

SEAN environmental contaminants SOP 2: field and laboratory guidelines for lichen tissue analysis

Version 1.0

Revision History Log:

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1 Introduction

This standard operating procedure (SOP) covers all the necessary information for SEAN staff and partners to plan and execute the field work to collect, dry, and ship lichen tissue to the University of Minnesota Research Analytical Lab for analysis of nitrogen, sulfur, and 25 additional elements in conformance with the protocols developed for the Tongass National Forest. Most of the text here was originally developed in Schirokauer et al. (2014):

Schirokauer, D., L. Geiser, A. Bytnerowicz, M. Fenn, and K. Dillman. 2014. Monitoring air quality in Southeast Alaska's National Parks and Forests: Linking atmospheric pollutants with ecological effects. Natural Resource Technical Report NPS/SEAN/NRTR—2014/839. National Park Service, Fort Collins, Colorado.

See SOP 5 for more details regarding specific analytes and data reporting procedures. Tasks related to this SOP include obtaining the permits required to perform the field sampling, gathering required supplies, observing collection standards, and methodically handling and preserving samples for shipping. It is important that all points of this SOP are read, clearly understood, and implemented as necessary starting six to nine months before field collection activities are planned. The project leader (currently, the SEAN ecologist) is responsible for ensuring this SOP is followed safely and accurately. Please refer to SOP 1 for operational safety considerations for each contaminants monitoring effort.

The next sampling event is planned for summer 2018. The current version of this SOP envisions that SEAN will maintain monitoring at a minimum of six sites: two in GLBA, three in KLGO, and one in SITK. An additional three sites adjacent to KLGO but outside park boundaries may also be included pending additional funding availability.

2 Field procedures

1. Permitting

Each SEAN park unit may require a permit for specimen collection activities. Contact the research permit coordinator for each park for current policy and application procedures. In some cases collections may be covered under an existing multi-year research permit. It is possible to obtain one permit that covers collection activities in all park units involved, but researchers should consult with permit coordinators for the preferred approach. Each year an Annual Investigator's Report is required. This report updates the details of the original study plan (if necessary), summarizes the study activities over the preceding year, and estimates funding expenditures. See these websites for more information on NPS Research Permit and Reporting System (RPRS) and GLBA-specific information on research in wilderness lands:

- - <https://irma.nps.gov/rprs/Home>
- - <http://www.nps.gov/glba/naturescience/research-permit-application-process.htm>

2. Supplies and personnel

Six to nine months before sampling is planned, the Project Leader should ensure that the field supplies required for collecting and shipping lichen tissue in a clean manner are organized and in working order (Table 2.1). At GLBA, a research vessel, usually the R/V Fog Lark, must be scheduled for sampling with a

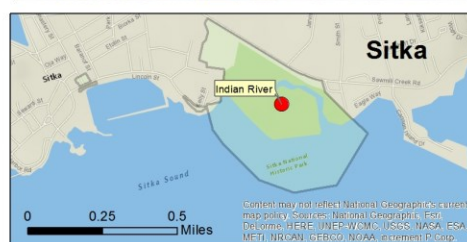
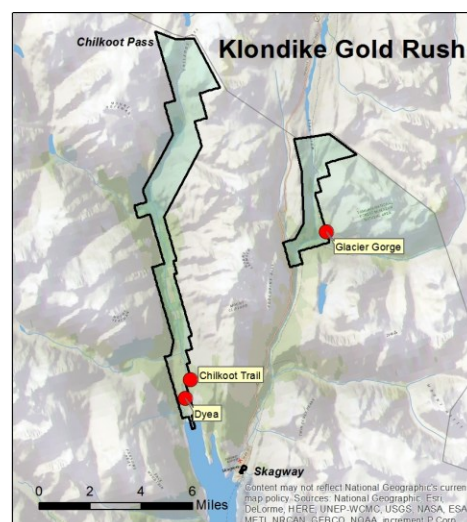
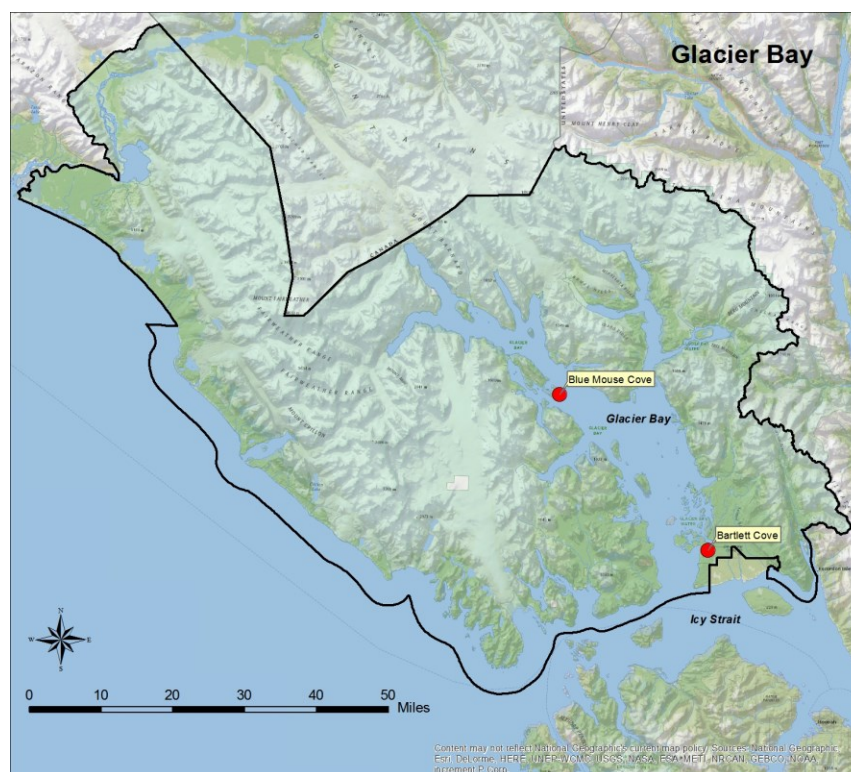
park-certified staff to captain. At SITK and KLGO, one person can accomplish field tasks, while at GLBA a minimum of two people are necessary for collection activities because a boat is required for every site except Bartlett Cove.

Table 2.1. Checklist for field collection.

-
- ☐ Drybag large enough for gear and samples
 - ☐ GPS and location description notes from USFS Lichen and Air Quality Database
 - ☐ 4" x 7" metalized polyester sample bags (Kapak bags), stored in gallon-sized Ziploc bags
 - ☐ One pair of non-powdered, disposable vinyl or nitrile gloves per plot per person
 - ☐ 100 g Pesola spring scale
 - ☐ (3) Rolls VWR $\frac{3}{4}$ " laboratory tape or blue painter's tape
 - ☐ (3) Black Sharpie markers
 - ☐ 100% cotton herbarium blotter sheets (11.5" x 16")
 - ☐ Dissecting kit
 - ☐ Waterproof field data sheets
-

3. Sampling sites

In GLBA, the Bartlett Cove site can be reached by foot in the front-country, while Blue Mouse Cove requires access via a vessel capable of shore landings (Figure 2.1). Currently, the SEAN conducts the majority of GLBA field work from the R/V Fog Lark, an 8.5 m Munson landing craft stationed in Bartlett Cove. In KLGO, the Dyea and Chilkoot Trail sites are reachable by road and foot travel. The Chilkoot Trail is accessed by walking approximately $\frac{1}{2}$ mile along the start of the Chilkoot Trail next to the Taiya River. In SITK, the single sampling location is within short walking distance from the Visitor Center.



Name	Park	Site code	Latitude	Longitude	Detailed location notes from USFS database
Blue Mouse Cove	GLBA	GLBA_BM	58.76447°	-136.44908°	Site 1A: Mainland. Glacier Bay NP, West Arm, small island southeast of Blue Mouse Cove (on Blue Mouse Island adjacent to West Arm). 545 ft. elev. (highest point, on map) - east of Hugh Milller Inlet, west side of island near small cove at south end (58.76447° - 136.44908°). Site 2A: Mainland. Glacier Bay NP, West Arm, south of throughfall samplers. Plot center is clump of dead ALCR nest in large limby spruce trees & a Tsme, - flat area at top of ridge from beach approx 300 ft uphill (58.7631° -136.44772°).
Bartlett Cove	GLBA	GLBA_BC	58.45660°	-135.86559°	Sites 1A/1B: Mainland. Glacier Bay NP, Bartlett Cove east of Admin site, along trail to Bartlett River, approx. 300 ft from admin trailhead between admin trailhead and trail coming down from road for public access; uphill from admin trailhead approx 50 ft (58.4566° -135.86559°). Site 2: Mainland. Glacier Bay NP, Admin site, trail to Bartlett Cove - 1/2 mile from admin site - uphill side of trail, large boulder along trail where plot center is located . Uphill approx 50 ft - small hemlock under 12" spruce tree - is plot center (58.45979° -135.86073°).

Dyea	KLGO	KLGO_DY	59.50263°	-135.35543°	Site 7: Mainland. Klondike Goldrush NHP, Dyea old town site- along trail to False Front from UTM that was taken at wooden trail marker, plot center is approx. 40 ft at 196 degrees from UTM - red pinflag at center (59.50227° -135.35529°). Site 8: Mainland. Klondike Goldrush NHP, Dyea old townsite in forest - at UTM on trail go into forest 46ft at 86 degrees to plot center. Trail is in False Front Area? At end of where the samplers are (59.50263° -135.35543°). Sites A/B/C/D: Mainland. Klondike Goldrush NHP. Terminal delta of the Taiya River floodplain (59.50477° -135.35608°).
Chilkoot Trail	KLGO	KLGO_CH	59.51165°	-135.34383°	Site 5: Mainland. Klondike Goldrush NHP, Between two throughfall samples- uphill side of the Chilkoot Trail on large rock cliffs (59.5168° -135.34171°.) Site 6: Mainland. Klondike Goldrush NHP, above the Chilkoot trail, near the Ogawa sampler- above the area where the throughfall samples are (59.51929° -135.34025°). Sites A/B/C/D/E: Mainland. Klondike Goldrush NHP. Saintly Hill, along the Chilkoot Trail (59.51165° -135.34383°).
Glacier Gorge	KLGO	KLGO_G G	59.57035°	-135.15169°	Site: Mainland. Klondike Goldrush NHP, w edge of tunnel @ mi 16 of Yukon Passs RR, 1 mi passed Glacier Station, on tr to old tunnel.
Indian River	SITK	SITK_IR	57.04769°	-135.31480°	Sites 1A/1B: Baranof Is. Sitka National Historical Park, north side of Indian River in forest at edge of estuary meadows

Figure 2.1. Long-term sampling locations in SEAN parks. Latitude and longitude coordinates are approximate. Samples were collected in the vicinity of the exact points given here. Location descriptions are transcribed directly from the USFS National Lichens and Air Quality Database.

4. Collection procedures at each site

2.4.1 Lichen species to collect

Three lichen species are collected at each site: *Hypogymnia enteromorpha*, *Hypogymnia inactive*, and *Platismatia glauca* (Figure 2.2). It is imperative to correctly identify the lichen and distinguish them from other lichen species that have similar characteristics. Training and use of the lichen photos and descriptions are useful in identifying the three lichen species to be collected. At the time this SOP was drafted no SEAN staff were trained in lichen identification, so field staff for subsequent collections should coordinate with USFS staff or other qualified lichen researchers to receive proper training.



Figure 2.2. Photos of lichen species used for elemental tissue analysis. From left to right: *Hypogymnia enteromorpha*, *Hypogymnia inactiva*, and *Platismatia glauca*.

2.4.2 Where to collect lichen

Lichen samples are collected outside of each monitoring plot but not more than 1 km (.65 miles) away from the plot perimeter. The target species should be collected from a minimum of 8 different locations near the site. Lichens attached to tree branches, shrubs or tree boles, and, if fresh, in the litter or on fallen branches, may be used.

2.4.3 How to collect lichen

Samples should be collected with the fingers. New non-powdered vinyl or nitrile gloves are worn at each site to prevent contamination of the samples. While wearing gloves the field crew should not touch anything brought with them onto the plot except the Kapak bag. Unused Kapak bags should be stored in a clean sealable plastic bag. New gloves will be used at each plot and replaced if they become torn or contaminated.

A sample consists of at least 14 grams of clean, dry lichen. Collect 3 bags of each of the 3 lichen species at every site for a total of 9 bags collected. The exception is the Upper Dewey site (an additional site that is not part of the permanent monitoring plots), where *Hypogymnia inactiva* is not found. At Upper Dewey, there will be a total of 6 bags of lichen collected. Do not mix lichen species in one bag. Samples should be collected as clean and free from bark and other foreign surface material as practical and will be cleaned carefully later in the office. Dusty, gritty, discolored, or decaying material should be avoided.

Place samples in metalized polyester Kapak bags and weigh on a 100-gram Pesola spring scale. Empty Kapak bags weigh 10 grams each. If the lichens are dry, the sample and bag together should weigh at least 24 g. If the lichens are wet, adequacy of the sample size should be judged by volume rather than weight. Dry lichens may weigh only 5 % of the wet weight. After enough material has been collected, press out excess air, fold the open edge of the bag over three times, and carefully seal with waterproof, removable, laboratory tape or painter's tape, which tends to be more reliable. The bag should be airtight. Sealed, airtight, sample bags should be placed in clean sealable plastic bag, which is sealed in turn, and placed in a shady location or inside a daypack so they do not overheat while the remaining work on the plot is performed.

2.4.4 Collecting field information

The following information should be recorded directly on the Kapak bag with an indelible marker: Plot name; date; substrate(s) (host species name and substrate location in order by the amount of sample in

the bag from that substrate. E.g., “*Pinus contorta* branches and boles”); target species acronym (Hypent, Hypina, or Plagla); collector’s initials; moisture status of sample at the time of collection (dry, damp, or wet). This information should also be recorded on a separate waterproof field sheet that will be scanned into a PDF as a project deliverable (EC_A; Table 4.1 in protocol narrative; Figure 2.3 in this SOP).

SEAN Airborne Contaminants						CONTACT	
Field data sheet						Project Leader: Chris Sergeant	
Version 2017-09-14						National Park Service - SEAN	
						3100 National Park Road	
Survey date range						Juneau, AK 99801	
Personnel						(907) 364-1591	
Page						christopher_sergeant@nps.gov	
Field collection data							
Plot name	Date	Time	Collector	Species	Moisture status	Substrate	Comments
- At each plot, use a new line for each species collected (Hypent, Hypina, or Plagla) - For dates, use mm/dd/yyyy format - For times, use 24-hour format - Moisture status: dry, damp, or wet - For substrate column, fill in what lichen were collected from (e.g. <i>Pinus contorta</i> branches and boles) - Plot names: Blue Mouse Cove = GLBA_BM, Bartlett Cove = GLBA_BC, Dyea = KLGO_DY, Chilkoot Trail = KLGO_CH, 'Glacier Gorge = KLGO_GG, Indian River = SITK_IR							

Figure 2.3. The SEAN airborne contaminants field data sheet. Printable versions of this sheet can be found on the SEAN Environmental Contaminants webpage toolbox.

Collect all paper field data sheets in a file over the course of field work. They will be turned into a deliverable in section 3, below.

2.4.5 Troubleshooting common problems in the field

If none, or only one, of the target species is present in sufficient quantities for collection, even if the sampling area is expanded to the 1 km maximum sampling radius, then no samples or only one sample should be collected. Do not substitute non-target species.

If more than 1 hour has been spent collecting but sample weight is still very low, get help from other crew members or switch to a more easily collected target species. Usually it’s a good idea to stop collecting after two hours and process the collection that has been made. The decision to send the sample to the laboratory will be made in the office after the sample is cleaned.

It is not acceptable for all of the sample material to come from one or two trees. The material must evenly represent at least eight locations. Expand the area of collection up to the maximum size allowed. If material is still too scarce, collect a different species or collect nothing.

Field collection methods require careful discrimination among species in the field and should only be performed by people who have completed lichen identification training. Because of the potential for lead and other heavy metal contamination, no smoking is allowed on the plot or in the vicinity of the plot during the visit.

2.4.6 Brief lichen identification training

This is only a brief discussion of considerations for lichen identification and does not replace training from an experienced collector.

Several species are easy to confuse with one another:

1. *Hypogymnia enteromorpha* and *apinnata*. Separated definitively by a P test though generally *H. apinnata* lacks the small side buds and can have a more appressed, “melted” look. Study examples in the herbarium and in *Macrolichens of the Pacific Northwest* (McCune and Geiser 2009). Because *H. apinnata* was described after the first round of monitoring was completed on the Tongass NF, it is assumed that historic collections were a mix of these two species. For this reason, it is not considered essential to separate the two species in new collections.
2. *H. occidentalis* and *enteromorpha*. *H. occidentalis* is usually narrower lobed and more appressed than *H. enteromorpha*. The upper surface of *H. occidentalis* has a dark, continuous margin and older parts are more rugose than *H. enteromorpha*. *H. occidentalis* and *enteromorpha* can be definitively separated in the office with a P test.
3. *H. inactiva* and *H. imshaugii*. These species are easily confused. Check insides of lobes in several places. *H. imshaugii* is white inside throughout; *H. inactiva* has a black floor.
4. *Platismatia glauca* and *norvegica*. *P. glauca* has no distinct ridges or veins.

5. Drying and cleaning lichen samples

Samples should be cleaned and air dried as quickly as possible after returning from the field, preferably the same day. Dry lichens make a crunch sound when the Kapak bag is squeezed; if the contents feel soft, they are damp (even if marked ‘dry’ by the field crew) and need to be dried to avoid decomposition of the samples. If unsure of the moisture status, dry the sample. Spread the lichens onto a clean 100% acid free blotter paper laid over a flat surface covered with clean plastic wrap. Label blotters so that sample identity is retained. Using tweezers from a dissection kit, and while wearing clean disposable gloves, clean the lichen specimens. Lichen are cleaned by removing insects, spiders, loose leaves and any twigs, bark or non-target lichens adhering to the lichen tissue.

Leave lichen on blotter paper to dry. Lichens and mosses should not be air-dried in areas subject to contamination (e.g., near cooking areas, roads, or in rooms where organic solvents are used, dust levels are high, or smoking is permitted). Kapak bags can be dried by crimping them open and leaving them upright, or by hanging them open on a line with a clothespin. Once dry, place the lichens back in the dried Kapak bags

and carefully reseal. Sealed bags should be airtight. Specimens must be thoroughly air dried to avoid fungal decay. Store dry samples in a clean, dry, dark place (bags are not opaque).

6. Sample delivery for laboratory analysis

Dried, resealed samples are mailed to a laboratory for elemental analysis. Bags should be packed closely, but without excessive crushing, in a sturdy cardboard box. Bags from several plots can be mailed in the same box. A packing list should be kept by the field crew specifying the plot number, forest, species, and mailing date of each sample.

Send specimens to:

Research Analytical Laboratory
University of Minnesota
135 Crops Research Building
1902 Dudley Avenue
St. Paul, Minnesota 55108

Please ensure that a contract (or agreement) is in place for analysis work costing more than the government credit card limit. Table 3.1 in the protocol narrative provides references and descriptions for the University of Minnesota laboratory procedures.

3 Submitting, validating, and accepting lichen field collection sheets (EC_A)

For this deliverable, the complete sets of paper field collection sheets that have accumulated over the survey year for all sites are scanned into a single PDF. The PDF is submitted through the validation / quality control iterations until accepted. It is stored in the repository and disseminated over the web.

1. Project leader (or delegate) tasks

1. Gather all of the field collection sheets that have been recorded during the field surveys for the year
2. Review sheets and fill in any missing information, if necessary
3. Arrange observer forms in chronological order (earliest sampling first)
4. Verify the set is complete. There should be at least one sheet for every sampled site. If a sheet is missing and cannot be found after diligent search, insert a sheet of paper in the stack stating the site name and “No Field Observer Form.”
5. Scan the entire packet into a single PDF file using a color scanner. Be sure the scanner is set for two-sided scanning.
6. Save the PDF on the SEAN network at
\\sean.glba.nps.gov\Data\SEAN_Data\Work_Zone\EC\EC_A\yyyy\EC_A_yyyy.PDF, where yyyy is the single year the samples were collected.
7. Request validation by sending a notification email to the Data Manager, specifying in the message body that deliverable EC_A, for year yyyy is ready; the Data Manager will know how to locate and work with the file named “EC_A_yyyy.PDF”.

2. Data manager tasks

1. On receipt of the submission, assign the next formal Submission Number to this file, as found in the master Submission_Log table.
 1. Use the “Update Submission Log” web tool at http://sean-dm.glba.nps.gov//0_submission_update.aspx.
 2. Complete Submission_Log details up through the Submission_Date column.
2. Copy the file into the staging area for validation at a subdirectory named with the submission number in \\sean.glba.nps.gov\Data\SEAN_Data\Staging\EC\EC_A.
3. Validate the submission according to current criteria.
4. Record validation summary data in the Submission_Log using the web tool.
5. If submission fails mandatory criteria, reply with a “failure email” that includes:
 1. The submission number assigned
 2. The deliverable ID
 3. The protocol ID
 4. Documentation listing all the specific mandatory criteria failed
6. If submission passes mandatory criteria, reply with a “success email” that includes:
 1. The submission number assigned
 2. The deliverable ID
 3. The protocol ID
 4. Documentation listing any specific optional criteria failed
 5. Request to certify deliverable as complete

3. Project Leader Tasks

1. On receipt of a failure email:
 1. Review the paper stack is in chronological order.
 2. Rescan or edit the PDF to ensure it is in the proper form.
 3. Resubmit for validation.
 4. If failure cannot be resolved, email the Data Manager that the submission is withdrawn.
2. On receipt of a success email, review any failed optional criteria:
 1. The following tasks are the responsibility of the Project Leader and may not be delegated.
 2. If the review of failed optional criteria indicates the validated data are acceptable reply with a “certification email” stating the deliverable is accepted and may be disseminated.
 3. If the validated data are unacceptable:
 1. Reply with a “withdrawal email”, stating the deliverable is withdrawn.
 2. Take remedial action to obtain a corrected deliverable.
 3. Restart the process from the beginning.

4. Data Manager Tasks

1. On receipt of a withdrawal email:
 1. Mark the withdrawal in the Submission_Log’s Status column using the web tool.
 2. Terminate the process.
2. On receipt of a certification email:
 1. Sensitivity is highly unlikely for this deliverable. But if sensitive:
 1. Mark the sensitive designation in the submission status column using the web tool.
 2. Notify the submitter of the situation.

3. Complete the deliverable tracking spreadsheet cells referring to dissemination with the word SENSITIVE.
4. Do not attempt to install the sensitive item in the auxiliary repository.
2. If not sensitive:
 1. Install this file in the NPS Data Store under Project Environmental Contaminants using current practices. Typically this is done by cloning an existing EC_A reference, updating the clone's metadata, and uploading a new file.
 2. Also install this file on the NPS web sites in the ...\\AUXREP\\EC\\EC_A folder, make a web link for it, test and deploy it using current practices.
 3. Mark the certification in the Status column in Submission_Log using the web tool.
 4. Update the deliverable tracking spreadsheet milestones with the date of completion for EC_A and propagate it through the web sites using current practices.

4 Submitting, validating, and accepting lichen tissue analysis laboratory technical report (EC_B)

For this deliverable, a laboratory technical report detailing all analytical results and the associated QA/QC procedures are provided to SEAN as a single PDF. The PDF is submitted through the validation / quality control iterations until accepted. It is stored in the repository and disseminated over the web.

1. Project leader (or delegate) tasks

1. Lab reports will most likely be sent electronically as a PDF. If the report employs some other medium, make a PDF file from it. Save the PDF on the SEAN network at \\sean.glba.nps.gov\\Data\\SEAN_Data\\Work_Zone\\EC\\EC_B\\yyyy\\EC_B_yyyy.PDF, where yyyy is the single year the samples were collected.
2. Request validation by sending a notification email to the Data Manager, specifying in the message body that deliverable EC_B, for year yyyy is ready; the Data Manager will know how to locate and work with the file "EC_B_yyyy.PDF".

2. Data manager tasks

1. On receipt of the submission, assign the next formal Submission Number to this file, as found in the master Submission_Log table.
 1. Use the "Update Submission Log" web tool at http://sean.dm.glba.nps.gov/0_submission_update.aspx.
 2. Edit in the Submission Log details up through the Submission_Date column.
2. Copy the file into the staging area for validation at a subdirectory named with the submission number in \\sean.glba.nps.gov\\Data\\SEAN_Data\\Staging\\EC\\EC_B.
3. Validate the submission according to current criteria.
4. Record validation summary data in the Submission_Log using the web tool.
5. If submission fails mandatory criteria, reply with a "failure email" that includes:
 1. The submission number assigned
 2. The deliverable ID
 3. The protocol ID

4. Documentation listing all the specific mandatory criteria failed
6. If submission passes mandatory criteria, reply with a “success email” that includes:
 1. The submission number assigned
 2. The deliverable ID
 3. The protocol ID
 4. Documentation listing any specific optional criteria failed
 5. Request to certify deliverable as complete

3. Project Leader Tasks

1. On receipt of a failure email:
 1. Rescan paper or edit the PDF to ensure it is in the proper form.
 2. Resubmit for validation.
 3. If failure cannot be resolved, email the Data Manager that the submission is withdrawn.
2. On receipt of a success email, review any failed optional criteria:
 1. The following tasks are the responsibility of the Project Leader and may not be delegated.
 2. If the criteria review indicates the validated data are acceptable reply with a “certification email” stating the deliverable is accepted and may be disseminated.
 3. Otherwise, when the validated data are unacceptable:
 1. Reply with a “withdrawal email”, stating the deliverable is withdrawn.
 2. Take remedial action to obtain a corrected deliverable.
 3. Restart the process from the beginning.

4. Data Manager Tasks

1. On receipt of a withdrawal email:
 1. Mark the withdrawal in the Submission_Log’s Status column using the web tool.
 2. Terminate the process.
2. On receipt of a certification email:
 1. Sensitive information may not be disseminated. (Sensitivity is highly unlikely for this deliverable.) If sensitive:
 1. Mark the sensitive designation in the submission status column using the web tool.
 2. Notify the submitter of the situation.
 3. Complete the deliverable tracking spreadsheet cells referring to dissemination with the word SENSITIVE.
 4. Do not attempt to install the sensitive item in the auxiliary repository.
 2. Otherwise, when not sensitive:
 1. Install this file in the NPS Data Store under Project Environmental Contaminants using current practices. Typically this is done by cloning an existing EC_B reference, updating the clone’s metadata, and uploading a new file.
 2. Also install this file on the NPS web sites in the ... \AUXREP\EC\EC_B folder, make a web link for it, test and deploy it using current practices.
 3. Mark the certification in the Status column in Submission_Log using the web tool.
 4. Update the deliverable tracking spreadsheet milestones with the date of completion for the EC_B and propagate it through the web sites using current practices.