and placed flat on a shaker table and agitated for 15 hr. Samples were centrifuged for 10 min at 2,500 rpm and then filtered through a pre-cleaned 0.45-μm filter into a pre-cleaned, acid-washed, poly centrifuge tube. Samples that were cloudy after the 0.45 μm filtration were filtered through a pre-cleaned 0.1-μm filter. After filtration, 400-μL of Seastar Baseline trace-metal grade nitric acid were added.

A set of additional extracts were made using samples of sediment from the Main Lake depression. For these additional samples, 400 μL of Seastar Baseline trace-metal-grade nitric acid were added to the sediment/DI water mixture prior to shaking (aside from the addition of the acid, all sample handling procedures were identical to those for nonacidified samples). The acidified extracts were prepared because pH has a significant impact on metal solubility and mobility. As a result, metal uptake from the water in the low-pH horse stomach could differ from that predicted using a DI water sediment extract. The acidified extracts were prepared to mimic the most acidic conditions that might be present in a horse stomach (see Merritt, 2003); however, it is not known that wild horses ever consumed sediment directly from the bed of the Main Lake depression. Samples were then transferred to the DRI Ultra-Trace Chemistry Laboratory for analysis.

Analytical Results

Differences in Main Lake Depression Dissolved Solids between July 2007 and February 2008

Dissolved solids concentrations in the Main Lake depression were much lower in the February 2008 samples (total dissolved solids [TDS] <1,000 mg/L based on the summation of individual major ion concentrations; see Table 2A of Appendix 3) than that observed in summer 2007 (TDS ≅ 31,000 mg/L, see Appendix 2). The high TDS concentrations observed in summer 2007 were likely caused by evaporative concentration during the spring and summer months; the February 2008 samples were collected at a time when evaporation was low and the Main Lake depression had received dilute inflow of rainwater, greatly increasing the volume of water in the Main Lake depression (the water depth in February 2008 was approximately 2 m, compared to approximately 0.3 m in summer 2007) (Figure 2a and b).

Evaporation concentrates solutes in the residual water by removing pure water while leaving behind dissolved solutes. However, as water evaporates and becomes more concentrated, chemical reactions, such as precipitation of minerals, occur. Because chloride is one of the last dissolved ions to precipitate from solution, it is very rarely lost from solution, so if chloride concentrations of a water at two stages of evaporation are known, the change in the chloride concentration can be used to estimate the amount of a solution that has evaporated. Using the mean concentration of chloride from the two depths sampled in February 2008 (55.6 mg/L) and the concentration reported for July 2007 (2,100 mg/L; see Appendix 2), an evaporative concentrations factor of approximately 38 was determined (i.e., the water in July 2007 was 38 times more concentrated than the water in February 2008).

Figure 2a. Photograph of Main Lake depression taken in summer 2007. Note the small volume of water in the depression, and manure visible in the foreground. The fencing visible in the photograph was erected after the summer 2007 wild horse deaths.

Figure 2b. Photograph of Main Lake depression taken in February 2008. Note the increase in water volume compared to the volume present in summer 2007 (Figure 2a).

Not all dissolved constituents measured in the February 2008 samples were measured in the July 2007 sample (e.g., bicarbonate, aluminum), so evaluating evaporative concentration for all ion concentrations between the two sets of samples cannot be conducted. However, concentrations of several major-ions can be evaluated. In some cases (e.g., sulfate, magnesium), applying the chloride-based evaporation factor to the February 2008 Main Lake depression data shows good matches to observed ion concentrations for the July 2007 samples. In other cases, applying the 38 times evaporation factor to the February 2008 sample produces concentrations higher than measured in the July 2007 sample (e.g., the sodium and potassium). However, as the Main Lake depression water is evaporated, minerals that incorporate sodium and potassium into their structures (e.g., clay minerals or alkali carbonates) are expected to form, removing sodium and potassium from solution.

In contrast, nitrate concentrations observed in the July 2007 samples were much higher than those calculated by applying the 38 times evaporation factor. As discussed below in the Nitrogen Isotope Values section, the higher-than-predicted concentrations of nitrogen appear to have resulted from nitrification (natural production of nitrogen) increasing nitrogen concentrations in addition to the evaporative concentration.

Organic Chemicals

Data for organic chemicals are given in Appendix 3. There were no positive results for glycols (components of currently used aircraft deicing agents), although many glycols undergo relatively rapid natural biodegradation. Laboratory half-lives for glycols are from one to 12 days in aerobic water and 0.2 to four days in soils (U.S. Environmental Protection Agency, 2000). Because of these very fast biodegradation rates for glycols and the large time period between the wild horses dying (July 2007) and collection of Main Lake depression samples for glycol analysis (February 2008), the lack of positive results for glycol analyses of Main Lake depression water, Main Lake depression sediment, and nearby sediments cannot definitively affirm that glycol-based deicing compounds were not the cause of the wild horse deaths. There were also no semivolatile organic compounds identified, and gasoline-range hydrocarbons were not identified in any of the samples.

Six drainage sediment samples tested positive for low concentrations of oil-range organic chemicals (15 to 90 mg/kg), and one of the samples also contained low

concentrations of diesel-range organic chemicals (13 mg/kg). These samples were collected in natural drainages between the airfield and the Main Lake depression. Five of the six samples containing detectable oil- or diesel-range organics were collected near roads for ease of access, but were collected a minimum of 5 m from the road in the upslope direction to minimize possible influence of direct runoff from the roads. It is likely that all six of the detected occurrences of organic chemicals are the result of small drips from vehicles driving on the NTTR road network and the airfield/industrial area (e.g., Lopes and Dionne, 1998; Bris *et al.*, 1999; Lau and Stenstrom, 2005). Neither the Main Lake depression water nor the Main Lake depression sediment tested positive for oil-range or diesel-range organic chemicals.

Inorganic Chemicals

As mentioned previously, the samples collected from the Main Lake depression in February 2008 are relatively dilute and do not now appear to contain dissolved concentrations of any individual compound sufficient to be acutely toxic to horses (results are given in Table 2 of Appendix 3). Although February 2008 arsenic concentrations in the Main Lake depression water (25.4 and 24.6 μg/L) are above the drinking-water standard for humans of 10 μg/L, they are below the recommended level for livestock of 200 μg/L (Soltanpour and Raley 1993).

The February 2008 nitrate (as N) concentrations in the Main Lake depression water samples (6.4 and 11.8 mg/L) are moderately high for natural waters, but lower than many of the observed nitrate concentrations in surrounding sediments. Only one sediment sample had a nitrate concentration above those listed by Leatham *et al*. (1983) for Nevada playas: sample 7 had a concentration of 1,927 mg/kg (as N). With the exception of sample 7, sediment nitrate concentrations ranged from below detection (sample 21) to 355 mg/kg (as N; sample 8). While the observed concentration range is large, it is not unusual to observe significant spatial variation in soil nitrate, even when samples are collected at the same depth. For instance, Leatham *et al*. (1983) observed a soil nitrate range equivalent to approximately 950 mg/kg (as N) in four samples from Frenchman Flat, NV, and a range of approximately 550 mg/kg in three samples from Yucca Lake, NV. Spatial variation in soil nitrate concentrations could result from spatial variation in nitrate deposition and water infiltration. It is also worth noting that the range in nitrate values observed in these samples is not anomalous in the context of other soil constituents. For example, chloride and nitrate concentrations in the sediment samples are well correlated, with a coefficient of determination (r^2) of 0.99, and r^2 values relating nitrate to magnesium and calcium are 0.92 and 0.89 (respectively); sample 7, with the highest observed nitrate concentration, also has a chloride concentration over four times higher than the next-highest value. This suggests that the observed range in nitrate concentrations is not an artifact of poor sampling or analysis, but rather a representation of the naturally occurring spatial variation of soil chemistry in a playa setting. As discussed previously, some sediment samples were processed and analyzed twice to give an indication of the variability present within each sample that was collected (approximately 1 kg of sediment was collected at each site, and approximately 4 g of this sample was used for the analysis). The results of the dual aliquots are shown in Appendix 3,

along with percent differences using the formula: percent difference $= \frac{|(a-b)|}{\frac{(a+b)}{2}} \cdot 100$, where *a* and *b* are the values for an analyte determined from the two aliquots of the same sediment sample. The average inter-aliquot difference for major ions was about 30 percent, and the average inter-aliquot difference for trace elements was about 29 percent. While there may be some laboratory error in each of the two aliquot analyses, most of the difference between aliquots is likely caused by intra-sample variability.

An issue complicating assessment of possible toxicity to wild horses on the NTTR is that the Main Lake depression water contains significant amounts of suspended solids. Even after field filtration through a 0.45-µm filter, the Main Lake depression water samples contained enough suspended sediment that they were opaque. Because metals tend to have positive charges and sediment particles tend to have negatively charged surfaces, under typical conditions for natural waters, many metals tend to adsorb strongly onto sediment particles (e.g., Leybourne, 2001). However, at low pH (as could be encountered in a horse stomach), the solubility of metals is greatly increased. As a result, introducing water with relatively low dissolved metals content, but with high suspended sediment content, into the acidic environment of the stomach could lead to an in-stomach solution with greatly elevated dissolved metals levels. Main Lake depression water subjected to acidification while they contained significant suspended material (after field filtration through a 0.45-µm filter) had aluminum concentrations of 21.7 and 28.4 mg/L (see Table 4A of Appendix 3), above the recommended level for livestock of 5.0 mg/L (Soltanpour and Raley, 1993), but no assessment has been made as to whether or not these levels would be acutely toxic; the necropsy of affected wild horses involved determination of several trace-element concentrations in kidney and liver tissue as well as serum, but aluminum was not one of the analytes. Aliquots of sediment samples from the Main Lake depression were leached with an acidic solution in addition to the standard DI leaching solution; the acidic leachates had significantly higher concentrations of many trace elements. However, as described previously, it is not known if the wild horses ever consumed sediment directly from the bottom of the Main Lake depression, and the necropsy results attributed the wild horse deaths to nitrogen poisoning rather than metal toxicity (CAHFS, 2007a).

Isotopes of Nitrogen and Oxygen in Nitrate

Stable isotopes are powerful tracers of the sources and sinks of biogeochemically important compounds. Nitrate, an important compound in the nitrogen cycle, contains nitrogen and oxygen. The variations in the isotopic composition of both its nitrogen and oxygen impart important information about how nitrate is formed and removed from biogeochemical systems such as soil, groundwater, and surface water. Stable isotopic variations are reported in delta (δ) notation, which gives the difference between the abundance ratio (the ratio of a given isotope to the most common isotope of that element, e.g., $^{15}N/^{14}N$) of an isotope in a sample relative to the ratio in an accepted standard (the standard for nitrogen is air N_2; the standard for oxygen is Vienna Standard Mean Ocean Water [VSMOW]). For example, $\delta^{15}N$ is defined as

$$([(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{standard}]/ (^{15}N/^{14}N)_{standard}) * 1{,}000 \qquad (1)$$

The difference between the isotopic content of a sample and the standard is reported in per mil (‰). The nitrogen standard, atmospheric N_2, has, by definition, a $\delta^{15}N = 0‰$; natural compounds typically have $\delta^{15}N$ values between -20‰ and +40‰, but in extreme circumstances, values can be greater than +100‰.

Nitrogen isotopes in nitrate

Nitrification is the oxidation of reduced nitrogen compounds by bacteria. Nitrate $\delta^{15}N$ variations are often interpreted as isotopic signatures of different sources of nitrogen that have been nitrified (Kendall, 1998). Many synthetic nitrogen compounds have $\delta^{15}N$ close to zero because they are synthesized by H_2 reduction of atmospheric N_2 ($\delta^{15}N = 0‰$) at high temperature facilitated by catalysts (Haber-Bosch process), with negligible isotopic change during the production process (Bateman and Kelly, 2007). For example, a commercially available urea, similar to the type used in deicers, analyzed for this study, had a $\delta^{15}N$ value of -1.0 ± 0.23‰ (n = 9) as determined using the standard TC/EA IRMS (thermal conversion, elemental analysis isotope ratio mass spectrometry) technique (Chang *et al*., 2004). Like urea, explosives and fertilizers are synthesized from air using the Haber-Bosch process and have $\delta^{15}N$ values near zero (Figure 3). The nitrogen in most explosives and post-detonation residues has negative $\delta^{15}N$ values while TNT has a slightly positive value (+5‰; McGuire *et al*., 1993; Pennington *et al*., 1999). Conversely, natural organic wastes are usually enriched in ^{15}N because metabolic processes preferentially use the ^{14}N isotope and compounds containing the heavier isotope are excreted. Manure nitrates typically have values between +10 to +25‰ (Högberg, 1997; Dijkstra *et al*., 2006) (see Figure 3).

Nitrates from the NTTR samples were all highly enriched in ^{15}N, that is, positive $\delta^{15}N$ values (see Appendix 3 and Figure 3). Nitrate extracted from sediments had $\delta^{15}N$ values of +18 ± 2‰, whereas spring waters had slightly lower $\delta^{15}N$ of +7.6‰ and +9.6‰, and the Main Lake depression water samples had nitrate $\delta^{15}N$ values that were highly enriched at +27‰ and +39‰. The elevated $\delta^{15}N$ values of the NTTR sediments are higher than those in typical desert soil nitrate, but similar $\delta^{15}N$ values have been measured for nitrate found in clay-rich soils in the Mojave Desert near the Barstow syncline (Böhlke *et al*., 1997a). The spring water nitrate $\delta^{15}N$ values are similar to natural Mojave ground water ($\delta^{15}N$ +6 to +11‰) (Böhlke *et al*., 1997b). Therefore, while the sediment and spring water $\delta^{15}N$ values are within the range of natural desert nitrates in the southwest, the Main Lake depression nitrate $\delta^{15}N$ values are atypical.

The high ^{15}N enrichment of the Main Lake depression nitrate relative to sediment and springs has three possible explanations. First, it could be the result of isotopic enrichment from denitrification, which leads to increases in $\delta^{15}N$ values as a function of nitrate loss. The second possible explanation is a source of new nitrogen whose isotopic composition is similar to the high $\delta^{15}N$ values observed in the Main Lake depression nitrates. The third explanation is preferential loss of ^{14}N during volatilization of ammonia (NH_3/NH_4^+) generated during the decomposition of organic matter or urea, which increases $\delta^{15}N$ values in the residual ammonium ion, and then the isotopically enriched residual ammonium ion undergoing nitrification.

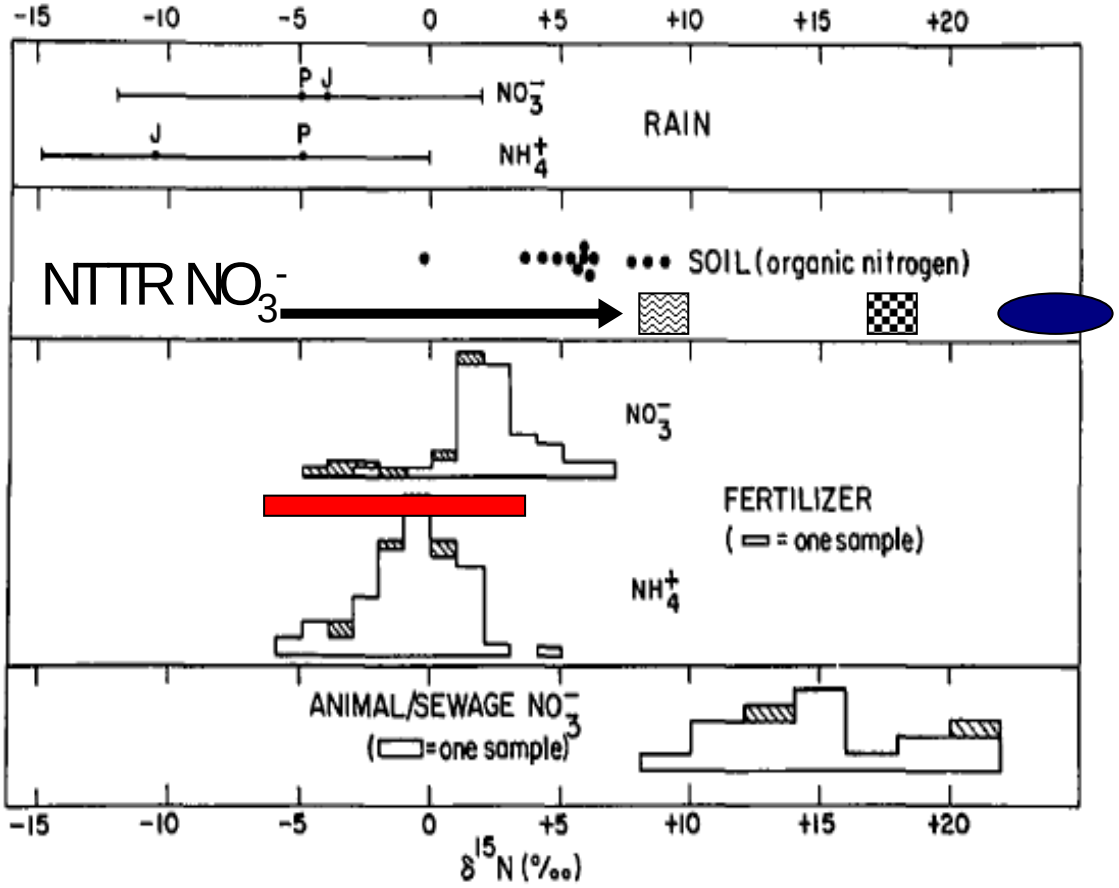

Figure 3. The $\delta^{15}N$ values of various nitrogen compounds as adapted from Heaton *et al*. (1986). The red bar shows the range of $\delta^{15}N$ for urea, explosives, and explosives residues. The NTTR nitrate samples include spring water (wave-filled rectangle), sediments (checker-filled rectangle), and Main Lake depression samples (blue oval).

The first scenario is unlikely since denitrification decreases nitrate concentrations, so any $\delta^{15}N$ enrichment would also be accompanied by lower nitrate concentrations, which is at odds with the high observed nitrate concentrations in the Main Lake depression water. Additionally, the $\delta^{18}O$ of nitrate oxygen increases by roughly twice that observed for $\delta^{15}N$ enrichment during typical terrestrial denitrification. This enrichment in $\delta^{18}O$ nitrate oxygen was not observed in the Main Lake depression nitrate (see detailed explanation in the 'Oxygen isotopes of nitrate' section below).

The second explanation for the high $\delta^{15}N$ values of the Main Lake depression nitrate is that it is derived from an isotopically enriched nitrogen source within the Main Lake depression, but not found in the surrounding sediments and springs. Urea from deicers, or other synthetic nitrogen compounds are unlikely because the $\delta^{15}N$ value of synthetic nitrogen compounds is about 0‰, which, when undergoing denitrification, would produce $\delta^{15}N$ values of 0‰ or less. During nitrification, bacteria preferentially use the ^{14}N isotope, so production of nitrate by nitrification in nutrient-rich waters (high urea and/or ammonium ion) would yield $\delta^{15}N$ values up to 35‰ lower than those of the initial ammonium ion source (Mariotti *et*

al., 1981); however, this effect diminishes when nutrient levels are low (e.g., Feigin *et al*., 1974; Shearer *et al*., 1978). Therefore, it does not appear that either denitrification of, or complete or partial nitrification of synthetic nitrogen compounds, including synthetic urea, were major contributors to nitrogen in the Main Lake depression water.

Similar mass-balance arguments also rule out any significant contribution of nitrate from nitrification of explosive residues or fertilizers. The biogeochemical breakdown of compounds such as TNT can change nitrogen isotopic composition (i.e., the residual product will have a $\delta^{15}N$ value different from the initial compound). For example, during the breakdown of TNT in soils, nitro moities (N-bearing molecule fragments) in TNT are reduced to amines, which are then bound to the soil. These amines have lower $\delta^{15}N$ values relative to that of the original TNT, leaving the residual nitro compounds with a higher $\delta^{15}N$ value than the unaltered TNT (Pennington *et al*., 1999). If TNT was the original source of nitrogen in the Main Lake depression, TNT by-product amines would be the source of nitrogen for nitrification and the $\delta^{15}N$ value of nitrate in the Main Lake depression water would have a $\delta^{15}N$ value close to 0‰. One scenario where TNT could generate nitrate with the high $\delta^{15}N$ values observed in the Main Lake depression water is bacterial oxidation of TNT by-products with high $\delta^{15}N$ values (Pennington *et al*., 1999). This would require, however, preferential oxidation of nitro compounds over ammonium, which seems unlikely given that ammonium oxidation is the most common nitrification pathway, and that the most common reaction of the nitro group in biological systems is reduction. In addition, none of the organic chemical screens performed for this study showed any other chemicals typical of decomposed explosives. Because fertilizers, including ammonium sulfates, ammonium nitrates, urea, and liquid ammonia also have nitrogen isotopic compositions that cluster around 0‰ (Heaton, 1986; Bateman and Kelly, 2007), they are also unlikely to have contributed any significant nitrate to the Main Lake depression water.

In contrast to synthetic nitrogen compounds, manure nitrates typically have $\delta^{15}N$ values of +10 to +25‰ (Figure 3; Högberg, 1997; Dijkstra *et al*., 2006), closer to those observed in the Main Lake depression nitrate. The $\delta^{15}N$ data suggest that the most probable explanation for the high $\delta^{15}N$ in the Main Lake depression nitrate is nitrification of manure. The Main Lake depression nitrate collected at 0.3 m depth had a $\delta^{15}N$ value of +27‰. Nitrate from deeper in the Main Lake depression (2 m) was further enriched with a $\delta^{15}N$ value of +39‰. The simultaneous enrichments of $\delta^{15}N$ and $\delta^{18}O$ (see below) in nitrate found in the deeper water suggest denitrification is important there because this process is known to isotopically enrich the residual nitrate. This is further supported by the decrease in nitrate concentration observed at 2-m depth since denitrification is a nitrate-loss process.

Volatilization of NH_3/NH_4^+ (either natural or synthetic), the third possible explanation, cannot be completely ruled out. At high pH, the chemical reaction: $NH_{3(g)} + H_2O \leftrightarrow NH_4^+ + OH^-$ proceeds to the left producing $NH_{3(g)}$. During this process, the $\delta^{15}N$ value of the remaining NH_4^+ becomes isotopically heavier (increases in value) because $^{14}NH_3$ (the lighter isotopic molecule) is preferentially lost from the system. The residual NH_4^+ with high $\delta^{15}N$ values can subsequently be nitrified, resulting in nitrate with elevated $\delta^{15}N$ values. A standard Rayleigh model (Lord Rayleigh, 1902) can be used to estimate the degree of NH_3 loss required to match the Main Lake depression nitrate $\delta^{15}N$ data (Criss, 1999)

$$\delta^{15}N = \delta^{15}N_o + \varepsilon \cdot \ln(f) \qquad (2)$$

where $\delta^{15}N_o$ is the initial NH_3/NH_4^+ $\delta^{15}N$ value, $\delta^{15}N$ is the $\delta^{15}N$ value observed in the Main Lake depression water nitrate, ε is the enrichment factor, which for ammonia volatilization is about -20‰ (Högberg, 1997), and f is the fraction of the original NH_3/NH_4^+ remaining (e.g., when 10 percent has been volatilized, the fraction remaining is 0.90). The question then becomes what $\delta^{15}N_o$ value to use as the initial source of NH_3/NH_4^+. In the present case, if it is assumed that the NH_3/NH_4^+ originated from synthetic urea ($\delta^{15}N_o$ = -1‰), then approximately 85 percent of the initial synthetic urea must have been decomposed and volatilized, followed by complete nitrification of the remainder. If it is assumed that manure was the nitrogen source (for this example, $\delta^{15}N$ = -20‰), then only 10 to 50 percent volatilization would be required to generate the nitrate $\delta^{15}N$ values observed in the Main Lake depression. Such a high degree of loss in the case of synthetic urea NH_3/NH_4^+ would be unlikely at the pH of the Main Lake depression, and the magnitude of the loss would also result in much less available nitrogen for nitrate production. The enrichment of manure nitrogen by volatilization followed by nitrification is the most reasonable explanation of the $\delta^{15}N$ data, and is also supported by oxygen isotopic values of the NTTR nitrate (discussed below).

Oxygen isotopes in nitrate

Oxygen isotopes in nitrate can provide additional insight on the source and sink processes of nitrogen (Amberger and Schmidt, 1987; Aravena *et al*., 1993; Durka and Voerkelius, 1996; Kendall, 1998). The $\delta^{18}O$ of soil nitrate is described by a mass-balance equation containing the $\delta^{18}O$ values of the two main nitrate inputs: atmospheric deposition ($NO_3^-{}_{atm}$) and nitrification by microbes ($NO_3^-{}_{bio}$)

$$\delta^{18}O\ NO_3^- = x\ \delta^{18}O\ NO_3^-{}_{atm} + (1-x)\ \delta^{18}O\ NO_3^-{}_{bio} \qquad (3)$$

where x is the mole fraction of $NO_3^-{}_{atm}$ and 1-x is the mole fraction of $NO_3^-{}_{bio}$. $NO_3^-{}_{bio}$ (nitrate produced by nitrification) is from oxidation of ammonium ion, where the ammonium ion can be derived from either a natural source (e.g., manure or plant and bacterial biomass) or human sources including artificial fertilizers and salts containing nitrogen (e.g., ammonium or urea).

Equation (3) contains three unknowns (x and two $\delta^{18}O$ values). The two $\delta^{18}O$ unknowns can be expanded and their values estimated. For $NO_3^-{}_{bio}$, two of the three oxygen atoms acquired by nitrogen during nitrification are derived from ambient water and the remaining oxygen atom from gaseous oxygen (O_2), so the mass-balance equation for determining the $NO_3^-{}_{bio}$ $\delta^{18}O$ is

$$\delta^{18}O\ NO_3^-{}_{bio} = 2/3\ \delta^{18}O\ H_2O + 1/3\ \delta^{18}O\ O_2 + \varepsilon \qquad (4)$$

where $\delta^{18}O$ of O_2 is assumed constant (+23‰) (Kroopnick and Craig, 1972), and ε, the enrichment factor occurring during nitrification, is often taken as zero (or self-cancelling when comparing nitrification samples from the same region).Water $\delta^{18}O$ values are highly variable in time and space because $\delta^{18}O$ values in precipitation vary depending on season, temperature, and origin points of individual storms (e.g., Pacific northwest versus Gulf of Mexico) (Bowen and Wilkinson, 2002). In addition, subsequent evaporation of rainfall from soils, streams, or lakes alters the $\delta^{18}O$ values that were present in the precipitation at the time it fell; these effects are amplified in low humidity desert regions (Craig *et al*., 1963; Criss,

1999). Estimating the $\delta^{18}O$ of the water presents a challenge because it requires knowing what water is used during the nitrification process. If nitrification is a continual process, limited only by water and ammonium ion availability, then nitrification can utilize waters across seasonal and evaporative cycles, thus incorporating waters with a range of $\delta^{18}O$ values into the bulk $NO_3^-{}_{bio}$.

For $NO_3^-{}_{atm}$, the most reliable $\delta^{18}O$ data suggest a range of values between +60 and +80‰, with the yearly average approximately +70‰ (Durka *et al*., 1994; Kendall, 1998; Burns and Kendall, 2002; Michalski *et al*., 2003). Current understanding of $NO_3^-{}_{atm}$ formation suggests that its $\delta^{18}O$ value is set by the mass balance between oxygen sources that are incorporated in $NO_3^-{}_{atm}$ during photochemical oxidation of NO_x ($NO_3^-{}_{atm}$ precursors). These oxygen sources are primarily O_3 and tropospheric water vapor (Michalski *et al*., 2003). The proportion of water vapor oxygen assimilated into $NO_3^-{}_{atm}$ changes over the course of a season, and the $\delta^{18}O$ value of the water vapor itself varies depending on the regional precipitation $\delta^{18}O$, local evapotranspiration, and temperature. Thus, the high variability in water $\delta^{18}O$ values over the course of a year introduces a high degree of uncertainty into the mass-balance equation (3).

It is possible to reduce this uncertainty by using the ^{17}O excess ($\delta^{17}O$), which is quantified by

$$\delta^{17}O = \delta^{17}O - 0.52\delta^{18}O \qquad (5)$$

For most physical processes, $\delta^{17}O \cong 0.52\ \delta^{18}O$ (Miller *et al*., 2002). Thus, for processes such as nitrification, evaporation, and condensation (precipitation), the $\delta^{17}O$ value is zero. Positive $\delta^{17}O$ values arise from other physical processes, primarily during ozone (O_3) formation or reactions involving ozone (Savarino *et al*., 2000; Thiemens *et al*., 2001; Michalski *et al*., 2003). Since $NO_3^-{}_{atm}$ obtains oxygen atoms from ozone during its formation, positive $\delta^{17}O$ values observed in $NO_3^-{}_{atm}$ originate from this mass-balance transfer (Michalski *et al*., 2003). Contributions of ozone to $NO_3^-{}_{atm}$ formation are less variable and are independent of changing $\delta^{18}O$ of tropospheric water vapors. These considerations allow one to formulate a second nitrate mass-balance equation similar to (3) but in terms of $\delta^{17}O$

$$\delta^{17}O\ NO_3^- = x \bullet \delta^{17}O\ NO_3^-{}_{atm} + (1-x) \bullet \delta^{17}O\ NO_3^-{}_{bio} \qquad (6)$$

Since meteoric waters and O_2 used in nitrification have $\delta^{17}O$ values of approximately 0‰, the $\delta^{17}O\ NO_3^-{}_{bio}$ term is zero so x can be readily solved

$$x = \delta^{17}O\ NO_3^- / \delta^{17}O\ NO_3^-{}_{atm} \qquad (7)$$

Here, $\delta^{17}O\ NO_3^-$ is the measured value for a soil or water sample and $\delta^{17}O\ NO_3^-{}_{atm}$ is the atmospheric value, which can be derived from measurements or modeling of regional atmospheric chemistry. The range in observed $\delta^{17}O\ NO_3^-{}_{atm}$ values is +20 to +30‰, with the annual average in the southwest U.S. being approximately +23‰ (Michalski *et al*., 2003, 2004b).

Oxygen isotopic analysis was conducted on the NTTR samples using the $AgNO_3$ thermal decomposition method (described by Michalski *et al*., 2002), with a $\delta^{18}O$ precision of ± 1‰ and a $\delta^{17}O$ precision of ± 0.3‰. Five of the six nitrate $\delta^{17}O$ values from sediments surrounding the Main Lake depression were clustered around +2.3‰ (+2.3 ± 0.2‰), with the remaining sample having a value outside this range at +1.4‰. Spring waters had lower $\delta^{17}O$

values (+1.7 and +0.46‰). $\delta^{17}O$ values in nitrate from the deep and shallow Main Lake depression water samples were +1.8‰ and +1.9‰, respectively.

With these values, the fraction of nitrate in the Main Lake depression water that was derived from nitrification can be estimated using Equation (6), and the $\delta^{18}O$ of water used during nitrification can be evaluated. For example, using the deep Main Lake depression sample ($\delta^{17}O\ NO_3^-$ = +1.76‰) and the $\delta^{17}O\ NO_3^-_{atm}$ of the southwestern U.S. (+23‰) results in 8 percent of the nitrate in the Main Lake depression is unprocessed, residual $NO_3^-_{atm}$ (+1.76‰/+23‰ = 0.08 = 8 percent) and 92 percent of the nitrate is from nitrification. Then, using these mole fractions (0.08 for $NO_3^-_{atm}$ and 0.92 for $NO_3^-_{bio}$), a seasonal average $\delta^{18}O\ NO_3^-_{atm}$ of +70‰, and the observed $\delta^{18}O\ NO_3^-$ values in the deep sample of +31.4‰, the $\delta^{18}O$ of the water used during the nitrification can be determined by Equation (3) [+31.4‰ = 0.08 (+70‰) + (0.92) $\delta^{18}O\ NO_3^-_{bio}$], resulting in $\delta^{18}O\ NO_3^-_{bio}$ = +28‰. Then using $\varepsilon = 0$ and $\delta^{18}O\ O_2$ = +23‰ and Equation (4) [+28‰ = 2/3 $\delta^{18}O\ H_2O$ + 1/3 (+23‰)], results in $\delta^{18}O\ H_2O$ = +30.5‰, a very isotopically enriched value (a large value greater than zero) indicative of significant evaporation.

The measured $\delta^{18}O$ value of water collected in February 2008 from the Main Lake depression water was +1.98‰, whereas the $\delta^{18}O$ value of the spring water from the surrounding mountains was -6.92‰. Assuming the spring water $\delta^{18}O$ value is representative of local precipitation, this suggests that the water in the Main Lake depression in February 2008 has been affected by evaporation, as evaporation causes the residual water's $\delta^{18}O$ value to increase. In addition, the calculation shown above for the nitrification water $\delta^{18}O$ value of the water contributing to nitrification in the Main Lake depression in summer 2007 had a $\delta^{18}O$ value of +30.5‰, indicating that the summer 2007 nitrification must have taken place after significant evaporation had occurred (i.e., enough evaporation to raise the water $\delta^{18}O$ value from +1.98‰ to +30.5‰). Since the Main Lake depression water evaporated into low-humidity desert air, a Rayleigh distillation model (Equation 2; in this case, the "$\delta^{15}N$"s would be replaced by "$\delta^{18}O$"s to yield $\delta^{18}O = \delta^{18}O_o + \varepsilon \cdot \ln[f]$) is a good approximation of the evaporation process. Using the deep Main Lake depression water as input water $\delta^{18}O$ (+1.98‰) and a water enrichment factor of -9.21‰ at 28 °C, the Rayleigh model suggests that nitrification occurred when evaporation had reduced the volume of water in the Main Lake depression to approximately five percent of its volume in February 2008 (i.e., $+30.5 = +1.98 + (-9.21) \cdot \ln(f)$; solving for *f* yields 0.045 ≅ 5%). The shallow Main Lake depression sample, with a calculated nitrification water $\delta^{18}O$ value of +21‰, yields similar results.

The two important concepts are 1) an accurate measure of the source mole fractions of nitrate using $\delta^{17}O$ measurements can be derived, and 2) from this, the source water isotopic composition and the timing of nitrification can be determined. In a general sense, the mole ratio of the two sources depends on environmental conditions, but in desert regions, this is limited by the availability of water and sources of ammonium ion. Given a limited geographic setting, the water availability can be assumed constant so that any change in the ratio is caused by a new source of available nitrogen.

The hypothesis that dumping or runoff of urea deicers into the Main Lake depression or its vicinity affected the nitrate concentration of the Main Lake depression water can be

tested by looking for changes in the $NO_3^-_{bio}/NO_3^-_{atm}$ ratio of the water relative to that in undisturbed sediments around the Main Lake depression. The $\delta^{17}O$ data suggest that natural nitrification in sediments accounts for 90 percent of the nitrate observed in the Main Lake depression water. The slight decrease in $\delta^{17}O$ values in the Main Lake depression water nitrate indicates that slightly more nitrification occurs in this environment (92 percent), which is not surprising given that nitrification in desert regions is often water limited. However, given uncertainties in the analytical technique (0.3‰) and assumptions in the model, it would not appear that a significant amount of additional nitrification occurs in the Main Lake depression itself. If a large influx of nitrate into the Main Lake depression at the time of the wild horse deaths in 2007 was caused by the nitrification of urea deicers, one would expect to observe a dramatic increase in the natural $NO_3^-_{bio}/NO_3^-_{atm}$ ratio and a negligible $\delta^{17}O$ signal. The $\delta^{17}O$ data do not support this hypothesis.

Nitrogen isotope summary

The multiple isotopic values are powerful forensic evidence that supports the following scenario for nitrogen input into the Main Lake depression water. Animal waste (manure, urine) with high $\delta^{15}N$ values decomposed and was partially volatilized. The remaining nitrogen was leached as ammonium ion into the Main Lake depression, but at relatively low concentrations. Nitrate from sediment and atmospheric deposition was also leached into the Main Lake depression at the same time. As water evaporated in the spring and summer, the ammonium ion concentrations increased, creating conditions favorable for nitrification, thus converting the ammonium ion into nitrate as evidenced by the high observed $\delta^{18}O$ values. Based on the $\delta^{17}O$ data, these high $\delta^{18}O$ values were not the result of atmospheric inputs. Nitrate concentrations increased to unhealthy levels as evaporative concentration of the water continued.

As water inflow in the fall, winter, and spring caused the amount of water Main Lake depression to grow (in terms of mass, and thus in terms of volume and surface area), there may have been some denitrification in the wet sediments that led to additional enrichments in the $\delta^{15}N$ and $\delta^{18}O$ values of the remaining nitrate (Aravena and Robertson, 1998; Boettcher *et al*., 1990; Fukada *et al*., 2003; Sigman *et al*., 2003). This last step introduces some ambiguity. One could hypothesize that nitrification occurred using existing Main Lake depression water ($\delta^{18}O$ = +1.8‰), not the highly evaporated water, which would have resulted in a nitrate $\delta^{18}O$ of approximately +7.3‰ (i.e., 0.08×70‰ + 0.92×1.8‰). The difference between this value and the approximate 30‰ $\delta^{18}O$ average observed in the Main Lake depression would require an approximate 23‰ $\delta^{18}O$ enrichment from denitrification. Since $\delta^{15}N$ enrichments from denitrification are about half those in $\delta^{18}O$, only roughly 12‰ of the +35‰ $\delta^{15}N$ enrichment observed in the Main Lake depression nitrate could be attributed to denitrification. This would suggest the original nitrogen source must have had a $\delta^{15}N$ value of approximately +25‰ before nitrification, again suggesting a manure source.

CONCLUSIONS

Twenty-two samples were collected at the NTTR in February 2008 to help determine possible causes of the death of 71 wild horses in July 2007. Samples included seven of water and 15 of sediment. This report provides a compilation of the data and a discussion of some results of interest.

Water in the Cactus Flat Main Lake depression was significantly less saline in February 2008 than in summer 2007, likely because of low evaporation and dilution by recent precipitation. Chloride concentrations suggest that the water in the Main Lake depression in July 2007 had been concentrated approximately 38-fold by evaporation, as compared to the water present in February 2008. Although TDS concentrations were not measured on either water, summation of know dissolved solids concentrations in the two samples shows that TDS was less than 1,000 mg/L in February 2008, but over 30,000 mg/L in July 2007. One sediment sample in a drainage channel near the Main Lake depression had a higher-than-expected level of nitrate, and some drainage channel sediments also tested positive for low levels of organic chemicals associated with motor oil (one sample also had a low level of diesel-type organic chemicals). However, the levels of nitrate in the Main Lake depression water and sediments were lower than the anomalous sediment concentration, and neither the water nor the sediments from the Main Lake depression contained detectable amounts of oil or diesel-type organic compounds. No samples collected for this study contained detectable amounts of glycol deicers or typical organic deicer additives. However, because of the time elapsed between the wild horse deaths in July 2007 and sampling by DRI in February 2008, the negative results for these analytes does not definitely affirm that glycol-based deicers were not present in the Main Lake depression at the time of the wild horse deaths.

The wild horse deaths at the NTTR in 1988 and 2007 were both attributed to nitrogen compounds (ammonia for the 1988 deaths, and nitrate/nitrite for the 2007 deaths). Given the concentrations of nitrate and nitrite measured in Main Lake depression water in July 2007 and the related toxicology report (CAHFS, 2007), the high nitrate/nitrite scenario seems reasonable. While the 1988 deaths were directly attributable to deicing chemicals disposal, the cause of the high nitrate and nitrite levels that likely caused the wild horse deaths in 2007 was initially less clear.

Based on a simple model of evaporation and insight from the nitrogen isotope data, it appears that the dilute water observed in the Main Lake depression in February 2008 could be altered by purely natural processes to yield the high dissolved solids concentrations (including nitrate and nitrite) observed in July 2007. Analysis of stable isotopes of nitrogen suggests that the most likely cause of the high nitrate in the Main Lake depression in July 2007 was a combination of two natural processes: evaporative concentration of natural nitrate, and addition of nitrate via nitrification of natural materials, including animal waste and natural soil nitrogen. As shown in Figure 2a, use of the Main Lake depression by the wild horse herd results in the deposition of herd manure around the depression. The Main Lake is a low point in the topographic basin, which includes a desert playa, and the depression forms an even lower point in the basin drainage system. Figure 2b, from the February 2008 sampling, shows that winter precipitation collects in the depression, so it is easy to envision transport of the herd manure deposited around the depression into the depression as water flows into the Main Lake and depression during the wet winter months.

It appears unlikely that human influence, such as contamination from urea or glycol-based deicing fluids played a significant role in the high nitrogen concentrations. However, because of the large time difference between the wild horse deaths and the sampling conducted by DRI, anthropogenic contamination cannot be definitively discounted since glycol deicers and other organic compounds can quickly degrade with time.

Knowledge of nitrate toxicity to horses is quite limited (CAHFS, 2007). More information is available for nitrate toxicity to livestock; however, direct application of livestock toxicity values to horses is not appropriate. This is because nitrate toxicity actually results from the conversion of nitrate to nitrite in an animal's digestive system; nitrite in the blood can convert hemoglobin to methemoglobin, which does not transmit oxygen to body tissues (Martinson *et al*., 2007). Although conversion of nitrate to nitrite does occur in horse cecums, the rumens of cattle and sheep are much more efficient at this process; as a result, horses are approximately 10 times less susceptible to high nitrate concentrations than are ruminants (Martinson *et al*., 2007). The issue is further complicated by the fact that nitrite is present in the Main Lake depression water, and this is more acutely toxic. Because there is little information regarding nitrate/nitrite toxicity to horses, it is not possible to predict the exact conditions that would lead to future wild horse deaths. Nitrogen and oxygen isotopes suggest that increases in nitrogen compounds in the depression from nitrification may have occurred at the very latest stages of evaporation, although evaporative concentration of nitrogen compounds also contributed to the high nitrate and nitrite concentrations. Additionally, assuming the wild horses have used the depression over many years, it would appear that the deaths from high nitrate concentrations in the depression are a rare occurrence. Monitoring of the chemical evolution of the water chemistry in the Main Lake depression as a function of water depth, in conjunction with reliable estimates of nitrogen toxicity in horses, would produce information that could be used to develop a strategy for managing herd access to the Main Lake depression at Cactus Flat.

RECOMMENDATIONS

Given this uncertainty, it is difficult to make explicit recommendations for management options to prevent further wild horse deaths. There are several possible options:

- The depression could be filled in. This would remove the water collection point, allowing the Main Lake playa to return to more natural conditions where winter precipitation would spread out over the playa and evaporate much more quickly. Exercising this option may require a new source of water for the herd.
- Access to the depression could be controlled by fencing and the herd could be allowed to continue to use the water source most years unless drought conditions reduce the volume of water in the depression. To best exercise this option, monitoring the water chemistry and depression depths over time would be required to determine conditions when access should be limited.
- The herd could continue to use the depression unmonitored as in previous years. The depression has been in existence for about 20 years and the herd has used this resource over many years with no previous reports of wild horse deaths. However, exercising this option could result in more wild horse deaths if drought conditions were to recur.

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APPENDIX 1. Final report of the California Animal Health & Food Safety Laboratory System regarding samples collected at the NTTR in July 2007. Samples were collected by the California Animal Health & Food Safety Laboratory System on behalf of the U.S. Bureau of Land Management; collection was concurrent with the sample described in Appendix 2.

Final Report Printed: 08/15/07

(This report supersedes all previous reports for this accession)

****
* Emailed Copy. *
* A signed original is on file. *
****

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ACCESSION#:T0701789
District:
County: NEVADA
Case Coordinator: RMOELLER

Submitter
MARIAN VANDERSCHRAAF DVM
CALIF DEPT OF FOOD AND AG
18830 ROAD 112
TULARE, CA 93274

Owner:
BUREAU OF LAND MANAGEMENT
LAS VEGAS FIELD OFFICE
4707 N TORREY PINES DR
LAS VEGAS, NV 89130

Agent or Collector:
Reference Number:

Species: NONAPPLICABLE
Herd/Flock ID:
Date Taken:
Date Received: 07/25/07

9 Specimens submitted: 5 pond, 2 dirt, and water-2

Approved by: Robert Moeller, DVM

L A B O R A T O R Y F I N D I N G S / D I A G N O S I S

1. Evaluation of environmental samples from Nellis Air Force Base:
- **a. Botulism toxin testing Dirt sample: negative for Botulinum toxin**
- **b. Anatoxin A testing (Water samples 1 and 2): Not detected.**
- **c. Microcystin testing (Water samples 1 and 2): not detected**
- **d. Salt screen (Water samples 1 and 2): see report, not significant**
- **e. Salt screen (Pond sample 5-9; pond scum): See report.**
- **f. Nitate/Nitrite levels Water sample 1: 5 ppm nitrate/not detected nitrite**
- **** g. Nitrate/Nitrite levels Water sample 2: 3670 ppm nitrate/50 ppm nitrite, probable toxic levels**
- **h. Extended heavy metal screen dirt samples (#3 & 4): See report**
- **i. Extended heavy metal screen on Pond samples 5-9: See report**
- **j. Organic compound screen on Water samples: Negative**
- **k. Nitrate screen on dirt(Sample 3 & 4) and pond scum (Sample 6 and 8):**
  - **Sample 6 pond scum: 3940 ppm nitrate and 848 ppm nitrite**
  - **Sample 8 pond scum: 3440 ppm nitrate and 825 ppm nitrite**
  - **Sample 4 Wet muck from edge at pond bank interface: 498 ppm nitrates**

A C C E S S I O N S U M M A R Y

Microcystin was not detected in the water samples. The salt screens of the water appear to have levels of the various elements at levels that would not be considered toxic. The pond scum samples (sample 5-9) have more elevated levels of the various elements but it is doubtful that the horse would be drinking a large amount of these samples. I am currently performing nitrate testing on the water samples (Sample 1 and 2), these results are pending.

08/03/07
The nitrate/nitrite levels in water sample 2 are very high. These levels are a concern and may be a factor in the deaths of the horses. The first water sample is low in nitrates, it is unknown why this has happened. I feel that this sample should be similar to the composite water sample. However it is possible that the nitrate may stratify in the water column resulting in the very high levels at various levels in the water. I would recommend that several water samples be taken at various depths in the pond to see if the water is stratifying. It is possible that the horses are coming to the pond and either mixing the water column or drinking at deeper depths that other animals are not drinking at which would result in the ingestion of possible toxic levels of nitrates. Water having this high of nitrates and nitrites would not be safe to drink for humans, cattle or sheep. Unfortunately, we known little about nitrates in horses and what would be toxic to them (I did a literature search (pubmed) and could not identify any articles dealing with nitrate toxicity in horses that have been written over the past 30 years). We are performing some organic screens on the water samples to see if we can identify a possible organic compound from which the nitrates could originate from.

08/08/07
The GC/MS screen was negative for possible organic compounds in the water.

08/15/07 Final report.

The pond muck (Sample 6 and 8) had very high nitrate and nitrite levels which could contribute to nitrate/nitrite toxicity. The dirt at the pond interface samples (Samples 4) contained 498 ppm nitrate and no nitrites. It is felt that these levels of nitrate and nitrite are toxic and may have contributed significantly to the death of the horses. From the samples submitted, I cannot determine the source of nitrates. It is possible that environmental conditions were just right to cause natural nitrogen fixing bacteria to multiply and elevate the levels of nitrates and nitrites in the water. It is possible that the poor water conditions and markedly depleted water hole may have had a high organic matter overload resulting in the production of nitrates and nitrites. I cannot also preclude nitrogen sources that could be manmade or natural. Further on the ground investigation for these sources will have to be performed to exclude these as possible sources of the nitrates and nitrites in the water. If a source is identified, please let me know since nitrate toxicity cases in horses are rare. If you wish more testing on some of the other samples please contact us as soon as possible.

T O X I C O L O G Y

Anatoxin-a was not detected in the submitted water samples at or above the indicated method detection limit. The samples were also negative for the listed microcystins.

The detected mineral contents of the various environmental samples are unremarkable. None of the metals included in our extended heavy metal screen are at sufficiently high concentrations to cause concern.

The detected nitrate/nitrite concentrations in water sample #2 (composite sample) would certainly be toxic for ruminants. The lack of data related to the toxicity of nitrates and nitrites to horses makes interpretation more problematic. Given the very high ocular fluid nitrate results and the rather high concentrations in the one water sample, nitrate/nitrite intoxication is possible in this case. Please note the higher nitrite concentrations detected in the "scum" samples. The relatively high nitrite concentrations re-enforce the suspicion of nitrate/nitrite intoxication.

No toxic compounds were detected using our gas chromatography - mass spectrometry (GC/MS) organic chemical screen for the two water samples. The GC/MS screen is designed to potentially detect a large number of organic compounds belonging to diverse chemical classes (pesticides, environmental contaminants, drugs and natural products).

Please note the pH values for the two water samples.

MDL = method detection limit (lowest concentration detectable by our test method).

HEAVY METALS- EXTENDED

Specimen Type WATER

Elements	As	Ba	Be	Cd
MDL	0.1 PPM	0.01 PPM	0.002 PPM	0.03 PPM
1-WATER	< 0.1 PPM	< 0.01 PP	< 0.002 P	< 0.03 PP
2-WATER	< 0.5 PPM	1.04 PPM	< 0.01 PP	< 0.15 PP

Elements	Co	Cr	Cu	Fe
MDL	0.03 PPM	0.03 PPM	0.01 PPM	0.02 PPM
1-WATER	< 0.03 PP	< 0.03 PP	< 0.01 PP	< 0.02 PP
2-WATER	< 0.15 PP	< 0.15 PP	0.07 PPM	53.2 PPM

Elements	Hg	Mn	Mo	Ni
MDL	0.1 PPM	0.004 PPM	0.04 PPM	0.03 PPM
1-WATER	< 0.1 PPM	< 0.004 P	< 0.04 PP	< 0.03 PP
2-WATER	< 0.5 PPM	1.81 PPM	1.0 PPM	< 0.15 PP

Elements	Pb	Tl	V	Zn
MDL	0.1 PPM	0.1 PPM	0.03 PPM	0.01 PPM
1-WATER	< 0.1 PPM	< 0.1 PPM	< 0.03 PP	< 0.01 PP
2-WATER	< 0.5 PPM	< 0.5 PPM	0.50 PPM	0.2 PPM

Specimen Type DIRT

Elements	As	Ba	Be	Cd
MDL	150 PPM	.5 PPM	.1 PPM	1.5 PPM
3-DIRT	< 150 PPM	132 PPM	1.4 PPM	< 1.5 PPM
4-DIRT	< 20 PPM	29 PPM	< .1 PPM	< 1.5 PPM

Elements	Co	Cr	Cu	Fe
MDL	1.5 PPM	1.5 PPM	.5 PPM	10 PPM
3-DIRT	< 1.5 PPM	18 PPM	12.0 PPM	17500 PPM
4-DIRT	< 1.5 PPM	< 1.5 PPM	3.6 PPM	1490 PPM

Elements	Hg	Mn	Mo	Ni
MDL	5 PPM	.2 PPM	10 PPM	1.5 PPM
3-DIRT	< 5 PPM P	368 PPM	< 10 PPM	14 PPM
4-DIRT	< 5 PPM	133 PPM	< 2 PPM	< 1.5 PPM

Elements	Pb	Tl	V	Zn
MDL	60 PPM	5 PPM	1.5 PPM	.5 PPM
3-DIRT	< 60 PPM	40 PPM	30 PPM	57.3 PPM
4-DIRT	< 15 PPM	< 5 PPM	< 1.5 PPM	6.2 PPM

Specimen Type WATER-POND

Elements	As	Ba	Be	Cd
MDL	2.5 PPM	0.25 PPM	0.05 PPM	0.75 PPM
5-POND	< 2.5 PPM	15.9 PPM	< 0.05 PP	< 0.75 PP
6-POND	< 5 PPM	43.6 PPM	0.4 PPM	< 1.5 PPM
7-POND	< 2 PPM	43.8 PPM	0.61 PPM	< 0.6 PPM
8-POND	< 5 PPM	80.5 PPM	1.0 PPM	< 1.5 PPM
9-POND	< 2 PPM	68.7 PPM	0.8 PPM	< 0.6 PPM

Elements	Co	Cr	Cu	Fe
MDL	0.75 PPM	0.75 PPM	0.25 PPM	0.5 PPM
5-POND	1.4 PPM	1.6 PPM	1.9 PPM	1560 PPM
6-POND	3.6 PPM	4.0 PPM	4.1 PPM	4160 PPM
7-POND	2.9 PPM	4.4 PPM	2.3 PPM	4460 PPM
8-POND	5.5 PPM	7.8 PPM	9.0 PPM	8330 PPM
9-POND	4.4 PPM	6.7 PPM	7.8 PPM	6970 PPM

Elements	Hg	Mn	Mo	Ni
MDL	2.5 PPM	0.1 PPM	1 PPM	0.75 PPM
5-POND	< 2.5 PPM	55.4 PPM	2 PPM	3.1 PPM
6-POND	< 5 PPM	162 PPM	< 2 PPM	6.6 PPM
7-POND	< 2 PPM	173 PPM	3 PPM	6.8 PPM
8-POND	< 5 PPM	300 PPM	5 PPM	12.5 PPM
9-POND	< 2 PPM	282 PPM	3.8 PPM	9.7 PPM

Elements	Pb	Tl	V	Zn
MDL	2.5 PPM	2.5 PPM	0.75 PPM	0.25 PPM
5-POND	< 2.5 PPM	5.1 PPM	4.0 PPM	6.2 PPM
6-POND	< 5 PPM	11 PPM	11.7 PPM	14.1 PPM
7-POND	< 2 PPM	11 PPM	8.7 PPM	20.0 PPM
8-POND	< 10 PPM	19 PPM	16.0 PPM	32.7 PPM
9-POND	< 10 PPM	15 PPM	12.8 PPM	28.3 PPM

ANATOXIN-A

Specimen Information Id	Type	Result	MDL
1-WATER	WATER	Not Detected	0.01 ppm
2-WATER	WATER	Not Detected	0.01 ppm

MICROCYSTINS

WATER SPECIMEN.ID	MDL	MICROCYSTIN LR	MICROCYSTIN LA	MICROCYSTIN YR	MICROCYSTIN RR
		1 ppb	1 ppb	1 ppb	1 ppb
1-WATER		Not Detected	Not Detected	Not Detected	Not Detected
2-WATER		Not Detected	Not Detected	Not Detected	Not Detected

NITRATE SCREEN

WATER SPECIMEN.ID	MDL	Nitrate	Nitrite
			1 ppm
1-WATER		Conf. Req'd	Not Detected
2-WATER		Conf. Rq'd	Conf. Req'd

DIRT SPECIMEN.ID	MDL	Nitrate	Nitrite
			10 ppm
4-DIRT		Conf. Req'd	Not Detected

WATER-POND SPECIMEN.ID	MDL	Nitrate	Nitrite
6-POND		Conf. Req'd	Conf. Req'd
8-POND		Conf. Req'd	Conf. Req'd

NITRATE CONFIRMATION

WATER		Nitrate	Nitrite
SPECIMEN.ID	MDL	1 ppm	1 ppm
1-WATER		5 ppm	Not Detected
2-WATER		3670 ppm	50 ppm

DIRT		Nitrate
SPECIMEN.ID	MDL	100 ppm
4-DIRT		498 ppm

WATER-POND		Nitrate	Nitrite
SPECIMEN.ID	MDL	1000 ppm	500 ppm
6-POND		3940 ppm	848 ppm
8-POND		3440 ppm	825 ppm

SALT SCREEN

Specimen Type WATER

Salts	Calcium	Magnesium	Phosphorus
MDL	0.05 PPM	0.05 PPM	0.05 PPM
1-WATER	23.0 PPM	1.70 PPM	< 0.05 PP
2-WATER	80.8 PPM	52.6 PPM	4.4 PPM

Salts	Potassium	Sodium	Sulfur
MDL	0.3 PPM	4 PPM	0.07 PPM
1-WATER	6.4 PPM	47 PPM	11.7 PPM
2-WATER	153 PPM	4800 PPM	624 PPM

Specimen Type WATER-POND

Salts	Calcium	Magnesium	Phosphorus
MDL	1 PPM	1 PPM	1 PPM
5-POND	2670 PPM	1050 PPM	79 PPM
6-POND	23800 PPM	2560 PPM	249 PPM
7-POND	7570 PPM	3230 PPM	227 PPM
8-POND	15600 PPM	5380 PPM	453 PPM
9-POND	13700 PPM	4790 PPM	358 PPM

Salts	Potassium	Sodium	Sulfur
MDL	6 PPM	80 PPM	1.4 PPM
5-POND	1110 PPM	6000 PPM	645 PPM
6-POND	2530 PPM	4130 PPM	369 PPM
7-POND	3170 PPM	6150 PPM	474 PPM
8-POND	5230 PPM	7290 PPM	477 PPM
9-POND	4500 PPM	6040 PPM	385 PPM

pH

Specimen Information ID	Type	Results
1-WATER	WATER	7.57
2-WATER	WATER	8.77

ORGANIC COMPND BY REQUEST

WATER SPECIMEN.ID	MDL	GC-MS Screen
1-WATER		Negative
2-WATER		Negative

B A C T E R I O L O G Y

CLOSTRIDIUM BOTULINIUM - TOXIN TESTING (T)

Specimen Information ID	Type	Results
4-DIRT	DIRT	Negative for Botulinum toxin

C L I N I C A L H I S T O R Y

Water samples from Nellis Air Force Base where horse die off has occurred.
Sample #1 Pond water sample
Sample #2 Composite water sample (top, middle, and bottom layers)
Sample #3 Dirt from lake bed
Sample #4 Wet muck at water/bank interface
Sample #5 Water (pond) scum
Sample #6 Pond water scum
Sample #7 Pond water scum
Sample #8 Pond water scum
Sample #9 Pond water scum
Request a mineral screen on water samples and dirt. Blue/green algae evaluation on water samples and pond scum.

C O N T A C T L O G S U M M A R Y

Report	Date Reported
Preliminary 4	08/08/07-
Preliminary 3	08/03/07-
Preliminary 2	08/01/07-
Preliminary 1	07/30/07-

S P E C I M E N S U M M A R Y

Specimen Type	Breed	ID	Age	Sex	Qty
WATER	ENVIRONMENTAL	Multiple IDs			2
DIRT	ENVIRONMENTAL	Multiple IDs			2
WATER-POND	ENVIRONMENTAL	Multiple IDs			4
WATER-POND	ENVIRONMENTAL	Multiple IDs			5

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APPENDIX 2. Excerpt of chemical data for a water sample collected from the Cactus Flat Main Lake depression on the NTTR in July 2007. Sample was collected on behalf of the U.S. Air Force; collection was concurrent with the samples described in Appendix 1.

TestAmerica
THE LEADER IN ENVIRONMENTAL TESTING

CSC Applied Technologies LLC
P.O. Box 569
Indian Springs, NV 89018
Attention: Cynthia Lang

Project ID: Gun Pit North End
Report Number: PQG0762

Sampled: 07/23/07
Received: 07/25/07

INORGANICS

Analyte	Method	Batch	Reporting Limit	Sample Result	Dilution Factor	Date Extracted	Date Analyzed	Data Qualifiers
Sample ID: PQG0762-01 (NS070723-01,2,3,4 - Water)								
Reporting Units: mg/l								
Chloride	EPA 300.0	P7G2505	50	**2100**	100	7/25/2007	7/25/2007	
Fluoride	EPA 300.0	P7G2505	1.0	**5.0**	10	7/25/2007	7/25/2007	
Nitrate/Nitrite-N	EPA 300.0	P7G2505	20	**1000**	100	7/25/2007	7/25/2007	
Nitrate-N	EPA 300.0	P7G2505	10	**1000**	100	7/25/2007	7/25/2007	
Nitrite-N	EPA 300.0	P7G2505	10	**18**	100	7/25/2007	7/25/2007	
Sulfate	EPA 300.0	P7G2505	50	**2100**	100	7/25/2007	7/25/2007	
Total Dissolved Solids	SM2540C	P7G2801	200	**31000**	10	7/27/2007	7/27/2007	
Sample ID: PQG0762-01 (NS070723-01,2,3,4 - Water)								
Reporting Units: pH Units								
pH	EPA 150.1	P7G2521	NA	**8.95**	1	7/25/2007	7/25/2007	HTI
Temp. at time of pH Analysis (°C)	EPA 150.1	P7G2521	NA	**20.3**	1	7/25/2007	7/25/2007	HTI

TestAmerica - Phoenix, AZ

Carlene McCutcheon
Project Manager

TestAmerica
THE LEADER IN ENVIRONMENTAL TESTING

CSC Applied Technologies LLC
P.O. Box 569
Indian Springs, NV 89018
Attention: Cynthia Lang

Project ID: Gun Pit North End
Report Number: PQG0762

Sampled: 07/23/07
Received: 07/25/07

METALS

Analyte	Method	Batch	Reporting Limit	Sample Result	Dilution Factor	Date Extracted	Date Analyzed	Data Qualifiers
Sample ID: PQG0762-01 (NS070723-01,2,3,4 - Water)								
Reporting Units: mg/l								
Barium	EPA 200.7	7G27136	0.010	**0.66**	1	7/27/2007	7/29/2007	
Beryllium	EPA 200.7	7G27136	0.0020	**0.0070**	1	7/27/2007	7/29/2007	
Cadmium	EPA 200.7	7G27136	0.0050	ND	1	7/27/2007	7/29/2007	
Chromium	EPA 200.7	7G27136	0.0050	**0.053**	1	7/27/2007	7/29/2007	
Copper	EPA 200.7	7G27136	0.010	**0.12**	1	7/27/2007	7/29/2007	
Iron	EPA 200.7	7G27136	0.040	**71**	1	7/27/2007	7/29/2007	
Magnesium	EPA 200.7	7G27136	0.020	**59**	1	7/27/2007	7/29/2007	
Manganese	EPA 200.7	7G27136	0.020	**1.9**	1	7/27/2007	7/29/2007	
Mercury	EPA 245.1	7G26065	0.00020	ND	1	7/26/2007	7/26/2007	
Nickel	EPA 200.7	7G27136	0.010	**0.060**	1	7/27/2007	7/29/2007	
Selenium	EPA 200.7	7G27136	0.010	**0.076**	1	7/27/2007	7/29/2007	
Zinc	EPA 200.7	7G27136	0.020	**0.30**	1	7/27/2007	7/29/2007	
Sample ID: PQG0762-01 (NS070723-01,2,3,4 - Water)								
Reporting Units: ug/l								
Antimony	EPA 200.8	7G27145	40	ND	20	7/27/2007	8/1/2007	RL1
Arsenic	EPA 200.8	7G27145	20	**540**	20	7/27/2007	8/1/2007	
Thallium	EPA 200.8	7G27145	20	ND	20	7/27/2007	8/3/2007	RL1

TestAmerica - Phoenix, AZ

Carlene McCutcheon
Project Manager

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APPENDIX 3. Chemical data from samples collected by DRI at the NTTR in February 2008.

Table 1. General sample descriptions for samples collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	Collection Date	Collection Time	UTM N (NAD 83)[1]	UTM E (NAD 83)[1]	Elevation (ft)	Elevation (m)
1	Main Lake depression water, 2 m depth	2/6/2008	9:20	522881	4188970	5,340	1,628
2	Main Lake depression water, 0.3 m depth	2/6/2008	10:45	522881	4188970	5,340	1,628
3	Main Lake depression sediment	2/6/2008	12:30	522881	4188970	5,340	1,628
4	Drainage sediment	2/6/2008	13:30	523095	4188751	5,317	1,621
5	Drainage sediment	2/6/2008	14:15	522984	4188885	5,316	1,620
6	Drainage sediment	2/6/2008	14:45	522987	4188885	5,316	1,620
7	Drainage sediment	2/6/2008	15:10	522692	4188908	5,310	1,618
8	Drainage sediment	2/6/2008	15:30	523000	4189191	5,328	1,624
9	Drainage sediment	2/6/2008	16:00	521543	4187128	5,343	1,629
10	Drainage sediment	2/6/2008	16:30	521365	4186168	5,361	1,634
11	Drainage sediment	2/6/2008	16:50	521491	4184865	5,418	1,651
12	Main Lake depression sediment	2/7/2008	7:30	522839	4188972	5,311	1,619
13	Main Lake depression sediment	2/7/2008	8:10	522815	4188961	5,317	1,621
14	Main Lake depression sediment	2/7/2008	9:25	522894	4188937	5,320	1,622
16	Cedar Wells Spring water	2/7/2008	11:30	566251	4173559	6,364	1,940
17	Rose Spring water	2/7/2008	13:00	558836	4177875	7,145	2,178
18	Corral Spring water	2/7/2008	14:45	554177	4182033	6,596	2,010
19	Silverbow Spring Tank water	2/7/2008	16:30	541960	4186893	5,965	1,818
20	Drainage sediment	2/8/2008	7:15	521555	4182892	5,476	1,669
21	Drainage sediment	2/8/2008	8:30	520887	4180677	5,474	1,668
22	Cactus Spring water	2/8/2008	11:15	516060	4174979	6,274	1,912
23	Drainage sediment	2/8/2008	12:20	523204	4189046	5,341	1,628

Note that there is no sample number 15

[1]UTM: Universal Transverse Mercator coordinate system

(N = northing, E = easting; NAD 83 = North American Datum of 1983)

Table 2A. Major-ion chemical data for water samples collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	Field pH	Lab pH	Field EC[1] (µS/cm)	Lab EC[1] (µS/cm)	Field DO[2] (mg/L)	Temp-erature (°C)	SiO_2 (mg/L)	HCO_3^- (mg/L)	CO_3^{2-} (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)	NO_3^- (as N) (mg/L)	NO_3^- (mg/L)	Na^+ (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	F^- (mg/L)	Br^- (mg/L)	NO_2^- (as N) (mg/L)	NH_3 (as N) (mg/L)	PO_4^{3-} (as P) (mg/L)	Dissolved P (mg/L)	Total P (mg/L)
1	Main Lake depression water, 2 m depth	8.47	8.69	1209	1210	0.47	4.5	-	550	NA	65	58	6.4	28.4	274	13.4	13.3	1.4	0.65	0.03	1.54	0.45	0.490	0.73	6.0
2	Main Lake depression water, 0.3 m depth	8.69	8.59	1139	1210	9.2	1.1	-	559	NA	46	39	11.8	52.2	259	12.7	12.3	1.2	0.75	0.02	0.091	0.36	0.512	0.84	4.9
16	Cedar Wells Spring water	7.55	7.93	702	715	5.56	11.3	-	325	NA	28	76	1.9	8.6	58.7	0.8	85.6	12.9	0.50	0.29	0.001	0.005	0.006	NA	0.010
17	Rose Spring water	7.22	7.85	634	649	4.47	12.8	-	316	NA	24	55	0.9	4.2	44.4	1.9	85.1	11.4	0.35	0.29	<0.001	0.003	0.008	NA	0.013
18	Corral Spring water	7.07	7.75	665	677	3.56	5.5	-	268	NA	37	83	0.1	0.4	76.1	2.8	67.8	7.0	0.95	0.40	<0.001	0.005	0.007	NA	0.010
19	Silverbow Spring Tank water	7.33	7.93	430	440	10.4	1.2	-	205	NA	22	33	0.3	1.2	45.2	2.0	43.2	7.8	0.36	0.20	<0.001	0.005	0.008	NA	0.016
22	Cactus Spring water	7.2	7.80	560	565	0.8	15.5	-	222	NA	26	76	0.0	0.0	56.3	2.6	59.5	7.6	0.71	0.17	<0.001	0.008	0.001	NA	0.002

NA: not applicable

[1]EC: electrical conductivity

[2]DO: dissolved oxygen

Table 2B. Major-ion chemical data for sediment samples (based on sediment extracts) collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	Lab pH	Lab EC[1] (µS/cm)	SiO_2 (mg/kg)	HCO_3^- (mg/kg)	CO_3^{2-} (mg/kg)	Cl^- (mg/kg)	SO_4^{2-} (mg/kg)	NO_3^- (as N) (mg/kg)	NO_3^- (mg/kg)	Na^+ (mg/kg)	K^+ (mg/kg)	Ca^{2+} (mg/kg)	Mg^{2+} (mg/kg)	F^- (mg/kg)	Br^- (mg/kg)	NO_2^- (as N) (mg/kg)	NH_3 (as N) (mg/kg)	PO_4^{3-} (as P) (mg/kg)	Dissolved P (mg/kg)
3	Main Lake depression sediment	9.51	456	412	1438	501.4	80.4	110	3.3	14.4	1005	54	11	2	15	<0.2	0.0	3.6	7.7	8.2
4	Drainage sediment	7.70	968	313	352	NA	145	3379	128	567	1399	158	489	19	4	0.2	0.4	1.1	0.4	0.6
5	Drainage sediment	7.89	797	333	451	NA	14.2	3024	11.8	52.3	1241	152	271	8	3	<0.2	1.1	25.0	0.6	0.8
6	Drainage sediment	9.18	335	371	1044	200.0	34.9	227	27.6	122	670	53	30	1	11	<0.2	0.2	1.2	3.9	4.1
7	Drainage sediment	7.31	2850	282	242	NA	1940	3802	1901	8416	3802	279	1862	74	5	0.6	0.2	1.8	0.1	0.3
8	Drainage sediment	8.43	966	314	666	14.8	461	1872	351	1552	1911	123	74	3	10	0.2	0.1	0.4	0.7	0.8
9	Drainage sediment	8.44	180	346	652	13.8	30.7	78.6	33.5	148	293	46	66	4	18	<0.2	0.1	0.3	3.7	4.0
10	Drainage sediment	8.52	150	345	668	21.7	3.8	77.1	7.0	31.0	265	46	48.8	3	18	<0.2	0.1	1.0	3.7	4.0
11	Drainage sediment	7.89	78	207	399	NA	3.8	9.0	4.9	21.8	80	46	58	4	2	<0.2	0.0	0.7	2.6	2.9
12	Main Lake depression sediment	9.43	414	364	1399	386.1	79.3	133.0	1.5	6.5	929	52	9	1	13	1.4	0.1	8.2	7.5	8.0
13	Main Lake depression sediment	9.29	436	364	1586	311.3	109	200	3.4	15.3	980	53	8	1	12	<0.2	0.1	12.7	6.5	7.1
14	Main Lake depression sediment	8.97	286	275	1290	131.0	21.2	83.6	9.0	39.7	636	53	15.3	2	8.9	0.2	0.7	7.0	4.8	5.2
20	Drainage sediment	7.74	159	198	408	NA	50.1	85.4	54.2	240	175	81	90	7	3	<0.2	0.1	0.9	3.6	3.9
21	Drainage sediment	7.83	106	191	587	NA	7.3	10.3	0.1	0.4	87	65	95	9	2	<0.2	0.2	4.8	2.6	3.3
23	Drainage sediment	9.25	304	377	1202	232.5	13.8	96.2	6.1	27.0	686	51	14	1	12	<0.2	0.1	0.6	4.2	4.5

NA: not applicable

All values reported were measured on soil extracts made with a 10:1 ratio (by mass) of deionized water:soil, and are converted to show mass in the soil

[1]EC: electrical conductivity

Table 3A. Nitrogen and oxygen isotope data for nitrate in water samples and oxygen isotope data for water collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	$\delta^{15}N$ (nitrate) (‰)	$\delta^{18}O$ (nitrate) (‰)	$\delta^{17}O$ (nitrate) (‰)	$\delta^{18}O$ (water) (‰)
1	Main Lake depression water, 2 m depth	38.55	31.42	1.76	1.98
2	Main Lake depression water, 0.3 m depth	27.39	25.05	1.88	-
16	Cedar Wells Spring water	9.61	14.46	1.69	-
17	Rose Spring water	7.58	9.39	0.46	-6.92

Dashes indicate analyte not tested in that sample

Table 3B. Nitrogen and oxygen isotope data for nitrate in sediment samples (based on sediment extracts) collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	$\delta^{17}O$ (‰)
4	Drainage sediment	18.8	19.95	2.42
5	Drainage sediment	ND	18.08	1.36
6	Drainage sediment	16.04	25.52	2.24
7	Drainage sediment	17.54	16.07	2.63
8	Drainage sediment	20.33	14.23	2.1
9	Drainage sediment	ND	ND	ND
10	Drainage sediment	ND	ND	ND
11	Drainage sediment	12.08	15.1	2.3
	Commercial urea	-1	-	-

ND: not detectable

Dashes indicate analyte not tested in that sample

Table 4A. Organic chemical data for water samples collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	TICs[1] (semivolatile)	TPH-E[2,3] (DRO)[4] mg/L	TPH-E[2,3] (ORO)[5] mg/L	TPH-P[2,6] (GRO)[7] mg/L	Diethylene glycol[8]	Ethylene glycol[8]	Propylene glycol[8]	Triethylene glycol[8]
1	Main Lake depression water, 2 m depth	none found	ND	ND	ND	ND	ND	ND	ND
2	Main Lake depression water, 0.3 m depth	none found	ND	ND	ND	ND	ND	ND	ND

Note that organic analyses were not performed on spring waters (samples 16, 17, 18, 19, and 22)
ND: non detect
[1]TICs: Tentatively identified compounds, analyzed by EPA Method SW8270; detection limit is 20 μg/L
[2]TPH: Total petroleum hydrocarbons, analyzed by EPA Method SW8015B
[3]-E: extractable
[4]DRO: diesel range organics, detection limit is 0.5 mg/L
[5]ORO: oil range organics, detection limit is 0.5 mg/L
[6]-P: purgable
[7]GRO: gasoline range organics, detection limit is 0.5 mg/L
[8]Analyzed by EPA Method 8015B, detection limit is 5 mg/L

Table 4B. Organic chemical data for sediment samples (based on sediment extracts) collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	TICs[1] (semivolatile)	TPH-E[2,3] (DRO)[4] mg/kg	TPH-E[2,3] (ORO)[5] mg/kg	TPH-P[2,6] (GRO)[7] mg/kg	Diethylene glycol[8]	Ethylene glycol[8]	Propylene glycol[8]	Triethylene glycol[8]
3	Main Lake depression sediment	none found	ND	ND	ND	ND	ND	ND	ND
4	Drainage sediment	none found	ND	ND	ND	ND	ND	ND	ND
5	Drainage sediment	none found	ND	ND	ND	ND	ND	ND	ND
6	Drainage sediment	none found	ND	50	ND	ND	ND	ND	ND
7	Drainage sediment	none found	ND	ND	ND	ND	ND	ND	ND
8	Drainage sediment	none found	ND	ND	ND	ND	ND	ND	ND
9	Drainage sediment	none found	13	90	ND	ND	ND	ND	ND
10	Drainage sediment	none found	ND	50	ND	ND	ND	ND	ND
11	Drainage sediment	none found	ND	32	ND	ND	ND	ND	ND
12	Main Lake depression sediment	none found	ND	ND	ND	ND	ND	ND	ND
13	Main Lake depression sediment	none found	ND	ND	ND	ND	ND	ND	ND
14	Main Lake depression sediment	none found	ND	ND	ND	ND	ND	ND	ND
20	Drainage sediment	none found	ND	15	ND	ND	ND	ND	ND
21	Drainage sediment	none found	ND	24	ND	ND	ND	ND	ND
23	Drainage sediment	none found	ND	ND	ND	ND	ND	ND	ND

ND: non detect
[1]TICs: Tentatively identified compounds, analyzed by EPA Method SW8270; detection limit is 1,300 μg/kg
[2]TPH: Total petroleum hydrocarbons, analyzed by EPA Method SW8015B
[3]-E: extractable
[4]DRO: diesel range organics, detection limit is 10 mg/kg
[5]ORO: oil range organics, detection limit is 10 mg/kg
[6]-P: purgable
[7]GRO: gasoline range organics, detection limit is 10 mg/kg
[8]Analyzed by EPA Method 8015B, detection limit is 15 mg/kg

Table 5A. Trace element data for water samples collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	Be (ppb)	Al (ppb)	V (ppb)	Cr (ppb)	Mn (ppb)	Fe (ppb)	Co (ppb)	Ni (ppb)	Cu (ppb)	Zn (ppb)	Sr (ppb)	Mo (ppb)	Ag (ppb)	Cd (ppb)	Sn (ppb)	Sb (ppb)	Ba (ppb)	Tl (ppb)	Pb (ppb)	U (ppb)	As (ppb)	Se (ppb)
1	Main Lake depression water, 2 m depth, centrifuged, lab filtered 0.1 μm, acidified	<10	52.6	32.2	<10	1.6	15.7	<10	<10	15.6	1.2	94.0	50.9	<10	<10	<10	1.8	13.3	<10	<10	5.1	25.4	<20
1A	Main Lake depression water, 2 m depth, field filtered 0.45 μm, acidified, centrifuged, lab filtered 0.1 μm	4.8	21674	52.2	2.0	955.3	2034	9.1	6.8	54.2	41.4	495.9	22.8	<10	1.2	<10	5.7	418.8	<10	46.3	6.8	30.3	<20
2	Main Lake depression water, 0.3 m depth, centrifuged, lab filtered 0.1 μm, acidified	<10	94.0	34.7	<10	3.1	30.5	<10	<10	10.2	1.1	76.0	26.8	<10	<10	<10	1.3	11.0	<10	<10	3.4	24.6	<20
2A	Main Lake depression water, 0.3 m depth, field filtered 0.45 μm, acidified, lab filtered 0.1 μm	5.4	28381	51.2	5.8	1172	6803	11.2	17.8	60.2	68.1	406.3	6.1	<10	<10	<10	<10	399.3	<10	53.6	5.7	23.5	<20
16	Cedar Wells Spring water	<1	<1	6.1	<1	1.7	<1	<1	<1	<1	1.4	1230	2.4	<1	<1	<1	<1	56.4	<1	<1	8.9	2.6	<5
17	Rose Spring water	<1	<1	<1	<1	<1	<1	<1	<1	<1	6.5	1110	1.6	<1	<1	<1	<1	4.2	<1	<1	13.5	1.1	<5
18	Corral Spring water	<1	<1	1.6	<1	<1	<1	<1	<1	<1	4.1	595.1	14.7	<1	<1	<1	<1	4.7	<1	<1	24.3	9.8	<5
19	Silverbow Spring Tank water	<1	1.5	4.0	<1	<1	10.9	<1	<1	2.1	1.5	316.4	1.1	<1	<1	<1	<1	34.6	<1	<1	3.5	14.9	<5
22	Cactus Spring water	<1	<1	<1	<1	143.4	387.5	<1	<1	<1	<1	983.8	12.1	<1	<1	<1	<1	35.8	<1	<1	9.2	<1	<5

Table 5B. Trace element data for sediment samples (based on sediment extracts) collected by DRI at the NTTR in February 2008.

Sample Number	Sample Description	Be (ppb)	Al (ppb)	V (ppb)	Cr (ppb)	Mn (ppb)	Fe (ppb)	Co (ppb)	Ni (ppb)	Cu (ppb)	Zn (ppb)	Sr (ppb)	Mo (ppb)	Ag (ppb)	Cd (ppb)	Sn (ppb)	Sb (ppb)	Ba (ppb)	Tl (ppb)	Pb (ppb)	U (ppb)	As (ppb)	Se (ppb)
3	Main Lake depression sediment	<10	2629	666	<10	89.9	1018	<10	<10	76.9	48.1	114	67.1	<10	<10	<10	<10	56.1	<10	<10	15.0	178	<50
3A	Main Lake Depression sediment, 1% HNO_3 extract	308	1641192	1294	176	146857	58561	1155	786	322	1791	65050	<100	<100	137	<100	<100	33314	<100	1565	196	656	<2000
4	Drainage sediment	<10	57.1	142	<10	<10	16.0	<10	<10	15.0	<10	4250	214	<10	<10	<10	<10	67.1	<10	<10	<10	62.3	<50
5	Drainage sediment	<10	42.0	358	<10	50.9	27.4	<10	<10	71.6	32.1	324	223	<10	<10	<10	<10	97.9	<10	<10	<10	134	<50
6	Drainage sediment	<10	231	512	<10	33.2	92.3	<10	<10	63.1	33.7	110	53.7	<10	<10	<10	<10	39.7	<10	<10	<10	151	<50
7	Drainage sediment	<10	16.2	315	<10	<10	<10	<10	<10	15.3	12.9	14096	288	<10	<10	<10	<10	164.2	<10	<10	<10	153	314
8	Drainage sediment	<10	980	1051	<10	23.9	396	<10	<10	47.5	<10	224	137	<10	<10	<10	<10	28.5	<10	<10	<10	335	<50
9	Drainage sediment	<10	62.5	323	<10	36.7	38.7	<10	<10	32.3	30.1	179	<10	<10	<10	<10	<10	56.3	<10	<10	<10	77.1	<50
10	Drainage sediment	<10	1424	643	<10	26.1	819	<10	<10	16.5	27.3	134	<10	<10	<10	<10	<10	14.8	<10	<10	<10	135	<50
11	Drainage sediment	<10	158	39.2	<10	34.9	55.1	<10	<10	29.1	20.6	120	<10	<10	<10	<10	<10	39.1	<10	<10	<10	11.1	<50
12	Main Lake depression sediment	<10	156	544	<10	117	109	<10	<10	97.9	58.0	268	56.8	<10	<10	<10	<10	98.6	<10	<10	16.4	142	<50
12A	Main Lake depression sediment, 1% HNO_3 extract	550	2270388	239	231	90832	80957	820	968	297	2153	55731	<100	<100	130	<100	155	34864	<100	1697	263	487	<2000
13	Main Lake depression sediment	<10	2486	677	<10	81.0	487	<10	<10	64.3	34.1	147	65.8	<10	<10	<10	<10	59.9	<10	<10	15.1	159	<50
13A	Main Lake depression sediment, 1% HNO_3 extract	458	2035221	1323	215	121865	75591	874	882	346	2042	63521	<100	<100	135	<100	<100	33198	<100	1751	234	552	<2000
14	Main Lake depression sediment	<10	176	756	<10	61.9	160	<10	<10	65.4	39.0	134	69.5	<10	<10	<10	12.7	56.6	<10	<10	14.5	272	<50
14A	Main Lake depression sediment, 1% HNO_3 extract	320	1465462	2694	153	196881	529978	1354	744	508	2611	82914	<100	<100	124	<100	<100	31909	<100	1519	183	743	<2000
20	Drainage sediment	<10	192	96.8	<10	41.3	81.5	<10	<10	44.5	40.5	141	<10	<10	<10	<10	<10	43.6	<10	<10	<10	50.0	<50
21	Drainage sediment	<10	54.9	75.8	<10	30.8	150	32.2	15.3	47.1	122	200	10.7	<10	<10	<10	<10	47.6	<10	<10	<10	21.9	<50
23	Drainage sediment	<10	1040	496	<10	55.3	517	<10	<10	75.4	76.3	157	39.8	<10	<10	<10	10.1	50.7	<10	<10	<10	141	<50

Except as noted, all values reported were measured on soil extracts made with a 10:1 ratio (by mass) of deionized water:soil, and are converted to show mass in the soil

Table 6. Dual-aliquot sample data for sediment major-ion analyses (based on sediment extracts).

Sample Description	Lab pH	Lab EC[1] (µS/cm)	SiO_2 (mg/kg)	HCO_3^- (mg/kg)	CO_3^{2-} (mg/kg)	Cl^- (mg/kg)	SO_4^{2-} (mg/kg)	NO_3^- (as N) (mg/kg)	NO_3^- (mg/kg)	Na^+ (mg/kg)	K^+ (mg/kg)	Ca^{2+} (mg/kg)	Mg^{2+} (mg/kg)	F^- (mg/kg)	Br^- (mg/kg)	NO_2^- (as N) (mg/kg)	NH_3 (as N) (mg/kg)	PO_4^{3-} (as P) (mg/kg)	Dissolved P (mg/kg)
6-Drainage sediment	9.18	335	371	1044	200	35	227	28	124	670	53	30	1.0	11	<0.2	0.2	1.2	3.9	4.1
6D-Drainage sediment	9.12	306	333	1103	172	29	198	20	89	672	49	14	1.4	9.9	<0.2	0.2	0.4	3.9	4.2
Percent difference	0.7	9.0	10.8	5.5	15.1	18.8	13.6	33.3	32.9	0.3	8.7	72.7	33.3	10.5	n/a	0.0	100.0	0.0	2.4
10-Drainage sediment	8.52	150	345	668	21.7	3.8	77.1	7.0	31	265	46	48.8	3.0	18	<0.2	0.1	1.0	3.7	4.0
10D-Drainage sediment	8.57	143	303	688	26.6	3.5	19.1	6.7	30	288	29	14.3	1.1	20	<0.2	0.1	0.2	4.7	5.1
Percent difference	0.6	4.8	13.0	2.9	20.3	8.2	120.6	4.4	3.3	8.3	45.3	109.4	92.7	10.5	n/a	0.0	133.3	23.8	24.2
14-Main Lake depression sediment	8.97	286	275	1290	131	21	84	9.0	40	636	53	15.3	2.1	9.00	0.2	0.7	7.0	4.8	5.2
14D-Main Lake depression sediment	8.95	270	255	1313	121	13	51	1.6	7	601	48	14.0	1.3	9	<0.2	0.7	7.0	4.9	5.4
Percent difference	0.2	5.8	7.5	1.8	7.9	47.1	48.9	139.6	140.4	5.7	10.2	8.9	47.1	1.5	n/a	0.0	0.0	2.1	3.8

All values reported were measured on soil extracts made with a 10:1 ratio (by mass) of deionized water:soil, and are converted to show mass in the soil
n/a indicates a percentage difference that cannot be calculated because at least one value is not a number
[1]EC: electrical conductivity

Table 7. Dual-aliquot sample data for sediment trace element analyses (based on sediment extracts).

Sample Description	Be (ppb)	Al (ppb)	V (ppb)	Cr (ppb)	Mn (ppb)	Fe (ppb)	Co (ppb)	Ni (ppb)	Cu (ppb)	Zn (ppb)	Sr (ppb)	Mo (ppb)	Ag (ppb)	Cd (ppb)	Sn (ppb)	Sb (ppb)	Ba (ppb)	Tl (ppb)	Pb (ppb)	U (ppb)	As (ppb)	Se (ppb)
5-Drainage sediment	<10	42	358	<10	51	27	<10	<10	72	32	324	223	<10	<10	<10	<10	98	<10	<10	<10	134	<50
5D-Drainage sediment	<10	96	288	<10	80	71	<10	<10	85	45	642	187	<10	<10	<10	<10	117	<10	<10	<10	115	<50
Percent difference	n/a	78.3	21.7	n/a	44.3	89.8	n/a	n/a	16.6	33.8	65.8	17.6	n/a	n/a	n/a	n/a	17.7	n/a	n/a	n/a	15.3	n/a
20-Drainage sediment	<10	192	97	<10	41	81	<10	<10	45	41	141	<10	<10	<10	<10	<10	44	<10	<10	<10	50	<50
20D-Drainage sediment	<10	186	71	<10	42	71	<10	<10	36	30	124	<10	<10	<10	<10	<10	39	<10	<10	<10	37	<50
Percent difference	n/a	3.2	31.0	n/a	2.4	13.2	n/a	n/a	22.2	31.0	12.8	n/a	n/a	n/a	n/a	n/a	12.0	n/a	n/a	n/a	29.9	n/a

All values reported were measured on soil extracts made with a 10:1 ratio (by mass) of deionized water:soil, and are converted to show mass in the soil

Table 8. List of analytical methods used for major-ion and trace element analyses.

METHODS SUMMARY FOR ACCREDITED TESTING

Analyte	Method	Description
Ortho-Phosphate	SM 4500-P F	Phosphomolybdate
Total Phosphorus	USGS I-4600-85 SM 4500-P F	Persulfate Digestion, Phosphomolybdate
Nitrite	SM 4500-NO3 F	Colorimeric, Automated
Nitrate	SM 4500-NO3 F	Colorimetric, Automated, Cadmium Reduction
Ammonia	SM 4500-NH3 F	Colorimetric, Automated Phenate
Fluoride	SM 4500 F C	Specific Ion Electrode
Bromide	EPA 300.0	Ion Chromatography
pH	SM 4500 H+ B	Electrometric
Alkalinity	USGS I 1030-85	Electrometric Titration
Conductivity	SM 2510 B	Electrometric
Chloride	EPA 300.0	Ion Chromatography
Sulfate	EPA 300.0	Ion Chromatography
Sodium	SM 3111B	Atomic Absorption
Potassium	SM 3111B	Atomic Absorption
Calcium	SM 3111B	Atomic Absorption
Magnesium	SM 3111B	Atomic Absorption
Silica	EPA 370.1	Colorimetric
Trace elements	EPA 200.8	ICP-MS

References:

1. *Methods for the Determination of Inorganic Substances in Environmental Samples,* EPA/600/R-93/100, August 1993, United States Environmental Protection Agency, Office of Research and Development, Washington DC 20460.
2. *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020 March 1979, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45468.
3. *Standard Methods For The Examination Of Water and Wastewater,* 18th edition, 1992, Editors A. E. Greenberg, L. S. Clesceri, A. D. Eaton, M. A. H. Franson, American Public Health Association, 1015 Fifteenth Street NW, Washington, DC 20005.
4. *United States Geological Survey, Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments,* Book 5, Chapter A1, 1985, Editors: M. W. Skougstad, M. J. Fishmann. L. C. Friedman, D. E. Erdmann, and S. S. Duncan, U.S. Government Printing Office, Washington D.C. 20402.
5. *Methods for the Determination of Metals in Environmental Sample*s, EPA/600/R-94/111,May 1994, United States Environmental Protection Agency, Office of Research and Development, Washington DC 20460.

APPENDIX 4. Chemical data from samples collected by U.S. Air Force at the NTTR in February 2008.

Table 1. Results of water chemistry analyses for samples collected by the U.S. Air Force at the NTTR in February 2008.

Sample ID	Sample Location	Analyte	Result	Unit Of Measure
085543-006	ROSE SPRING (BKGD)	Nitrite	0.033	mg/L
085543-006	ROSE SPRING (BKGD)	Nitrogen, Nitrate/Nitrite	0.715	mg/L
085543-006	ROSE SPRING (BKGD)	Nitrate	0.93	mg/L
085542-006	#1 HORSE POND	Nitrite	1.4	mg/L
085542-006	#1 HORSE POND	Nitrate	6.38	mg/L
085542-006	#1 HORSE POND	Nitrogen, Nitrate/Nitrite	7.7	mg/L