TEEM Lichen Project Interim Report:

Mapping the characteristics of air pollutant deposition patterns in the Athabasca Oil Sands Region using epiphytic lichens as bioindicators

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1.0 Introduction

1.1 CONTEXT

Rapid industrial development in the Athabasca Oil Sands Region (AOSR) of northeastern Alberta has led to increased concern around nitrogen- and sulphur-based emissions in this region. Emissions from the oil sands industry are of interest because extended periods of enhanced deposition of these pollutants and their products can alter ecosystems (e.g., lichen fertilization response to ammonia deposition, van Dobben et al. 2001; van Dobben and ter Braak 1998 and ammonia toxicity to crop and tree species, Krupa 2003) directly through soil and surface water acidification (Fenn et al. 1998; Reuss and Johnson 1986) or eutrophication (Fenn et al. 2003). Lichens are excellent bioindicators because they are sensitive to dry and wet deposition of airborne pollutants (especially compounds containing nitrogen and sulphur, Farmer et al. 1992; Nash and Gries 2002) and thus serve as an early warning indicator for potential air pollution damage to higher plants (Gries 1996). Lichens have been shown to be affordable and effective biomonitors for nitrogen (N) and sulphur (S) in the AOSR in studies supported by the Wood Buffalo Environmental (WBEA; Berryman et al. 2004; CE Jones and Associates 2006). The overall objective of this project was to document the patterns of atmospheric deposition of N and S at the regional scale, using epiphytic lichens as bioindicators of air pollution.

Oil sands operations are the primary source of air pollution in the AOSR. There are two main types of oil sands operations in the AOSR at this time: surface mine sites and in-situ (e.g., steam-assisted gravity drainage) sites. The surface mine sites require a large diesel fleet for moving materials, and some companies have on-site bitumen upgraders. Suncor Energy Inc. (Suncor) and Syncrude Canada Ltd. (Syncrude) have been conducting oil sands mining, extraction and upgrading operations since 1968 and 1978, respectively. Until recently, these two companies were the only major sources of local anthropogenic air pollution in the region. In the past decade there has been extensive industrial development in the AOSR, including new mining, mining expansion, and new in-situ operations. In addition, there are a number of mining and in-situ projects that are under development, but not yet operational or fully operational. These industrial activities emit acid-forming compounds, such as sulphur dioxide (SO_2) and nitrogen oxides (NO_x), and airborne particulates containing trace metals (Pauls et al. 1996). Sources of these emissions include point sources such as stacks associated with upgrading operations (with emissions resulting from combustion of petroleum coke and natural gas), and area sources such as mine fleets (mainly diesel powered), and fugitive dust from exposed materials (tailings ponds, dikes, road surfaces) and mining activities (Dillon Consulting Ltd. 2001).

SO_2 emissions reached their highest in 1994 at approximately 480 tonnes per day in the AOSR (Golder Associates 2003). Since then, emissions controls have been implemented at the main upgrading operations to reduce overall SO_2 and particulate emissions. In 2008, SO_2 emissions in the AOSR were estimated at approximately 280 tonnes per day and NO_x emissions at approximately 180 tonnes per day (Table 1-1; see accompanying CALPUFF report by M. Davies, Table E-1, page viii). The values above are estimates derived primarily from information contained in Environmental Impact Assessments (EIAs) and in Environment Canada's National Pollutant Release Inventory (NPRI), as actual emissions rates are difficult to measure or are not measured. EIAs in particular tend to overestimate emissions, therefore in order to address these data limitations, individual operators in the AOSR were contacted to confirm emissions estimates used for this project and to fill in data gaps. Thus, emissions estimates presented in Table 1-1 represent the best information on these parameters available for the study period. In addition, the emissions context in the region is constantly shifting, due to both new project development and to implementation of emissions controls; these shifts are reflected in Table 1-1.

Table 1-1: Summary of SO_2 and NO_x emissions (tonnes per day) from source types in the CALPUFF model domain, from 2003 to 2008. For a detailed description of this domain and of methods of emission estimation, see the accompanying CALPUFF report.

	Source type/location	2003	2004	2005	2006	2007	2008
			Year 1	Year 2	Year 3	Year 4	Year 5
		(t/d)	(t/d)	(t/d)	(t/d)	(t/d)	(t/d)
Anthropogenic Emission Sources							
SO_2	Stacks north of Fort McMurray	263.3	309.3	300.3	286.6	300.8	273.5
	Stacks south of Fort McMurray	3.3	3.9	4.1	4.7	3.8	8.6
	Stacks south of Air Weapons range	5.8	6.8	7.3	9.5	8.2	5.8
	Area Sources (Mines)	1.2	1.2	1.1	1.4	1.4	1.9
	Non-Industry Sources	0.3	0.3	0.3	0.3	0.3	0.3
	Sum	**273.9**	**321.5**	**313.1**	**302.4**	**314.4**	**290.0**
NO_x	Stacks north of Fort McMurray	60.2	67.1	68.9	74.5	79.2	69.3
	Stacks south of Fort McMurray	17.6	16.2	19.2	21.2	20.2	23.8
	Stacks south of Air Weapons range	12.2	13.2	15.0	15.0	16.8	16.8
	Area Sources (Mines)	42.1	43.1	43.2	51.4	57.0	73.7
	Non-Industry Sources	16.0	16.0	16.0	16.0	16.0	16.0
	Sum	**148.2**	**155.5**	**162.2**	**178.1**	**189.3**	**199.7**
Wildfire Emission Sources							
Number of notable wildfires in the region		Outside model simulation period	1	0	3	2	No wildfire data
SO_2	Wildfires (emissions are annualized)		3.5	0.0	1.3	8.1	
NO_x	Wildfires (emissions are annualized)		10.6	0.0	3.9	19.4	

1.2 LICHENS AS BIOINDICATORS OF AIR POLLUTION

Lichens lack vascular system and roots and therefore, obtain their nutrients from the atmosphere, while also readily accumulating atmospheric contaminants. Elemental content in lichen is not influenced by the soil nutrients and is a true reflection of atmospheric deposition. Unlike vascular plants, lichens lack a cuticle or specialized guard cells to control the exchange of water, nutrients, gases, and contaminants with the external environment. Consequently, concentrations of elements in lichen typically reflect atmospheric sources of nutrients and contaminants. Lichen elemental content has been used in many studies to map pollutant deposition or to characterize pollution gradients in relation to sources (some examples include: Addison and Puckett 1980; Bargali and Mikhailova 2002; Bruteig 1993; Gartner Lee et al. 2006; Gombert et al. 2003; Sloof & Wolterbeek 1991; Søchting 1995; Zakshek et al. 1986).

Many lichen species respond to very low levels of sulphur- or nitrogen-based pollutants, and lichen elemental content reflects depositional loading of these pollutants (Søchting 1995; Palomäki et al. 1992). Pollutants such as SO_2 can reduce lichen abundance and species richness in a community (Nash and Gries 2002). Furthermore, pollutants, especially sulphur-based compounds, can cause morphological modifications (i.e., shrubbier, compact growth; discolouration; and increased production of asexual propagules) to exposed lichens (Kauppi 1983; Nash and Gries 1995; Sigal and Nash 1983). Previous

studies in the AOSR indicated that pollutants associated with oil sands industrial emissions, including N and S, were typically enhanced in lichens and mosses near the main emissions sources (Addison and Puckett 1980; Berryman et al. 2004; CE Jones and Associates 2006; Dabbs 1985; Marsh et al. 2005, draft report; Pauls et al. 1996; Peterson and Douglas 1977).

Many lichens can accumulate high concentrations of elements without showing signs of damage (Nimis et al. 2002). Accumulation of elements in lichen occurs by particulate trapping, active uptake of anions, passive absorption of cations, and ion exchange (Nieboer et al. 1978). Lichens are subject to continual wetting and drying cycles (depending on rain and fog events). When lichens are wet, nutrients and contaminants deposited on the lichen surface are absorbed, and are later concentrated in the lichen when dry (Nieboer et al. 1978). However, particulate trapping and diffusion of gases into the lichen can occur when the lichen is either wet or dry. In addition, during periods of rain, the elements stored in the lichen can be slowly leached. Although lichen elemental concentrations reflect atmospheric deposition, they do not simply reflect total deposition, as lichens are living organisms with multiple factors influencing elemental flux in the lichen. The residence of elements in lichen is varies by element. Macronutrients (i.e., nitrogen, S, potassium, magnesium, calcium) are relatively mobile, and their concentrations of certain elements in lichen can change seasonally (Boonpragob et al. 1989; Farmer et al. 1992). However, the absorption and leaching (or flux) of N and S compounds can vary by lichen species, by time of year, and by site conditions and these flux rates are not well known for lichen species in the AOSR. Trace metals (e.g., cadmium, lead, zinc) accumulate in lichen over time and are less mobile than macronutrients; however, trace metals are slowly released from the lichen with time (Garty 2001). Despite this complexity in factors controlling elemental fluxes in lichens, lichens have been used as accurate and effective indicators of deposition in many places worldwide, and are extremely useful in locations such as the AOSR where installation of air quality samplers is constrained by access limitations.

1.3 STUDY OBJECTIVES

WBEA has identified that using lichens as bio-indicators of atmospheric deposition represents a relatively cost-effective approach to collecting reliable, high-density information across the AOSR in order to characterize the extent and patterns of the regional deposition fields, especially in areas where air-quality monitoring stations are absent. This lichen project was implemented in the summer of 2008 to map atmospheric deposition patterns in the AOSR using epiphytic lichens (i.e., those lichens growing on trees) as receptors. The project uses N and S concentrations (where the concentration represents the total percent N or S of the dry lichen weight) in epiphytic lichens to document regional spatial deposition patterns of these elements. The project was designed to accomplish four main objectives:

1. Develop a geospatial model to:
    a. produce predictive maps of total S and N in two epiphytic lichen species, in order to document patterns and extent of relative air-pollutant deposition in the AOSR ; and,
    b. determine key site-level factors related to patterns of N and S loading in lichens that can be used to improve the above predictive maps.

2. Compare N and S concentrations in lichen to predicted deposition values from the CALPUFF atmospheric dispersion model, in order to evaluate concordance between model predictions to related empirical data.

3. Collect epiphytic lichens at sites where passive and continuous air-quality sampling is present, in order to determine if there is a relationship between N and S concentrations in lichen and wet/dry deposition/concentration measures of N and S species.

4. Use receptor modeling and source-apportionment analysis to identify specific source types that contribute to elemental enrichment in lichens, including diesel fleets, bitumen upgrading, tailings

impoundments, in-situ bitumen extraction operations, light vehicle traffic, and biogenic and long-range transport sources.

This project is comprehensive and involves many scientific collaborators that are essential to its success. Dr. Sagar Krupa is leading the receptor modeling and source apportionment component of this project and will be reporting separately on its status and results.

2.0 Project Status Update

2.1 LICHEN SAMPLES

2.1.1 Field program

The field sampling program was implemented in August-September 2008. A field crew of four spent 48 days in the field and visited 359 sites throughout the AOSR (Figure A-1; Appendix A). Most sites were accessed by helicopter, with sites visited by ground where road access allowed. At each site, lichen samples from two species (*Hypogymnia physodes* and *Evernia mesomorpha,* as present at each site) were collected, and site measurements were taken to characterize the vegetation community, topography and potential exposure to air pollutants. Lichen samples were frozen at the end of each day following collection. A total of 822 lichen samples were collected during this field program.

2.1.2 Sample processing and laboratory analyses

All 822 lichen samples were cleaned and processed during the fall of 2008 and winter of 2009. The cleaning process included removing all foreign materials (e.g., bark, etc.) to achieve a pure lichen sample for subsequent processing and analyses. Cleaned samples were sent to the University of Minnesota Research Analytical Laboratory (UMRAL) in the winter of 2009. Lichen samples were divided, splitting each sample (for both species) to have sufficient mass for the following laboratory analyses:

1. N and S analyses at UMRAL; and,
2. trace element analyses for the receptor modeling and source apportionment work.

The samples splits used for N and S analyses were oven dried, ground and analyzed by dry combustion for total N and S concentrations in lichen. The sample splits for trace-element analyses were stored frozen at UMRAL prior to analysis. (Note: trace-element analyses were initiated subsequent to completion of N and S analyses, as N and S results were used to select a sub-set of lichen samples for the trace-element work.)

The cleaning, processing, sample splitting and N and S analyses took much longer than anticipated in the original workplan schedule. As a result, final results from UMRAL N and S concentrations in lichen were not received until late summer 2009. Quality assurance (QA) and quality control analyses (QC) were completed in September 2009 and resulted in a request for re-run analyses of multiple samples. Due to this timing, the statistical analyses and reporting have been delayed by approximately four months. Similarly, selection of the sub-set of samples for trace-element analyses as part of the receptor modeling/source apportionment work was not possible until all re-run analyses and QA/QC for the N and S component were complete; thus, this component of the work has also been delayed substantially.

2.2 DATA MANAGEMENT AND DOCUMENTATION

A database has been developed for all data related to this study, with the exception of the receptor modeling/source apportionment study component led by Dr. Krupa. This database includes:

- site level data for all 359 field sampling sites;
- lichen sample data and data on N and S concentrations in lichens for all 822 lichen samples; and,
- air quality data for sites where lichen samples were co-located with other air-quality samplers (including Air Monitoring Stations; TEEM remote sites, both lake and jackpine/aspen monitoring sites; and sites with Ion Exchange Resin samplers). Air-quality data were provided through collaboration with other WBEA researchers, key collaborators include: Dr. Andrzej Bytnerowicz, Dr. Mark Fenn, Martin Hansen (AMS and TEEM passive data).

A draft of the database and associated metadata files are being sent with this interim report and a final database will be submitted with the final project report. The project database is designed for easy data entry following field data collection and could be adapted to incorporate multi-year data if any sites are revisited for future monitoring.

Samples of *Evernia mesomorpha* from all sites where foliar and soil samples were collected have been sent to Dr. Bernhard Mayer for isotope analysis (Table C-1; Appendix C). *E. mesomorpha* was selected for the isotope analyses as it is a better accumulator of N and S than *Hypogymnia physodes*. I addition, *E. mesomorpha* was generally more abundant at sample sites; therefore, there tends to be more sample available for this species. Once Dr. Mayer completes the isotope analyses, we will also incorporate these data into the project database.

A detailed lichen protocol has been drafted describing the study design, field sampling methods, sample processing/cleaning methods and laboratory methods. This protocol document will be included in the final report to TEEM and can easily be adapted to a manual format if TEEM pursues this work as a long-term monitoring program. This protocol will be continually updated to the project end and a draft is included here (Appendix D).

2.3 STATISTICAL ANALYSES AND REPORTING

Statistical analyses began in December of 2009. Statistical analyses involve two key collaborators: Dr. Jay Ver Hoef, a geospatial statistician in Fairbanks, AK, and Dr. Greg Brenner, with Pacific Analytics, in Scio, Oregon. Dr. Ver Hoef is leading the spatial statistical analyses that support the N and S mapping for the study area and he is making comparisons between N and S concentrations in lichen and CALPUFF output variables for N and S compounds. Dr. Brenner is leading the analyses comparing lichen chemistry to measured air quality at co-location sites, and he is also analyzing potential differences in N and S concentrations in lichen between forest-edge and forest-interior sampling sites (see Section 3.1.4).

Currently, due to the delays discussed above in the laboratory analyses, data analyses are still ongoing and near completion. Consequently, all results presented here are preliminary at this time, as we are still refining the analyses and statistical results. Reporting is ongoing, and final reporting on results from this project (with the exception of the receptor modeling and source apportionment component) is expected by the end of September 2010.

2.4 CALPUFF

Mervyn Davies is the lead of the CALPUFF modeling component of this program. The CALPUFF modeling is complete and the CALPUFF output datasets have been finalized and were integrated in the lichen database. The draft report for the CALPUFF modeling component has been completed, and is

attached (see CALPUFF report). Spatial statistical analyses comparing N and S concentrations in lichen to CALPUFF output are still underway, and near completion.

2.5 SOURCE APPORTIONMENT

Dr. Sagar Krupa developed a team of analytical chemists to work on the receptor modeling and source apportionment component of this program. As part of this program, the analytical methods for analysis of trace elements in lichen were tested in fall 2009/winter 2010 using a subset (n=20) of *Hypogymnia physodes* samples from the 2002 TEEM lichen pilot study (Figure A-2, Appendix A). The tested analytical methods have been refined, and form the basis for trace-element analysis in the 2008 lichen samples. Results from the analyses of these 20 *H. physodes* samples will be summarized in a separate report by Dr. Krupa and other key collaborators working on the project.

The first batch of 49 *Hypogymnia physodes* samples from the 2008 sample event was processed and analyzed for trace elements in the winter of 2010. These 49 samples (Table C-2, Appendix C; Figure A-3, Appendix A) were selected based on targeting samples with either:

- high N and S concentrations in lichen;
- low N and S concentrations in lichen;
- high N:S ratios in lichen; or
- low N:S ratios in lichen.

A second batch of 20 lichen samples was selected in February 2010. These samples were selected based on preliminary maps of N and S concentrations in lichen, which showed three key areas of high N and S:

1. near the main oil sands operations;
2. to the far north of the study area; and
3. to the east near the Saskatchewan/Alberta border.

These 20 samples were selected from these elevated N and S areas and are currently being analyzed for trace elements (Table C-2, Appendix C; Figure A-3, Appendix A). Additional samples will be selected during 2010 for trace-element analyses, and progress and results from this work will be reported separately to TEEM by Dr. Krupa.

3.0 Methods

3.1 STUDY DESIGN

3.1.1 Stratified-grid sampling design

Epiphytic lichen was collected in forested sites within a large study area (approximately 7,068,600 ha) surrounding the major oil sands operations. The study area was defined as a circle of 150 km radius, centred on the current main emissions sources (i.e., the Syncrude and Suncor upgraders). Sample sites were located on a stratified grid, with a higher sampling intensity closer to emission sources (Figure A-1, Appendix A).

Lichen sampling was stratified according to five distance strata from the study-area centre: 0-15 km; 15-30 km; 30-65 km; 65-100 km; and 100-150 km (Figure A-1, Appendix A). These strata were designed to account for general trends of N and S in lichen samples from previous studies, collected in 2002 and

2004 (Berryman et al. 2004; CE Jones and Associates 2006; Marsh et al. 2005, draft report). The first two strata (0-15 km and 15-30 km) were selected to cover the previously noted high-deposition area within 30 km of the main emission sources. The third stratum, 30-65 km, was selected based on sites that occasionally showed elevated levels of N and S in lichen samples, primarily to the north and south of the main industrial areas along the Athabasca River Valley, where atmospheric deposition may be higher due wind-flow patterns and/or inversions. Finally, the last two strata (65-100 km and 100-150 km) were selected to represent the areas furthest from the main oil sands operations that may be closer to or at background levels of N and S in lichens.

Our final study design resulted in 359 total sites distributed across the five distance strata (Table 3-1; Figure A-1, Appendix A). Of these 359 sites, 80 sites were sampled to establish spatial autocorrelation estimates for mapping of N and S in lichens across the study area (hereafter referred to as "autocorrelation sites"). These autocorrelation sites were paired spatially with base sites and were distributed evenly across the distance strata, resulting in 16 autocorrelation sites per stratum.

Table 3-1: Number of sample sites for each distance stratum, autocorrelation sites, special interest sites and other sites which includes sites where air quality data are collected and the forest edge/interior paired sites.

Site Type	Number of Sites
Base Sites by Distance Strata	
0-15 km	27
15-30 km	29
30-65 km	28
65-100 km	35
100-150 km	30
Autocorrelation sites, 16 per stratum	80
Special interest areas	82
TOTAL	**311**
Other Site Types (includes Edge/Interior and co-located sites)	48
GRAND TOTAL	**359**

3.1.2 Intensive sampling around special interest areas

Additional intensive sampling was implemented north and south along the Athabasca River Valley, around Ft. McMurray, and southeast of Ft. McMurray (Table 3-1; Figure A-1, Appendix A). The study design incorporated more intensive sampling in these areas for the following reasons:

1. Emissions from the main operations along the Athabasca River may be funneled north and south along the river valley, extending the range of pollutant deposition (Berryman et al. 2004; M. Davies, pers. com.);
2. There is an increasing number of new or developing mine sites adjacent to the Athabasca River Valley and north of the original Syncrude and Suncor operations (thus north of the study centre point;

3. A north-south deposition pattern centred along the Athabasca River Valley is consistent with CALPUFF modeled deposition from recent EIAs (Synenco Northern Lights 2006);
4. Micro-climatic conditions in the river valley (such as fog formation and inversions that may create a fumigation effect of emissions from the main upgrading stacks at Suncor and Syncrude) may enhance deposition in this area and near Ft. McMurray;
5. This area includes the town of Ft. McMurray, with its growing population, and the heavily traveled Highway 63, which runs from Ft. McMurray north to the mine sites and south to Edmonton; and
6. The area south of Ft. McMurray is subject to increasing in-situ development and special-interest sampling sites were specifically located around the Long Lake in-situ project.

With the exception of the Long Lake operation, we did not sample intensively specifically around relatively small-scale and newly developing mining operations in the region as this project was designed to map spatial patterns of N and S in lichen based on current deposition only. These smaller and newly developing operations were not major contributors to overall emissions for the region at the time of field sampling in 2008. Installing additional sampling sites based on "expected" expansion and emissions could be done if TEEM intends to develop this study into a long-term monitoring program, where repeated sampling would be conducted to characterize change in deposition patterns over time.

3.1.3 Co-location of lichen sampling with air quality samplers

Lichen sampling was co-located with air-quality samplers where possible (Figure A-6, Appendix A; Tables C-3 and C-4; Appendix C). This included sampling from sites with:

- passive MAXXAM samplers for nitrogen dioxide (NO_2, ppb) and SO_2 (ppb, Figure A-7; Appendix A);
- Dr. Andrzej Bytnerowicz from the US Department of Agriculture (USDA) passive samplers for nitric acid (HNO_3, $\mu g/m^3$) and ammonium (NH_3, $\mu g/m^3$; see Figure A-8, Appendix A); and
- ion exchange resin (IER) samplers for nitrate (NO_3^-, $kg^{-1}ha^{-1}$), ammonium (NH_4^+, $kg^{-1}ha^{-1}$) and sulphate (SO_4^{2-}, $kg^{-1}ha^{-1}$; see Figure A-9, Appendix A).

In addition, lichen samples were collected near the WBEA continuous Air Monitoring Stations (AMS) where sufficient trees and lichen were present to allow for collection (Tables C-3 and C-4, Appendix C; Figure A-10, Appendix A). This co-location effort was implemented as a preliminary assessment to determine if N and S concentrations in lichen relate to atmospheric deposition or concentration measurements of N- and S-based pollutants. At all of the co-location sample sites, lichens were sampled, processed, prepared, and analyzed according to the methods described below in Section 3.2.

All air-quality sampler types were not present at all co-located sites. In addition, not all air-quality measurements were available for the 2007 and 2008 sample periods, therefore statistical comparisons of measured air-quality data with lichen chemistry were sometimes limited, due to limited air- quality data and small sample size (see Tables C-3 and C-4, Appendix C).

Table 3-2: Number of co-located sites separated by site type, including WBEA AMS sites, TEEM lake sites, TEEM monitoring sites, and the Syncrude lysimeter site.

Co-located Sites	Number of Sites
WBEA Continuous Air Monitoring Stations	11
WBEA Monitoring on lake edges	8
Monitoring Equipment at Syncrude Mildred Lake Operations	1
TEEM Sites	11
Total	**31**

3.1.4 Standardized sampling

The two epiphytic lichen species targeted for sampling only grow on trees and shrubs (boles and branches) and were most abundant on conifers in the AOSR. Our sampling approach was standardized to focus on three vegetation classes:

1. conifer-dominated upland forests;
2. conifer-dominated mixedwood upland forests; and,
3. conifer-dominated wetland forests.

This standardized approach to vegetation types was adopted to minimize within-site and between-site variability, and to improve resultant mapping precision of N and S in lichen. All sampled vegetation types contained conifers to ensure that the target epiphytic lichens would be present for collection. The conifer-dominated upland class included three major vegetation community types: black spruce- (*Picea mariana*) /white spruce-(*Picea glauca*) dominant (and mixed variants of these); jack pine-(*Pinus banksiana*) dominant; and white spruce-dominant stands. The conifer-dominated mixedwood stands included predominantly jack pine/aspen (*Populus tremuloides*) and white spruce/aspen mixes. Aspen-dominated stands were not sampled because aspen is a relatively poor substrate for epiphytic lichens and thus these stands lack sufficient lichen for sampling. Conifer-dominated wetlands included: black spruce-dominant bogs and fens, and mixed black spruce and tamarack (*Larix laricina*) fens.

Vegetation information and mapping was compiled for the study area prior to field sampling, and the sampling grid was adjusted so that sites fell in one of the three vegetation classes described above. The vegetation map was based on Alberta Ground Cover Classification (AGCC) mapping produced for the Cumulative Environmental Management Association's (CEMA)'s Sustainable Ecosystems Working Group. This mapping was derived from an integration of two sources:

1. AGCC derived from 1998-1999 Landsat imagery, through supervised classification conducted by Alberta Sustainable Resource Development (ASRD); and
2. Alberta Vegetation Inventory (AVI) produced through air-photo interpretation for AlPac's Forest Management Agreement Area. AVI classes were subsequently converted to equivalent AGCC classes by the University of Alberta.

These products were merged by ASRD to create seamless coverage of the proposed study area (excluding Wood Buffalo National Park and a portion of Saskatchewan. The AGCC classification map covers approximately 94.7% of the study area, with the outermost edges (100 to 150 km sample stratum) to the north (in Wood Buffalo National Park) and to the east (in Saskatchewan) not mapped.

Nevertheless, this classification is the most complete and consistently mapped vegetation information for the proposed study area.

It should be noted that the accuracy of the AGCC data is limited at the scale of application of this project (where sampling sites represent approximately 0.01-0.1 ha). Mapping accuracy for this project was quantified by comparing site-level vegetation data (measured in the field) to AGCC data for each sample site, with accuracy defined by concordance between field data and the AGCC mapping, using the three vegetation classes defined above. Overall, accuracy for the AGCC map was 50.2%, with highest accuracy for conifer wetlands (75.4%) and lowest for conifer mixedwood stands (18%; see Table 3-3).

Table 3-3: Number of sampled lichen sites that fall within each vegetation type as defined by Alberta Ground Cover Classification Method (AGCC), and the accuracy of the AGCC map for each vegetation class.

Vegetation Class	Number of Sampled Sites	AGCC Map Accuracy
Conifer-dominated uplands (CU)	139	36.6%
Conifer-dominated mixed wood Upland (CMU)	50	18.0%
Conifer-dominated wetlands (CW)	170	75.4%
Total	**359**	**50.2%**

Stand Edge vs. Interior

As noted above, it was important in this study to standardize sampling as much as possible, in order to minimize variability and improve mapping precision. For the standard program, we restricted sampling to forest interiors, as forest edge characteristics varied greatly across the study area. Forest interiors were defined as an area at least 15 m from any stand edge. In addition, we targeted mature to old forest stands where lichen communities have had time to establish and develop, so that sufficient lichen was available for collection without severely impacting the lichen population.

A topic of interest for this project was to characterize potential differences in deposition along large, natural stand edges versus forest interiors, using N and S concentrations in lichen as an indicator. Large, natural forest edges that have maximal leaf area and exposure to advective air movement were hypothesized to receive higher levels of deposition than forest interiors (De Jong and Klaassen 1997; Draaijers et al. 1994; A. Legge, pers. com.; M. Davies, pers. com.). In order to explore this issue, we sampled 13 forest edge/interior paired sites (Table 3-1), targeting sites with edges facing the main emissions sources, and at least 100 m of fetch (open area) on the windward, emissions side of the exposed edge. This type of stand edge was rare in the landscape and, therefore, it was not possible to evenly disperse the paired edge/interior sites throughout the study area (Figure A-4, Appendix A). Consequently, we took an opportunistic sampling approach for locating the edge/interior pairs, and sampled them when we found them in the landscape (mainly based on aerial reconnaissance). In order to try to control variation in this program component, edge/interior pairs were located along an approximate transect to the northeast of the main emissions sources. Thus, with edge characteristics and direction from emissions sources controlled. The forest edge/interior paired sites were sampled following similar methodology as the standard methods described below. However, lichens were collected from both the interior site and the edge site; at the edge site, lichens were only collected from trees along the stand edge, primarily on branches on the edge side of the trees, and measurements specific to the edge were recorded.

3.2 FIELD PROGRAM

3.2.1 Site and sample plot location

All sites were pre-selected using GIS, stratified by distance from the main mining operations and located within one of the three target vegetation classes described above. A list of sites was generated with associated UTM coordinates and vegetation class, and this list was used for site location during field sampling. Most sites were accessed by helicopter, with sites located near industrial development or population centres accessed by vehicle. Accessibility was a limiting factor for the field program, because there were few areas where a helicopter could land directly near the pre-determined site location. To sample the large number of sites across the study area, we had to limit the amount of time spent getting to a site, in order to ensure we could visit all sites and achieve a representative region-wide sample. We took a two-tiered approach for site location to improve field efficiency and ensure a representative, robust, and non-biased sampling. This approach is described below.

The steps for site location were as follows:

1. fly to the original site location using the pre-field UTM coordinates;
2. confirm the mapping of vegetation class at the original site location, or, in the case of incorrect mapping, determine correct vegetation class at the original site location;
3. locate the nearest suitable landing area to the original site location, from the air;
4. determine if the original site can be accessed in less than 5 minutes walking from the landing area (decision made in the air by team leads). If yes, proceed to step “a”, below. If no, proceed to step 5:
    a. land, and locate the original site, using high-accuracy GPS for navigation. If the location of the original site is not in a forest interior, as defined above, adjust the location of plot centre to position it within the forest interior.
5. from the air, locate the nearest helicopter landing area, this landing area was directly adjacent to a forest stand that was:
    a. in the same vegetation class as the original site, or, in the case of adjusted vegetation class (step 2), in the adjusted class;
    b. in a mature stand that has sufficient lichen for collection of at least one of the target lichen species;
    c. in a forest interior, as defined above; and,
    d. the field crew walked in the direction of the original pre-determined sample sites from the helicopter landing spot, the crew walked until they reached an area that was at least 15 m from any stand edge and within the forest interior of a conifer-dominated stand, ideally in the same vegetation stratum as the original site.

Once an original or adjusted plot centre was located, the centre was temporarily established with flagging tape, to provide a reference point for the field crew. Sampling area was variable depending on the stand density and abundance of lichen for collection, but was generally between 0.01 and 0.1 ha.

3.2.2 Stand measurements

At each stand, the location (UTM, NAD 83) of plot centre was recorded using high-accuracy (external-antenna) GPS. Photographs of the stand and target lichen species were taken at each site for documentation (see Access database file for photos of each site). Slope and aspect were recorded at the site, with elevation estimated from the GPS position. The ecosite phase or wetland type was classified for each stand and the plant community type was described (following classifications in: Beckingham and Archibald 1996; Vitt et al. 1996). The forest stand was characterized by visually estimating percent canopy cover of trees and by estimating percent composition of tree species in both the canopy and sub-

canopy. Canopy closure was characterized using a convex densiometer at the plot centre (see Protocol in Appendix D for details on sampling methods).

Additional measurements were recorded at sites sampled along forest edges. Large forest edges represent a transition zone from one vegetation community to another and therefore tended to have a mixed composition of species and variable canopy coverage, often with many tall shrubs in the understory. To characterize the edge we recorded:

- edge orientation to the main emissions sources;
- percent composition of edge tree species and tall shrubs if present; and
- approximate size and shape of opening adjacent to edge (estimate and characterization of fetch).

Densiometer measurements to characterize canopy closure were taken at the plot centre, located directly on the edge border.

3.2.3 Sampling lichen

We focused on two target epiphytic lichen species, *Hypogymnia physodes* and *Evernia mesomorpha,* for this study. Both species are abundant throughout the forested areas of the AOSR, are pollution-tolerant and are good accumulators of N, S and trace elements (Berryman et al. 2004). In addition, *H. physode*s has been documented as a very effective bio-indicator for air pollution world-wide (Pfeiffer and Barclay-Estrup 1992; Rhoades 1999). *E. prunastri*, a morphologically similar species to *E. mesomorpha*, has been shown to be an effective bio-indicator in the Pacific Northwest, particularly for N (Geiser and Neitlich 2007). We selected these two lichen species because they are common across the study area. We did not sample species sensitive to air pollutants for this study, as they do not serve as adequate bio-indicators for our study objectives because many sensitive species are missing from areas exposed to high air pollution deposition (e.g., *Usnea* and *Bryoria* species are missing in stands near Suncor and Syncrude operations; see Berryman et al. 2004). In this study, it was essential to sample lichen across the deposition gradient, and therefore we targeted pollution-tolerant species that were present at most forested sites.

Both target lichen species did not always occur in sufficient abundance at each site for collection. Therefore, we collected a sample of at least one target lichen species per site, collecting both species where possible (Table 3-4). The sample area for lichen collection varied depending on the stand density and the abundance of lichen available for collection, as described above. The crew began collecting the target lichen species from trees nearest to the plot centre and sampled from a minimum of six trees, but typically sampled 12-18 trees per site. Thus, the sampled area varied depending on the stand density and lichen abundance. A larger sampling area was required to obtain sufficient lichen sample in forest stands that were less dense or where lichen was sparse. In most cases, lichen was not collected directly near roads, the stand edge, or major developed area unless there was no other choice, or where the objective was to sample these areas (e.g., AMS sites were typically near disturbances, or we intentionally sampled forest edges for the edge/interior comparisons).

Table 3-4: Summary of lichen samples by site and by species, and summaries of field duplicates (hereafter referred to as "dupes") collected by site and species.

	Total # samples	# sites where collected	# sites with field duplicates	% of sites with at least one duplicate collection	% of samples with duplicate collections
Evernia mesomorpha	414	356	58	16.3%	14%
Hypogymnia physodes	408	355	53	14.9%	13.0%
All samples across both species	822	359	79	22.2%	13.5%

The lichen sampling protocol followed methods for the U.S. Forest Service Air Quality Monitoring Program in the Pacific Northwest, with a few modifications (Geiser 2004). We collected 10-15 grams dry weight of each lichen species into clean, unused sealable bags made from clear saran polyester film (see Protocol in Appendix D for details). Sample weight was determined by a spring-scale, with bag weights deducted. If the lichens were wet during collection, a greater amount of lichen was collected and the amount of lichen was judged by volume rather than weight. Lichens were collected from tree branches only, not tree boles, at a height of at least 1.5 m from the forest floor, since lichens growing at lower heights may be less exposed to atmospheric deposition (e.g., may be covered in snow during part of the year). In order to standardize collection conditions across sites, lichens were typically not collected from shrubs (there were a couple exceptions where tall willows were sampled), litter or fallen branches, since many sites lacked these components, and because lichen on litter may be exposed to varying levels of atmospheric deposition.

All attempts were made to collect only live lichens, avoiding extremely dusty, discoloured, or decaying lichen individuals, and to avoid biasing sampling toward exceptionally robust individuals. However, near the main emissions sources and industrial developments, lichens were often discoloured, dusty, and stunted, and by necessity some of these individuals were included in samples at these locations (decaying lichen tissue was removed from samples during processing). Powderless latex gloves were worn when collecting to prevent introduction of off-site contamination to samples. New gloves were used at each site, and were replaced if they become torn or potentially contaminated. While wearing gloves, the field crew did not touch any non-site materials (e.g., equipment, clothing, skin) except the clean sample bag. Once sufficient lichen was collected, the sample bag was sealed to prevent any contamination during storage and transport.

Duplicate lichen collections (referred to as field dupes) for each lichen species were collected at a subset of sites, evenly spread across the study area, where 13.5% of the sample total (822 samples; see Table 3-4) were field duplicates. These duplicate collections followed the same protocol as above, collecting from the same host tree species and substrate locations, in similar amount (mass/volume) as the first collection. The purpose of field duplicate collection is to assess the proportion of within-site variability in elemental content (i.e., %N and %S) attributable to the field collection method. The lichen collection protocol is designed to achieve a composite sample that is representative (in range of ages, sizes and elemental contents) of the lichen population at the site. By collecting a duplicate sample using identical collection methods, we can assess the extent to which these ranges are captured by the sampling method employed. The less variability there was among field duplicates, as reflected in analytical results, the more likely that the lichen sample is representative of the population mean.

3.3 LICHEN SAMPLE PROCESSING

Samples were frozen the same day of collection to prevent decomposition of damp or wet lichen. Samples were maintained in a frozen state during storage and transport to the processing/cleaning location. Prior to processing, lichens were removed from the polyester sample bags and air-dried in paper bags. The lichen samples were then processed to remove bark, twigs, needles, non-target lichen species, insects, etc. This procedure was performed in a contaminant-free environment using new gloves and a glass surface that was cleaned between each sample. We processed a minimum of 4 grams (dry weight) of lichen for each sample, with the remaining unprocessed lichen archived for potential future use. Processing and cleaning the lichen samples was time-consuming (approximately 90 minutes per sample) since twigs and bark were not easily separated from the lichen during field collections. Processed lichen samples were sealed in new polyester sample bags and sent to the University of Minnesota Research Analytical Laboratory (UMRAL) for further preparation and analysis.

3.4 LABORATORY ANALYSIS

Samples were split at the University of Minnesota Research Analytical Laboratory (UMRAL), reserving up to two grams of un-ground sample for the receptor modeling and source apportionment component of the study. The un-ground sample was placed in labelled polyester samples bags and was stored in the freezer at UMRAL. The remaining sample split was intended for the total N and total S analyses. Each lichen sample was passed through a stainless steel grinder with a 20-mesh sieve to mix the sample thoroughly. Ground samples were dried at 65˚ C for two hours and cooled in a desiccator before weighing for analysis. UMRAL laboratory techniques are described in detail on their website at http://ral.cfans.umn.edu/.

3.4.1 Total sulphur

Total S concentration was determined by dry combusting 0.100-0.150 g of sample covered with a tungsten oxide compound in an oxygen atmosphere at 1350˚ C in a Leco Corp. Sulphur Determinator (Model No. S144-DR). The SO_2 evolved from the sample was determined by a non-dispersive infrared detector, which was empirically calibrated with three LECO plant reference materials (LECO 1026, orchard leaves; LECO 1025, orchard leaves; LECO 1010, tobacco leaves) and with an in-lab check of dried rose leaves (RLV). Instrument precision was poor for measurements of S concentrations near the method detection limit (MDL). The MDL is the concentration of an analyte in a lichen sample that gives a signal equal to two times the standard deviation of the background emission at that wavelength.

3.4.2 Total nitrogen

Total N concentration was determined by dry combustion of a 150-500 mg sample in a gel capsule using a LECO FP-528 Nitrogen Analyzer. Each gel capsule was dropped into an 850˚ C furnace purged with O_2 gas. The combustion products (CO_2, H_2O and NO_2) were filtered, cooled by a thermoelectric cooler to condense most of the water, and collected into a large ballast. A 3 cc aliquot of the ballast combustion product was integrated into a helium carrier stream. The stream first passed through a hot copper column to remove O_2 and convert NO_x to N_2. A reagent tube then scrubbed the remaining CO_2 and H_2O from the stream. N_2 content was measured by a thermal conductivity cell against a helium background and the result was displayed as weight percentage of N. The laboratory ran three calibration standards with LECO plant reference materials (LECO 1006, rice flour; LECO 1026, orchard leaves; and, LECO 1052, ethylenediaminetetraacetic acid) and with an in-lab check of dried rose leaves (RLV).

3.4.3 Reference materials and blanks (quality assurance analysis)

Reference materials, lab checks, and acid blanks were all used in the quality-assurance analysis for the UMRAL analytical methods. Methods used in the quality-assurance analysis are listed below. A full report of the quality assurance results are not presented here, but will be presented with the final report.

NIST and lichen Standard Reference Materials
Two National Institute of Standards and Technology (NIST) standard reference materials (SRM 1547, peach leaves and SRM 1515, apple leaves) were analyzed with each batch of 10 samples in the total N and total S analyses. NIST standards have established concentration ranges. The lichen standard (CRM 482, *Pseudevernia furfuracea*) was also analyzed with each batch in the N and S analyses. The European Commission certified this lichen Reference Material. Analysis results from the NIST standards and the lichen standard were used to assess laboratory accuracy. As noted above, LECO calibration standards and an in-house standard of rose leaves (RLV - uncertified) were also run for both S and N analyses.

The laboratory accuracy was evaluated by comparison of the laboratory measurements of the NIST and LECO standard reference materials to established values for these materials. The percent **inaccuracy** of the UMRAL method was calculated as:

$$\% \text{ inaccuracy} = 100(x_{obs} - x_{true})/ x_{true}$$

where x_{obs} was the UMRAL measurement of a given element in the standard and x_{true} was the published standard value.

Laboratory replicates

Replicate analyses of systematically selected (i.e., every 10^{th} sample) digests were run on 10% of lichen samples for S and N analyses. These repeated measures of the same sample were used to determine laboratory precision, as expressed by relative percent difference (RPD), using the equation:

$$RPD = ABS|(x_1 - x_2)| / (\text{mean } x_1, x_2) *100$$

where ABS is the absolute value; x_1 is the first lab measurement and x_2 is the repeated lab measurement. Re-runs of lichen samples were also considered lab replicates and were used to estimate laboratory precision.

Acid blanks

One or multiple acid blanks were analyzed with each analytical batch of 10 lichen samples. These acid blanks were only run for N analyses, as there was no potential source of S contamination for the total S analyses. These blanks passed through the same digestion/analytical procedures for the N analyses as the lichen samples. The blanks were used to detect and quantify any potential contamination of the samples from the analytical reagents and air leaks. No contamination was present if all measurements of the acid blanks were below the MDL. Contamination occurred when elemental concentrations in blanks were above the MDL. The mean concentrations of elements were calculated for blanks with values above the MDL. The blank mean for that element was then compared to the grand mean of the element concentrations in all lichen samples by calculating the percent of the grand mean for each element:

One or multiple acid blanks were analyzed with each analytical batch of 10 lichen samples, and were used to detect and quantify any contamination of the samples from the analytical reagents. These acid blanks were only run for N analyses, as there is no potential source of S contamination for the total S

analyses. These blanks pass through the same digestion/analytical procedures as the lichen samples. It is deemed that no contamination was present if all measurements of the acid blanks were below the MDL. For elements with acid-blank concentrations above MDLs, the mean element concentrations for all blanks above the MDL was calculated. The blank mean for that element was then compared to the grand mean of the element concentrations in all lichen samples, by calculating the percent of the grand mean for each element, indicating the relative impact the contamination on the laboratory measurements in lichen samples:

% of Grand Mean = [$mean_{blanks}$/grand $mean_{lichens}$] *100

The relative impact the contamination on the laboratory measurements in lichen samples was evaluated using this measure.

3.5 SPATIAL MODELING

The spatial model was developed to achieve three primary objectives:

1. To test and estimate the effects of all available model covariates (e.g., explanatory variables listed below) on N and S loading in lichens. Results from this model were used to interpret patterns of N and S enrichment in lichen across the region (objective #2, below).
2. To predict N and S concentrations in lichens at unmeasured locations for the entire study area, using predictive models. The predictive models only included covariates that were available for the entire study area (e.g., any variables that could be derived from GIS layers) and that were shown to be significant to elemental loading in lichen based on initial results from the covariate modeling at the 359 sample sites, described above.; and,
3. Compare spatial patterns of N and S concentrations in lichens to CALPUFF-modeled concentration/deposition predictions (see section 3.7 for more details on methodology).

Our analysis used a geostatistical spatial model incorporating data from all 359 sample sites, , with Universal Transverse Mercator (UTM) positions providing x and y coordinates for each site in the model. Explanatory variables included distance to centre point (where the centre point was the point equidistant between Suncor and Syncrude main stacks), distance to Syncrude main stack, distance to Suncor main stack, direction from centre point, direction from Syncrude main stack, direction from Suncor main stack, vegetation type (i.e., ecosite phase or wetland type), canopy closure, elevation, aspect, slope, proximity to fire (either within or adjacent to an area burned within the past 10 years; 32 of the 359 sites were within or near a fire), and exposure, if the site was on a natural forest edge (6 of the 359 sites were on a natural edge, these are sites that were not edge/interior pairs). The dependent variable in the model was lichen chemistry, N and S concentrations for each lichen species (*Evernia mesomorpha* and *Hypogymnia physodes),* resulting in four spatial models. Dependent and distance variables were transformed with the natural logarithm (ln). Direction variables were transformed using a sine rotation where the bearing to the centre point, Suncor main stack and Syncrude main stack were transformed following: sine(π[bearing + r/8]), r = 0, 1, 2, or 3). We used a spatial linear model (e.g., Ver Hoef and Cressie 2001), with an exponential autocovariance model,

$$C_\theta(h) = \theta_1 I(h = 0) + \theta_2 \exp(-h/\theta_3)$$

where h is the distance between any two sites, I(a) is the indicator function (equal to 1 if the expression a is true, otherwise it is 0), and the vector θ contains three parameters: the nugget θ_1, partial sill θ_2, and range θ_3. Restricted maximum likelihood (REML) was used to estimate θ because ML is more biased than REML (Mardia and Marshall 1984; Ver Hoef and Cressie 2001). All regression and covariance parameters were fit using PROC MIXED in SAS and degrees of freedom used the Satterthwaite option (Satterthwaite 1941).

The first step in building the spatial model was to assess relationships between explanatory variables and lichen data at the site level. To do this, we first screened all of the explanatory variables for co-linearity, and assessed outlier points in the lichen data using a cross-validation approach. If there were significant outliers, they were deleted from the analysis. A stepwise regression was run using the spatially explicit model to determine which explanatory variables were important to N and S enrichment in lichens, with non-significant ($\alpha \geq 0.05$) variables removed from the model. The final model was determined following the step-wise removal process. Model residuals were checked using Q–Q plots (Wilk and Gnanadesikan, 1968) and appeared to be normally distributed in all cases. Akaike Information Criteria (AIC; Akaike 1973) indicated that there was no evidence of anisotropy in the residuals. A spatial version of R^2 was computed to assess model fit. Results from this model were used to interpret patterns of N and S enrichment in lichens across the study area, and formed the basis for the development of the predictive model.

As part of the model building effort, we estimated the three covariance parameters in the model. The first covariance parameter included: the variance associated with spatial autocorrelation. Spatial autocorrelation refers to the degree of similarity among a set of spatially located data (e.g., lichen samples). Spatially autocorrelated data are not "independent" samples and thus require a spatially explicit model that can quantify variability due to spatial autocorrelation. The second variance parameter was related to within-site variability due to the lichen collection sample methods. A field duplicate collection of lichen was made at a subset of sites to quantify within-site variability. Finally, the third variance component was related to laboratory error and was quantified by replicating elemental analysis for lichen samples (laboratory replicates run every 10 samples, described above). These variances parameters were accounted for (or "filtered out") when developing the predictive model and for making predictions of N and S concentrations in lichens for mapping purposes.

The second step in the modeling was to use results from the first step to inform the development of a spatial model to predict N and S concentrations in lichen for the entire study area. The predictive model was made using model parameters that were identified as important to elemental loading in lichens in the model-building described above (note: important parameters varied by element, N and S, and lichen species). However, these parameters were only used in the predictive model development if they were available for every prediction point on the landscape (data had to be mapped and available in GIS layers). Note that the implication of this constraint is that the accuracy and precision of the GIS data layers directly affects the power of the predictive model (in contrast to the site-level model, in which relationships were based on observed data). The same modeling approach described above was used for the predictive model development.

There are well-known difficulties in making predictions on the log-transformed data and then back-transforming to the original scale (e.g., Cressie 1993, p. 136). Therefore, a Bayesian approach to conditional simulation (see below) was used following De Oliveira et al. (1997) for a log transformation and the exponential covariance, but using the reference prior described by Berger et al. (2001). Predictions were made on a grid of 2332. A total of 200 simulations were run on the prediction grid for each N and S, by lichen species. The predictions at each location were back-transformed from the log scale, and then means over the 200 simulations were used to create maps.

Conditional simulation is an approach that creates multiple simulations (or predictive maps) which collectively provide an estimate of both the local and global variability. This method is preferred over regular Kriging because:

1. Kriging tends to smooth (or average) prediction data locally; and,
2. conditional simulation allows development of more accurate maps that enable calculation of area in the study region above a set threshold value; for example, hectares above an "elevated" threshold for N concentrations in lichen.

3.6 MAPPING

Individual maps for each element (N and S) and lichen species were generated based on the average of the applicable 200 conditional simulations, and preliminary maps are presented in this interim report. Subjective intervals based primarily on natural breaks were created to best graphically represent spatial patterns in lichen chemistry. These maps provide a basis of understanding for the regional "deposition field" for N and S based on lichen chemistry. Predicted N and S concentrations in lichens do not provide absolute deposition values, but provide a regional picture of spatial patterns of average lichen N and S accumulation, which is a measure relative N and S deposition. It is important to note that the maps based on the conditional simulations represent a set of standardized environmental conditions determined by sampling opportunities/constraints and protocols: in this case, conifer-dominated forest interiors.

3.7 CALPUFF

3.7.1 CALPUFF modeling

The CALMET/CALPUFF model system was used to provide predictions of sulphur- and nitrogen-compound concentrations and deposition for comparison to N and S concentrations in lichens. The model system was applied for the five-year period preceding the lichen collection program (October 2003 to September 2008). Emissions of SO_2 and NO_x were determined for a 290 by 700 km area that included WBEA's airshed in the northern portion and the Lakeland Industry and Community Association (LICA) airshed in the southern portion (this area of model application is larger than the lichen study area, as modeling was in part supported by work conducted for Alberta Environment; see CALPUFF report for more details). The source and emission inventory for the simulation was based on information collected from recent project EIAs, the NPRI database, and discussions with the operators of major emission sources located in the WBEA airshed. This inventory information is summarized in Table 1-1.

Meteorological data used for this assessment were based on the application of the CALMET meteorological preprocessor model. The CALMET model used mapped terrain information and land-cover properties, surface meteorological data from 15 sites (i.e., airports and WBEA ambient air-quality stations), and information from the Pennsylvania State/National Centre for Atmospheric Research MM5 mesoscale meteorological model. The 2003 to 2006 MM5 data were obtained from Alberta Environment (AENV), and the 2007 and 2008 MM5 data were generated as part of this study. The CALMET model produced three-dimensionally varying wind, temperature and turbulence fields across the model domain for the five-year simulation period. The CALMET output was used by the CALPUFF model.

The CALPUFF model was used to simulate the transport, dispersion, chemical transformation, and deposition of S and N compounds for the project study area. This model accounts for near-source effects such as transitional plume rise, partial plume penetration as well as longer-range effects such as chemical transformation, wet scavenging, and dry deposition. The model was used to predict hourly, daily, monthly, seasonal, and annual average ambient concentrations, and deposition (wet and dry), of the following S and N compounds: SO_2, SO_4^{-2}, nitric oxide (NO), NO_2, NO_x (the sum of NO and NO_2), HNO_3 and NO_3^- (see Table 3-5). These predictions were made at the 359 lichen sampling sites, 67 ambient continuous and passive ambient air-quality monitoring sites (some of which are included in the 359 lichen sites, due to co-location of lichen sampling and air-quality monitoring sites), and an additional 8,657 Cartesian grid locations across the CALPUFF model study area.

The performance of the CALPUFF model system was gauged by comparing 1-hour, 24-hour and annual average SO_2 and NO_2 concentrations with the corresponding measurements at the WBEA continuous ambient air-quality monitoring stations (Figure A-10; Appendix A). On the whole, the model predicted reasonable concentrations at most of the continuous monitoring stations, high concentrations where high concentrations were measured and low concentrations where low concentrations were measured.

All methods for the CALPUFF modeling are reported in the draft CALPUFF report. The CALPUFF modeling and associated reporting is complete at this time and will be finalized upon review by TEEM.

Table 3-5: Summary of CALPUFF output variables

Output Type	Chemical Compound	Comment
Ambient Concentration	SO_2	Can be provided for 10-hour, 24-hour and annual averaging periods ($\mu g/m^3$)
	SO_4^{-2}	
	NO	
	NO_2	
	HNO_3	
	NO_3^-	
Wet Deposition	SO_2	Combined to provide Total Wet Sulphur deposition (kg S ha^{-1} yr^{-1})
	SO_4^{-2}	
	NO	Combined to provide Total Wet Nitrogen deposition (kg N ha^{-1} yr^{-1})
	NO_2	
	HNO_3	
	NO_3^-	
Dry Deposition	SO_2	Combined to provide Total Dry Sulphur deposition (kg S ha^{-1} yr^{-1})
	SO_4^{-2}	
	NO	Combined to provide Total Dry Nitrogen deposition (kg N ha^{-1} yr^{-1})
	NO_2	
	HNO_3	
	NO_3^-	
Total Deposition (Wet plus Dry)	SO_2	Combined to provide Total Dry Sulphur deposition (kg S ha^{-1} yr^{-1})
	SO_4^{-2}	
	NO	Combined to provide Total Dry Nitrogen deposition (kg N ha^{-1} yr^{-1})
	NO_2	
	HNO_3	
	NO_3^-	

3.7.2 Comparing CALPUFF output to lichen chemistry

The output variables shown in Table 3-5 (hereafter referred to as CALPUFF variables) were summarized annually, where annual summaries were cumulative from the nominal date to the date of lichen sampling completion (for example, total N deposition 2005 represents a cumulative sum of N deposition from October 2005 to August 2008). In addition, the CALPUFF output variables were summarized by two seasons: Summer (May - September) and Winter (October - April), and were cumulatively summed by season from the nominal date to the date of lichen sampling completion.

Due to the large number of output variables generated by the CALPUFF modeling, the initial task in the CALPUFF comparison analysis was to develop a statistical method to find the CALPUFF variable that best related to N and S concentrations in lichen. This was accomplished through spatial regression, in which each CALPUFF variable was modeled as a potential predictor of lichen chemistry, where N-based

CALPUFF variables were compared to N concentrations in lichen and S-based CALPUFF variables were compared to S concentrations in lichen. This analysis was conducted separately for both lichen species. Since most CALPUFF variables were highly correlated (see Tables 7-1 through 7-4 in the CALPUFF report) often the predictive power was similar across variables and across years. This means in practice that correct selection of a single CALPUFF variable for comparative analysis to lichen chemistry is of reduced importance, as, for example, most CALPUFF sulphur variables behave similarly, and thus results from comparative analysis of one CALPUFF S variable are likely to be very similar to those from another CALPUFF S variable. Given this similarity, the analytical approach that we are using to conduct comparative analyses of CALPUFF variables focuses on two CALPUFF variables per analysis (for each element [N and S] and each lichen species):

1. the CALPUFF variable with the best relationship with lichen chemistry, based on the AIC statistic from the models, where the lowest AIC value indicated the best predictor; and,
2. the CALPUFF variable representing annual total deposition (including background and wildfire deposition) for the period October 2007-August 2008 (the approximately one-year period preceding the lichen sampling). This latter variable was selected for inclusion as lichens have the ability to absorb all forms of N and S (hence total deposition including background and fire) and, since both of these elements are mobile macronutrients, the residence time in the living lichen is unclear. The N and S concentrations in lichen are likely most reflective of a 1-year exposure period (hence the October 2007-August 2008 time period).

The next step in the analyses was to determine how the best CALPUFF predictor variable (as described above) and lichen chemistry differ spatially and to asses which factors (explanatory variables) were most related to these differences. Explanatory variables were the same as those described in section 3.5 above. The dependent variables for these analyses were the “differences” between selected CALPUFF variables and corresponding lichen chemistry. To create these “difference” variables, each variable was standardized (both CALPUFF and lichen chemistry) by subtracting the mean and then dividing each value by the standard deviation of the mean. This resulted in each variable ranging between -2 and 2 (i.e., this standardization approach creates variables that are normally distributed with a mean of zero and a variance of 1). After standardizing, differences were taken for the standardized CALPUFF variable and standardized lichen concentration (i.e., %N and %S): [CALPUFF model variable] - [lichen concentration]. In this case, the lichen concentration represents the “benchmark” for comparison, where we are evaluating the CALPUFF model variable deviation from measured lichen concentration. Using this approach, we would expect this difference to be zero in most cases because the average of each variable is zero (from standardizing), and if the variables had exactly the same spatial pattern, the differences would be zero everywhere in the study area. Where the difference is not zero, this indicates divergence between the CALPUFF variable and lichen concentrations.

These differences were then modeled spatially. A backward-stepwise regression-modeling procedure was used, where non-significant explanatory variables (p-values greater than 0.05) were removed from the model. Final models were used to interpret key factors related to differences between the CALPUFF variable and lichen concentrations at each of the 359 lichen sites. Predictions of differences were made on a grid of 2332 using the spatial model and an ordinary Kriging approach. These predicted differences were then mapped to indicate areas where divergences between CALPUFF variable and lichen chemistry were greatest.

At this time, the CALPUFF variables for comparative analyses (with lichen chemistry) have been selected, as above, but these analyses have not been finalized. This report provides an example of our proposed analytical approach, to illustrate interpretation and presentation methods. The completed analyses will be provided in the final project report. Note that the final report will also contain a more comprehensive description of statistical analytical methods used in these analyses.

3.8 COLOCATION – COMPARISONS OF AIR QUALITY MEASUREMENTS TO LICHEN CHEMISTRY

At co-located sites, N and S concentrations in lichens were compared to air-quality data from various samplers using two-sided correlation tests based on Spearman's and Kendall's correlation coefficients. Both Spearman and Kendall are coefficients of rank correlation follow a non-parametric approach using ranks. These comparisons of lichen chemistry to air-quality measurements were made for averages by season (winter, May-September; and summer, October - April) and for annual averages (October – September) for air concentrations and deposition measurements. Seasonal and annual averages of air quality measurements were made for years 2007 and 2008, starting in the winter of 2007 through the summer of 2008, as this time period is most relevant to potential N and S loading prior to the lichen sampling. Due to the single sample period for lichen, these comparisons do not allow evaluation of deposition rates or short-period fluctuations in air-quality data (e.g., month-to-month changes), nor do they allow for the use of direct air-quality data to inform estimates of elemental flux periods in lichens. To achieve this, transplant studies or experimental studies would be necessary.

This portion of the statistical analyses is still ongoing, and complete methodology and results will be presented in the final report. The next phase of these analyses will be to select a subset of air- quality measures (or compounds) that have strong and significant correlations with lichen chemistry, in order to develop predictive modeling. Models will be established to predict a given air-quality measure (e.g., NO_2 concentrations, ppb, at sites with MAXXAM passive samplers) based on lichen N chemistry and site characteristics. Explanatory variables used in model development will include: lichen chemistry; distance from centre point, direction from centre point, vegetation type, elevation, slope, aspect, and canopy closure. The intent of this model is to use lichen chemistry as a predictive tool to estimate concentrations or deposition of various N and S compounds at remote sites where air-quality samplers are not present.

Further, we will evaluate relationships between lichen chemistry, air-quality measures and lichen response measures at a given site. At a subset of sites (20 sites), we conducted selected lichen response measures, and will be evaluating how these measures relate to air quality, as a measure of potential pollution effects to lichen. These measures included:

1. Twig and bole bark pH, to characterize the substrate that *E. mesomorpha* was growing on. N and S deposition has been show to change bark pH (Kermit and Gauslaa 2001; van Herk 2001; Wolseley and Pryor 1999).
2. Stress ratio of *E. mesomorpha* individuals sampled from the same locations as used for bark pH testing. Stress ratio is calculated based on lichen age, size and weight (see Protocol in Appendix C for more details; B. McCune, unpublished data).
3. Chlorophyll content of *E. mesomorpha* individuals sampled from the same locations as used for bark pH testing.

Note that results from these analyses will be included in the final report, and are included solely as a preliminary assessment of how some of these measures may be useful for long-term monitoring of air pollution effects to lichens.

4.0 Preliminary Results - Key Findings

It is important to note that statistical analyses are still ongoing at the time of this interim report; although these analyses are nearing completion, and every effort has been made to include the most current information and interpretations in this report, the reader should be aware that final results, figures and

tables are subject to change. Thus, the following discussion is limited to an overview of the key findings; all final results will be presented in the final report.

4.1 PATTERNS OF NITROGEN AND SULPHUR IN LICHEN

We analyzed lichen samples from 359 sites in the region for N and S concentrations. We collected two lichen species (*Hypogymnia physodes* and *Evernia mesomorpha*) from nearly every site (Table 4-1 presents sample numbers and summary statistics by lichen species and analyzed element). Results show that *E. mesomorpha* was a better overall indicator (accumulator) of N and S deposition; this was also shown in the2002 pilot work (Berryman et al. 2004). However, patterns of N and S in *H. physodes* were very similar to those in *E. mesomorpha*.

Table 4-1: Summary of measured N and S concentrations in *Evernia mesomorpha* and *Hypogymnia physodes*. n = number of sites where the corresponding species was collected. Min = minimum N or S concentration value; Max = maximum N or S concentration value; Mean = average of N or S concentration value; Max = maximum N or S concentration value; Mean = average of N or S concentrations across all samples for the corresponding species; and StDev = standard deviation of the average across all samples for the corresponding species. All values other than n are based on % dry weight of N or S in lichen

Element	N	Min	Max	Median	Mean	StDev
Evernia mesomorpha						
Nitrogen	356	0.514	2.832	0.874	0.959	0.274
Sulphur	356	0.043	0.362	0.084	0.095	0.039
Hypogymnia physodes						
Nitrogen	355	0.565	2.676	0.773	0.816	0.194
Sulphur	355	0.038	0.206	0.070	0.071	0.019

For reasons given above, discussion in this interim report focuses on results from spatial modeling for *E. mesomorpha*. Preliminary maps for both lichen species are presented in Appendix B, including both observed and predicted N and S concentrations in lichen for the study area. The highest N and S concentrations in lichen were in the vicinity of the main oil sands operations (see Figures B-3, B-4, B-5 and B-6, Appendix B). The distance and direction from the main oil sands development were key factors related to N and S enrichment in lichen (see Tables 4-2 and 4-3 for spatial regression results for N and S concentrations in *Evernia mesomorpha*; these models have R^2 =0.51 and 0.57, for N and S, respectively). N and S concentrations in lichen generally decreased with increasing model distance from the main oil sands emissions-source region, with the lowest concentrations found to the west (i.e., predominantly upwind) of this region. Elevated concentrations of N and S extended along a northern corridor from the main oil sands emissions-source region towards the Athabasca delta, south of Lake Athabasca (see Figures B-3, B-4, B-5 and B-6, Appendix B). Elevated N levels in this area have also been documented by passive-sampler measurements of ambient air concentrations of nitric acid and ammonia (Bytnerowicz et al. 2010). Lichen N and S concentrations were also more elevated to the east than to the west, with higher N concentrations at sites near the Alberta-Saskatchewan border. Potential causes of this observed N and S enrichment at the eastern study-area boundary are not known at this time. Elevated concentrations are possibly due to an influence of forest fires in Saskatchewan, as this area is dominated by jack pine uplands characterized by frequent large wildfires. Results from the receptor modeling and source apportionment component of this project will assist in identifying source types contributing to elevated N and S levels near the Athabasca delta and the Alberta-Saskatchewan border.

These strong patterns of N and S deposition as indicated by lichen are related to distance and direction from the main oil sands operations (see Tables 4-2 and 4-3), but the actual factors driving these

deposition patterns are key variables we were unable to measure or characterize at our sites, such as meteorological influences, atmospheric chemistry, and emissions and dispersion characteristics. The spatial modeling conducted for this study incorporated distance and direction variables as surrogates for these unmeasured factors that are relevant to the regional deposition field, as direct data on meteorology and atmospheric chemistry were not available for study sites

Other site-level factors, such as proximity to fire and vegetation type, were more related to local variation of N and S concentrations in lichen (see Tables 4-2 and 4-3). Proximity to fire (as indicated by sampling either within or adjacent to areas burned within 10 yrs) was related to slightly higher concentrations of N in lichen (see positive estimate in Table 4-2 for *Evernia mesomorpha*, based on the natural log transformation of N concentrations). This trend likely represents a local enrichment effect related to N emissions from the actual fire event, but may also be related to post-fire site conditions, such as vegetation type, exposure, and changes in nutrient processing.

Site vegetation was classified during field sampling and was significantly related to N and S concentrations in lichens (Tables 4-2 and 4-3 for *Evernia mesomorpha*). Lichens in conifer-dominated uplands (pine and spruce forests) had highest N and S concentrations. This is likely related to the high surface area that these forest canopies present for both dry and wet deposition, which, during rain events, can wash off and load lichen with N and S (as throughfall wash). We sampled few sites in stands with significant hardwoods in the overstory (Table 3-3), but lichens at these sites had highest levels of S and N compared to conifer-only upland types. The lowest values for N and S concentrations in lichens were in forested wetlands (bogs and fens), which were dominated by black spruce in bogs and black spruce and larch in fens. The stature of these trees was much smaller compared to upland stands, and the overstory canopy cover is substantially lower; therefore, there was less canopy surface area for accumulation of deposition compounds, and less resultant deposition to lichens through throughfall wash.

Table 4-2: Spatial model regression results examining relationships between N concentrations in *Evernia mesomorpha* and measured covariates (i.e., effects) at all sites where this lichen was collected (R^2=0.51). SE = standard error of the model estimate; df = the degrees of freedom based on the total number of samples, including field and laboratory duplicates; T = the T statistic from the regression model. The vegetation type represents a categorical variable in model, where the model uses jackpine lichen upland was the reference for this variable.

Effect	Estimate	SE	df	T	P-value
Intercept	2.53	0.23	467	10.92	<0.001
Log Distance to Centre Point	-1.39	0.15	467	-9.01	<0.001
Log (Distance to Centre Point)^2	0.19	0.02	467	7.66	<0.001
(UTM E)^2	-0.05	0.01	467	-4.70	<0.001
(UTM N)^2	-0.03	0.01	467	-2.44	0.015
Vegetation type (ecosite phase)					
jack pine lichen, upland	0.00	NA	NA	NA	NA
jack pine-aspen with blueberry, upland	-0.03	0.04	467	-0.91	0.363
wooded bog, wetland	-0.21	0.03	467	-6.66	<0.001
jack pine-black spruce with Labrador tea (mesic), upland	-0.17	0.04	467	-4.32	<0.001
aspen with low-bush cranberry, upland	0.03	0.03	467	0.89	0.372
balsam poplar-aspen with dogwood, upland	0.18	0.06	467	2.85	0.005
balsam poplar-white spruce with horsetail, upland	0.16	0.07	467	2.40	0.017
wooded fen, wetland	-0.22	0.03	467	-7.34	<0.001
black spruce-jack pine with Labrador tea (sub-hygric), upland	-0.30	0.06	467	-5.09	<0.001
white spruce-black spruce with Labrador tea and horsetail, upland	-0.21	0.04	467	-5.22	<0.001
Fire	0.09	0.03	467	2.77	0.006

Table 4-3: Spatial model regression results examining relationships between S concentrations in *Evernia mesomorpha* and measured covariates (i.e., effects) at all sites where this lichen was collected (R^2=0.57). See Table 4-2 for definitions of variables. The vegetation type represents a categorical variable in model and therefore the jackpine lichen upland was the reference for this variable.

Effect	Estimate	SE	df	T	P-value
Intercept	0.36	0.22	459	1.64	0.102
Log Distance to Centre Point	-1.29	0.13	459	-9.77	<0.001
Log (Distance to Centre Point)^2	0.15	0.02	459	8.07	<0.001
UTM E	0.26	0.10	459	2.53	0.012
UTM N	0.03	0.01	459	2.30	0.022
(UTM E)^2	-0.03	0.01	459	-4.32	<0.001
Vegetation type (ecosite phase)					
jack pine lichen, upland	0.00	NA	NA	NA	NA
jack pine-aspen with blueberry, upland	-0.08	0.05	459	-1.53	0.127
wooded bog, wetland	-0.25	0.04	459	-5.86	<0.001
jack pine-black spruce with Labrador tea (mesic), upland	-0.23	0.05	459	-4.50	<0.001
aspen with low-bush cranberry, upland	0.01	0.05	459	0.27	0.787
balsam poplar-aspen with dogwood, upland	0.22	0.08	459	2.58	0.010
balsam poplar-white spruce with horsetail, upland	0.05	0.09	459	0.59	0.555
wooded fen, wetland	-0.25	0.04	459	-6.17	<0.001
black spruce-jack pine with Labrador tea (sub-hygric), upland	-0.44	0.08	459	-5.59	<0.001
white spruce-black spruce with Labrador tea and horsetail, upland	-0.21	0.05	459	-3.81	<0.001
Log Distance to Centre Point*Sine Bearing from Centre Point	0.22	0.06	459	3.52	<0.001
Log (Distance to Centre Point) ^{2}*Sine Bearing from Centre Point	-0.08	0.02	459	-3.12	0.002

Results from the regression analyses indicated that certain site-level variables were important predictors for lichen chemistry (total N and S concentrations) and as a result, these variables were used in making predictive maps of N and S concentrations in lichen for the entire study area (including non-sampled areas). See Figure B-1 (in Appendix B) as an example of a preliminary map for N in *Evernia mesomorpha* based on a predictive model (R^2= 0.31) with the following GIS data predictors: distance and direction from main mining operations, elevation (where elevation is serving as a surrogate for vegetation type), and proximity to fire (see Table 4-4). Prediction error was greatest in this map directly in the centre, where we were unable to sample within actual mine footprints (due to lack of ecosystems supporting epiphytic lichens and restricted access) and on the far edges of the study area, where distances between sample sites were greater (see prediction error in Figure B-9, Appendix B). The predictive mapping for S in *E. mesomorpha* follows the same general patterns (Figures B-2 and B-10, Appendix B). Such maps inform overall patterns of N and S deposition in the region and identify low- and high-deposition areas that may be relevant for future long-term monitoring efforts. Further, with repeated sampling over time, this approach can be integrated into long-term monitoring to assess temporal “changes” in patterns of N and S deposition as indicated by lichen.

Table 4-4: Predictive spatial regression model for N concentrations in *Evernia mesomorpha* based on GIS variables (R^2=0.31). See Table 4-2 for definitions of variables

Effect	Estimate	SE	df	T	P-value
Intercept	2.72	0.29	472	9.39	<0.001
Log Distance to Centre Point	-1.53	0.19	472	-8.01	<0.001
Log (Distance to Centre Point)^2	0.22	0.03	472	7.10	<0.001
UTM E	0.25	0.10	472	2.61	0.009
(UTM E)^2	-0.06	0.01	472	-4.34	<0.001
(UTM N)^2	-0.05	0.02	472	-2.67	0.008
Elevation	0.00	0.00	472	-3.84	<0.001
Fire	0.14	0.07	472	1.97	0.049
Log Distance to Centre Point*Sine Bearing from Centre Point	0.16	0.06	472	2.69	0.007
Log (Distance to Centre Point) ^{2}*Sine Bearing from Centre Point	-0.06	0.02	472	-2.71	0.007

Table 4-5: Predictive spatial regression model for S concentrations in *Evernia mesomorpha* based on GIS variables (R^2=0.44). See Table 4-2 for definitions of variables.

Effect	Estimate	SE	df	T	P-value
Intercept	1.08	0.35	467	3.10	0.002
Log Distance to Centre Point	-1.80	0.23	467	-7.84	<0.001
Log (Distance to Centre Point)^2	0.25	0.04	467	6.65	<0.001
UTM E	0.38	0.12	467	3.25	0.001
(UTM E)^2	-0.07	0.02	467	-4.45	<0.001
(UTM N)^2	-0.05	0.02	467	-2.56	0.011
Elevation	0.00	0.00	467	-4.07	<0.001
Log Distance to Centre Point*Sine Bearing from Centre Point	0.28	0.07	467	3.85	<0.001
Log (Distance to Centre Point) ^{2}*Sine Bearing from Centre Point	-0.10	0.03	467	-3.68	<0.001

We present preliminary predictive maps for each element and by lichen species in Appendix B; final maps will be included in final report. Large-scale patterns of N and S concentrations in both lichen species are similar, and provide a basis for understanding the regional deposition field for N and S based on lichen chemistry. Concentrations of N and S in lichens do not provide absolute deposition values, but provide a regional picture of spatial patterns of average lichen N and S accumulation, which is a measure that is reflective of overall N and S deposition.

4.1.1 Edge/interior comparisons

N and S concentrations in both lichen species showed slightly higher levels along natural forested edges than forest interiors. These natural forest edges were selected specifically to have a fetch of at least 100 m (e.g., adjacent to a very open sedge fen) and they were facing the main emissions sources. This particular type of forest edge is not common in the AOSR. After accounting for site level variables in both edge and interior paired sites (i.e., distance and direction from the centre point, and vegetation type), regression results indicated significant differences in both N and S concentrations in lichen between the stand edge and interior.

In *Evernia mesomorpha*, S concentrations on edges were on average increased by 0.024%, in comparison to stand interiors (P-value = 0.001 for the estimate; Model F-statistic = 8.78, on 8, 17 df; P-value < 0.001, R^2 = 0.81). In *Hypogymnia physodes*, S concentrations on edges were higher on average by 0.019%, in comparison to stand interiors (P-value <0.0001 for the estimate; Model F-statistic = 9.32, on 8,17 df; P-value = < 0.001; R^2=0.81). This difference in S concentrations in lichen between stand edges and interiors is fairly substantial, and should be considered when looking at predictions of S concentrations in lichen (Figures B-7 and B-8, Appendix B) as these predictions are relevant to conifer-dominated forest interiors. However, it is important to note that the type of natural forest edge we sampled was rare in the landscape, with the more common forest edge existing due to cut-lines and cut-blocks. These anthropogenic forest edges are likely more similar to stand interiors in terms of deposition characteristics, due to the abrupt edge (lower overall canopy surface area along the edge) and lack of fetch.

Nitrogen concentrations in *Evernia mesomorpha* on edges were on average increased by 0.022% in comparison to stand interiors (P-value < 0.001; Model F-statistic = 7.14, on 8,17 df; P-value < 0.001, R^2=0.77). In *Hypogymnia physodes*, N concentrations on edges were on average increased by 0.015% in comparison to stand interiors (P-value <0.001; Model F-statistic = 8.42, on 8,17 df; P-value < 0.001, R^2 = 0.80). These observed differences are relatively small compared to absolute N concentrations, and are approximately one order of magnitude less than the differences observed in S concentrations.

4.2 CALPUFF

4.2.1 Summary of CALPUFF modeling results

Total S and N deposition predicted by CALPUFF modeling is presented in Figure E-3, in the Executive Summary of the accompanying CALPUFF report. These plots represent the cumulative five-year average annual deposition for these elements for the period October 2003 to September 2008. The maximum S deposition during this period was near the upgraders that are the main sources of SO_2 emissions in the project study area. The maximum N deposition was located near the upgraders and the mine areas that are the main sources of NO_x emissions in the project study area.

The deposition estimates presented in Figure E-3 of the CALPUFF report include emissions from six wildfires that occurred in the study area during the 2004 to 2007 wildfire seasons. The main contribution due to the wildfires appears south of Fort Chipewyan, due to the relatively large Old Fort wildfire that occurred in July 2007. While the wildfires did not have a strong influence on the overall S and N deposition patterns as predicted by CALPUFF, they can produce local contributions that may influence local ambient air quality and lichen tissue measurements.

4.2.2 Preliminary results for comparing CALPUFF output to lichen chemistry

CALPUFF predictions of N and S compounds were related to N and S concentrations in lichen (see Figures 4-1 and 4-2 as examples for *Evernia mesomorpha*). Using spatial modeling, we evaluated where CALPUFF and lichen chemistry diverged most, and found that the main factors related to these differences included vegetation type (i.e., ecosite phase), proximity to fire, and distance and direction from the main mining operations (Tables 4-6 and 4-7 for *Evernia mesomorpha*). Enrichment of N and S concentrations in lichen was significantly related to vegetation type (see above Tables 4-4 and 4-5); however, the CALPUFF model output does not show fine-scale differences in N and S deposition due to vegetation type, as the vegetation input dataset has a 5 km^2 resolution. Further, even though the CALPUFF model output included deposition estimates from fire, lichen chemistry appears to be more sensitive to local N enrichment from fire, and is a better indicator of these local-scale patterns (Table 4-6).

Table 4-6: Spatial regression model (R^2=0.40) for testing factors related to log differences between N concentrations in *Evernia mesomorpha* and CALPUFF predicted annual total N deposition with background and wildfire (kg $ha^{-1}yr^{-1}$). See Table 4-2 for definitions of variables. The vegetation type represents a categorical variable in model and therefore the jackpine lichen upland was the reference for this variable.

Effect	Estimate	SE	df	T	P-value
Intercept	-9.10	1.20	470	-7.58	<0.001
Log Distance to Centre Point	6.06	0.75	470	8.03	<0.001
Log (Distance to Centre Point)^2	-1.00	0.12	470	-8.14	<0.001
(UTM E)^2	0.27	0.07	470	4.02	<0.001
(UTM N)^2	0.29	0.05	470	5.35	<0.001
Vegetation type (ecosite phase)					
jack pine lichen, upland	0.00	NA	NA	NA	NA
jack pine-aspen with blueberry, upland	0.13	0.14	470	0.92	0.356
wooded bog, wetland	0.77	0.12	470	6.24	<0.001
jack pine-black spruce with Labrador tea (mesic), upland	0.62	0.14	470	4.34	<0.001
aspen with low-bush cranberry, upland	0.09	0.13	470	0.69	0.488
balsam poplar-aspen with dogwood, upland	-0.73	0.24	470	-3.04	0.003
balsam poplar-white spruce with horsetail, upland	-0.26	0.24	470	-1.10	0.274
wooded fen, wetland	0.83	0.11	470	7.49	<0.001
black spruce-jack pine with Labrador tea (sub-hygric), upland	1.11	0.23	470	4.73	<0.001
white spruce-black spruce with Labrador tea and horsetail, upland	0.80	0.15	470	5.34	<0.001
Fire	-0.34	0.12	470	-2.86	0.004

Table 4-7: Spatial regression model (R^2=0.26). for testing factors related to log differences between S concentrations in *Evernia mesomorpha* and CALPUFF predicted annual total S deposition with background and wildfire (kg $ha^{-1}yr^{-1}$). See Table 4-2 for definitions of variables. The vegetation type represents a categorical variable in model and therefore the jackpine lichen upland was the reference for this variable.

Effect	Estimate	SE	df	T	P-value
Intercept	-0.46	0.13	462	-3.65	<0.001
UTM E	-0.35	0.16	462	-2.17	0.031
UTM N	-0.11	0.05	462	-2.06	0.040
$(UTM N)^2$	-0.13	0.03	462	-4.02	<0.001
(UTM E)(UTM N)	-0.08	0.04	462	-2.00	0.046
Vegetation type (ecosite phase)					
jack pine lichen, upland	0.00	NA	NA	NA	NA
jack pine-aspen with blueberry, upland	0.22	0.13	462	1.73	0.084
wooded bog, wetland	0.65	0.12	462	5.50	<0.001
jack pine-black spruce with Labrador tea (mesic), upland	0.65	0.13	462	4.88	<0.001
aspen with low-bush cranberry, upland	0.05	0.12	462	0.38	0.701
balsam poplar-aspen with dogwood, upland	-0.40	0.22	462	-1.84	0.066
balsam poplar-white spruce with horsetail, upland	0.21	0.21	462	0.99	0.324
wooded fen, wetland	0.72	0.10	462	6.84	<0.001
black spruce-jack pine with Labrador tea (sub-hygric), upland	1.01	0.22	462	4.60	<0.001
white spruce-black spruce with Labrador tea and horsetail, upland	0.48	0.14	462	3.46	0.001
Log Distance to Centre Point*Sine Bearing from Centre Point	0.19	0.08	462	2.31	0.021

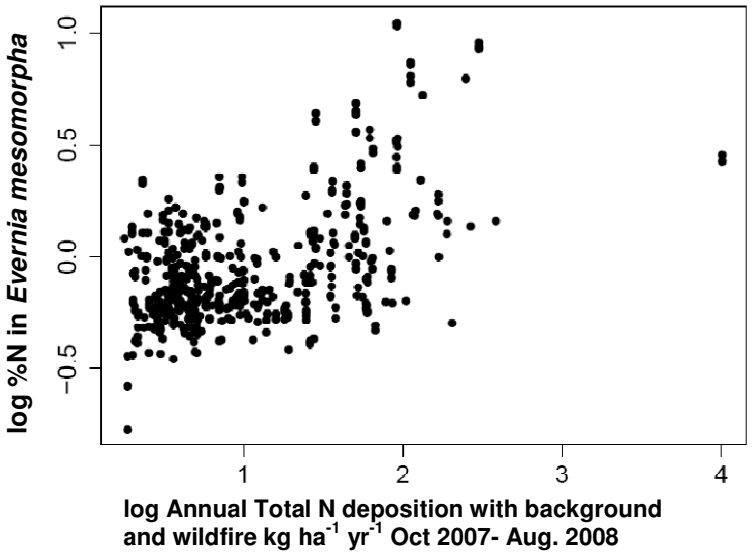

Figure 4-1: Scatter plot of log-transformed N concentrations in *Evernia mesomorpha* by the log transformed CALPUFF variable, annual N-deposition including background and wildfires for the period October 2007-August 2008.

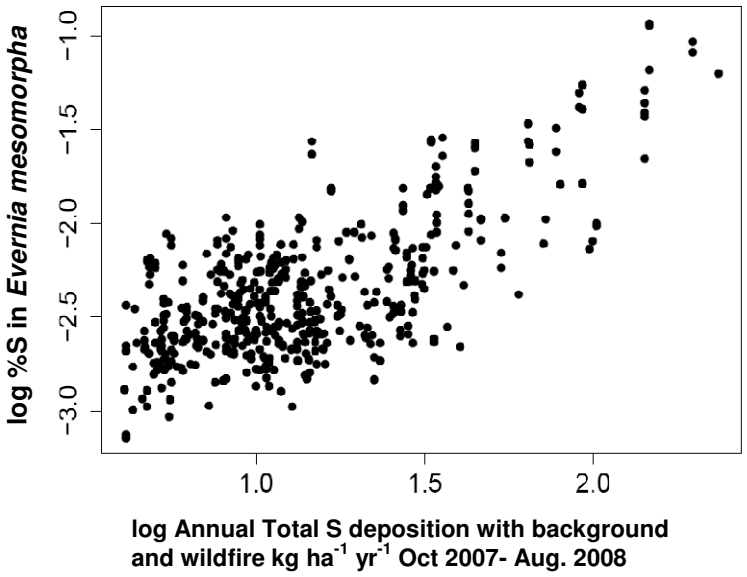

Figure 4-2: Scatter plot of log-transformed S concentrations in *Evernia mesomorpha* by the log transformed CALPUFF variable, annual S-deposition including background and wildfires for the period October 2007-August 2008.

Predictions of these differences between lichen chemistry and CALPUFF output were mapped on the log scale for:

- N concentrations in *Evernia mesomorpha* and CALPUFF-predicted annual total N deposition with background and wildfire (Figure 4-3); and,
- S concentrations in *E. mesomorpha* and CALPUFF-predicted annual total S deposition with background and wildfire (Figure 4-4).

The values near zero in these maps (light-green colours) indicate areas of relatively good agreement between lichen chemistry and CALPUFF predictions. Higher(more positive, or red-coloured) values are associated with areas where CALPUFF predictions suggest higher relative N and S deposition than observed in sampled lichens, while lower(more negative) values are associated with areas where CALPUFF predictions suggest lower relative N and S deposition than observed in sampled lichens (Figures 4-3 and 4-4).

In general, the information presented in Figures 4-3 and 4-4 show areas where lichen chemistry measurements indicate:

- Lower deposition than would be predicted by the CALPUFF model. For N, these areas are in direct proximity to current mining and upgrading operations, primarily to the north of the centre point for the Suncor and Syncrude main stacks. For S, these areas are primarily directly to the northwest and east of the main emissions sources.
- Higher deposition than would be predicted by the CALPUFF model. For N, this occurs in isolated areas at greater distances from the central mining and upgrading operations, and may be due to local fire effects not captured by the CALPUFF modeling. For S, these area occur primarily at the northern, eastern and southwestern margins of the study area.

Overall, observed differences between CALPUFF output and predicted lichen chemistry are more prominent for S than for N. CALPUFF generally predicts highest S deposition immediately adjacent to the upgrading operations. In some cases, these modeled high deposition values are supported by S concentrations in lichen, particularly in the north-south axis from the upgraders. This comparison suggests that CALPUFF does not universally over-predict S deposition at near-source locations, but does over predict in specific areas to the northwest and east of the upgrading operations, at least as indicated by comparison to lichen chemistry.

Since lichens are biological organisms, continually exposed to atmospheric deposition, they integrate pollutant compounds over time and capture local variability in N and S deposition in the landscape. In contrast, the CALPUFF model predicts larger scale gradients of N and S deposition for the region based on major emissions sources. Note that as with all models, the performance of the CALPUFF model is limited by the accuracy of its input datasets, and some of the uncertainty and limitations of the model output may be directly related to data limitations such as inaccurate and coarse-scale vegetation data, and incomplete emissions inventory data. If the input datasets utilized in CALPUFF were improved, model performance in terms of representing regional deposition would likely improve.

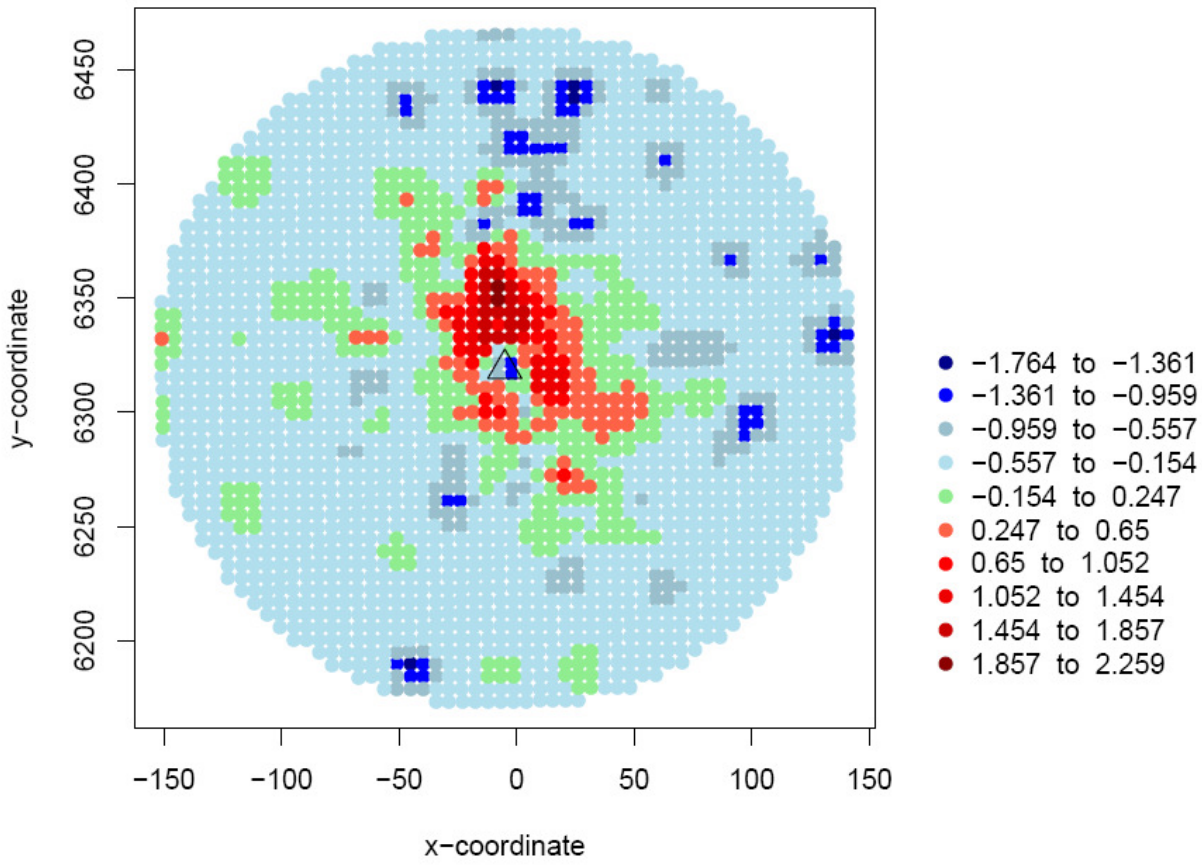

Figure 4-3: Map of predicted log differences between N concentrations in *Evernia mesomorpha* and CALPUFF-predicted annual total N deposition with background and wildfire (kg $ha^{-1}yr^{-1}$). Prediction points are based on Universal Kriging and are shown on x,y coordinates for the study area. The triangle represents the centre point between the Suncor and Syncrude main upgrader stacks

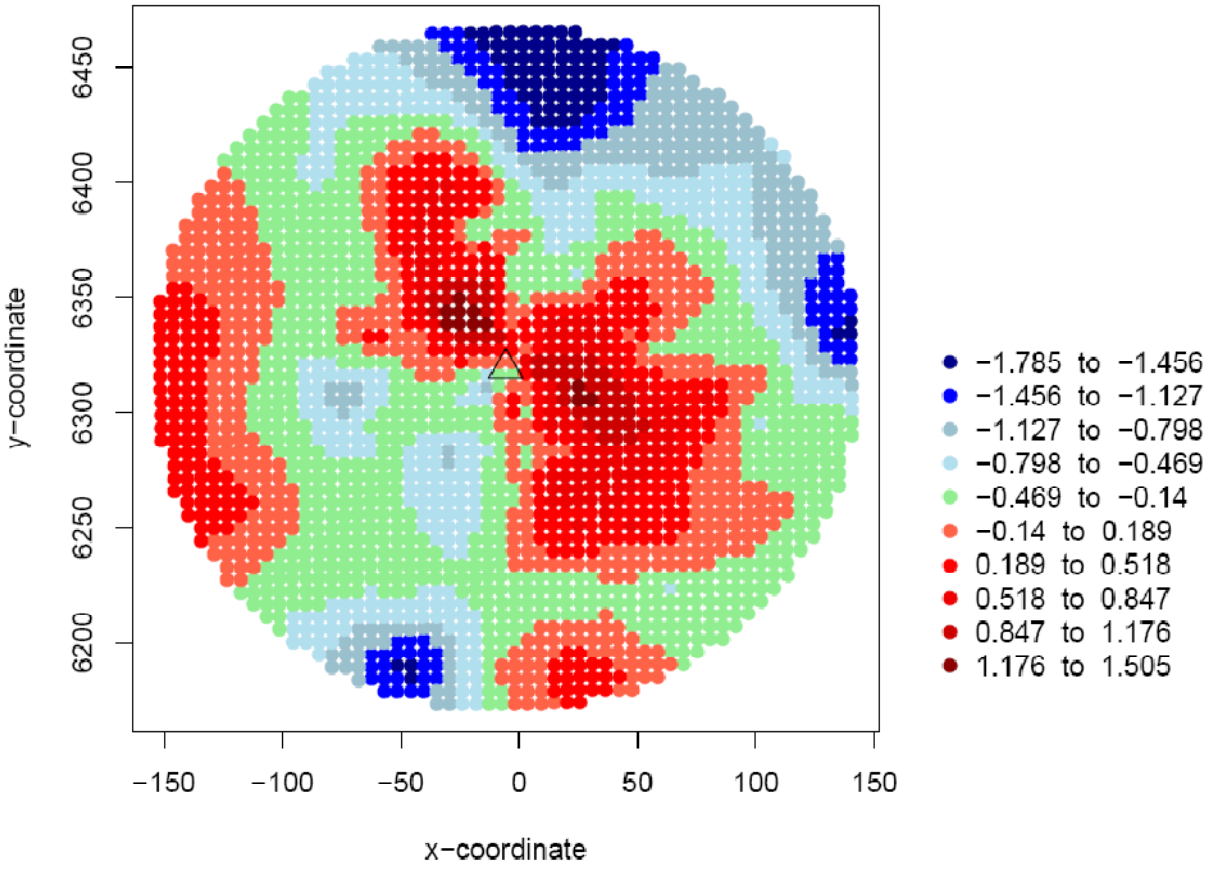

Figure 4-4: Map of predicted log differences between S concentrations in *Evernia mesomorpha* and CALPUFF-predicted annual total S deposition with background and wildfire (kg ha^{-1}yr^{-1}). Prediction points are based on Universal Kriging and are shown on x,y coordinates for the study area. The triangle represents the centre point between the Suncor and Syncrude main upgrader stacks.

It is important to emphasize again that these analyses and interpretations are not final, and should be treated as examples of how these study objectives will be addressed. The updated and complete analytical comparison of CALPUFF predictions and lichen observations will be presented in the final report.

4.3 CO-LOCATION SITES

Sampling and measurement of N and S concentrations in lichen contribute to the understanding of spatial patterns of deposition in the AOSR. The relationship between lichen chemistry and direct air-quality or deposition measurements can be better understood through building correlative relationships between lichen chemistry and measured air-quality data. We explored these relationships through co-locating a subset of lichen sampling sites with existing air-monitoring devices installed in the AOSR (see section 3.1.3). Initial results from these analyses are presented here for two types of passive air- quality samplers:

1. MAXXAM passive samplers that measure concentrations (ppb) of NO_2 and SO_2 on a monthly (bi-monthly in winter) basis; and,
2. USDA passive samplers that measure concentrations (ppb) of HNO_3 and NH_3 on a monthly basis (bi-monthly in winter).

Of all the study components presented in this interim report, the co-location analyses are at the earliest stages, and are most preliminary. These analyses are still ongoing for comparisons to all air-quality measures (see Table C-3, Appendix C), and results will be presented in the final report. Initial findings are presented below.

S and N concentrations in both lichen species were significantly related to measurements of SO_2 and NO_2 respectively, across all time periods (Table 4-8). Relationships were best for N and S concentrations in *Evernia mesomorpha*, as this species tends to be a better overall accumulator of N and S deposition in the AOSR than *Hypogymnia physodes*. SO_2 and NO_2 concentrations were measured across 22 sites, spanning the deposition gradient (Figure A-7, Appendix A).

N concentrations in both lichen species were significantly related to HNO_3 concentrations (with the exception of Winter 2007 for *H. physodes*), however strongest relationships were with *E. mesomorpha*. N concentrations in *E. mesomorpha* were weakly related to NH_3 concentrations, with significant relationships for the winter 2007 period and the annual average for 2007-2008. In contrast, N concentrations in *H. physodes* were not significantly related to NH_3 concentrations for any time period (Table 4-9). HNO_3 and NH_3 concentrations were measured at 31 sites in the region (Figure A-8, Appendix A).

Overall, these initial findings indicate significant relationships between some passive-sampler measures of N and S compounds and lichen chemistry. It is our intention that further analyses of these datasets will allow us to develop predictive models relating select air-quality measurements to lichen chemistry, with the goal being to enable prediction of a given air-quality measure (e.g., NO_2 concentration) from lichen chemistry data, for sites where air-quality monitoring equipment is not present. This could serve as an effective tool to utilize lichen as a surrogate measure for air-quality parameters that are difficult to measure intensively across the region, and thus augment the WBEA air-quality measurement dataset.

Although it is recognized that lichen chemistry may not perfectly reflect atmospheric deposition, it is also true that there are many uncertainties and limitations with direct air or deposition measurements as well. For instance, passive filters collect gaseous dry deposition only, and are more prone to variations in wind flow (passive filters tend to over-sample at higher wind velocities or turbulence and under-sample at low wind velocities or very stable conditions, and these relationships vary according to the gas species being deposited). Passive concentration data does not yield direct deposition information, but must be converted to deposition estimates through modeling. Ion-exchange resin samplers mainly predominantly wet deposition, but also collect through-fall, which characterizes an unknown component of dry deposition captured but not retained by the tree canopy (predominantly insoluble or relatively low-solubility particulate matter). Therefore, we expect relationships between lichen chemistry and air-quality measurements to be imperfect, based on limitations or inherent properties of all receptors. Nevertheless, co-location of lichen samples with direct air measurements has been successfully demonstrated in the past (e.g., Bruteig 1993; Fenn et al. 2007) and it is hoped that this will be a valuable addition to the TEEM air-quality monitoring program, allowing linkages to be established between air measurements and lichen chemistry and response.

Table 4-8: Correlation coefficients, Spearman and Kendall, for relationships between lichen chemistry (%S and %N in both lichen species) and SO2 and NO2 concentrations (ppb) measured by MAXXAM passive samplers at 22 sites. SO2 and NO2 were measured on a monthly (bi-monthly in winter) basis and these measurements were averaged to represent summer, winter and annual averages for 2007 and 2008. Correlation coefficients in bold font indicate a significant relationship based on a P-value of 0.05 or less.

	Spearman	**Kendall**	**Spearman**	**Kendall**
SO_2 (ppb)	*Evernia mesomorpha* %S		*Hypogymnia physodes* %S	
Summer 2007	0.72	0.55	0.52	0.37
Winter 2007	0.79	0.64	0.51	0.41
Summer 2008	0.65	0.51	0.62	0.46
Annual 2007-2008	0.74	0.56	0.51	0.44
NO_2 (ppb)	*Evernia mesomorpha* %N		*Hypogymnia physodes* %N	
Summer 2007	0.59	0.46	0.71	0.51
Winter 2007	0.86	0.72	0.84	0.72
Summer 2008	0.74	0.58	0.75	0.54
Annual 2007-2008	0.88	0.72	0.86	0.72

Table 4-9: Correlation coefficients, Spearman and Kendall, for relationships between N concentrations (%N) in both lichen species and concentrations (µg/m3) of NH3 and HNO3 measured by USDA passive samplers at 31 sites. NH3 and HNO3were measured on a monthly basis (bi-monthly in winter) and these measurements were averaged to represent summer, winter and annual averages for 2007 and 2008. Correlation coefficients in bold font indicate a significant relationship based on a P-value of 0.05 or less.

	Spearman	**Kendall**	**Spearman**	**Kendall**
NH_3 (µg/m^3)	*Evernia mesomorpha* %N		*Hypogymnia physodes* %N	
Summer 2007	0.29	0.20	-0.10	-0.10
Winter 2007	0.44	0.31	0.06	0.05
Summer 2008	0.34	0.21	0.08	0.06
Annual 2007-2008	0.32	0.26	0.03	-0.01
HNO_3 (µg/m3)				
Summer 2007	0.61	0.42	0.35	0.28
Winter 2007	0.56	0.41	0.33	0.22
Summer 2008	0.53	0.35	0.38	0.29
Annual 2007-2008	0.56	0.39	0.42	0.28

4.4 SOURCE APPORTIONMENT

A total of 69 lichen samples (*Hypogymnia physodes*) for selected sites across the study area with both high and low N levels in the lichen have been selected and analyzed so far for trace elements. Samples

were being analyzed for a suite of trace elements and for both lead and mercury isotopes. Additional samples will be selected for analysis to complete the dataset. Results will provide fingerprints of elemental composition in lichen that can be linked to results from source type characterization, in order to determine which sources are contributing to elemental enrichment in lichen. Analysis is ongoing for this work and reporting for this project component will be provided in separate documentation.

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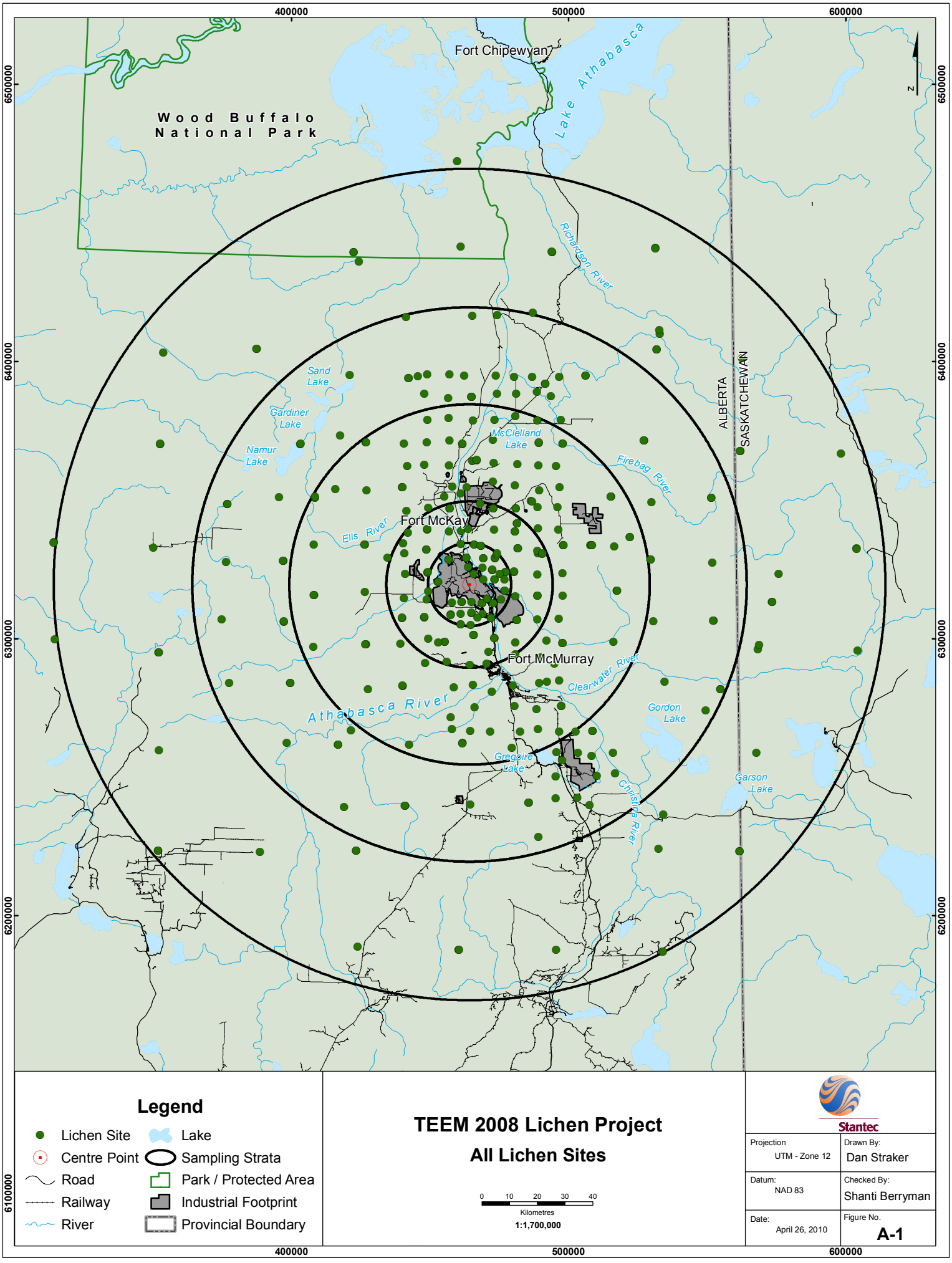
Appendix A:
Site Maps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Firebag River
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SASKATCHEWAN
Ells River
Fort McKay
Fort McMurray
Clearwater River
Athabasca River
Gordon Lake
Gregoire Lake
Garson Lake
Christina River
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Lichen Site
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Industrial Footprint
Provincial Boundary
TEEM 2008 Lichen Project
All Lichen Sites
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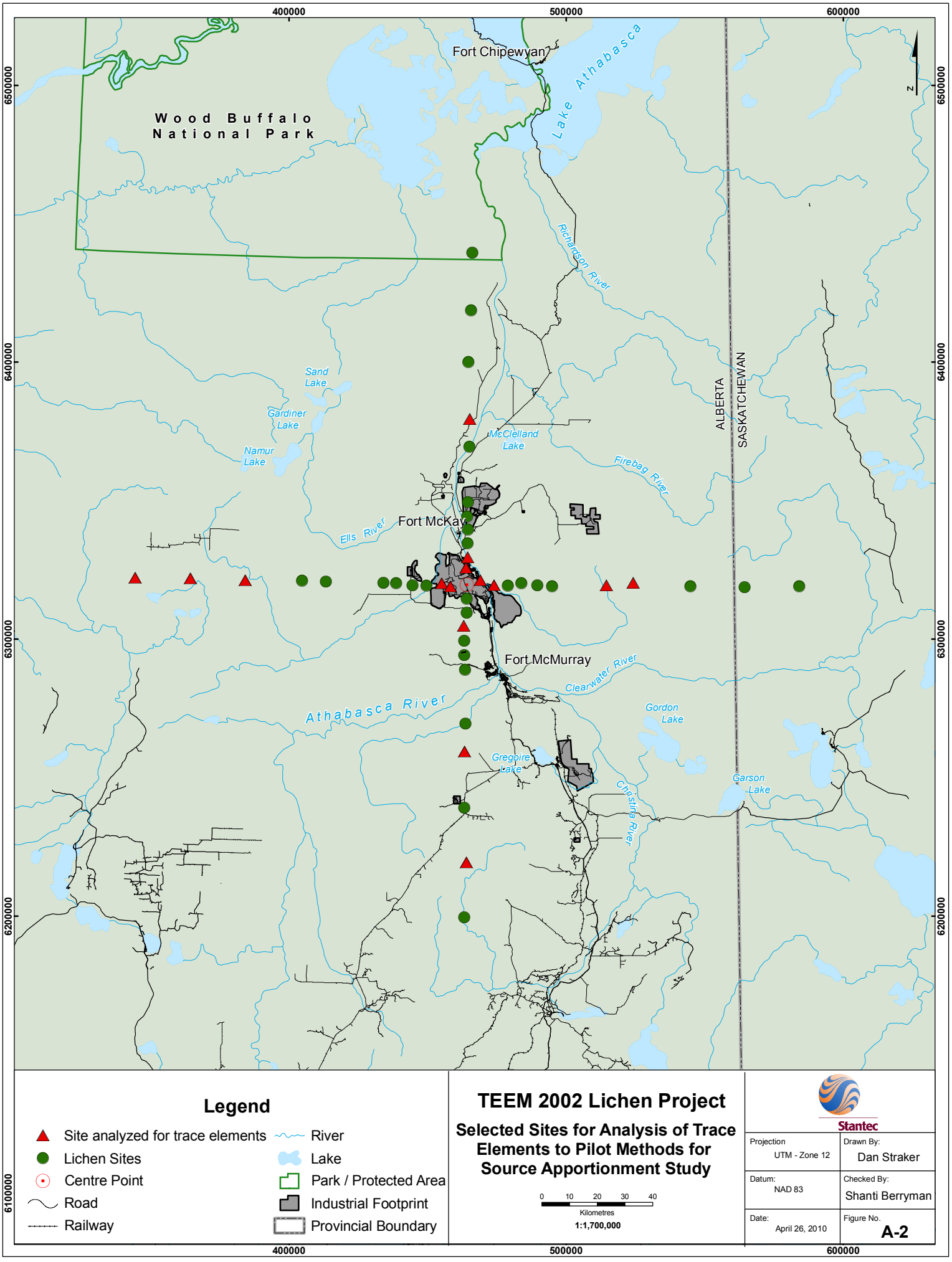

Legend

- Site analyzed for trace elements
- Lichen Sites
- Centre Point
- Road
- Railway
- River
- Lake
- Park / Protected Area
- Industrial Footprint
- Provincial Boundary

TEEM 2002 Lichen Project

Selected Sites for Analysis of Trace Elements to Pilot Methods for Source Apportionment Study

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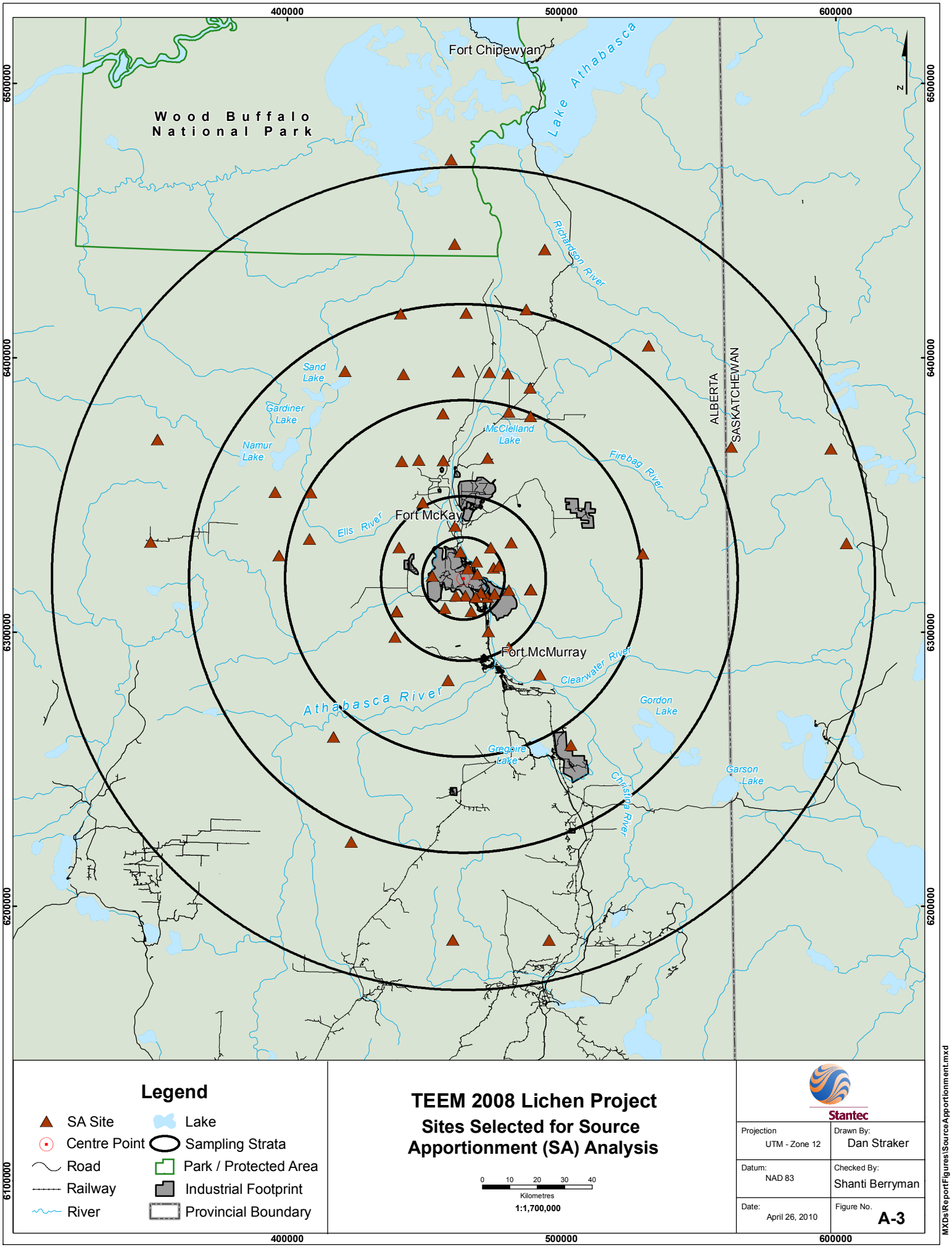
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ALBERTA
SASKATCHEWAN
Ells River
Fort McKay
Fort McMurray
Clearwater River
Athabasca River
Gordon Lake
Gregoire Lake
Garson Lake
Christina River
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TEEM 2008 Lichen Project
Sites Selected for Source Apportionment (SA) Analysis
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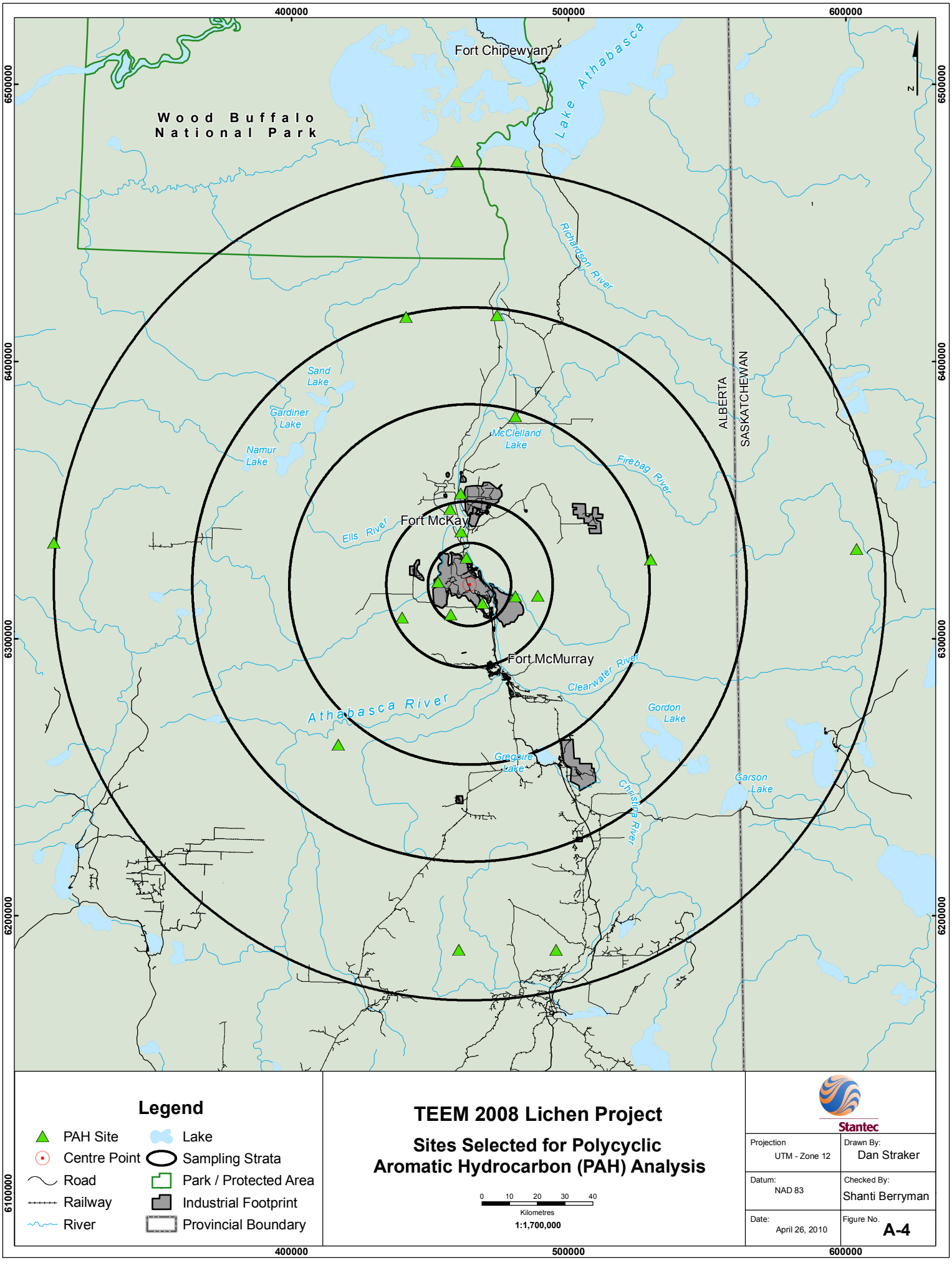
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McClelland Lake
Firebag River
ALBERTA
SASKATCHEWAN
Ells River
Fort McKay
Fort McMurray
Clearwater River
Athabasca River
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Gregoire Lake
Garson Lake
Christina River
Legend
PAH Site
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Industrial Footprint
Provincial Boundary
TEEM 2008 Lichen Project
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Projection
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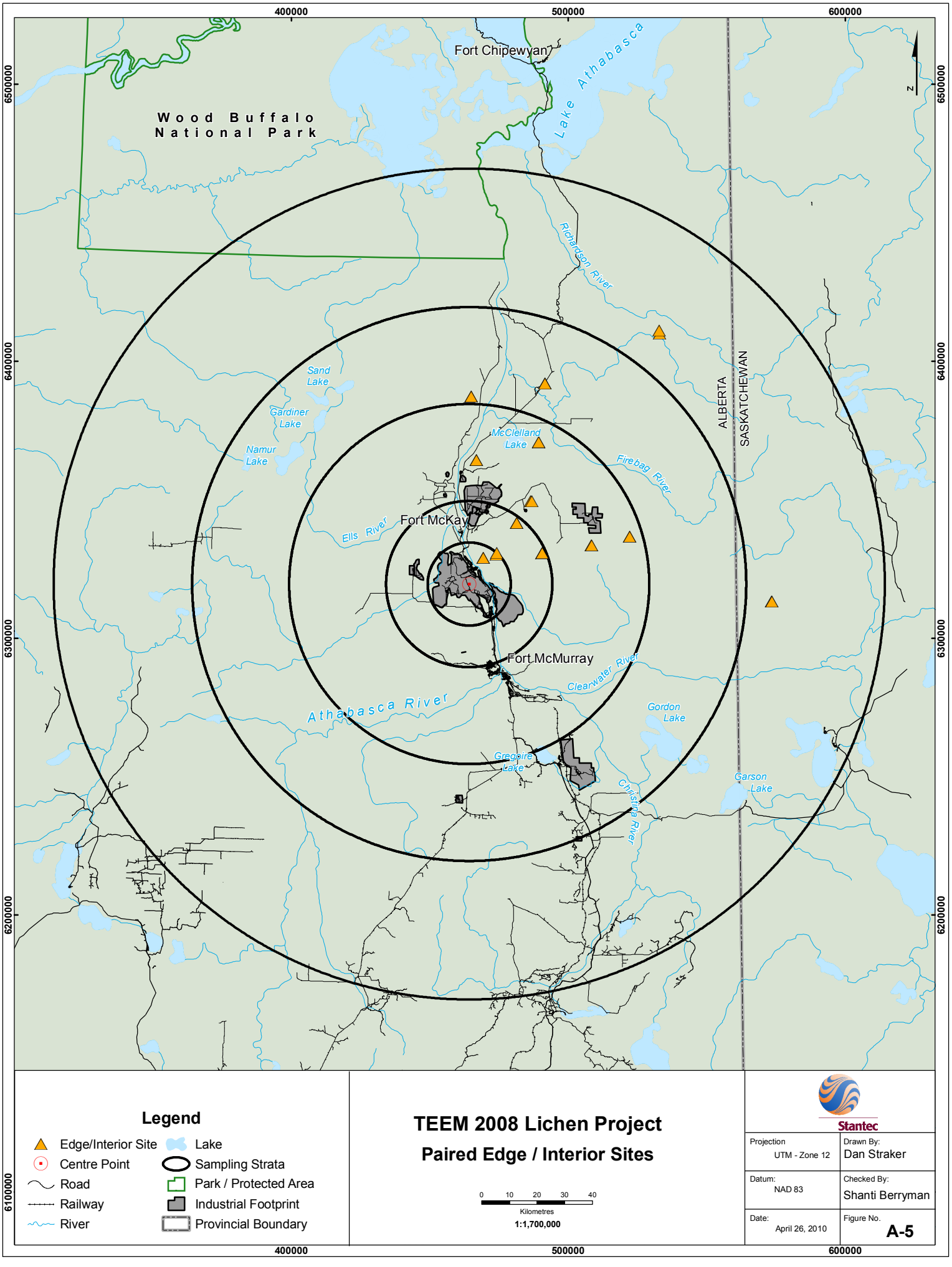

Legend

- Edge/Interior Site
- Centre Point
- Road
- Railway
- River
- Lake
- Sampling Strata
- Park / Protected Area
- Industrial Footprint
- Provincial Boundary

TEEM 2008 Lichen Project

Paired Edge / Interior Sites

0 10 20 30 40
Kilometres
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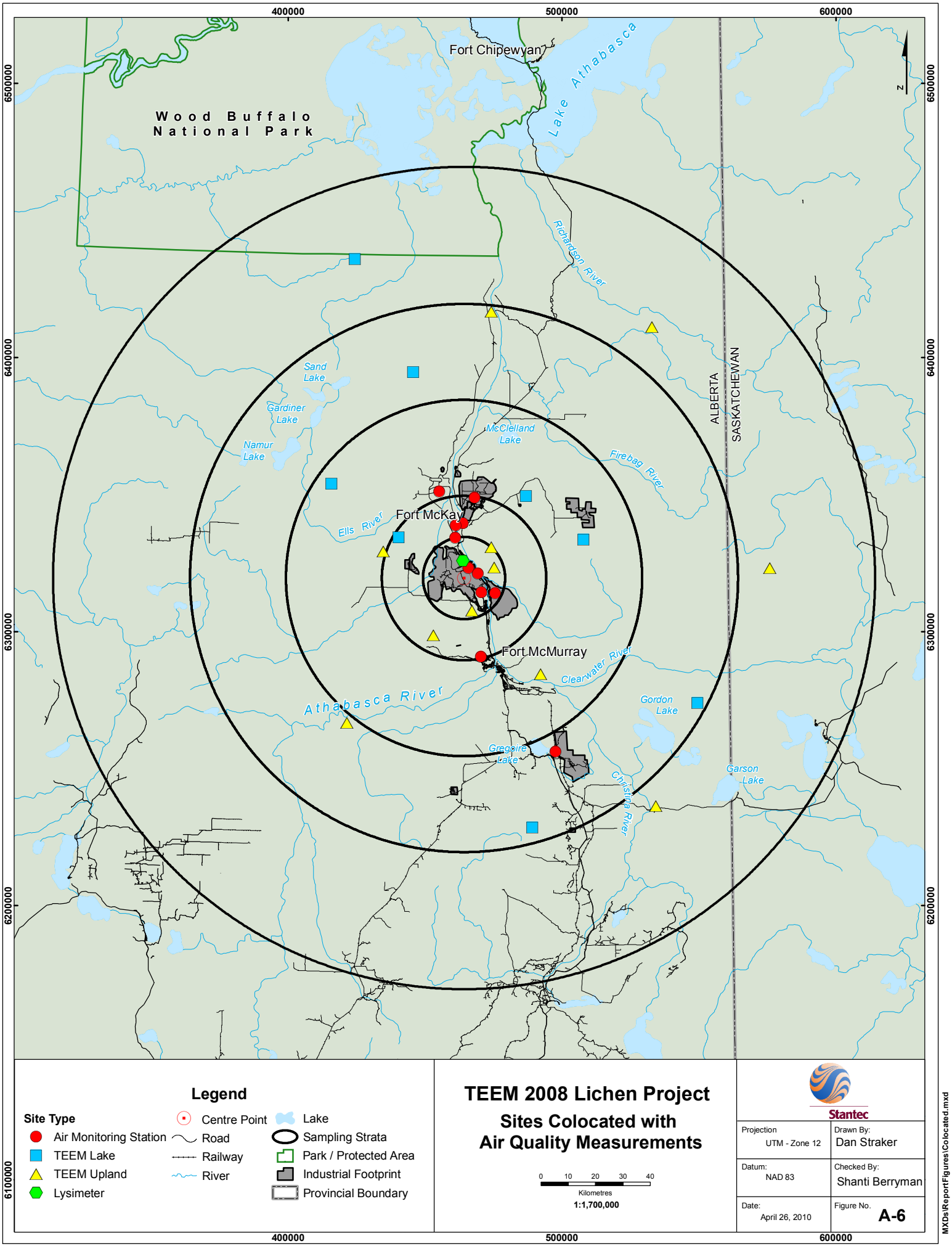

Legend

Site Type

- Air Monitoring Station
- TEEM Lake
- TEEM Upland
- Lysimeter
- Centre Point
- Road
- Railway
- River
- Lake
- Sampling Strata
- Park / Protected Area
- Industrial Footprint
- Provincial Boundary

TEEM 2008 Lichen Project

Sites Colocated with Air Quality Measurements

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Kilometres
1:1,700,000

Stantec

Projection: UTM - Zone 12	Drawn By: Dan Straker
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Date: April 26, 2010	Figure No. A-6

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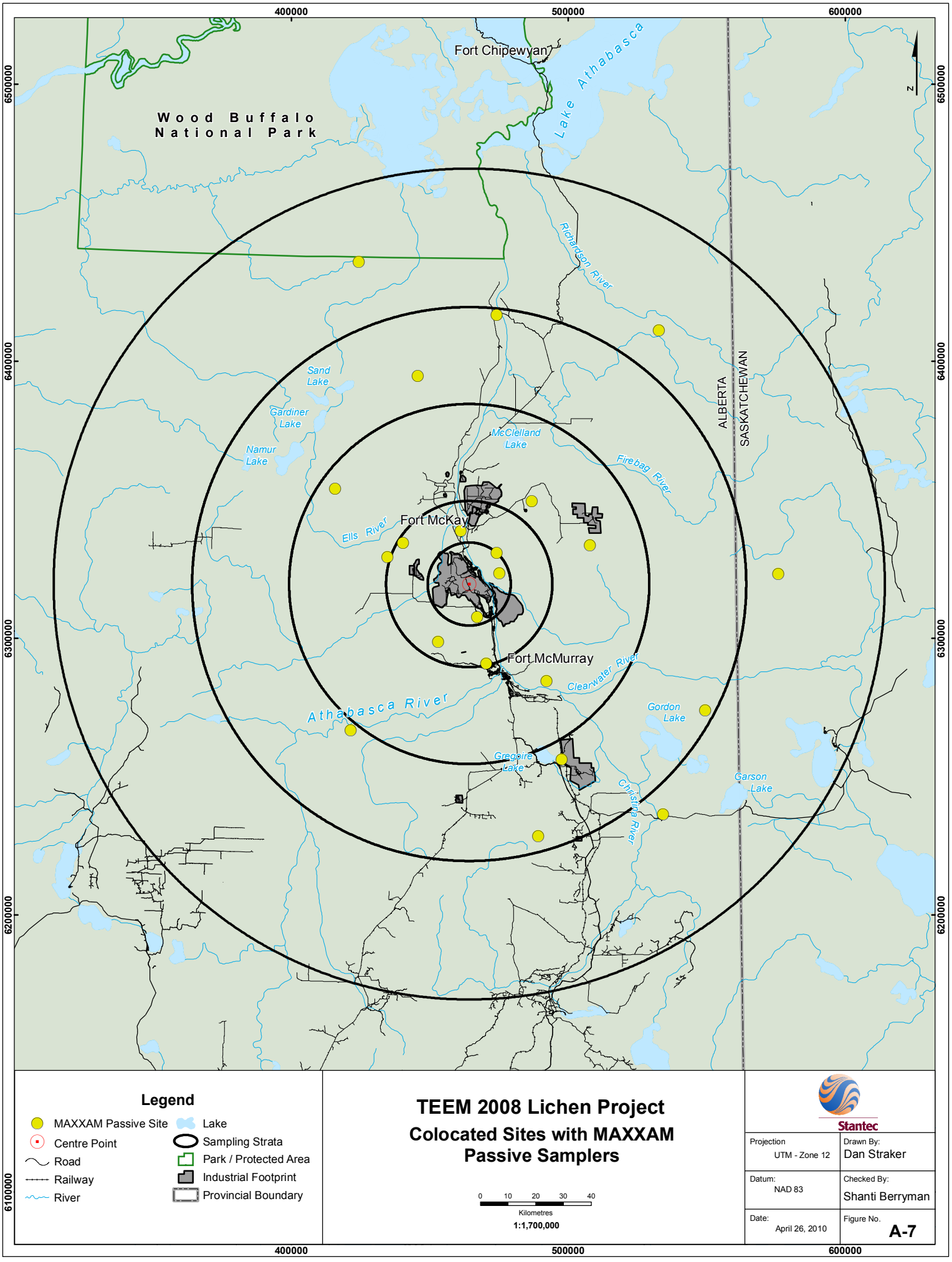


Legend

- MAXXAM Passive Site
- Centre Point
- Road
- Railway
- River
- Lake
- Sampling Strata
- Park / Protected Area
- Industrial Footprint
- Provincial Boundary

TEEM 2008 Lichen Project

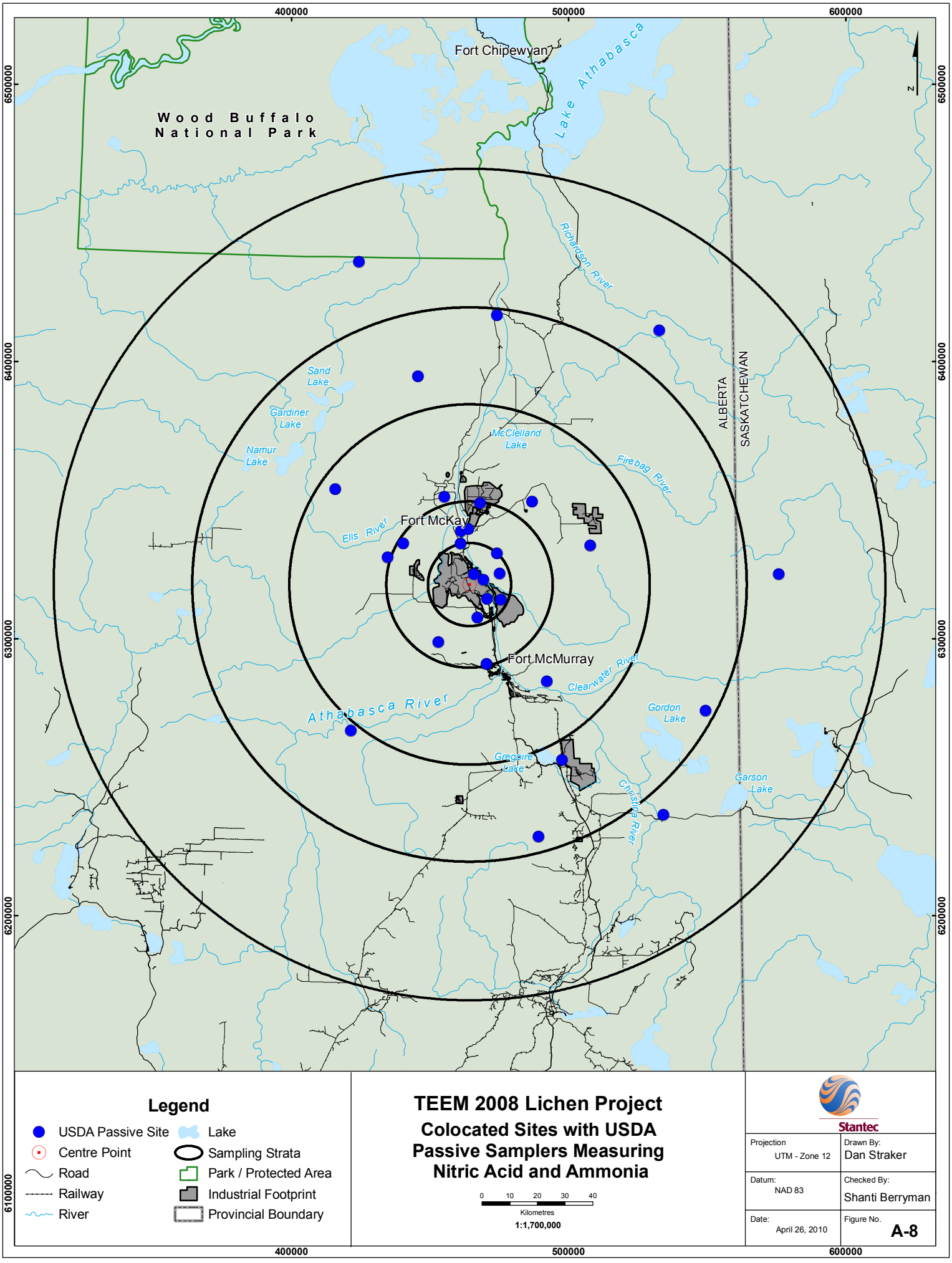
Colocated Sites with MAXXAM Passive Samplers

0 10 20 30 40
Kilometres
1:1,700,000

Stantec

Projection: UTM - Zone 12	Drawn By: Dan Straker
Datum: NAD 83	Checked By: Shanti Berryman
Date: April 26, 2010	Figure No. A-7

MXDs\ReportFigures\PassiveMAXXAM.mxd



Legend

- USDA Passive Site
- Centre Point
- Road
- Railway
- River
- Lake
- Sampling Strata
- Park / Protected Area
- Industrial Footprint
- Provincial Boundary

TEEM 2008 Lichen Project

Colocated Sites with USDA Passive Samplers Measuring Nitric Acid and Ammonia

0 10 20 30 40
Kilometres
1:1,700,000

Stantec

Projection: UTM - Zone 12	Drawn By: Dan Straker
Datum: NAD 83	Checked By: Shanti Berryman
Date: April 26, 2010	Figure No. A-8

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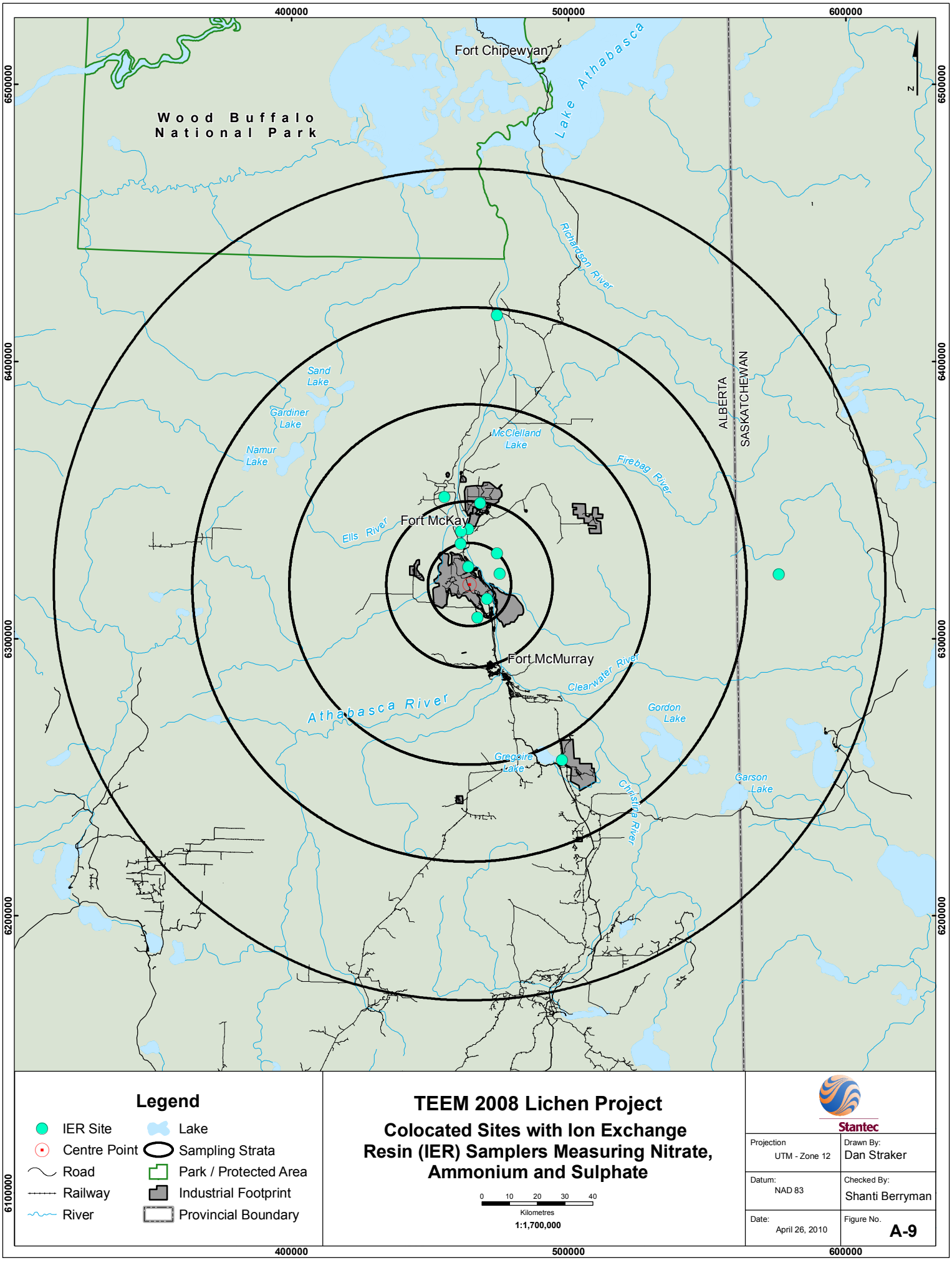

Legend

- IER Site
- Centre Point
- Road
- Railway
- River
- Lake
- Sampling Strata
- Park / Protected Area
- Industrial Footprint
- Provincial Boundary

TEEM 2008 Lichen Project

Colocated Sites with Ion Exchange Resin (IER) Samplers Measuring Nitrate, Ammonium and Sulphate

0 10 20 30 40
Kilometres
1:1,700,000

Stantec

Projection: UTM - Zone 12	Drawn By: Dan Straker
Datum: NAD 83	Checked By: Shanti Berryman
Date: April 26, 2010	Figure No. A-9

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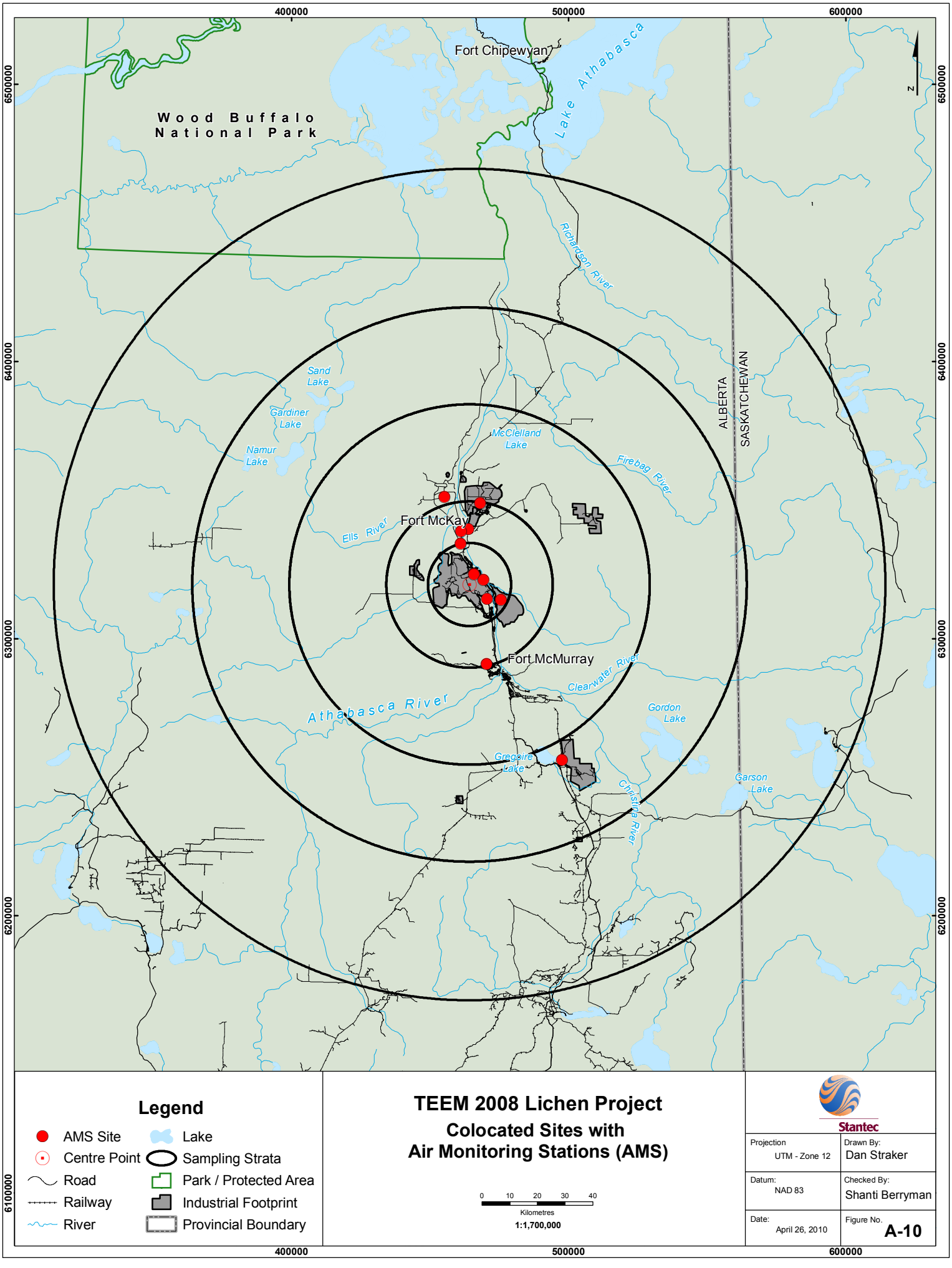
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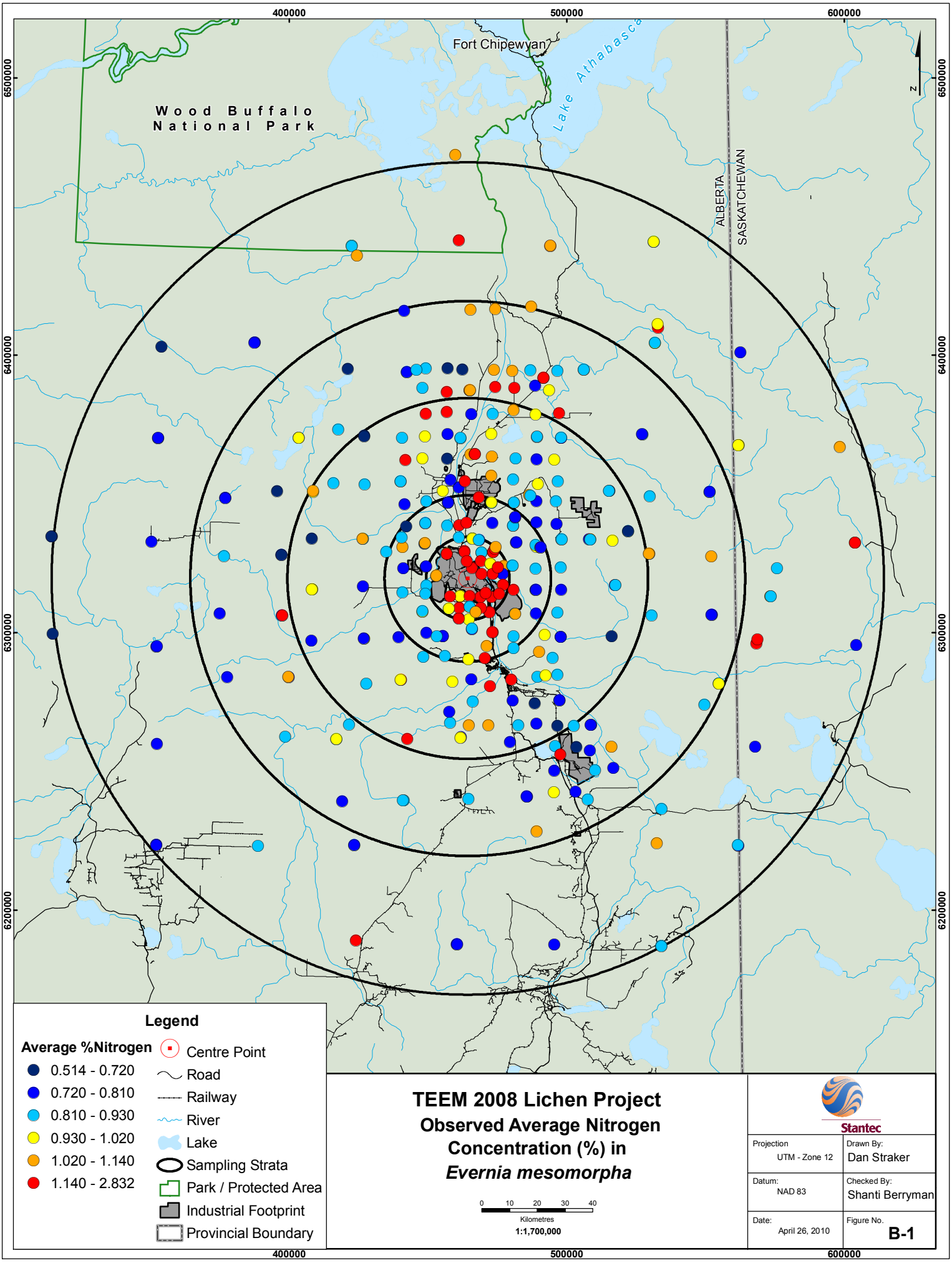
500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
N
Richardson River
Sand Lake
Gardiner Lake
Namur Lake
McClelland Lake
Firebag River
ALBERTA
SASKATCHEWAN
Ells River
Fort McKay
Fort McMurray
Clearwater River
Athabasca River
Gordon Lake
Gregoire Lake
Garson Lake
Christina River
Legend
AMS Site
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Industrial Footprint
Provincial Boundary
TEEM 2008 Lichen Project
Colocated Sites with
Air Monitoring Stations (AMS)
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
A-10
MXDs\ReportFigures\AMS.mxd

Appendix B:
Observed and Predicted Nitrogen and Sulphur Values in Lichen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500000
600000
6500000
6400000
6300000
6200000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Average %Nitrogen
0.514 - 0.720
0.720 - 0.810
0.810 - 0.930
0.930 - 1.020
1.020 - 1.140
1.140 - 2.832
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Industrial Footprint
Provincial Boundary
TEEM 2008 Lichen Project
Observed Average Nitrogen Concentration (%) in
Evernia mesomorpha
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-1
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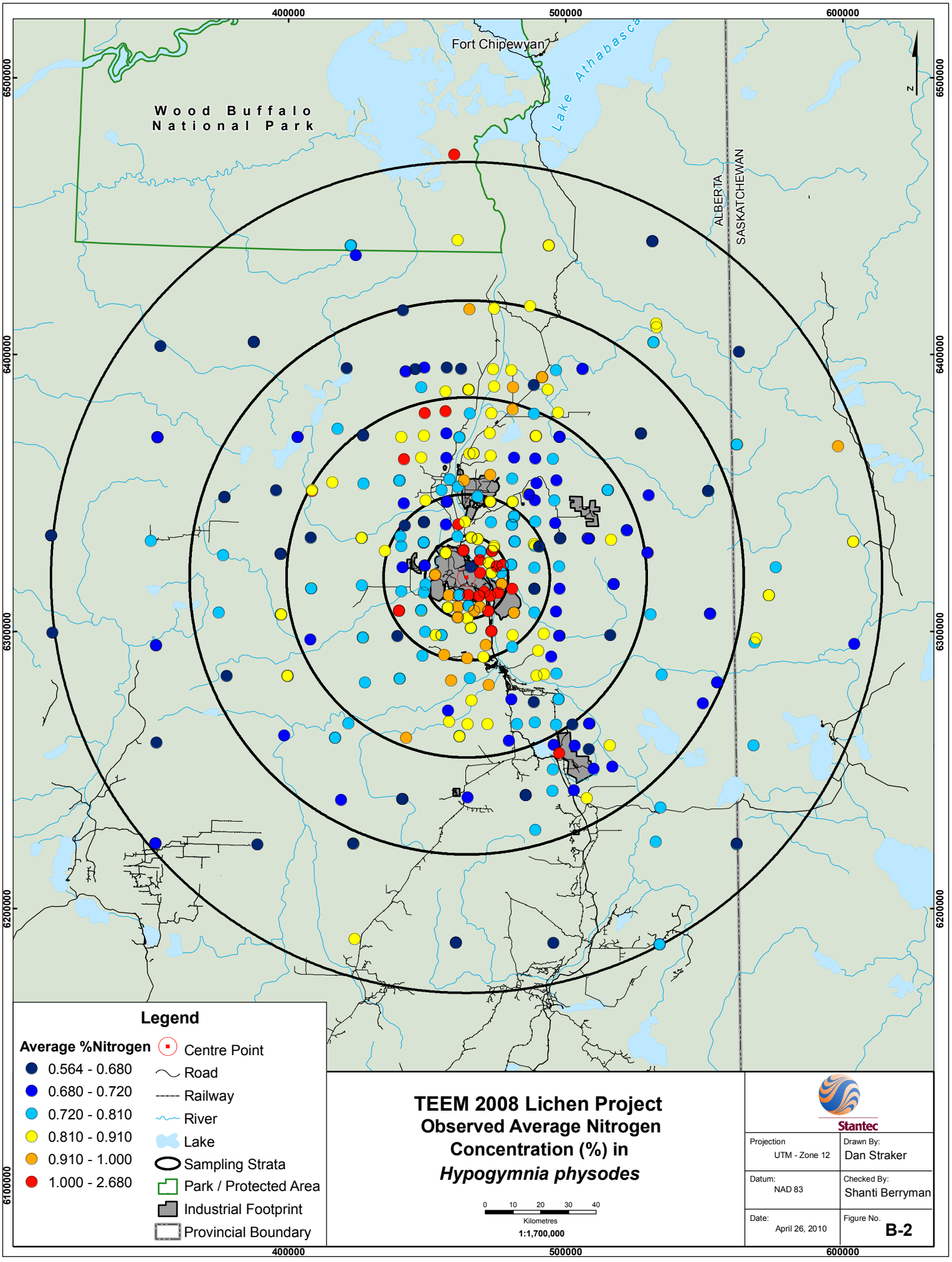
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500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Average %Nitrogen
0.564 - 0.680
0.680 - 0.720
0.720 - 0.810
0.810 - 0.910
0.910 - 1.000
1.000 - 2.680
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Industrial Footprint
Provincial Boundary
TEEM 2008 Lichen Project
Observed Average Nitrogen Concentration (%) in
Hypogymnia physodes
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-2
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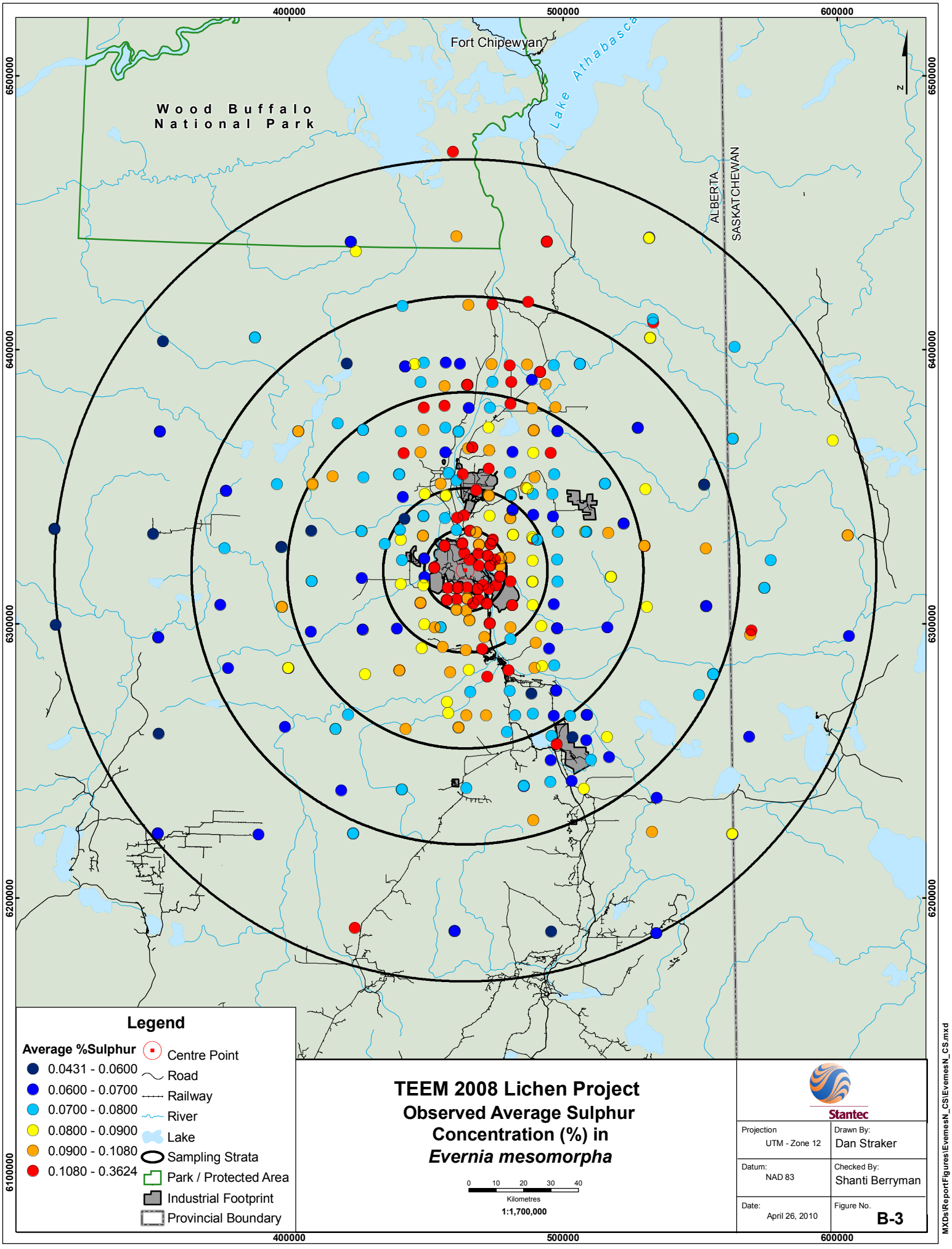
400000
500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Average %Sulphur
0.0431 - 0.0600
0.0600 - 0.0700
0.0700 - 0.0800
0.0800 - 0.0900
0.0900 - 0.1080
0.1080 - 0.3624
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Industrial Footprint
Provincial Boundary
TEEM 2008 Lichen Project
Observed Average Sulphur Concentration (%) in
Evernia mesomorpha
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-3
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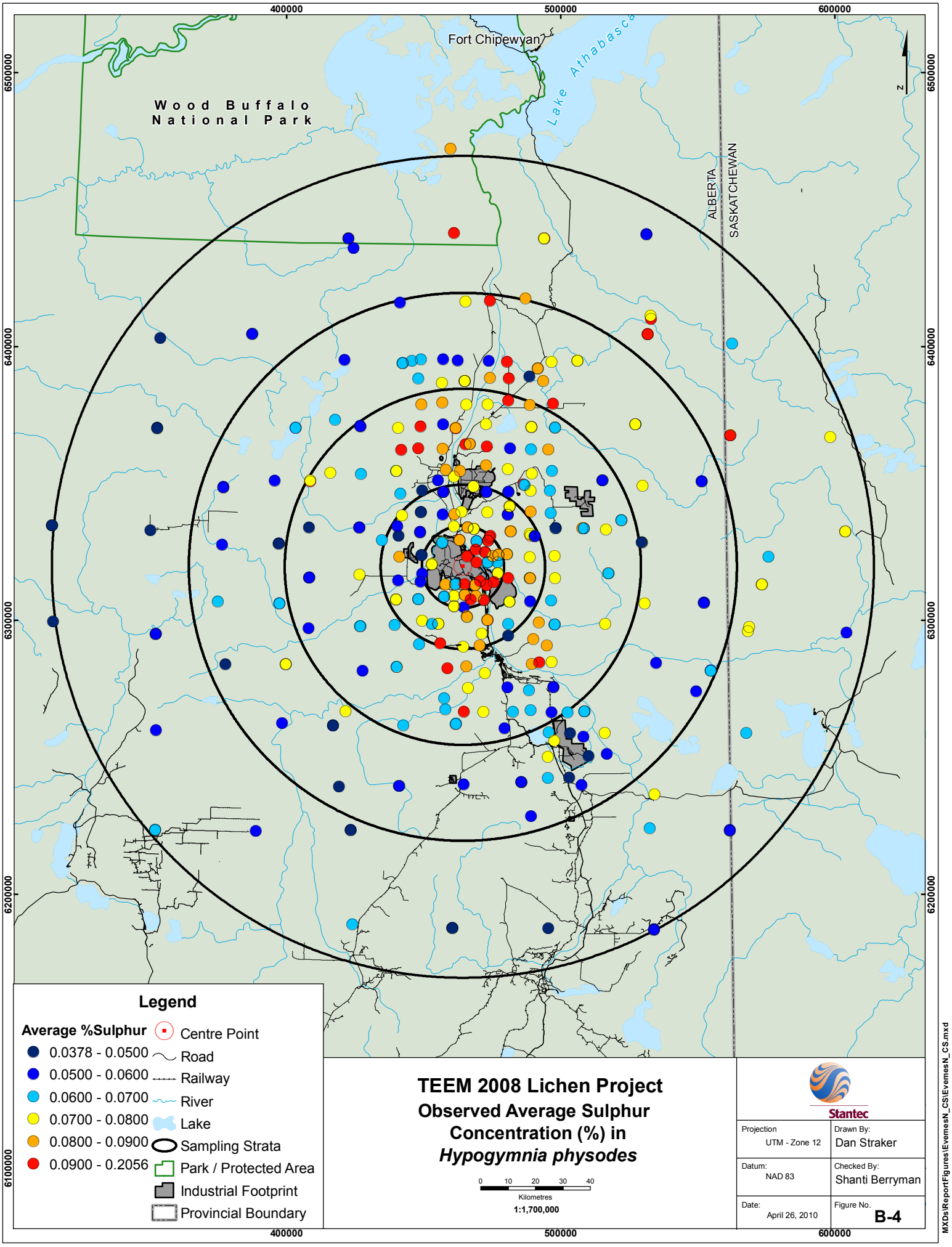
400000
500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Average %Sulphur
0.0378 - 0.0500
0.0500 - 0.0600
0.0600 - 0.0700
0.0700 - 0.0800
0.0800 - 0.0900
0.0900 - 0.2056
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Industrial Footprint
Provincial Boundary
TEEM 2008 Lichen Project
Observed Average Sulphur Concentration (%) in
Hypogymnia physodes
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-4
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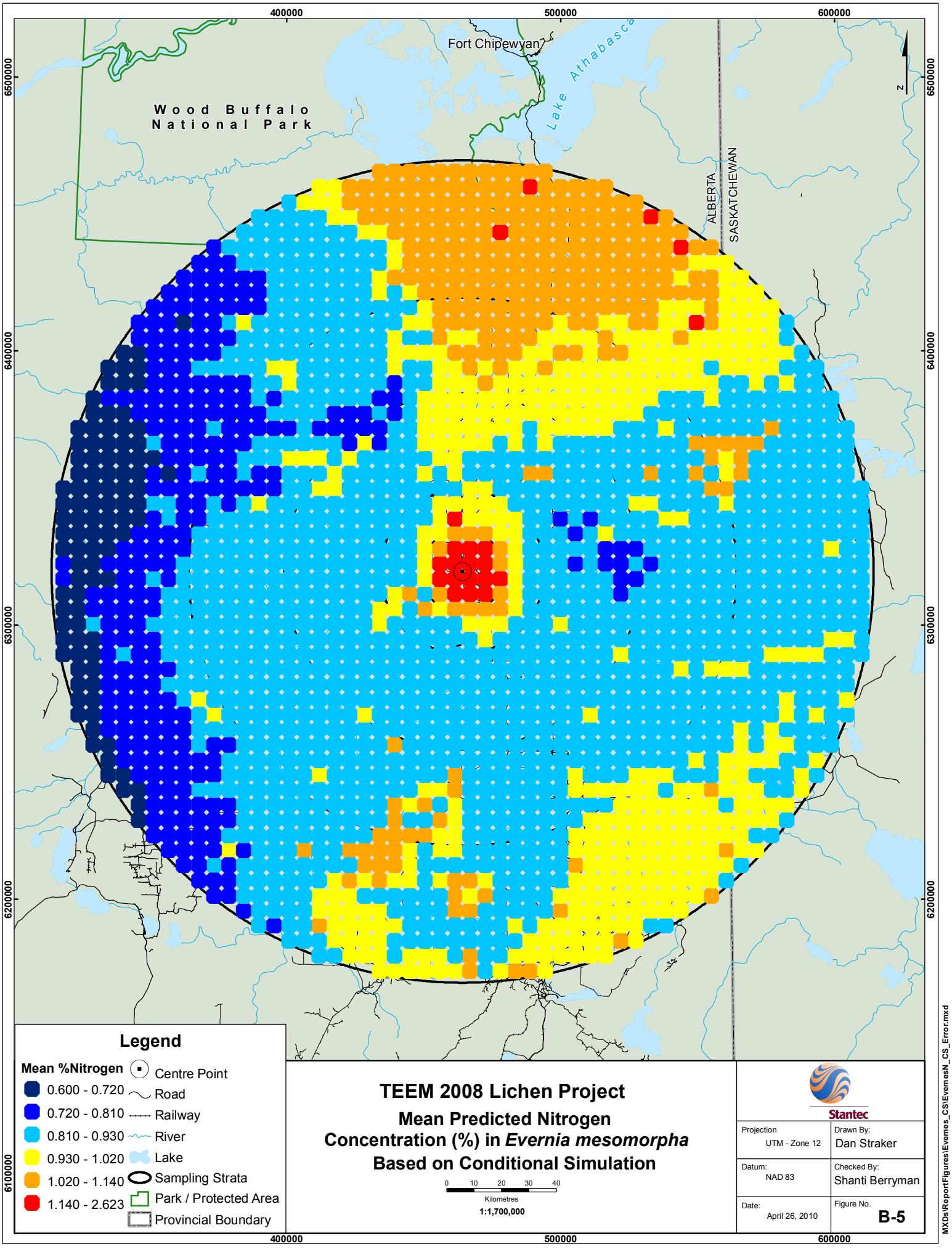
400000
500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Mean %Nitrogen
0.600 - 0.720
0.720 - 0.810
0.810 - 0.930
0.930 - 1.020
1.020 - 1.140
1.140 - 2.623
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Mean Predicted Nitrogen Concentration (%) in Evernia mesomorpha Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-5
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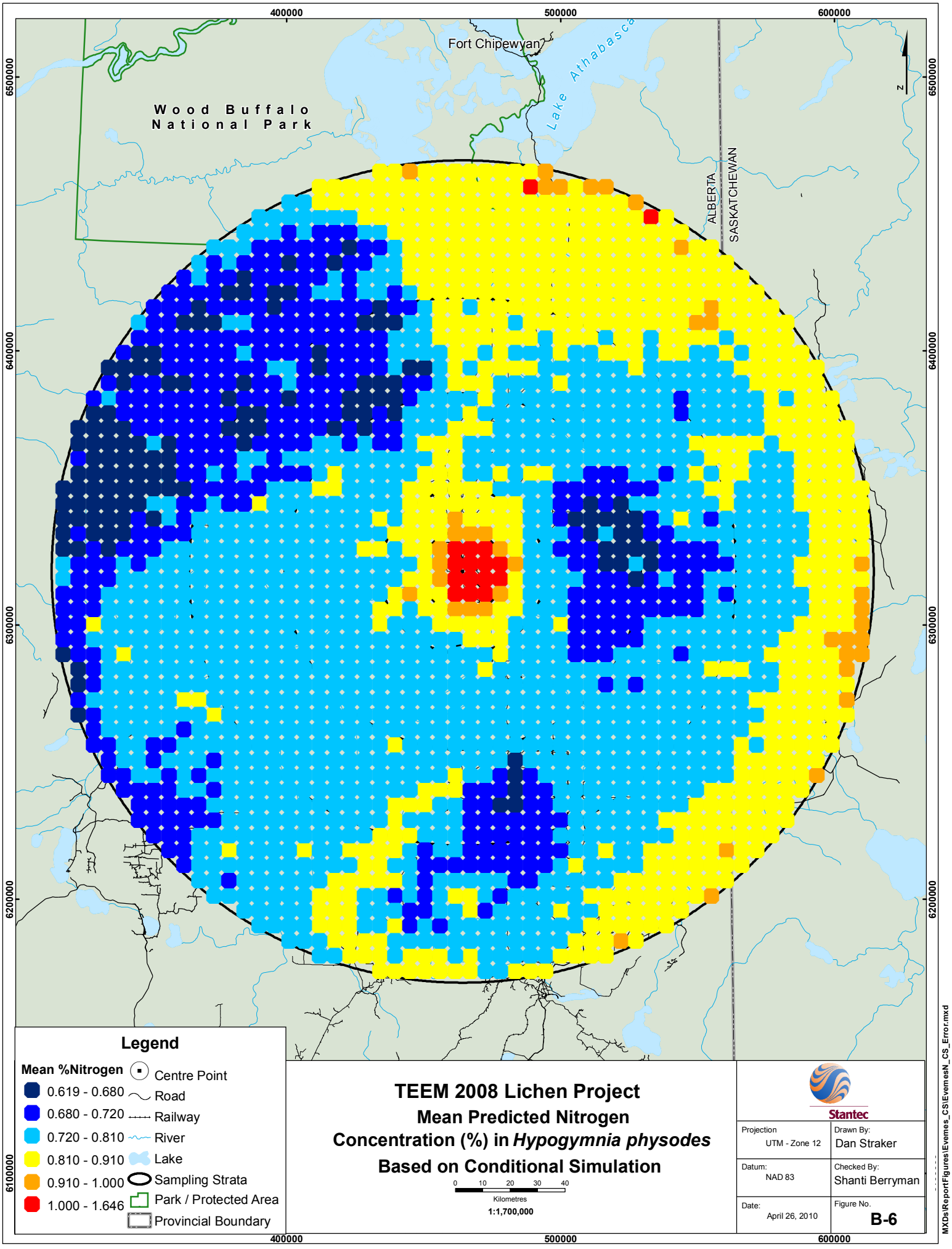
400000
500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Mean %Nitrogen
0.619 - 0.680
0.680 - 0.720
0.720 - 0.810
0.810 - 0.910
0.910 - 1.000
1.000 - 1.646
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Mean Predicted Nitrogen
Concentration (%) in Hypogymnia physodes
Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-6
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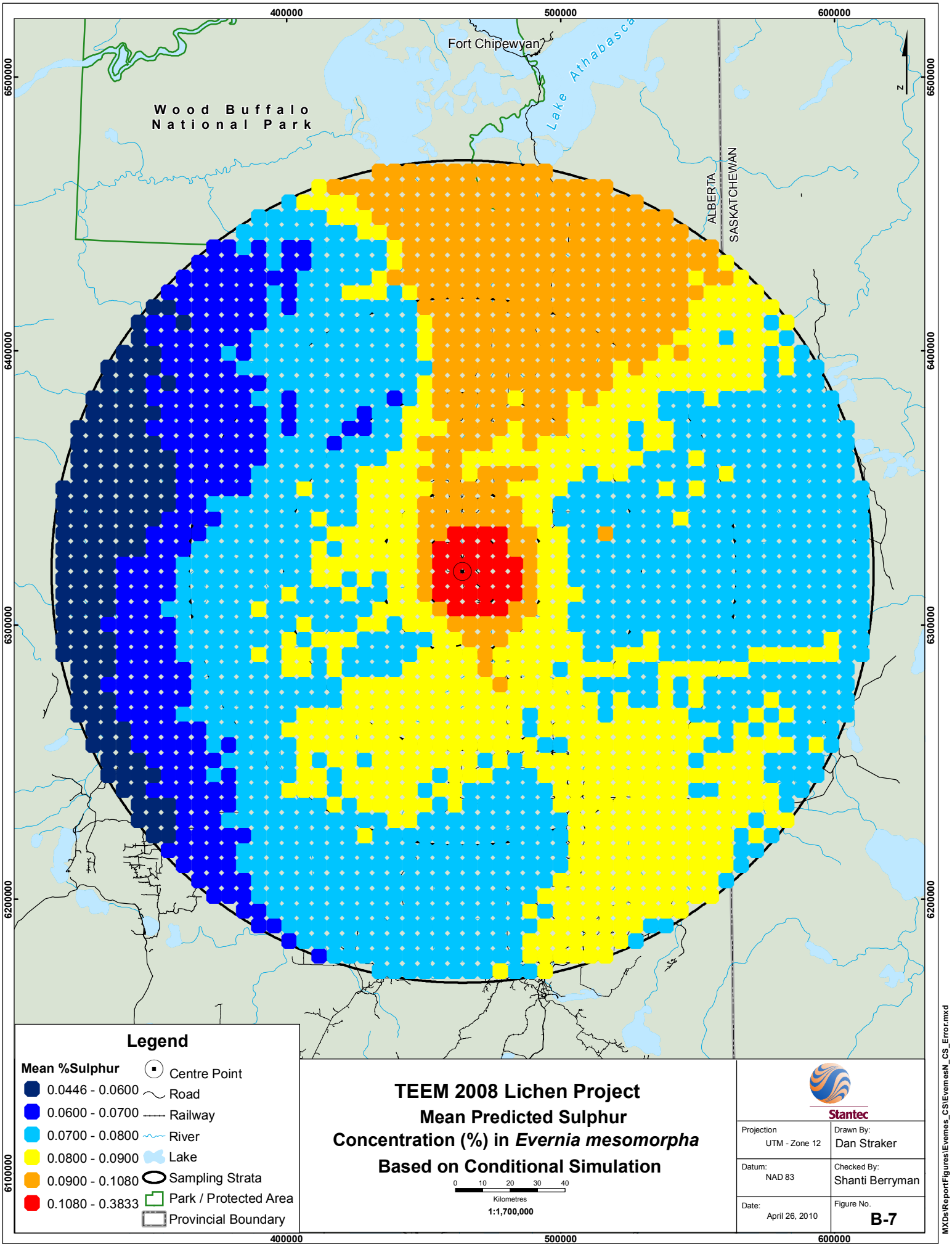
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500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Mean %Sulphur
0.0446 - 0.0600
0.0600 - 0.0700
0.0700 - 0.0800
0.0800 - 0.0900
0.0900 - 0.1080
0.1080 - 0.3833
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Mean Predicted Sulphur
Concentration (%) in Evernia mesomorpha
Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-7
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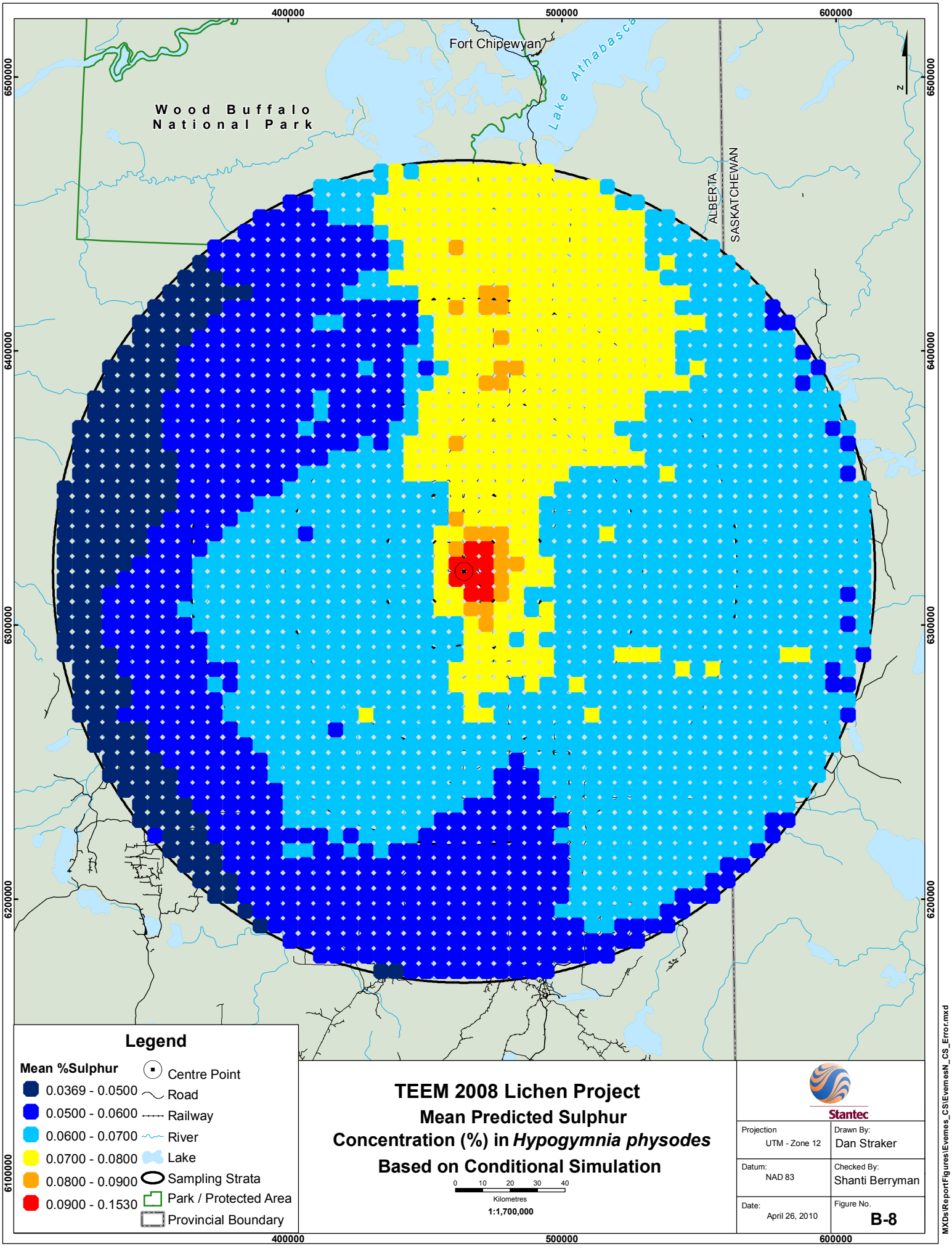
400000
500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Mean %Sulphur
0.0369 - 0.0500
0.0500 - 0.0600
0.0600 - 0.0700
0.0700 - 0.0800
0.0800 - 0.0900
0.0900 - 0.1530
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Mean Predicted Sulphur
Concentration (%) in Hypogymnia physodes
Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-8
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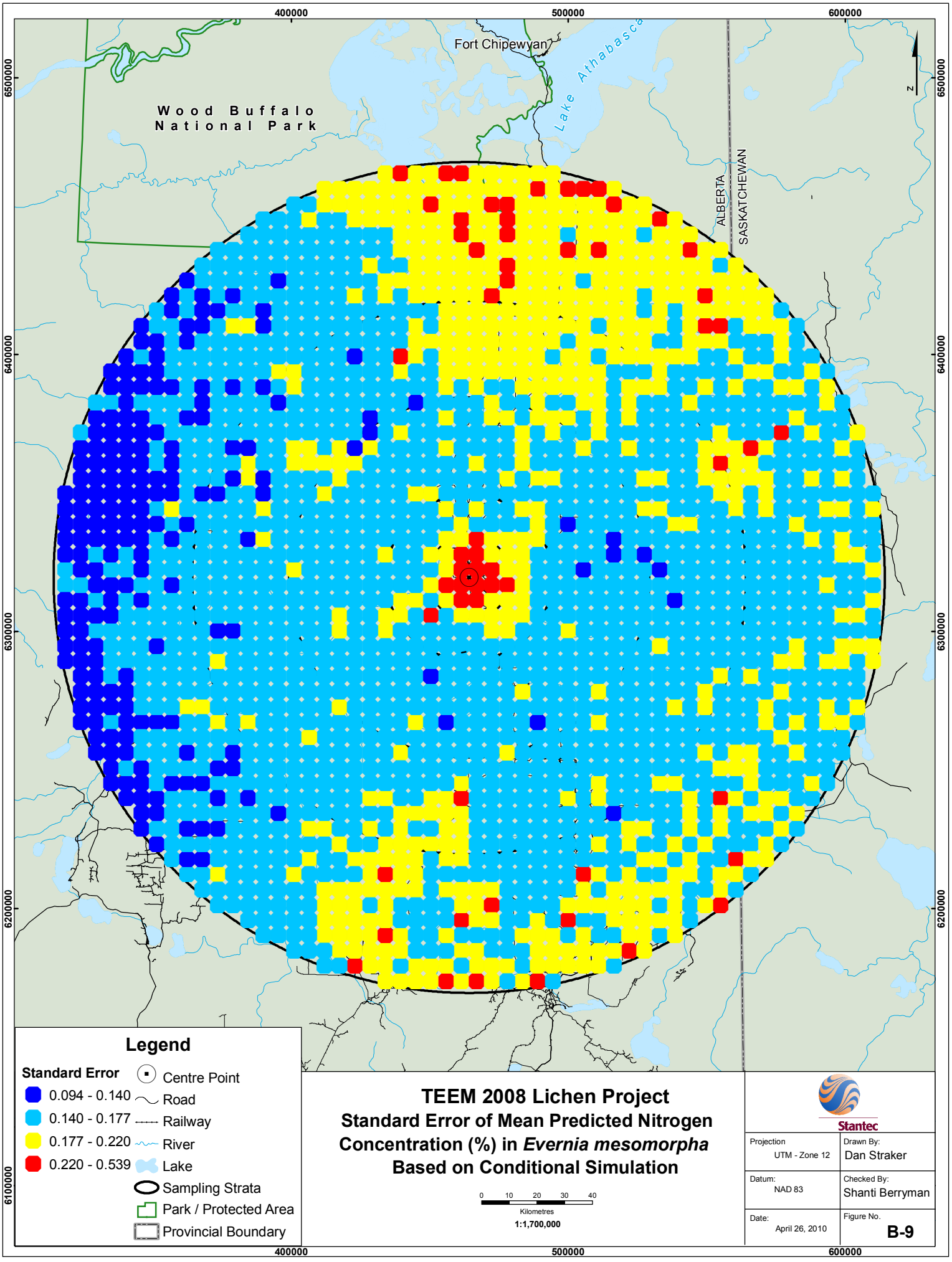
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500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Standard Error
0.094 - 0.140
0.140 - 0.177
0.177 - 0.220
0.220 - 0.539
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Standard Error of Mean Predicted Nitrogen Concentration (%) in Evernia mesomorpha Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-9
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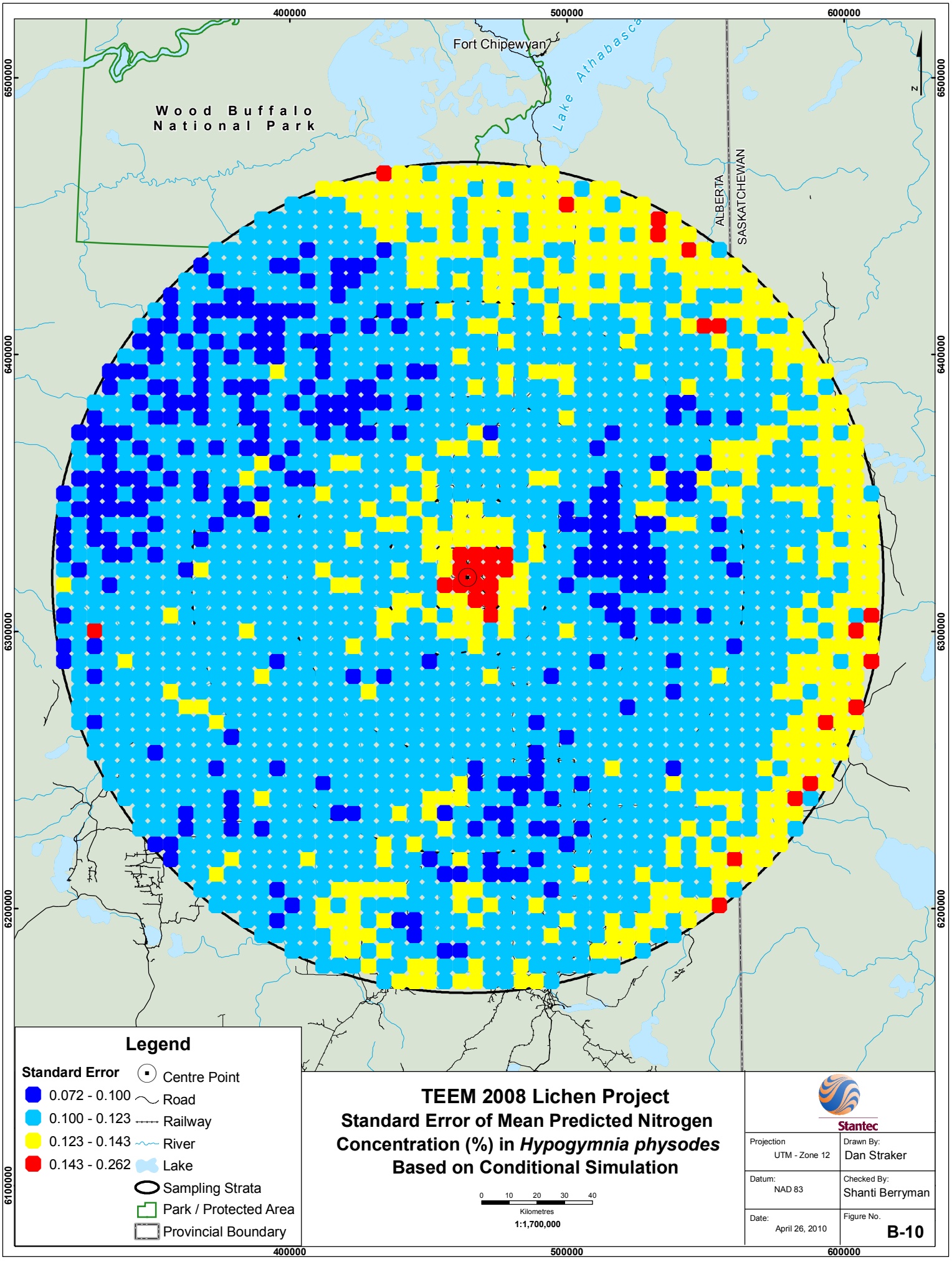
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500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Standard Error
0.072 - 0.100
0.100 - 0.123
0.123 - 0.143
0.143 - 0.262
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Standard Error of Mean Predicted Nitrogen Concentration (%) in Hypogymnia physodes Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-10
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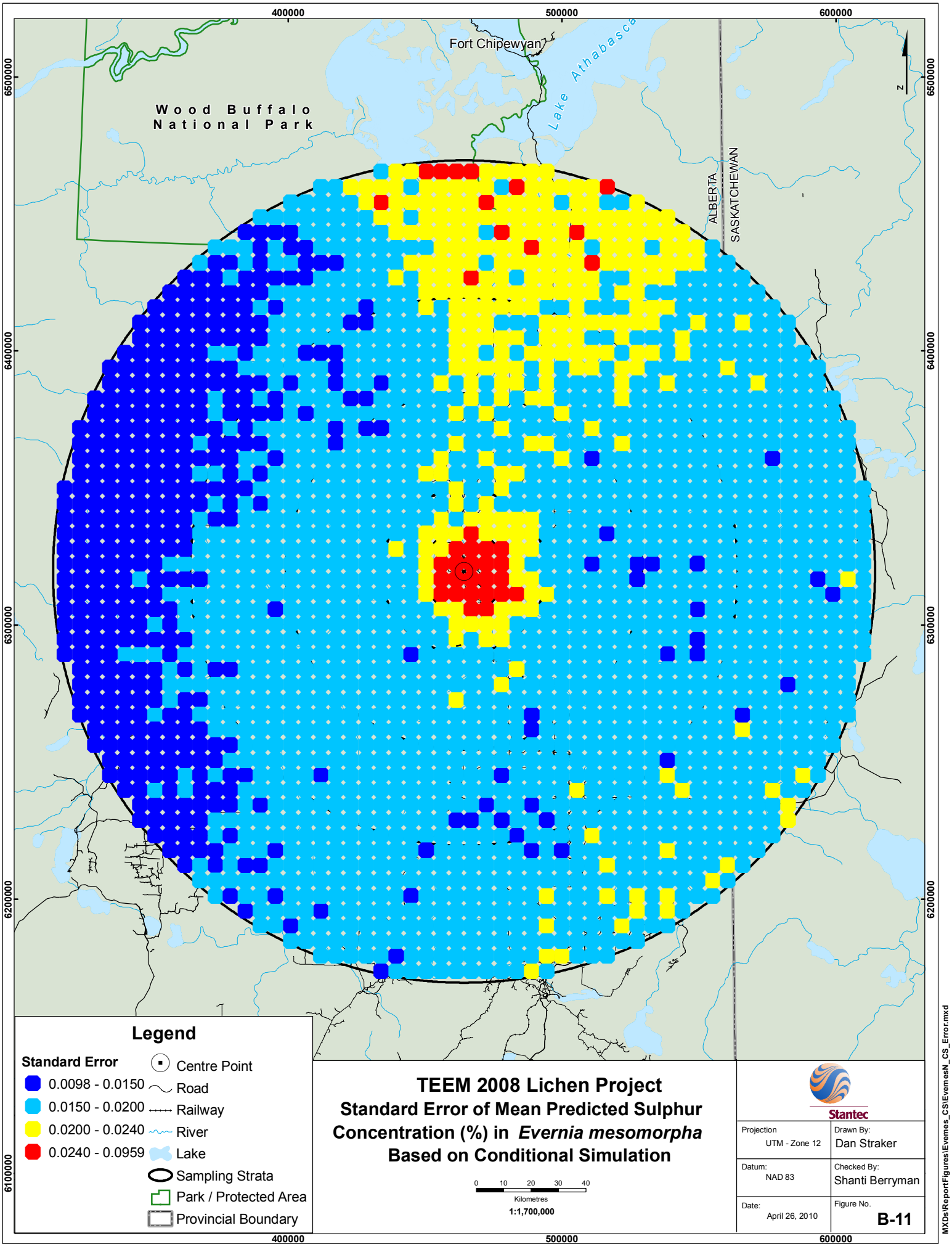
400000
500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Standard Error
0.0098 - 0.0150
0.0150 - 0.0200
0.0200 - 0.0240
0.0240 - 0.0959
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Standard Error of Mean Predicted Sulphur Concentration (%) in Evernia mesomorpha Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-11
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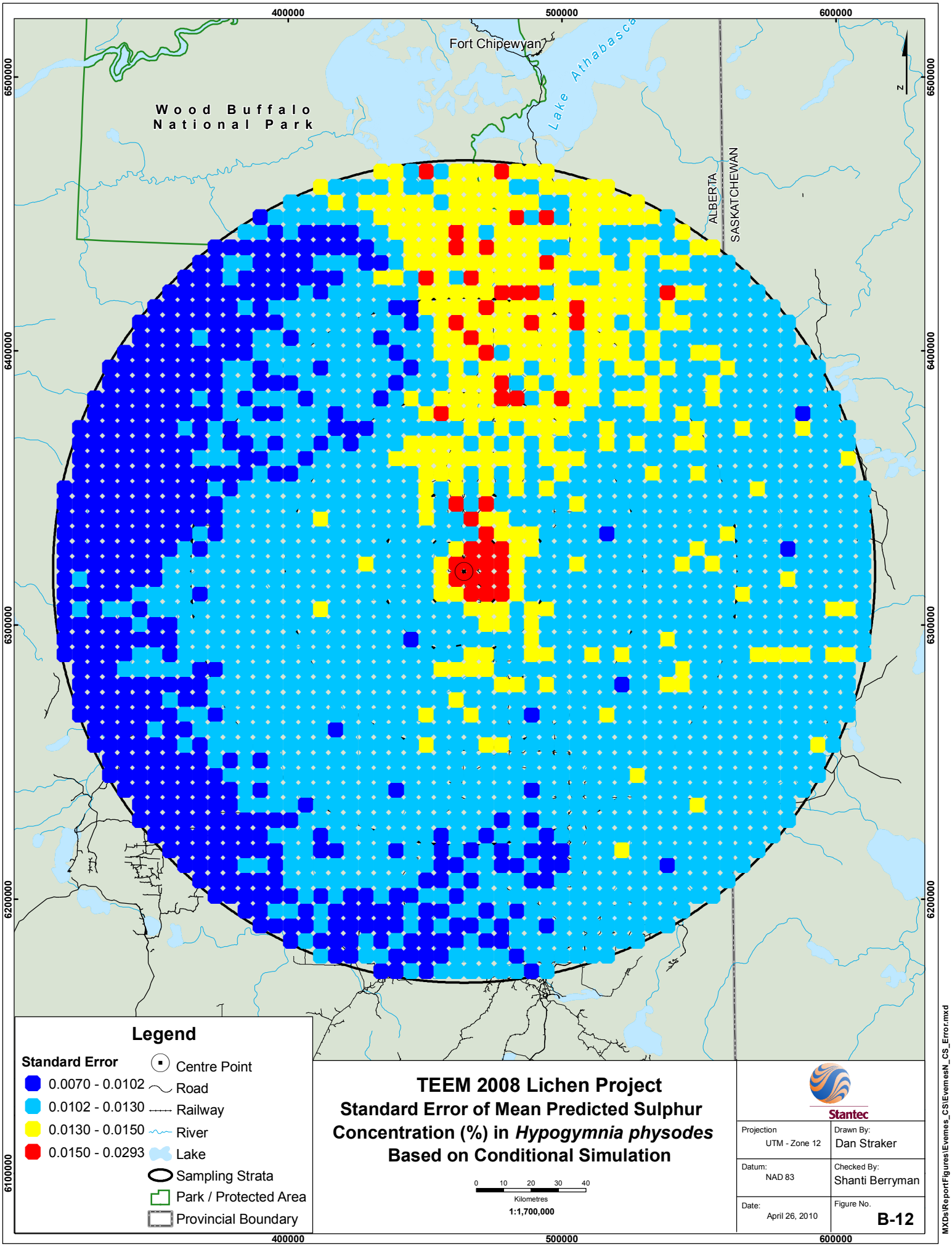
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500000
600000
6500000
6400000
6300000
6200000
6100000
Fort Chipewyan
Lake Athabasca
Wood Buffalo National Park
ALBERTA
SASKATCHEWAN
N
Legend
Standard Error
0.0070 - 0.0102
0.0102 - 0.0130
0.0130 - 0.0150
0.0150 - 0.0293
Centre Point
Road
Railway
River
Lake
Sampling Strata
Park / Protected Area
Provincial Boundary
TEEM 2008 Lichen Project
Standard Error of Mean Predicted Sulphur Concentration (%) in Hypogymnia physodes Based on Conditional Simulation
0 10 20 30 40
Kilometres
1:1,700,000
Stantec
Projection
UTM - Zone 12
Drawn By:
Dan Straker
Datum:
NAD 83
Checked By:
Shanti Berryman
Date:
April 26, 2010
Figure No.
B-12
MXDs\ReportFigures\Evemes_CS\EvemesN_CS_Error.mxd

Appendix C:
Summary Tables

Table C-1: List of sites where tree foliage (one branch per tree, from 5 trees per site), soil samples and lichen samples were collected for Dr. Bernhard Mayer to complete isotope analyses. Site type includes: TEEM –remote jack pine and aspen monitoring sites; AMS – active Air Monitoring Stations; Base – base site for the study design; and, Other –lysimeter site near Syncrude. Soil horizons are as follows: A – the uppermost upland mineral soil horizon, directly under the organic forest floor; AB – a mixture of 'A' and directly underlying 'B' horizons; Ah – A horizon enriched with organic matter; LFH – refers to a combination of the organic horizons that form the forest floor occurring at the soil surface; F – the organic horizon consisting of partially decomposed organic material; FH – a combination of the F and H horizons; and, none – no soil of this type was collected.

		Soil Samples		**Foliar Samples**	**Lichen Samples**	
Site Name	**Site Type**	**Mineral Soil**	**Organic Soil**	**Tree Species (5 samples)**	***Evernia mesomorpha***	***Hypogymnia physodes***
205INTERIOR	TEEM	AB	LFH	*Pinus banksiana*	1	1
210	TEEM	AB	none	*Pinus banksiana*	2	2
212	TEEM	AB	none	*Pinus banksiana*	2	2
213	TEEM	AB	none	*Pinus banksiana*	2	1
65B27	Base	AB	LFH	*Picea glauca*	1	1
AH3	TEEM	AB	LFH	*Pinus banksiana*	1	2
AH7	TEEM	AB	none	*Pinus banksiana*	1	1
AH8R	TEEM	AB	none	*Pinus banksiana*	1	1
AMS1	AMS	A	F	*Picea mariana*	1	1
AMS10	AMS	Ah	FH	*Picea mariana*	1	1
AMS11	AMS	AB	LFH	*Picea glauca*	1	1
AMS12	AMS	AB	FH	*Pinus banksiana*	1	1
AMS13	AMS	AB	LF	*Picea mariana*	1	1
AMS14	AMS	AB	LFH	*Picea mariana*	1	1
AMS15	AMS	AB	LF	*Pinus banksiana*	1	1
AMS2	AMS	B	FH	*Picea mariana*	1	1
AMS5	AMS	AB	F	*Pinus banksiana*	1	1
AMS6	AMS	B	FH	*Picea mariana*	1	1
AMS9	AMS	AB	FH	*Picea mariana*	2	2
H2	TEEM	AB	none	*Pinus banksiana*	1	1
H4EDGE	TEEM	none	none	*Pinus banksiana*	1	1
H4INTERIOR	TEEM	AB	none	*Pinus banksiana*	2	2
L1	TEEM	AB	none	*Pinus banksiana*	1	1
L7	TEEM	AB	none	*Pinus banksiana*	2	2
SYN1	Other	AB	FH	*Pinus banksiana*	2	none

Table C-2: List of the sub-set of *Hypogymnia physodes* samples selected for trace-element analysis as part of the receptor modeling and source apportionment component of this project. Batch 1 consisted of 49 samples; batch 2 was a subsequent set of 20 samples selected based on observed elevated nitrogen (N) and/or sulphur (S) concentrations in *H. physodes.*

Site	Batch	Total % Sulphur	Total % Nitrogen	N:S Ratio	Distance to centre point (km)	Comments
15AC1	1	0.118	0.769	6.54	13	high S; low N:S
15AC1	1	0.103	0.702	6.81	13	high S; low N:S
15AC5	1	0.086	1.037	12.13	7	high N
15AC9	1	0.103	1.004	9.71	14	high S
15AC16	1	0.078	1.087	14.00	11	high N
30AC20	1	0.084	0.705	8.41	22	low N:S
30AC24	1	0.048	0.602	12.67	25	low N
30AC26	1	0.101	1.191	11.77	21	high N
30AC30	1	0.073	1.050	14.46	27	high N
30B9	1	0.085	0.661	7.77	25	low N:S
30B10	1	0.094	1.150	12.24	17	high N
30B27	1	0.076	1.055	13.80	27	high N
65AC46	1	0.093	1.050	11.26	64	high N
65B9	1	0.049	0.846	17.17	31	high N:S
65B11	1	0.043	0.665	15.30	58	low S
65B31	1	0.066	0.619	9.43	33	low N
65SI1	1	0.108	0.945	8.79	38	high S
65SI22	1	0.119	0.915	7.66	63	high S; low N:S
65SI23	1	0.098	0.796	8.10	64	low N:S
65SI30	1	0.106	1.040	9.85	48	high S; high N
65SI31	1	0.103	0.827	8.07	46	high S; low N:S
65SI32	1	0.085	0.697	8.23	44	low N:S
65SI34	1	0.104	0.914	8.75	45	high S
65SI58	1	0.047	0.755	16.09	30	high N:S
100AC50	1	0.048	0.827	17.17	78	high N:S
100B3	1	0.052	0.609	11.71	99	low N
100B4	1	0.056	0.616	11.10	75	low N
100B8	1	0.050	0.574	11.43	87	low N/S
100B11	1	0.051	0.649	12.78	76	low N/S
100B13	1	0.047	0.620	13.20	68	low N
100B20	1	0.045	0.775	17.24	75	high N:S
100B32	1	0.038	0.687	18.20	66	low S; high N:S
100SI66	1	0.051	0.868	16.89	76	high N:S
100SI74	1	0.044	0.612	13.78	74	low S; low N

Site	Batch	Total % Sulphur	Total % Nitrogen	N:S Ratio	Distance to centre point (km)	Comments
100SI80	1	0.045	0.706	15.73	72	low S
150AC74	1	0.039	0.707	18.25	132	low S; high N:S
150AC78	1	0.040	0.574	14.35	151	low S; low N
150AC79	1	0.039	0.697	17.88	122	low S; high N:S
150B4	1	0.098	0.787	8.01	108	low N:S
150B8	1	0.107	0.808	7.58	109	high S; low N:S
150B18	1	0.045	0.610	13.48	136	low N
150B21	1	0.045	0.651	14.59	104	low S
150B28	1	0.040	0.564	14.01	151	low S; low N
AMS1	1	0.088	1.131	12.81	19	high N
15B25	1	0.087	1.063	12.20	8	high N
30B21	1	0.048	0.772	16.23	26	high N:S
150B26	1	0.044	0.626	14.16	151	low S; low N
150B27	1	0.048	0.781	16.18	115	high N:S
H2	1	0.104	0.933	8.97	12	high S
100B1	2	0.074	0.970	13.18	97	north hotspot, both N and S
100B2	2	0.083	0.876	10.62	101	north hotspot, both N and S
100SI67	2	0.112	0.861	7.71	77	S hotspot north of McClelland Lake
150B1	2	0.111	0.875	7.89	122	north hotspot, both N and S
150B10	2	0.071	0.832	11.65	140	east hotspot
150B2	2	0.079	0.897	11.34	124	north hotspot, both N and S
150B32	2	0.082	1.067	13.06	153	north hotspot, both N and S
150B9	2	0.076	0.977	12.92	142	east hotspot
15B1	2	0.087	1.540	17.72	10	hotspot near main mines
15B12	2	0.103	1.491	14.46	8	hotspot near main mines
15B2	2	0.105	1.422	13.52	11	hotspot near main mines
15B4	2	0.111	1.598	14.36	6	hotspot near main mines
212	2	0.111	0.978	8.79	12	hotspot near main mines; TEEM site
65SI19	2	0.081	1.131	13.95	61	N hotspot near Aurora North
AH3	2	0.113	0.831	7.38	45	hotspot east of Fort McMurray; TEEM site
AMS11	2	0.178	2.680	15.03	5	hotspot near main mines
AMS12	2	0.103	1.327	12.86	13	hotspot near main mines
AMS2	2	0.136	0.622	4.56	4	hotspot near main mines
AMS5	2	0.145	2.137	14.72	8	hotspot near main mines
H4INTERIOR	2	0.112	0.945	8.46	15	hotspot near main mines; TEEM

Table C-3: List of sites where air quality data are measured as part of WBEA programs. An "x" indicates this type of air quality sampler was present at the site during the 2008 lichen field program. Site type includes: TEEM – TEEM remote jack pine and aspen monitoring sites; Lake – remote lake sites; AMS – active Air Monitoring Stations (see Table C-4 for more details at AMS sites); and, Other –lysimeter site near Syncrude. The Passive MAXXAM samplers measure concentrations (ppb) of NO_2 and SO_2 on a monthly (bi-monthly in winter) basis; the USDA passive samplers measure concentrations (ppb) of HNO_3 and NH_3 on a monthly basis (bi-monthly in winter); and the Ion Exchange Resin Samplers (IER) measure NO_3^-, NH_4^+, and SO_4^{2-} on a seasonal basis (summer = May - September; winter = October - April) and on an annual basis (October – September). IER samplers were deployed in both open and throughfall positions. Dates in the table reflect data collection period for the data used in this study.

Site	Site Type	Data Collection Period for this Study		
		MAXXAM Passive Sampler	USDA Passive Sampler	Ion Exchange Resin Sampler
205INTERIOR	TEEM	Jan 07 – Jan 09	Jul 05 – Sep 08	-
210	TEEM	Jan07 - Jan 09	Sep05 - Sep 08	-
212	TEEM	May07 - Jan 09	May07 - Sep 08	May 08 – Apr 09
213	TEEM	Jan07 - Jan 09	Sep05 - Sep 08	May08 - Apr 09
AH3	TEEM	Jan07 - Jan 09	May05 - Sep 08	-
AH7	TEEM	Jan07 - Jan 09	May05 - Sep 08	-
AH8R	TEEM	Jan07 - Jan 09	Nov05 - Sep 08	-
AMS1	AMS	Jan07 - Jan 09	Jul05 - Sep 08	May08 - Apr 09
AMS10	AMS	-	Jul05 - Sep 08	May08 - Apr 09
AMS11	AMS	-	Jul05 - Sep 08	-
AMS12	AMS	-	May05 - Sep 08	-
AMS13	AMS	-	Jul05 - Sep 08	May08 - Apr 09
AMS14	AMS	May08 - Jan 09	Nov07 - Sep 08	May08 - Apr 09
AMS15	AMS	-	Jan 08 – Sep 08	May08 - Apr 09
AMS2	AMS	-	Jul05 - Sep 08	-
AMS5	AMS	-	May05 - Sep 08	May08 - Apr 09
AMS6	AMS	Jan07 - Jan 09	Jul05 - Sep 08	-
AMS9	AMS	-	Jul05 - Sep 08	May08 - Apr 09
BM10	Lake	Aug08 - Jan 09	Oct06 - Sep 08	-
BM11	Lake	Aug08 - Jan 09	Dec06 - Sep 08	-
BM7	Lake	Aug08 - Jan 09	Sep06 - Sep 08	-
H2	TEEM	Jan07 - Jan 09	May05 - Sep 08	May08 - Apr 09
H4INTERIOR	TEEM	Jan07 - Jan 09	May05 - Sep 08	May08 - Apr 09
L1	TEEM	Jan07 - Jan 09	May05 - Sep 08	-
L7	TEEM	Jan07 - Jan 09	May05 - Sep 08	May08 - Apr 09
NE10	Lake	Aug08 - Jan 09	Oct06 - Sep 08	-

Site	Site Type	Data Collection Period for this Study		
		MAXXAM Passive Sampler	USDA Passive Sampler	Ion Exchange Resin Sampler
NE11INTERIOR	Lake	Aug08 - Jan 09	Oct06 - Sep 08	-
NE7	Lake	Aug08 - Jan 09	Oct06 - Sep 08	-
SM8	Lake	Aug08 - Jan 09	Oct06 - Sep 08	-
SYN1	Other	-	Jul 08 – Sep 08	May 08 – Apr 09
WF4	Lake	Aug 08 – Jan 08	Oct 06 – Sep 08	-
Total Number of sites		**22**	**31**	**13**

Table C-4. Relevant nitrogen and sulphur parameters measured at WBEA continuous air-monitoring stations, where 'x' indicates a parameter measured from January 2007 to December 2008 (measurements at the AMS15 site were only taken in 2008). Lichen samples were co-located with these AMS sites. TEMP = temperature; TRS = total reduced sulphur.

Site	NO (ppb)	NO_2 (ppb)	NO_x (ppb)	PM2.5 (ug/m3)	SO_2 (ppb)	TEMP @ 2 m (°C)	TRS (ppb)	Wind Direction @ 10 m (deg)	Wind Speed @ 10 m (km/hr)
AMS1	x	x	x	x	x	x	x	x	x
AMS10	x	x	x	x	x	x			
AMS11					x	x		x	x
AMS12	x	x	x	x	x	x	x	x	x
AMS13	x	x	x	x	x	x	x	x	x
AMS14	x	x	x	x	x	x	x		
AMS15	x	x	x	x	x	x	x	x	x
AMS2					x	x		x	x
AMS5					x	x			
AMS6	x	x	x	x	x	x	x	x	x
AMS9						x	x	x	x

Appendix D:
Manual for Monitoring Air Quality Using Lichens in Boreal Forest of Alberta

1.0 Lichen Sampling Manual

1.1 BIOMONITORING FOR AIR POLLUTION IN ATHABASCA OIL SANDS REGION

Air emissions and fugitive dust (pollutants) resulting from mining and mine-related activities, and the potential effects of these pollutants on surrounding ecosystems, are of increasing scientific and environmental interest. However, in many contexts, characterization of off-site deposition of pollutant compounds can be extremely difficult. Specific challenges are posed by the lack of technology capable of characterizing air quality and pollutant deposition across a large landscape, due to high costs associated with deploying and maintaining air-monitoring equipment, and to location constraints relating to requirements for continuous, alternating-current power. Because of these limitations, it is often logistically and economically prohibitive to implement air-quality sampling (passive or continuous) throughout a large remote area at a spatial density that is sufficient to reliably characterize patterns and levels of atmospheric deposition.

One option to address the limitations noted above is to characterize (and potentially monitor) pollutant deposition using biological receptors (e.g., vegetation tissues). This approach presents a cost-effective method to characterize spatial patterns of pollutant deposition across a large study area, and can be used either in isolation or in conjunction with direct passive or active air-quality measurements. Lichens are naturally occurring biological receptors with specific characteristics that lend themselves to reliable use in air-pollution assessment and monitoring programs. Analysis of elemental content in lichens collected near remote industrial emissions sources can help to determine spatial patterns of atmospheric pollutant deposition, including fugitive dust from mining and milling operations and atmospheric emissions from processing and mine fleet operations.

Rapid industrial development in the Athabasca Oil Sands Region (AOSR) of northeastern Alberta has resulted in increased emissions of sulphur and nitrogen compounds, (e.g., sulphur dioxide [SO_2] and nitrogen oxides [NO_x]), and airborne particulates containing trace metals (Pauls et al. 1996). Extended periods of enhanced deposition of these pollutants and their products can alter ecosystems both directly and through soil and surface-water acidification or eutrophication. There is a recognized lack of empirical data for historic and current pollutant deposition fields in the AOSR, and this lack of information represents a critical gap in regional air-quality and ecological monitoring and modeling programs. Thus at present we are unable to confirm predicted deposition patterns at the regional scale, which would help to establish a more reliable link between air-pollutant emissions and their possible effects on ecosystems. Lichens are a cost-effective indicator for collecting reliable, high-density information across the AOSR region and to help overcome logistical challenges (associated with limited access) for air-pollutant measurement and monitoring programs within this region.

This document has been prepared to describe in detail methods used for sampling and analysis in the TEEM Lichen Bioindicator project, both simply to document these methods, and to allow replication of these methods if this project is expanded (through repeated sampling) as a monitoring component of the TEEM program.

1.2 FIELD PREPARATION

The following equipment is required before beginning the field sampling portion of the program:

- Disposable powder-free (powderless) nitrile gloves
- one pair/sampler/site + 30% additional as contingency
- can be ordered from Lab Safety Supply (http://www.labsafety.com)

- Sample bags (with air tight lock)
- one/each species/site + 50% for field replicates and additional contingency
- can be ordered at: http://www.nashvillewraps.com/ShowDetail.ww?Sku=suzp812, item description: 6x9x2-1/2" Clear Zipper Top Bag - #: SUZP812
- Large Ziploc freezer bags
- Garbage bags
- Permanent black felt markers (Sharpie pens)
- 2-3 Pensola spring scales (60 or 100g)
- Stainless-steel knife (optional)
- Data sheet (Section 1.2.2).
- Digital camera
- Freezer (optional – for sizing see Section 1.4)

Items should be ordered beforehand to ensure readiness for field sampling start date. Lead time for acquisition of powder-free gloves, sample bags, scales and freezer may be up to a few weeks in advance of the field project, for arrival in Fort McMurray. These items will be necessary in the cleaning process as well, so check all steps in the manual to estimate how many of each will be needed. Other items are generally available, and can be replenished as needed.

1.2.1 Use of Gloves

Contamination of lichen sample

The parameters measured from the lichen samples make it necessary to keep them totally free from contamination of any sort. This includes contamination from:

- Skin
- Clothing
- Tools (scale, pens, notebooks)
- Any part of the human environment
- Cross-contamination with other samples

For this reason it is very important to use the proper field equipment and field procedures listed below to avoid contamination of the lichen sample. If lichen sample comes into direct contact with any part of the human environment or any part of the natural environment outside of its own site in any way it is compromised and must be discarded. A lichen sample may only come into contact with parts of the natural environment on the site where it is collected. Refrain from opening sample bags once they have been shut.

Powderless (Powder-Free) nitrile gloves must be used to avoid contamination of lichen samples. To keep them from becoming contaminated themselves, these gloves must not contact any part of the human environment. This includes but is not exclusive to: equipment, clothing, skin, tools etc. Once at a site, gloves may come into contact with anything that naturally occurs within that environment. If at any time gloves do come into contact with any part of the human environment, are torn, or are contaminated in any way, they must be removed and discarded and a new pair of gloves equipped.

Equipping gloves

When picking up a glove grasp by the wrist portion of the glove rather than by the finger or palm portion of the glove and carefully pull over your other hand. Take the second glove by the exterior of the wrist portion with the fingers of the gloved hand and stretch the glove over the uncovered hand.

Glove and lichen sample bag preparation

Before going into the field prepare sampling equipment beforehand to save time. Gloves, sample bags, and garbage bags must be removed from original packaging and double bagged in air tight Ziploc freezer bags. To prepare bags of powder free nitrile gloves:

1. Estimate how many gloves will be necessary (one pair of gloves / sampler / site). Different sizes of gloves may be necessary depending on who is sampling. About 50 gloves (25 pairs) will fit into one Ziploc bag. If you have different sizes of gloves, label Ziplocs by size of glove.
2. Open enough boxes of gloves for the estimated amount of gloves.
3. Open each Ziploc bag.
4. Equip gloves as instructed above.
5. With gloved hands, transfer gloves from original packaging to Ziploc bags.
6. Proceed to fill all bags roughly half full (~50 gloves).
7. Once transfer of gloves into Ziploc bags is complete, squeeze the Ziplocs to remove excess air and seal them.
8. Place Ziploc bag of gloves inside another Ziploc so that each bag is double bagged.

1.2.2 Field card datasheet preparation

Prepare datasheets on waterproof paper with fields to record the following types of data while in the field:

- Site Name
- GPS coordinates
- Date
- Observers
- Site Type
- Distance strata
- Vegetation stratum (based on Alberta Ground Cover Classification)
- Community type (Upland classification keyed from Natural Resources Canada Ecosites of Northern Alberta; Section 7: Boreal Mixedwood and Wetland classification keyed from Wetlands guide) and general notes on the ecosite and vegetation
- Slope and Aspect
- Densiometer measurements
- Lichen sample inventory information including:
- Species
- Field Duplicate (Y/N)
- Weight of sample (w/o bag)
- Moisture (dry, damp, wet)
- Time required to collect sample
- Substrate sample collected from (plant species and location on plant)
- Comments regarding anything of note on site, oddities, proximity to human disturbances, abundance or lack of lichen etc.

British Columbia standardized Ground Inspection Form (GIF) cards can be used to list various site and vegetation related data such as vegetation species, layer within vertical structure and percent cover at each site as well.

1.2.3 Identifying Target Species

Evernia mesomorpha and *Hypogymnia physodes* are two epiphytic lichen species that can be used effectively to assess human caused air pollution in this region. Both grow sporadically on tree branches and boles. *Hypogymnia physodes* (Hypphy) is mat-like in shape with a puffy smooth texture, a white surface, black underside and lobed ends.

Figure D-1: *Hypogymnia physodes* (Hypphy)

Hypphy can easily be mistaken with *Parmelia sulcata* (Parsul) which is of a similar size and has the same mat shape and white surface with lobed ends. The key to distinguishing the two is the flatter texture and concave dimpled surface of Parsul rather than the puffy, smooth texture of Hypphy.

Figure D-2: *Parmelia sulcata* (Parsul)

Figure D-3: *Evernia mesomorpha* (Evemes)

Figure D-4: *Ramalina farinacea* (Ramfar)

The other target species, *Evernia mesomorpha* (Evemes) is bushy and light green with rounded mostly dichotomous branching. It can easily be confused with *Ramalina farinacea* (Ramfar) which shares its colour and branching patterns. The key to distinguishing these is that Evemes branches are rounded and more cylindrical while Ramfar branches are flattened.

Moisture content in lichen sample

These target lichen species are capable of absorbing a large quantity of water greater than 500% of their weight, so special attention must be paid to the moisture content of the sample before, during and after collection. If the sample is collected during or after a rain event it will most likely be **wet** and have absorbed at least 500% of its mass in water. If a sample is wet it will be flaccid and fragile. When the lichen sample is wet, expect to sample at least 1500% of the target amount of clean dry lichen sample. For instance if the target is to attain 5 grams of clean dry lichen sample, approximately 75 grams of wet lichen sample is the minimum amount required.

If there has been no recent rain event (within 24 hours), the sample will most likely be **damp**, meaning that it has absorbed some moisture but is not saturated. A damp sample will neither be flaccid like a wet sample nor rigid and crunchy like a dry sample. Most commonly samples will be damp as a number of environmental factors in the region attribute moisture to lichen including frequent and abundant amounts of precipitation and humidity/condensation. For damp lichen sample, approximately 500% of the target weight is the minimum amount required to attain the target amount of clean, dry lichen.

Very rarely is a sample **dry**. It takes a minimum of 3+ warm days without precipitation, in a low humidity microclimate and direct sunlight for these species to be dry in natural conditions of this region. It is rare for these conditions to be met as rain events occur frequently throughout summer and humidity is relatively high in this region. A **dry** lichen sample is quite crunchy and very light in terms of weight. In this case, roughly 250% of the target amount is required to achieve the target amount of clean, dry lichen.

The moisture content of these target lichen species makes the required weight of the sample highly variable. Good judgment must be exercised on the part of the sampler when deciding how much lichen sample to collect in the field. Discretion must be used to collect more or less sample appropriately depending on the moisture content of the lichen sample. Once samplers are more experienced, the size of damp and wet samples can be judged by volume rather than weight.

1.3 FIELD SAMPLING

Once on a site collect all site data and vegetation data relating to the data sheet. Prepare lichen sample bags by labeling with site name, species and date before equipping gloves. Once gloves are equipped, collection of lichen sample can begin. A few things to remember when sampling:

- Try to retain the whole of the lichen individual complete with the thallus (lichen branch) that connects to the tree branch. This is more difficult when the sample is dry as it tends to become more brittle as it loses moisture.
- Pay close attention to contamination of gloves, if you notice yourself or another sampler contaminating their gloves be sure to point it out. It takes a while to get used to.
- If not carrying a sample bag while sampling, deposit collection into a sample bag often as lichen will compact in the storing hand making the sample much more difficult to clean later. Alternatively, have all samplers carry their own sample bag to deposit into.
- Most lichen sample is attainable by use of fingers. Although unnecessary for these particular lichen species, a stainless steel knife may be used to aid in sample collection but must be cleaned and stored in soapy water or alcohol after and in between each site.
- Dispose of as much large non lichen tissue debris (ie. bark, twigs, etc.) as you collect on site as it will save time in the cleaning process, and helps to more accurately estimate the amount of actual lichen sampled.
- Collect from at least 6 trees within the site so that the sample is representative of the entire population. If sample cannot be collected from 6 trees then this should be noted on the data sheet.
- Collect only from branches of standing (live or dead) trees above 1m (3 ft.) Do not collect from the bole of the tree, downed trees, or from the litter of forest floor, unless that is the only sample attainable on the site. If sample must be taken from one of these sources it must be noted on the data sheet.

NOTE: Lichen is sampled from branches because it is expected that branches are more exposed to deposition sources than boles and therefore more representative in demonstrating deposition patterns. These species decompose rapidly on the forest floor and therefore measure of deposition from these samples cannot be relied on.

- Don't forget to look up, not all lichen of these species grows at eye level. It can often be found on the tops of higher up branches, and may require breaking off branches to collect.

1.3.1 Labeling and storing samples in the field

Once enough lichen sample has been collected, have one sampler remove their gloves and weigh the bag using a Pesola spring scale to ensure that enough lichen has been collected. If there is enough lichen sample then seal bags and remove gloves. Label the bag with the following information:

- Site name
- Species
- Identify whether sample is a field duplicate or not
- Date
- Moisture (wet, damp, dry)
- Collector(s)
- Weight of sample (in grams)

Label this information boldly and clearly with a consistent convention (ie. label each parameter in the same place on each bag, consistently use either upper or lower case etc.) for every sample to avoid confusion. If preferred, much of this information can be labeled on the bag before collection begins, but be sure to double check all parameters at the end of collection. Once lichen sample bags are correctly labeled, place them together into one 'Site' Ziploc bag (or more if necessary) that is labeled with the site name and date and the number of samples in the bag. To ensure that samples are not lost throughout the day, place them in a larger garbage bag as they are collected. Do not open lichen sample bags once they have been removed from their site until ready for drying and cleaning process.

1.4 ORGANIZING AND STORING SAMPLES

At the end of each sampling day, inventory all samples and enter all data labeled on sample bags into an excel spreadsheet. Sort samples into separate sub-categories (eg. all sites beginning with 15AC, all samples beginning with 15B) and place each sub-category into separate garbage bags.
Freeze or dry lichen samples as soon as possible after collection, because if they contain any moisture and are left at room temperature they will begin to decompose within 24 hours.

When choosing a freezer for storage, use the dimensions of an average filled lichen sample bag multiplied by the number of samples to be collected and add 20-30% for overhead to calculate the volume of freezer necessary. An average sample will have the dimensions 22.5cm (or 9") tall x 15cm (6") wide x 6.5cm (2.5") deep. Place all 'Site' Ziploc lichen samples in the freezer in their sub-categorized garbage bags.

1.4.1 Shipping samples

When shipping samples, follow these criteria:

1. Use only coolers or rubberized totes
2. Number each box used for shipping. Inventory all samples as they are put in each box and provide an inventory sheet within each box and keep one for your own records

3. Keep samples cool with non leaking ice packs or a reasonable substitute. Nalgene bottles filled with water and frozen will not leak if sealed properly. If using Nalgene bottles, do not fill bottles to the top as water will expand by 10% when frozen. If water does leak it may contaminate lichen samples.
4. If samples take longer than 3 days to ship to their destination there is an increased risk of decomposition, find an alternative shipping method.
5. Seal each box with tape so that it is airtight and there is no chance of lid coming off.

Each time samples are shipped there is an increased risk of disorganization and potential loss of samples, so only ship samples when necessary. Provide a copy of this manual with any shipment so that the recipient is aware of how to store samples appropriately, and how they are organized.

2.0 Lichen Cleaning

2.1.1 Station set-up

Set up stations for lichen drying and cleaning prior to receiving samples so that processing can begin when samples arrive. Ensure there are no obvious contaminant or pollution sources in the area, air or otherwise. Make sure a large amount of space is available and that the drying and cleaning stations are separated from one another to reduce confusion during sample processing.

Drying station

- For the drying station the following equipment is mandatory:
- Powder free nitrile gloves (1 glove/sample)
- Black felt permanent marker (sharpie pens)
- # 2 paper bags
- Clothesline (or something to hang samples from)
- Clothespins
- Freezer

Samples must be kept frozen prior to drying. Prepare Ziploc plastic bags of powder free gloves according to instructions outlined above in section 1.2.1. Inspect paper bags to make sure no holes or leaks are present in the bags. Hang a clothesline or a suitable alternative nearby so that samples may be hung to dry. This line must be very secure, so that it can hold multiple sample bags during the drying process.

Cleaning station

Order and prepare the following equipment ahead of scheduled cleaning time in a clean environment:

Amount	Equipment	Suggested Order Link
1 per sample	Sample bags	http://www.nashvillewraps.com/cellophane-bags/zip-lock-bags/sku-suzp812.html
1 per station	Pesola spring scale (30g with 0.25g increments)	http://www.benmeadows.com/search/Pesola/20661/?type=brand
1 per station	Forceps (Teflon coated)	http://www.amazon.com/Chemware-PTFE-Coated-Forceps-Nickel-plated-Length/dp/B0015SJ6KG
1 per station	Scalpel handle and blades (stainless steel)	http://www.amazon.com/SCALPEL-HANDLE-3-STAINLESS-STEEL/dp/B001D72R46/ref=sr_1_2?ie=UTF8&s=industrial&qid=1269297759&sr=1-2
1 per station	Scalpel blade	http://www.amazon.com/Nasco-SCALPEL-BLADE-10/dp/B001D769JK/ref=sr_1_2?ie=UTF8&s=industrial&qid=1269297559&sr=1-2
1 pair per sample	Powder free nitrile gloves	http://www.amazon.com/Kimberly-Clark-Gloves-Nitrile-Purple-Powder-Free/dp/B001429LWG/ref=sr_1_1?ie=UTF8&s=industrial&qid=1267472252&sr=1-1

1 pint / 30 samples	Alcohol with no coloring or additives; put alcohol in a clean spray bottle	http://www.amazon.com/VI-JON-CUMBERLAND-SWAN-ALCOHOL-ISOPROPYL/dp/B001B5JT8C/ref=sr_1_1?ie=UTF8&s=hpc&qid=1269297214&sr=8-1
4 / sample	Kimwipes	http://www.amazon.com/KIMBERLY-CLARK-KIMWIPES-Delicate-Wipes/dp/B003085W7U/ref=sr_1_2?ie=UTF8&s=hpc&qid=1269297263&sr=1-2
1 / station	Flat glass surface (2' x 3')	Suggest to purchase locally from a glass or window store

Use bright light surrounding the cleaning area as the cleaning process requires focus on a small area for extended periods of time. Use reading glasses or magnifying glasses to help focus and reduce strain on eyes. Hang the spring scale above the cleaning station at a comfortable height to use while cleaning, or use a digital scale with accuracy of at least 0.1g. Prepare spray bottle with alcohol, and place box of Kimwipes next to cleaning station. These will be used in between every lichen sample to clean surface and tools (use as many Kimwipes as necessary to thoroughly clean the area and to clean up any residual alcohol). Bags of gloves (1 pair/sample) should also be prepared by the method outlined above in section 1.2.1 and should be readily available.

Lichen dust

A major concern when cleaning dried lichens is that they are very dusty, mainly from the asexual propagules that break off the lichen in the bag and during cleaning. Human allergies to this dust have been documented, resulting in minor skin irritation and minor respiratory problems. For this reason, it is recommended to wear the following while cleaning samples:

- respirator, or simple dust mask
- hat or hair cover
- coveralls
- shoe covers (paper or otherwise)

Wearing this protection in the cleaning area protects personal clothing from lichen dust. An air purifier will also reduce the amount of dust in the air and help maintain a clean environment during the lichen cleaning process. Clean all equipment and surfaces used between sample cleanings using the alcohol and clean Kimwipes. Flat glass (roughly 2 feet x 3 feet) is the recommended surface for cleaning samples, as it is easily cleaned. Stainless steel or Teflon coated forceps and scalpels are recommended to aid in the cleaning process and these tools must also be cleaned with alcohol and Kimwipes between samples.

2.1.2 Air drying lichen samples

At this point, both the drying and cleaning stations should be set up and the lichen samples are stored in a freezer (Note: a standard chest freezer is fine). Samples in the freezer should be organized in clean large plastic bags or containers by distance stratum (i.e., 15, 30, 65, 100 and 150) and site type classes (B, AC, TEEM, Lake, AMS or other). Remove approximately 40 samples at a time from the freezer in preparation for drying. For ease of organization and sample inventory, remove samples from within the same subcategories of distance strata and site type (e.g., all bags from 65 AC) and work with each subcategory one at a time.

The air drying process takes at least four days and drying times can be doubled if samples are very wet. Estimate how many samples you can clean per day and adjust how many samples are being dried accordingly as the process continues.

Each sample must be transferred into a paper bag for drying. Remove 8-12 lichen sample bags from their 'Site' Ziploc bags, and keep the 'Site' Ziploc bag handy. Set aside very wet frozen samples as they need to thaw slightly (approximately 4-6 hours at room temperature or until flexible) before they can be transferred to a paper bag successfully without breaking through the paper bag. It may be necessary to double-up paper bags or transfer to a new paper bag for extremely wet samples to ensure the bottom of the bags do not break due to high moisture content.

Labeling paper bags

Copy all information from a lichen sample bag onto a clean paper bag consistently with site name, species, duplicate (if not a field duplicate, then leave this blank), date sampled, and number of grams sampled according to its corresponding sample bag (Figure D-5**Error! Reference source not found.**). Site name, species, and duplicate are not enough information to identify samples if labeling mistakes are made during the drying processing. Labeling whether a sample is wet or dry will help to distinguish those samples that require more drying time. Once a paper bag is labeled, place it back into the larger 'site' Ziploc containing the corresponding lichen sample bag and leave the 'site' Ziploc bag open. In doing this, both the labeled paper bag for drying, and the lichen sample bag itself are kept together which will help to avoid confusion during the transfer process.

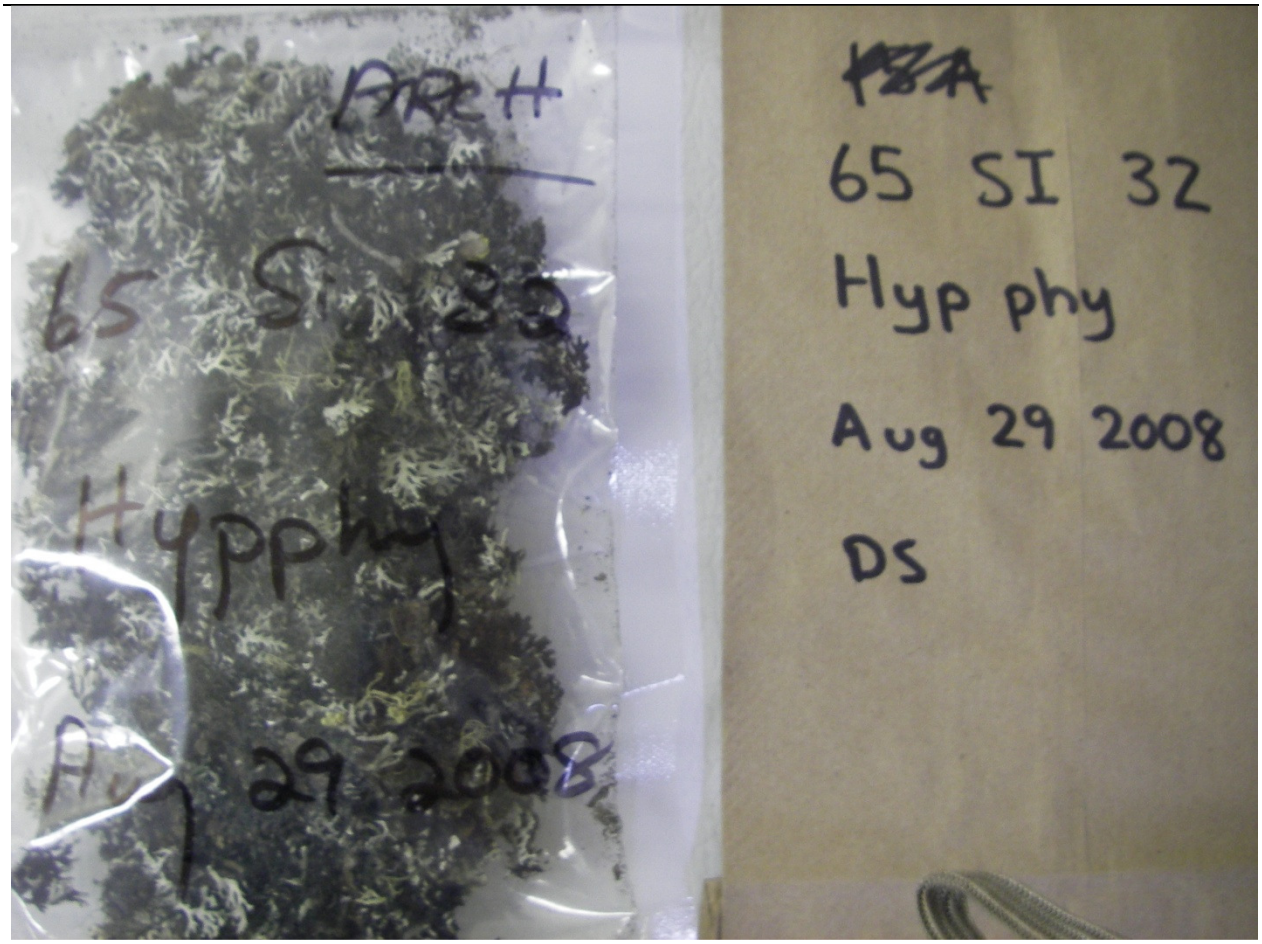

Figure D-5: Labeling paper bag for drying lichen sample. All information from field lichen sample bag (left) is copied onto drying lichen bag (right).

Before transferring the lichen sample to the paper bags, check the label of each paper bag against the lichen inventory spreadsheet. This process will help identify any inconsistencies in labeling between lichen samples and the inventory spreadsheet before the lichens are transferred from the plastic sample bag to the paper bag for drying. If there are inconsistencies in the label for the lichen sample and inventory, set these samples aside in a "problem bag" and place back in freezer. Follow-up to clarify labeling problems for these samples and once reconciled, then proceed with drying.

Transfer lichen into paper bag

Take these 8-12 lichen samples inside their 'Site' Ziploc bags along with their corresponding paper bags to the transfer area. Remove all lichen sample bags and labeled paper bags from one 'Site' Ziploc bag. Open each lichen sample bag for that site. Take corresponding paper bag by the top and open using a rapid wrist flick without putting hands on the inside of the clean bag, and leave the paper bag open standing on table next to open lichen sample bag. DO NOT put your hand inside the paper bag or the lichen sample bag to open as this has the potential to introduce contaminants.

1. Remove one nitrile powder-free glove from bag of gloves and equip on preferred hand.
2. With ungloved hand hold the lichen sample bag, and with gloved hand guide lichen sample into the clean paper bag (note: you can tip the lichen sample bag into the paper bag, but be careful not to lose lichen in this process; use gloved hand to ensure all lichen goes into the paper bag).

REMEMBER: Any lichen sample that falls outside of the paper bag is considered contaminated and cannot be used. DO NOT take any lichen that has touched the floor, table, skin or clothing and put in paper bag; this lichen must be discarded to ensure a clean sample.

3. Once all lichen sample has been transferred to the paper bag, gently fluff the lichen to increase air circulation during the drying process; be careful not to break apart lichen individuals while doing this.
4. Fold top of paper bag that contains lichen sample three times at ~ ½ inch intervals so that lichen will not fall out.
5. Reseal the used lichen sample bag, and label sample bag at top as used. Put all used field lichen sample bags from each subcategory into garbage bags labeled “xx transfer sample bags” where xx is the subcategory (e.g., “65AC transfer sample bags”). Save these empty bags as a reference if there are problems in labeling/naming of samples that come up during the drying/cleaning process.
6. Remove used glove and discard. Check paper bag off on inventory spreadsheet and note that it is now drying by noting the date under ‘drying’ column. It is important to document the process steps for each sample.
7. Hang paper bag from folded portion on clothesline and let dry for at least four days. It is important that paper bags with lichen sample are closed securely and hung securely. If they fall from the line, the lichen sample must not come out of the bag; if it does, the sample is lost due to potential contamination.

   NOTE: If the lichen is too wet to hang, consider setting the paper bag on the table to dry for the first day or two, fluffing once or twice to increase air circulation, and then hang for the remaining period of time to complete the drying process. It may be necessary to put material into a new labeled paper bag as well.

Once lichen sample is completely air dried, the lichen cleaning process can begin.

2.1.3 Cleaning lichen samples

Take 8-12 air dried lichen samples from same sites down from the drying clothesline, put in a box and take to cleaning station. Keep samples from the same site together to avoid inventory confusion. If more than one person is cleaning lichen, have one person clean all lichen samples from a given site (includes the different lichen species and/or field duplicates). Mark on the inventory sheet which samples are dried and being taken to be cleaned to keep track of sample processing for each individual sample. Before entering the cleaning room, equip any health and safety equipment including respirator, coveralls, hats, shoes etc. and activate air purifier.

Lichen cleaning steps

Repeat these steps for cleaning each lichen sample:

1. Label two new plastic sample bags for each lichen sample (one as “Clean” and one as “Archive”) with site name, species, dupe, field collection date, as well as the cleaners initials and cleaning date. The “Clean” bag will contain all cleaned lichen and the “Archive” bag will contain the remaining unclean sample at the end of the cleaning process.
2. For each sample, record on the inventory spreadsheet: cleaning start time, date, and cleaners initials.
3. Open the bag labeled “Clean” and hang it from a suspended Pesola scale and place the “Archive” bag on side of table.
4. Spray glass surface with alcohol and clean using Kimwipes. Wipe away excess alcohol with Kimwipes. Wipe down forceps and scalpel with alcohol and Kimwipes.

5. Unfold paper bag and hold over glass surface and without touching the lichen, empty lichen sample onto the glass surface. REMEMBER if any lichen sample touches anything but the glass surface it is considered contaminated and must be disposed of.
6. Prepare any other equipment that is needed (reading glasses, air purifier, lights etc.) as once cleaning begins gloved hands cannot touch anything but forceps, scalpel and lichen.
7. Without touching finger or palm section of clean gloves, equip gloves on each hand. For help in avoiding contamination while equipping gloves, follow guidelines from section 1.2.1.

Cleaning should be conducted in an ergonomic and comfortable fashion as cleaning a sample can take between 45 minutes to 2 hours. Remove all non-lichen material (i.e., bark, twigs, other plants) from the lichen sample and put aside in a disposal pile. Remove all lichen species that are not the targeted species from the sample (e.g., often *Usnea* sp. and *Bryoria* sp. are mixed in with *Evernia mesomorpha* samples). Deposit all clean lichen into the "Clean" sample bag hanging from Pesola scale, and continue this process until the target cleaned lichen weight is met.

Amount of lichen to clean

Depending on how much lichen sample there is to work with, more or less lichen can be discarded during the cleaning process. Typically, the more sample there is initially, the easier it is to clean the required amount, but if there is relatively little lichen sample initially, be conservative with how much lichen is disposed of during cleaning.

It is essential that you take caution not to break apart the lichen individuals too much during cleaning. Make the best effort to retain a fairly representative "cleaned" lichen sample by not biasing the cleaning process. To achieve this, try to clean across the range of lichen individuals present, do not bias cleaning to the largest or healthiest individuals. Further, try to clean the complete individual, rather than tearing off bits and pieces the lichen

The targeted dry weight of the cleaned lichen sample will depend on the laboratory analyses targeted for the lichen sample. As the field collection method targets a composite lichen sample that is representative of the lichen population at a given site, it is important to maintain that "representative" composite sample during the cleaning process. To achieve this, clean as much sample as is possible (and realistic), targeting an ideal 7 g of cleaned lichen for each sample. This will results in a representative clean lichen sample and is sufficient sample for most laboratory analyses. The minimum sample that should be cleaned is 4g, and only spend 2 hrs cleaning to reach this minimum. If more than two hours of cleaning has elapsed and 4g have not been attained, 3g or more becomes the minimum at the cleaner's discretion. However, with less clean lichen sample, there is an increasing risk of not representing the lichen population and the integrity of the sample decreases.

Archiving uncleaned dried lichen sample

Once enough clean lichen sample has been attained, the rest of the un-cleaned lichen sample can be placed in the "Archive" bag. At this point, note the following information in the sample inventory sheet:

- Weight of "Clean" lichen sample (this is the weight of the sample minus the sample bag weight)
- Mark the sample in the inventory as cleaned
- Enter cleaning end time, clean date and total time spent cleaning
- Record lichen cleaner's name or initials in "Cleaner"

Close the Clean and Archive lichen sample bags and dispose of gloves. Weigh the Archive sample bag and note the condition of the archive lichen sample by visually assessing the amount of actual target lichen (not debris or other lichen species) in the sample bag by the following key:

A	> 90% lichen
B	> 70% < 90% lichen
C	> 40% < 70% lichen
D	< 40% lichen (uncleanable)

Be sure to label the weight of the lichen on the Clean and Archive sample bags. Cross check labeling of Clean and Archive sample bags against inventory before filing lichen samples. See Table 1 below for an example of how a lichen sample cleaning inventory looks after samples have been cleaned.

Table D 1: Example of a lichen sample inventory datasheet.

Site Name	Field Duplicate	Species	Date Collected	Field Weight	Field Moisture	Dry Date	Clean Date	Clean Time Start	Clean Time End	Total Time	Clean Weight	Cleaner	Archive Weight	Archive Condition	Problem / Comments
150AC65		evemes	19-Aug	19	dry										
150AC65		hypphy	19-Aug	16	dry										
150AC66		evemes	19-Aug	20	dry	14-Sep	23-Sep	8:00	9:45	1:45	8.5	SB	12	B	
150AC66		hypphy	19-Aug	23	dry	14-Sep	23-Sep	10:00	12:00	2:00	4	BB	3	C	not much archive
150AC67		evemes	18-Aug	20	dry										
150AC67		hypphy	18-Aug	13	dry										
150AC67	Y	evemes	19-Aug	16	damp	13-Sep	23-Sep	13:00	14:30	1:30	7	SB	8	A	

Storing clean and archived samples

Label new separate large plastic bags for Clean and Archive lichen sample bags for each subcategory (e.g., “65AC Clean” or “30SI Archive”) for storage. Place Clean and Archive samples into their appropriate plastic bag and keep these bags in freezer for storage. The empty paper bag left over from drying can be kept with the appropriate bag that holds used lichen sample bags that were set aside during the drying process. Keeping all used bags will allow for double checking of labeling and inventory in case of labeling errors and inconsistencies in the inventory.

After storage repeat all steps of section 2.1.3 for cleaning each lichen sample.

3.0 Related Field Sampling and Analyses

3.1 COLLECTING CANOPY FOLIAGE SAMPLES

Foliar samples can be collected for isotope analysis. Results from these analyses can be compared with results from other lichen related analyses to gain an understanding of the relationship of direct deposition within the canopy to throughfall deposition accumulated in epiphytic lichens.

3.1.1 Pre-field preparation

Collecting foliar samples is a two person job and should not be attempted alone. Attempting to use a pole pruner alone could cause damage to the device and is also dangerous for the user and bystanders. Before going into the field to collect foliar samples ensure that you have the proper equipment and that it is functional:

- Pole pruner with addable sections (8 or more poles ~5 ft each in length)
- Enough screws and nuts for the number of pole sections
- Blade head attachment with rope
- Large Ziploc freezer bags (1 / sample)
- Sharpie
- Hard hat
- Safety Glasses

Check to see that each pole section is straight by looking down it like a telescope. If a bend in the pole is noticable it should not be used. Only if necessary, one or two slightly bent poles can be used but must be marked so they can be used near the top (closer to blade head) of the assembly. This is because poles near the bottom are under strain from each pole above it, and a bent pole is more likely to fail under this strain.

Check that the head functions properly. Test this while it is unattached to any sections to make sure it is sharp enough to cut a branch, and to get an understanding of how the device works before using it in the field. Use by placing branch inside cutting area and pulling on the attached rope.

3.1.2 In-field preparation for collecting foliage samples

Selecting foliage samples

Foliage samples from high in the canopy are hard to retrieve due to the difficulty of using an extendable pole pruner at heights greater than 10 meters. Target the highest branch from five representatives of mature dominant trees of the dominant tree species in the stand. The highest branch of dominant trees, with foliage at the top of the canopy, are the most likely to receive atmospheric deposition within a stand. Select high branches that are perpendicular to the main pollution source, or at least facing the suspected pollution source. Select trees that are somewhat close together, because assembling and disassembling the pole pruner is a time consuming task and, though difficult, it is more efficient to move from tree to tree with the pole pruner fully extended.

Assembling pole pruner

Once you have selected your 5 sample trees and branches, plan the order in which you want to cut them. It is a hassle to take apart and reassemble the pole pruner, so plan as extensively as possible how to

move from tree to tree without having to disassemble the pole pruner. Once five branches have been chosen, begin assembling the pole pruner:

Get all equipment ready in front of the first tree that a sample will be taken from.
Attach the blade head to a pole section using appropriate screw and nut, and make sure the rope is attached firmly to head section. If there are any slightly bent pole sections, use them here.
Attach each additional section in the same fashion using a screw and nut. Assemble it vertically by balancing assembled sections against a tree branch. Do not assemble on the ground as the apparatus is too fragile to lift from a horizontal position.

NOTE: Only attach as many sections as are needed. Each additional section makes the device more difficult to use and manage. Have someone assist in assembly by managing the rope to make sure it doesn't get tangled while assembling.

3.1.3 Handling assembled pole pruner

The key to handling the pole pruner is to keep underneath it, do not fight or try to control with arm strength; balance it by moving and staying under it. If it starts to fall in one direction, take a quick step in that direction. The person holding the rope can also help control by guiding the head. If it feels like you're losing control, try to guide it into the closest tree so that it doesn't come all the way down.

CAUTION: The pole pruner is difficult to manage and easily bent when equipped with all its extensions. The handler must also be aware not to jostle the head portion too much or get it caught on branches when moving up and down, as this section becomes quite dangerous if it comes off the pole portion.

Clipping foliage sample

Once assembled one person holds the rope attached to the head and one person takes the assembled pole pruner (Figure D-6). Spot the branch that is to be sampled and lift the pole pruner to that height. The target is to cut the branch roughly 2-4 feet from the terminus of the branch. The rope holder can get a better view by standing back and can communicate how close or far the head is to contacting the branch. The rope holder must also pay attention to which side their rope is on in relation to the blade head. If the rope becomes wrapped around the pole or there are branches between it and the blade head, the assembly will not work.

Once the target branch is in the blade head, the pole will feel somewhat stuck to the branch. To confirm that the head is on the branch jiggle the pole up and down and watch the branch to see if it shakes accordingly. Once confirmed, the rope holder stands next to the pole pruner and pulls along the axis of the poles as much as possible to decrease angular strain on the pole assembly, while the person holding the pole pruner stabilizes themselves and the pole as best as possible as they have to counter act the force of the rope pulling down.

Figure D-7: Handling pole pruner. One person holds the rope while the other handles the pole in order to control it

If the branch is clipped successfully, it will fall and may get caught on another branch while falling. To dislodge the cut branch, swing the rope against the branch, or if more extreme measures are required, use the pole pruner to knock or shake nearby branches. Once the sample is retrieved, clip it into smaller sections so that they fit into a Ziploc bag. Keep those sections that have more foliage (needles); not all of the sample needs to be retained, just enough to fill a Ziploc bag. Label this bag with site name, date, collector, tree species and tree number (1-5) and set it aside.

Moving with assembled pole pruner

Before moving to the next sample tree, plan ahead how you can move from tree to tree. It can be rested easily on a branch facing you, or directly nestled into the foliage of a tree. Make sure the canopy above is free of branches so that it won't get caught while moving. Look for trees that it can be rested on in case it starts to fall while moving. Once all of these aspects have been assessed, begin moving to the next sample tree while controlling the poles and handling the head using the rope as mentioned above. If required, remove a few pole sections from the bottom of the assembly. This may be easier than trying to maneuver a fully extended pole for longer distances. Repeat steps for cutting and bagging foliar samples at each sample tree.

Processing samples

Keep samples in a freezer, or failing that a fridge, so that they don't decompose. Samples should not be left at room temperature in their Ziploc sample bags for more than a day, otherwise there is a risk of decomposition of the sample from inherent moisture.

3.2 COLLECTING ORGANIC AND MINERAL SOIL SAMPLES

Organic and mineral soil samples can be taken to compare with other site related analyses.

3.2.1 Preparation

The following equipment is required for taking soil samples:

- shovel
- medium sized knife (6" blade) or trowel
- Large Ziploc bags (2 per site)

Most upland sites in this region have a sandy mineral soil quite near the surface with a shallow organic layer. In wetland or muskeg, the organic layer is very deep, and mineral soil is unreachable. Do not attempt to take mineral soil samples in muskeg.

Once on site, to find an unbiased point where to take organic and mineral soil samples, generate a random compass bearing and distance between 1 and 5 metres from plot centre. Use a knife or trowel to cut a 9" x 9" square into the organic layer. Place shovel along one of the edges and take the shovel length worth of soil. Carefully pull this soil from the hole keeping on the shovel with layers in order.

The dark layer of soil at the top is the organic layer. Remove and discard all living material (any fresh leaves, needles, roots or lichen) from the soil on the shovel and using hands, separate and place organic layer into a Ziploc bag. Roughly ¼ of the volume of the Ziploc bag is the minimum amount required for most soil analyses. If there is too much organic soil sample for the Ziploc bag try to keep a representative sample of all organic layers (not just top or bottom of organic layers). Place into Ziploc bag and mark with site name, date, collector, soil type (organic), and horizon(s) (L, F or H or a combination of the three).

Take remaining mineral soil and place in ziploc bag. Again, if there is too much try to maintain a representative sample by having all layers in the sample and attempt to retrieve enough soil to fill at least ¼ the volume of the Ziploc bag. Label this bag with site name, date, collector, soil type (mineral), and horizon(s) (A, B, Ah, Ae or a combination).

At the end of the day, place samples in freezer or send to lab for analysis.

3.3 TESTING BOLE BARK PH IN THE FIELD USING FLATHEAD ELECTRODE

The following section describes the methods required for testing the pH of bole bark of live standing trees using a flathead electrode. These measurements can be compared to results of pH testing of twigs and the stress ratio of *Evernia mesomorpha* individuals.

3.3.1 Pre-field Preparation

Before going into the field, review the steps for creating KCl solution below. The following equipment is required for testing bole bark pH in the field:

- Hanna Instruments 9026 pH meter
- Cole Parmer flat head electrode
- 25 mM KCl solution (prepared using instructions below and stored in a spray bottle)
- Deionized water in a capped bottle or squirt bottle
- Electrode storage solution
- pH buffer solutions (7.00 and 4.00)
- 4 beakers (2 small for calibration, 2 larger for rinsing and mixing solutions)

Creating a diluted solution from an aqueous stock solution

When deciding how much solution to make, consider that each sampling / spraying will take between 5-10 ml of KCl solution depending on absorbancy of bark. To dilute a stock solution of KCl from an aqueous solution of KCl use the following calculation.

$$V_s = \frac{V_d * C_d}{C_s}$$

Where V_s = Volume of stock solution required[1], V_d = volume of desired solution, C_d = Concentration of desired solution, and C_s = Concentration of stock solution.

Creating a diluted solution from solid powder stock

To create an aqueous solution of KCl from dry powder, use the following calculation:[2]

$$M_s = C_d \times V_d \times W_F$$

Where M_s = Mass of powder required (in grams), C_d = Concentration of desired solution (in Molar ie. 25 millimolar = 0.025 Molar), V_d = volume of desired solution (in Litres), and W_F = Formula Weight (KCl = 74.55 g/mol)

eg. To create 1 Litre of 0.025 Molar (or 25 milliMolar) KCl solution use the following calculation:

0.025 M x 1 L x 74.55 g = 2 g of dry powder in 1 L of deionized water solution = 0.025 M KCl

3.3.2 Testing bole bark pH in the field

Calibrating the Electrode

[1] Some stock solutions express concentration as a percentage or in parts per million. In this case 1000 ppm = 1 g/mol = 1 Molar
[2] Assumes that dry powdered KCl solution is = 1 Molar or 100% concentration

Electrode and reader should be calibrated once at each site. The following are instructions for calibrating a Hanna Instruments 9026 pH meter:

1. Pour pH 7 and pH 4 buffer solution into two separate beakers.
2. Plug electrode into reader and turn on
3. Press CAL button
4. Remove protective cap (keep upright to keep storage solution)
5. Put electrode into pH 7 solution and wait for reader to stop flashing "*NOT READY*"
6. If the reader calibrates correctly it should show "*READY"* and a "CFM" symbol will appear at the top of the screen. If the reader flashes "*WRONG"* then there is a problem and the instrument may require cleaning (to clean place electrode in cleaning solution for 30 mins)
7. Press CFM button to confirm reading
8. Remove electrode from ph 7 solution and rinse in regular or deionized water before immersing into ph 4 solution
9. Repeat steps 5 and 6 for ph 4 solution

The reader is now calibrated and you can begin taking measurements.

Measuring pH of Bole Bark

Measure the pH of 5 mature living trees of one species per site. If conducting lab measurements of twig bark pH and stress ratio of *Evernia mesomorpha* be sure to number each tree in datasheet recording this number consistently for all related studies using these same trees. To increase the probability of comparing pH between sites, measure the pH of 5 trees of each of two species at each site (10 trees / site). Below are instructions for measuring pH of bole bark on live trees:

1. Pick a spot on the bole of a mature tree of the desired species at chest height, clear of any obvious organisms or debris that would interfere with direct measurement of the actual bark (lichen, moss, etc.)
2. Spray bark around the chosen spot from ~7cm away with approximately 5-10 mL of KCl solution (2-3 sprays)
3. Wait 5 seconds
4. Spray the same spot a second time (1 spray)
5. wait 3 seconds
6. Begin timing and apply tip of electrode to bark surface gently

NOTE: (if the electrode is pressed too hard it is not measuring the substrate on which lichens grow and is instead measuring the deeper tissues underneath the bark)

7. Once pH stabilizes (reading should stabilize after 60-75 seconds and fluctuation should become minor ie. +- 0.01 every few seconds) record pH reading and the time taken for reading to stabilize.

NOTE: If the reading takes longer than 120 seconds to stabilize, repeat all steps on a different part of the tree

3.4 TESTING PH OF TWIG BARK USING GEL ELECTRODE

Testing twig bark for pH has been conducted in various studies to relate to epiphytic lichen chemistry. Because epiphytic lichens grow on the surface of twig bark, the pH of this bark surface can be related to certain elements of lichen chemistry (eg. Total Nitrogen and Sulphur content).

3.4.1 Sample Collection

The following equipment is required for sampling twigs:

- Paper Lunch Bags
- Sharpies for labeling
- Pruning Shears
- Ruler with mm increments

Collect 2-3 twig samples from 5 mature trees / site of the same tree species. When collecting twig samples, use shears to cut a section at least 6 cm in length (length can be shortened in lab or in field) and ideally between 4-6 mm in diameter in order to standardize the surface area of bark that is exposed to the environment. Keep samples from each tree in separate paper bags (2-3 samples / 1 bag and 1 bag/ tree) and assign each tree/bag a number (1-5). If conducting related studies that use these same trees, make sure the numbering from 1-5 corresponds correctly across all studies.

Collect samples with some lichen on them because the chemistry of a branch with no lichen present won't be representative of a branch that is capable of hosting lichen. If planning on conducting a Stress Ratio test for *Evernia mesomorpha*, collect twig specimens with samples of this species. Place twig samples in paper bag and label with site name and give each tree a number, and species info.

To increase the probability of comparing pH measurements between sites collect samples for each of two different tree species if possible (5 trees / site x 2 tree species = 10 trees / site). Also, try to collect from the same tree species from site to site to increase the probability to compare measurements across sites. If there isn't a second tree species available, one tree species will do. The idea is that when a site only has one tree species, measurements from other sites can still be compared for that species at least.

3.4.2 Lab Preparation

The following equipment is required to conduct measurements of pH on twig samples:

- Hanna instruments 9026 reader
- Hanna electrode (HI1230B)
- KCl solution (instructions for creating from aqueous or solid are below)
- deionized water
- storage solution
- pH buffer solutions (7.00 and 4.00)
- 6+ beakers (2 x 100ml for calibration of pH meter, 2 larger (~ 400mL) for mixing and measuring solution, 1 for melting paraffin wax (~200mL), and 1 for rinsing electrode in between measurements)
- test tubes or vials with caps (can be ordered from Prolab Scientific pk. of 125 x 12mL)
- test tube rack
- plastic syringe w/ volume (or graduated cylinder or pipet)
- Tweezers
- Pruning Shears
- Ruler (flexible, 10-30cm with mm increments)

- Paraffin wax (in blocks or sheets)
- Hot plate and pot

Creating a diluted solution from an aqueous stock solution

When deciding how much solution to make, consider that each sampling / spraying will take between 5-10 ml of KCl solution depending on absorbancy of bark. To dilute a stock solution of KCl from an aqueous solution of KCl use the following calculation.

$$V_s = \frac{V_d * C_d}{C_s}$$

Where V_s = Volume of stock solution required[3], V_d = volume of desired solution, C_d = Concentration of desired solution, and C_s = Concentration of stock solution.

Creating a diluted solution from solid powder stock

To create an aqueous solution of KCl from dry powder, use the following calculation:[4]

$$M_s = C_d \times V_d \times W_F$$

Where M_s = Mass of powder required (in grams), C_d = Concentration of desired solution (in Molar ie. 25 millimolar = 0.025 Molar), V_d = volume of desired solution (in Litres), and W_F = Formula Weight (KCl = 74.55 g/mol)

eg. To create 1 Litre of 0.025 Molar (or 25 milliMolar) KCl solution use the following calculation:

0.025 M x 1 L x 74.55 g = 2 g of dry powder in 1 L of deionized water solution = 0.025 M KCl

3.4.3 Testing pH of Samples

Calibrating the Electrode (for a Hanna Instrements 9026 pH meter)

Electrode and reader should be calibrated before every session of testing. Recalibrate every hour if conducting testing continuously. The following are instructions for calibrating a Hanna Instruments 9026 pH meter:

1. Pour pH 7 and pH 4 buffer solution into separate 100mL beakers.
2. Plug electrode into reader and turn on
3. Press CAL button
4. Remove protective cap from electrode (remove while electrode is upright to keep storage solution in the cap)
5. Rinse electrode head in deionized or tap water
6. Put electrode into pH 7 solution and wait for reader to stop flashing "*NOT READY*"
7. If the reader calibrates correctly it should show "*READY"* and a "CFM" symbol will appear at the top of the screen. If the reader flashes "*WRONG"* then there is a problem and the instrument may require cleaning (see instructions for cleaning electrode)
8. Press CFM button to confirm reading

[3] Some stock solutions express concentration as a percentage or in parts per million. In this case 1000 ppm = 1 g/mol = 1 Molar, and 100% = 1 Molar
[4] Assumes that dry powdered KCl solution is = 1 Molar or 100% concentration

9. Remove electrode from pH 7 solution and rinse in deionized or tap water before immersing into pH 4 solution
10. Repeat steps 5 to 8 but with pH 4 solution instead of pH 7

The meter is now calibrated and you can begin taking measurements

Preparing samples

The target is to measure the pH of the substrate on which the lichen is growing, more specifically the bark surface. If twigs have been left at room temperature for an extended period of time, the bark may be dry and become detached from the inner wood tissue which is not desirable. Try to conduct lab analysis within days of collection, failing that freeze samples for later testing.

Repeat the following steps for each twig being tested one at a time:

1. Cut twig to 6 cm in length (if sample is not between 5-7 mm in width, leave twig at a longer length. The idea is to achieve a standard surface area of bark on twig (= 2 x PI x radius x length)
2. If desired, analyze and note the species of lichen present on each twig
3. Remove all lichen from twig using tweezers trying not to remove as much bark as possible

4. NOTE: If conducting stress ratio analysis on *Evernia mesomorpha*, remove the most robust individuals and place in a separate appropriately labeled coin envelope with “Evemes” and site name, twig #, initials of lab tech and date.

5. Prepare melted paraffin wax by placing un-melted wax into a beaker and immersing in a hot water bath on a hot plate.

6. NOTE: do not place wax near direct heat or in the microwave as it is highly flammable. It must be melted in a hot water bath.

7. Once wax is melted, using tweezers dip a few millimeters of each end of twig into wax in order to seal the inner wood tissue of the twig
8. Place twig into test tube, and label test tube with unique twig ID. The unique twig ID is made up of the site name, species of tree in two letter code, ‘T’ for twig, and finally the previously assigned tree number from 1-5. (eg. the unique twig ID for a twig from the 4th black spruce at site 205 would read 205-Sb-T4)

9. Once all twigs have been placed in test tubes, fill ~20-30 twig tubes with 10 ml of 25 mM KCl solution, capping each one afterwards. The reason 20-30 are filled is so they can be measured as close to one hour after saturation as possible, because the process involved with each measurement with the pH reader takes 1-2 minutes.

10. Shake saturated twigs in tubes every 15 mins. to release ions from bark

After one hour of saturation in KCl solution begin measurement. If twigs are left in KCl solution for too long, ions will be drawn from the inner wood tissue and the pH of the solution will no longer be a measurement of the pH of the surface bark.

Measuring pH of samples

1. Ensure pH meter is calibrated if you haven’t already done so
2. Measure samples in the same order as they were prepared in order to keep saturation time between each twig consistent
3. With tweezers, remove twig from tube and set aside leaving KCl solution inside tube

4. Turn calibrated electrode on (only for first sample measurement, meter does not need to be turned on and off for every sample)
5. Insert electrode into tube with twig KCl solution
6. Wait until measurement stabilizes and record measurement
7. Remove electrode and place in deionized water until ready to measure next twig.
8. Place twig back into tube with solution and replace cap for archiving purposes if desired.

   NOTE: Testing the archived solution at a later date will not give the target result as this method is designed to extract ions from the bark surface, whereas elongated saturation will draw ions from the inner wood tissue.

9. Rinse electrode and place in water between measurements and repeat process for each twig.

   NOTE: Do not leave electrode stored in either tap or deionized water, or dry for more than 30 mins as it can damage electrode. Store only in storage solution with cap on if leaving for more than 30 mins at a time

3.5 CALCULATING STRESS RATIO FOR *EVERNIA MESOMORPHA*

3.5.1 Introduction

Evernia mesomorpha is an epiphytic lichen species that grows abundantly in the Boreal forest region of Alberta. The relationship between the weight, age and size of an *Evernia mesomorpha* (Evemes) individual can be used to indicate the level of stress that individual may be undergoing caused by the growing conditions of its environment. Stressed individuals tend to grow slowly and more compacted with shorter branches than healthier specimens. Therefore a stressed individual would be smaller and heavier than a healthy individual of the same age. There are several factors that can lead to an individual being stressed including atmospheric pollution and lack of light or nutrients. Methods for calculating and analyzing this ratio have been adapted from McCune and Stone (1990) who use a different species of this genus (*Evernia prunastri*) to calculate a stress ratio.

3.5.2 Sampling evemes individuals

This study is meant to be done in conjunction with the testing of twig pH, as evemes individuals are removed from twigs that have been sampled for testing twig pH. Read section 3.4 pertaining to testing twig pH before conducting evemes stress ratio calculations. The following equipment is required once twigs have already been sampled and are returned to lab:

- Coin Envelopes
- Forceps
- Scalpel
- Ruler (10 centimetres long with 1 millimetre increments)
- Scale (accurate to 1 ten-thousandth of a gram)
- Dissecting scope

As mentioned in the testing twig pH section, all lichens must be removed from the twig bark surface. While removing lichen, set aside the largest evemes individual from each twig and place in a labeled coin envelope. Label Coin envelope with a unique identifier for the evemes individual that refers to the twig it originates from (incorporating site name, tree species, 'E' for evemes, and twig number), as well as the date the evemes individual was processed in lab and initials researcher. For instance, the unique ID for an evemes removed from a twig collected at site 205 from the 4th sample tree of black spruce would be "205-BS-E4", where 205 refers to the site name, BS refers to the species of tree collected from, E refers to evemes and 4 refers to the 4th sample tree of black spruce on that site. The twig sample for this would simply be referred to as "205-BS-T4" in this case, where T stands for tree.

3.5.3 Measuring paramaters of evemes individuals

Calculating surface area of an individual

For each evemes individual take dimensional lengths using a standard ruler with millimetre increments. First measure the longest axis of the individual and record that, and then measure the axis perpendicular to the longest axis. From this, calculate the surface area for an ellipsoid in cm^2 using this formula:

$$\text{Surface Area of Evemes} = \pi \times \frac{\text{Longest dimension}}{2} \times \frac{\text{Perpendicular dimension}}{2}$$

Aging an individual

Evemes individuals can be aged by counting branches of thalli, using methods adapted from McCune and Stone (1990). Starting at the base of the individual and using a probe following a single branch, count every occurrence of branching thalli until the end of that branch is reached. The red dots in Figure D-9 show examples of branching thalli in evemes. Each red dot would count as 1 year. The base of the evemes can be identified as the portion that is physically attached to the bark surface of the twig and where all branches of the individual meet. It is difficult to identify once removed from the bark surface, but possible. Repeat the aging process three times following different branching patterns from the base to their terminus. Average the three values and use this as an approximation for the age of the individual.

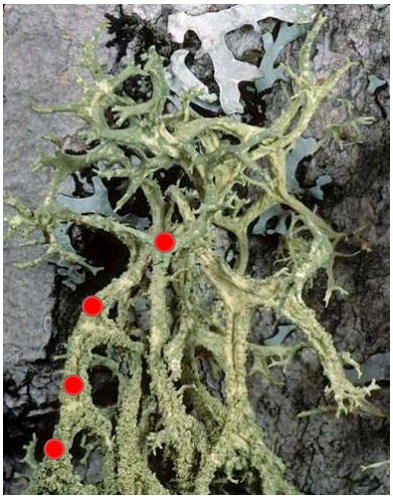

Figure D-8: Branching in *Evernia mesomorpha*

Weighing an individual

To weigh evemes individuals, use a scale that is accurate to ten-thousandth of a gram. Using tweezers, place evemes on scale and record the weight. Scales with this type of accuracy tend only to be available in labs, do not attempt to use less accurate scales.

4.0 Laboratory Analyses

4.1 TOTAL NITROGEN AND SULPHUR CONTENT IN LICHEN

Laboratory techniques for the University of Minnesota Research Analytical Laboratory are described in detail on their website at http://ral.cfans.umn.edu/. The laboratory first split samples, reserving up to two grams of un-ground sample for the receptor modeling and source apportionment component of the study. The un-ground sample was placed in labelled samples bags and stored in the freezer at UMRAL. The remaining sample split was intended for the total N and total S analyses. Each sample was passed through a stainless steel grinder with a 20-mesh sieve to mix the sample thoroughly. Ground samples were dried at 65˚ C for two hours and cooled in a desiccator before weighing for analysis.

4.1.1 Total sulphur

Total percent sulphur was determined by dry combusting 0.100-0.150 g of sample covered with a tungsten oxide compound in an oxygen atmosphere at 1350˚ C in a Leco Corp. Sulphur Determinator (Model No. S144-DR). The SO_2 evolved from the sample was determined by a non-dispersive infrared detector, which was empirically calibrated with three LECO plant reference materials (LECO 1026, orchard leaves; LECO 1025, orchard leaves; LECO 1010, tobacco leaves) and with an in-lab check of dried rose leaves (RLV). Instrument precision was poor for measurements of sulphur concentrations near the method detection limit (MDL). The MDL is the concentration of an analyte in a lichen sample that gives a signal equal to two times the standard deviation of the background emission at that wavelength.

4.1.2 Total nitrogen

The combustion method for total nitrogen used a LECO FP-528 Nitrogen Analyzer. A 150-500 mg sample was weighed into a gel capsule and dropped into an 850˚ C furnace purged with O_2 gas. The combustion products (CO_2, H_2O and NO_2) were filtered, cooled by a thermoelectric cooler to condense most of the water, and collected into a large ballast. A 3 cc aliquot of the ballast combustion product was integrated into a helium carrier stream. The stream first passed through a hot copper column to remove O_2 and convert NO_x to N_2. A reagent tube then scrubbed the remaining CO_2 and H_2O from the stream. N_2 content was measured by a thermal conductivity cell against a helium background and the result was displayed as weight percentage of nitrogen. The laboratory ran three calibration standards with LECO plant reference materials (LECO 1006, rice flour; LECO 1026, orchard leaves; and, LECO 1052, EDTA) and with an in-lab check of dried rose leaves (RLV).

4.1.3 Reference materials and blanks (quality assurance analysis)

Reference materials, lab checks, and acid blanks were all used in the quality assurance analysis for the UMRAL analysis methods. Methods used in the quality assurance analysis are listed below.

NIST and lichen Standard Reference Materials

TwoNational Institute of Standards and Technology (NIST) standard reference materials (SRM 1547, peach leaves and SRM 1515, apple leaves) were analyzed with each batch of 10 samples in the total N and total S analyses. NIST standards have concentration ranges established by the National Institute of Standards and Technology. The lichen standard (CRM 482, *Pseudevernia furfuracea*) was also analyzed with each batch in the nitrogen and sulphur analyses. The European Commission certified this lichen Reference Material. Analysis results from the NIST standard and the lichen standard was used to assess laboratory accuracy. Three LECO calibration standards of were run with the sulphur analyses, including:

LECO 1026, orchard leaves; LECO 1025, orchard leaves; LECO 1010, tobacco leaves. Three LECO calibration standards of were run with the nitrogen analyses, including: LECO 1006, rice flour; LECO 1026, orchard leaves; and, LECO 1052, EDTA. An in house standard of rose leaves (RLV) was also run for both sulphur and nitrogen analyses, this standard is not certified.

The laboratory accuracy was evaluated by comparison of the laboratory measurements to established values for these NIST and LECO standard reference materials. Inaccuracy of laboratory measurements was assessed for each standard based on reported element concentrations in the standards. The percent **inaccuracy** of the UMRAL method was calculated as:

% Inaccuracy = 100(*xobs* – *xtrue*)/ *xtrue*

where *xobs* was the UMRAL measurement of a given element in the standard and *xtrue* was the published standard value.

Laboratory replicates

Replicate analyses of selected sample digests were run every 10 lichen samples for sulphur and nitrogen analyses. This determined the laboratory precision for repeated measures of the same sample. Relative percent difference (RPD) between the laboratory replicates was determined to assess laboratory precision using the equation:

RPD = ABS|(x1 – x2)| / (mean x1, x2) *100

where ABS is the absolute value; x1 is the first lab measurement and x2 is the repeated lab measurement. Re-runs of lichen samples were also considered lab replicates and were used to estimate laboratory precision.

Acid blanks

One or multiple acid blanks were analyzed with each analytical batch of 10 lichen samples. These acid blanks were only run for nitrogen analyses, as there is no potential source of S contamination for the total S analyses. These blanks pass through the same digestion/analytical procedures for the nitrogen analyses as the lichen samples. The blanks were used to detect and quantify any contamination of the samples from the analytical reagents. No contamination was present if all measurements of the acid blanks were below the MDL. Contamination occurred when elemental concentrations in blanks were above the MDL. The mean concentrations of elements were calculated for blanks with values above the MDL. The blank mean for that element was then compared to the grand mean of the element concentrations in all lichen samples by calculating the percent of the grand mean for each element:

% of Grand Mean = [$mean_{blanks}$/grand $mean_{lichens}$] *100

The relative impact the contamination on the laboratory measurements in lichen samples was evaluated using this measure.

4.2 ANALYZING CHLOROPHYLL CONTENT IN *EVERNIA MESOMORPHA*

Analyses were conducted by the USDA Forest Service in Riverside, California. For most sites five samples of *Evernia mesomorpha* were analyzed, though some sites had three. When there was enough tissue, two samples were taken per envelope and a mean value was calculated based on these two samples. A site mean based on the 5 sample means was then calculated.

Chlorophyll is a vital pigment found in the photosynthetic plastids of the algal partner of the lichen symbiosis. Twenty mg ± ten mg of tissue per sample were collected and placed in test tubes containing 5 ml dimethyl sulfoxide (DMSO) for extraction. The samples were incubated in test tubes for 45 minutes at 60 °C, removed from the incubating oven, and diluted with an additional 5 ml DMSO. Optical density (OD) was measured in a spectrophotometer at 415, 433, 645, 665, and 750 nm. Chlorophyll *a* and *b* concentration was calculated using the equation:

$$\text{mg chlorophyll } g^{-1} \text{ air dry weight} = \frac{(20.2*(OD_{645}) + 8.02*(OD_{665}) \times 10 \text{ ml}}{1000 \text{ ml} \times \text{g air-dry weight}}$$

Another measure of photosynthetic pigment health is the ratio of OD at 433:415, or the ratio of chlorophyll *a* to phaeophytin *a* pigments. Acidification of the chlorophyll *a* molecule can cause the removal of the central magnesium, converting the pigment to phaeophytin, a brown pigment utilized in the electron chain transport system of photosynthesis. This conversion reduces the photosynthetic capacity of the lichen thallus. An OD_{433}:OD_{415} curve developed Ronen and Galun (1984) allows us to calculate the chlorophyll *a*: phaeophytin *a* ratio using the equation:

$$\% \text{ phaeophytin } a = -104.8 \times \ln(OD_{433}:OD_{415}) + 39.715$$

Methods references:

Ronen, R., Galun, M., 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. Environmental and Experimental Botany 24, 239-245.