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DIVERSITY AND SOIL-TISSUE ELEMENTAL RELATIONS OF VASCULAR PLANTS OF CALLAHAN MINE, BROOKSVILLE, MAINE, U.S.A.

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abstract. Metal-contaminated soils provide numerous stressors to plant life, resulting in unique plant communities worldwide. The current study focuses on the vascular plants of Callahan Mine in Brooksville, ME, USA, a Superfund site contaminated with Cu, Zn, Pb, and other pollutants. One hundred and fifty-five taxa belonging to 50 families were identified, with the Asteraceae (21%), Poaceae (11%), and Rosaceae (9%) as the most species-rich families. Ninety-six species encountered at the Mine were native to North America (62%), including 11 taxa (7%) with rarity status in at least one New England state. Fifty-one species were non-native (33%), including nine taxa (6%) considered invasive in at least one New England state. We characterized how the plant community changed across different habitats at the Mine, from disturbed and exposed (waste rock piles, tailings pond) to inundated and relatively undisturbed (wetland, shore), and documented concurrent shifts in the ionic content of the soils across the habitats. We found substantial differences in both the plant community and soil chemical features among habitats. Habitats separated out along a single axis of an ordination of the plant community, with wetland and shore habitats at one extreme and tailings pond and waste rock-pile habitats at the other. The first principal component axis of the 21 soil variables was significantly predicted by the ordination of the plant community, indicating a gradient of increasing organic matter, Fe, Mg, Mn, total N, Na, and K roughly parallel to the gradient of increasing wetland vegetation. None of the plant species tested accumulated substantial concentrations of metals in their leaf tissue except *Salix bebbiana* and *Populus balsamifera*, which accumulated 1070 ppm and 969 ppm Zn in dry leaf tissue, respectively—approximately one-third of the concentration considered as hyperaccumulation for Zn.

Key Words: edaphic ecology, geobotany, habitat restoration, metal pollution, phytoremediation, plant-soil relations, Superfund sites

Edaphically extreme habitats, such as serpentine outcrops, guano deposits, alkaline flats, and metal-enriched mining sites pose unique challenges to plant life (Rajakaruna and Boyd 2008). The stressors faced by plants of such habitats can include: water stress due to the rocky and often shallow nature of the substrate, generally low levels of essential nutrients, extremes of pH, and elevated concentrations of ions, including heavy metals. Although trace levels of some heavy metals are required by plants as micronutrients (Ahmad and Ashraf 2011; Marschner 1995), high levels can interfere with essential physiological processes and cause toxicities (Hansch and Mendel 2009; Peralta-Videa et al. 2009). Some heavy metals, such as copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn), regulate various biological processes in plants (Epstein and Bloom 2004), but when they occur in excess they can disrupt critical biological processes (Chaffai and Koyama 2011; Kabata-Pendias 2001). Thus, most plants exclude metals at the root level by binding them to organic acids or ligands or storing them within vacuoles in the roots, where they cannot interfere with important physiological processes (Gall and Rajakaruna 2013; Hossain et al. 2012). However, metal-hyperaccumulating plants are able to take up high concentrations of heavy metals from the soil and translocate them into above-ground tissue at concentrations exceeding, in most cases, 0.1% of total dry leaf tissue mass (Rascio and Navari-Izzo 2011; van der Ent et al. 2012). The Brassicaceae (Gall and Rajakaruna 2013), Caryophyllaceae (Verkleij and Prast 1988), Asteraceae (O'Dell and Rajakaruna 2011), Rubiaceae (Reeves 2003), and Fabaceae (Page et al. 2006) are families known to consist of species able to tolerate metals either through exclusion or accumulation.

Understanding the ecology of metal-contaminated sites is becoming critical as increasing pollution exposes more land to heavy metals and other contaminants (Boyd 2004; Ensley 2000; McGee et al. 2007; Wuana and Okieimen 2011). Metal-enriched habitats and their locally adapted biota are also undergoing drastic changes due to natural and human-induced stressors (Williamson and Balkwill 2006), even those resulting from recent efforts to remediate metal mines abandoned for long periods of time (Jacobi et al. 2011; Palmer et al. 2010). Thus, floristic surveys in support of conservation efforts should be encouraged. These should document the wealth of biological diversity continually being lost from such sites worldwide, particularly those metal-tolerant plants that could be used for phytoremediation (Baker et al. 2010; Whiting et al. 2004). Although there are many metal-enriched sites in northeastern North America (Rajakaruna, Harris, and Alexander 2009), including 118 EPA-designated Superfund sites in New England (Environmental Protection Agency 2013), the sites are under-explored for both their botanical diversity and the occurrence of species with unusual metal-accumulating physiologies (Rajakaruna, Harris, and Alexander 2009). Studies conducted at Pine Hill, an Ni-enriched serpentine quarry on Little Deer Isle, ME, suggest both a unique bryophyte (Briscoe et al. 2009) and vascular flora (Pope et al. 2010) compared to Settlement Quarry, an adjacent granite outcrop. Harris et al. (2007) also found a unique lichen flora at Pine Hill, including two species new to New England and an additional three new to Maine. Rajakaruna et al. (2011) recently showed a unique composition of lichens at the Cu-, Zn-, and Pb-enriched Callahan Mine in Brooksville, ME, consisting of taxa that are often found in metal-enriched sites worldwide.

In this study, we compiled a list of vascular plants growing at Callahan Mine (hereafter also, the Mine) and examined how the plant community and soil ionic content varied across five distinct habitats at the Mine (tailings pond, waste rock piles, shore, wetland, and 'in between;' see Figure 1 and Materials and Methods for habitat descriptions). We hypothesized that: (a) diversity would be lowest in the disturbed and exposed habitats (waste rock piles and tailings pond) and the often inundated wetland habitat, compared to less disturbed and less exposed habitats (shore, 'in between'); (b) species composition and life forms would be distinct among the different habitats with herbaceous, annual, and non-native species dominating the more disturbed and highly exposed habitats, compared to native and perennial herb, shrub, and tree species in the less disturbed and less exposed habitats and the wetland habitat; and (c) substantial differences in soil variables would be present across habitat types, and would correlate with variation in the plant communities (although not necessarily causing, or caused by, variation in the plant community).

We collected descriptive data on the ionic content of leaf tissue from select plant taxa growing at Callahan Mine to assess if there are species that show unusual physiologies with respect to metal

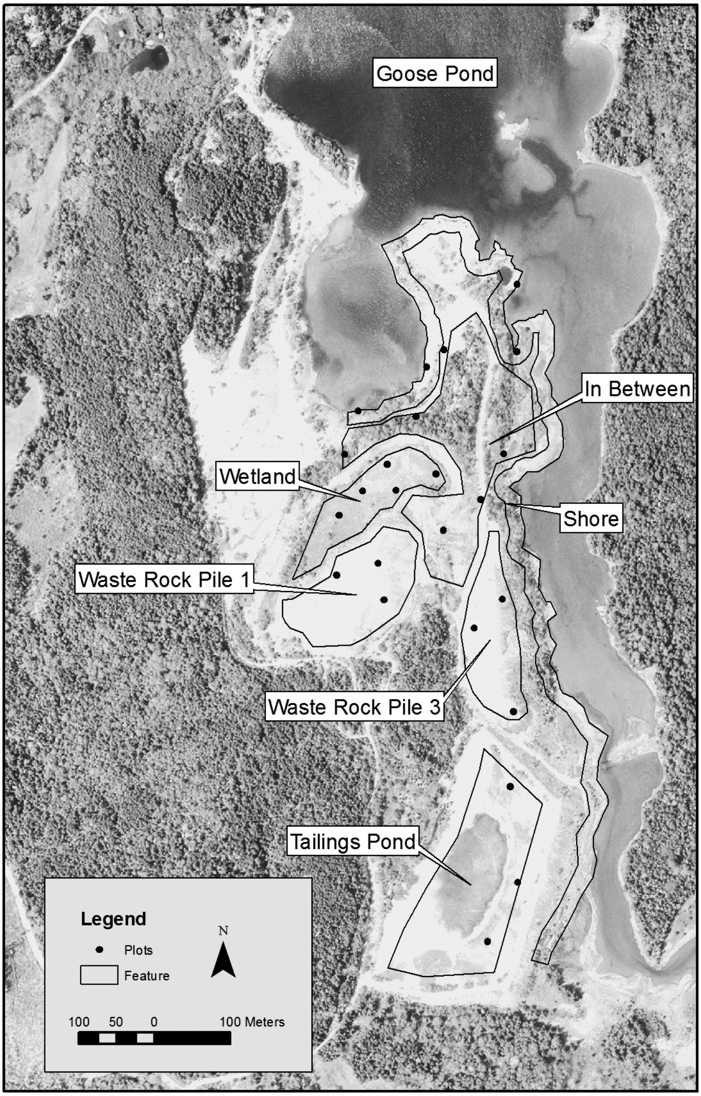

Figure 1. Map of Callahan Mine featuring the sampling plots placed within the five habitats chosen for the vegetation survey.

accumulation and that could be utilized in the restoration of metal-contaminated sites in New England. To informally place the flora of the Mine within a regional context, we calculated the proportion of species that were native to North America and to New England.

Finally, we compared the species list from the Mine to those previously reported from two adjacent rock outcrops (Pope et al. 2010) to see if the metal-tolerant plants at the Mine were more abundant at nearby Pine Hill (serpentine outcrop), relative to Settlement Quarry (granitic outcrop). We expected the flora at the Mine to be more similar (in terms of species composition, measured by Bray-Curtis dissimilarity) to the flora of Pine Hill than to the flora of Settlement Quarry.

MATERIALS AND METHODS

Site description. Callahan Mine is a former intertidal open-pit mine in Brooksville, Hancock County, ME (44°20′ N, 68°48′W; WGS 84; Figure 1). It has been mined intermittently since 1880, with the most intensive mining taking place from 1968–1972 (Environmental Protection Agency 2009; Rajakaruna et al. 2011). Today, the 150-acre site is composed of the ore pad where rocks from the Mine were crushed to a fine sand, three waste rock piles where non-ore-bearing rocks were piled, and a tailings pond made up of refuse (fine-textured soil particles) from the chemical separation of mineral and non-mineral particles. Callahan Mine was listed as a Superfund site in 2002 by the Environmental Protection Agency (2002) due to elevated levels of organic contaminants and heavy metals, including Cu, Zn, Pb, and Cd. Remediation efforts that began in 2010 at the Mine have restricted access to the northern part of the site. Therefore, this study focused on the southern portion of the Mine, including waste rock piles 1 and 3, the tailings pond, and the wetland, areas that were also surveyed during the recent lichen study (Rajakaruna et al. 2011; Figure 1).

Floristic survey. We stratified Callahan Mine into five separate habitats: tailings pond, waste rock piles 1 and 3, wetland, shore, and a section without distinct geographical features referred to as 'in between' (Figure 1). Within each habitat, five 10 × 10 m plots were placed (six within the waste rock piles) using the Geographic Information System (GIS) random point generator (ArcGIS 10.1 Spatial Analyst, 'Create Random Points' tool). Two of the random plots on the tailings pond were not included in any analyses, as remediation efforts had recently removed vegetation. We avoided selecting plots from any areas within the Mine that were currently undergoing remediation or were planned for such activities in the future. Randomly generated plots devoid of any vegetation were also not selected for the survey. Within each plot, vascular species were recorded, and the percent cover of each species was calculated within a grid of twenty-five 2 × 2 m subplots to obtain a percent cover measure per 10 × 10 m plot. Percent cover data were used to calculate species diversity indices and other species-habitat associations. A few species not encountered within the plots were identified from throughout the study area and included in the species list for Callahan Mine. They were not included in the diversity indices we calculated for individual plots. Plants were identified using Haines (2011). Voucher specimens were deposited at the herbarium of College of the Atlantic, Bar Harbor, ME (HCOA).

Soil analysis. Soil samples were collected in August 2011 from the four corners and center of each 10 × 10 meter plot from up to 10 cm below the surface using a stainless steel trowel. Samples were air-dried for 2 weeks and stored in plastic bags. Soil pH was measured with the 1:2 soil-to-solution method, with distilled water and 0.01 M $CaCl_2$ (Kalra and Maynard 1991). Organic matter was measured by loss on ignition at 375°C. Using a 1 M potassium chloride solution, nitrate and ammonium nitrogen were extracted and analyzed colorometrically by a Dual-Channel Automated Ion Analyzer (OI Corporation, TX). Calcium, K, Mg, Na, P, and S were extracted with 1 M neutral ammonium acetate (Kalra and Maynard 1991) and determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Phosphorus was determined colorometrically by the Ion Analyzer. Electrical conductivity (EC) was measured by a saturated media water extraction (Gavlak et al. 2003). Aluminum, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, and Zn were extracted in 0.005 M DPTA to a pH of 7.3 for 2 h and determined by ICP-OES. Analyses were conducted by the Analytical Laboratory at the University of Maine in Orono (UMO).

Tissue analysis. Ten to fifteen fully expanded and mature leaves were collected from throughout the Mine from five to ten widely spaced individuals of *Achillea millefolium* subsp. *lanulosa*, *Betula papyrifera*, *Festuca rubra* subsp. *rubra*, *Galium mollugo*, *Hypericum perforatum* subsp. *perforatum*, *Juncus gerardii*, *Lotus corniculatus*, *Lupinus polyphyllus* var. *polyphyllus*, *Morella caroliniensis*, *Onoclea sensibilis*, *Phragmites australis*, *Populus balsamifera*, *Salix bebbiana*, *Silene vulgaris* subsp. *vulgaris*, *Spiraea alba* var. *latifolia*, *Stellaria graminea*, *Thlaspi arvense*, *Typha latifolia*, *Vaccinium angustifolium*, and *Vicia villosa* subsp. *villosa*. The unequal sample size was due to the leaf size differences among the target species (more leaves were collected from species with smaller leaves in order to have adequate mass for tissue analyses) or due to their relative abundance at the Mine (common species were collected more often). The species selected were from plant families known to contain metal accumulating taxa (e.g., Asteraceae, Brassicaceae, and Caryophyllaceae) or from those found in high abundance at the Mine (e.g., Betulaceae, Fabaceae, Poaceae, and Salicaceae). Leaves were rinsed with distilled water, washed in 0.1 M HCl solution, and rinsed again twice in distilled water. Samples were dried in a forced-draft oven for 48 h at 80°C. One composite tissue sample from each species, consisting of tissue from five to ten individuals, was sent to the Analytical Laboratory at UMO. To determine tissue concentrations (ppm) of Ca, K, Mg, P, Al, B, Cu, Fe, Mn, Zn, Ni, Cr, Cu, Cd, Pb, and Mo, samples were dry-ashed at 450°C for 5 h and dissolved in 50% HCl; concentrations were determined using ICP-OES. Direct combustion analysis at 1150°C in pure oxygen with detection by thermal conductivity in the combustion gases was used to estimate total N (TN %) content of tissue.

Statistical analyses. All statistical analyses were conducted using R (R Core Team 2013). To compare the complete flora of Callahan Mine with those of nearby Pine Hill and Settlement Quarry, we calculated the proportion of shared species relative to the total number of species in a given site pair (also known as Jaccard's similarity index). To compare the higher taxonomy between these sites, we used a taxonomic variant of Jaccard's index (Δ_T; Bacaro et al. 2007) which is a measure of the similarity of a pair of taxonomic trees. To estimate diversity of the plant community across habitats at the Mine, we calculated species richness and the Shannon diversity index ($-\Sigma_i (p_i \cdot \ln p_i)$, where p_i is the proportional abundance of the ith species) for each plot. To test for differences in diversity among habitats, we used a GLM (generalized linear model) with a quasi-Poisson distribution (for species richness) and a normal linear model (for Shannon diversity). To examine species composition across habitats, we used non-metric multidimensional scaling (hereafter, nMDS) with Bray-Curtis distance for an unconstrained ordination of the plant community (ter Braak 1995). Two sites of the 'waste rock pile' habitat were removed prior to the ordination as they were devoid of vegetation. Habitat membership was regressed against site scores from the ordination, and the coefficient of determination (R^2) was calculated. A permutation test was then used to assess the probability that site scores and habitats were non-randomly associated: the vector of habitat membership was permuted and R^2 calculated with each permutation. A p-value was calculated by asking what proportion of the permuted R^2 was greater than the observed R^2. This procedure follows ter Braak (1995) and is implemented in the vegan package (Oksanen et al. 2013). To facilitate visualization of plant community structure (i.e., plant life form and family membership across habitats), we partitioned species scores from the ordination into separate subsets based upon life form and family membership. For each subset, we calculated the centroid and a 95% confidence ellipse. Species scores in an nMDS ordination are essentially weighted means of site scores (e.g., a mean of site scores weighted by the number of times the taxon appears in the sites). Each species score can be viewed as an optimum—the point in ordination space where the abundance of that taxon is maximal. In nMDS, the rate of decline in abundance from the optimum is not uniform in every direction (the taxon may decline in abundance more quickly in one direction than another). Therefore, the centroid of species scores for a subset of taxa (e.g., a clade) should not be interpreted as the point where the abundance of this clade is maximal, but instead as the central tendency of the optima of the taxa in that clade. To examine soil ionic content across habitats, we used principal components analysis (PCA) to reduce log-transformed soil variables (pH was not log-transformed) into orthogonal eigenvectors. Variables were scaled and centered prior to PCA. The first six principal components explained ~92% of the variance in the soil data; subsequent axes were not considered further. We used a one-way MANOVA with an approximate F-test to determine whether habitats explained a substantial amount of variation in the PCA axes. To assess soil ionic content across variation in the plant community, we fit PCA axes to the nMDS ordination using an analogous procedure to that described above for habitats. Essentially, each PCA axis was regressed against the corresponding site scores of the nMDS axes. It is important to note that the PCA axes were analyzed individually; we assessed the degree to which each PCA axis could be predicted, given the ordination.

RESULTS

We collected 155 taxa and identified 148 to full species (Appendix). Seven taxa were only identified to genus as our collections were made before or after peak flowering and the vegetative samples were not sufficient for identification to species. A total of 50 families were encountered at Callahan Mine. Ninety-six species encountered at the Mine were native to North America (62%) whereas fifty-one species were non-native (33%), including nine taxa (6%) considered invasive in at least one New England state (New England Wild Flower Society 2012). Within the Mine, the shore and 'in between' habitats were the most species rich, with 66 and 53 taxa, respectively; the tailings pond and the waste rock piles were the least species rich, with 20 and 21 taxa, respectively (Appendix). Eleven species (7%) were listed as rare, threatened, endangered, or special concern in at least one state in New England (New England Wild Flower Society 2012).

Woody vegetation was abundant across the Mine but was predominantly associated with the shore and 'in between' habitats—although several tree species were common across habitats (such as *Betula papyrifera* and *Picea* spp.). The wetland community consisted of typical wetland species such as *Glyceria striata*, *Torreyochloa pallida*, and *Typha latifolia*; annual and perennial forbs (predominately of the Asteraceae, such as the goldenrods *Euthamia graminifolia* and *Solidago rugosa*); and a few woody species of the Rosaceae (*Prunus virginiana*, *Rosa palustri*s, and *Rubus idaeus*). The shoreline was dominated by a mix of hydrophyllous and maritime species such as *Juncus gerardii*, *Phragmites australis*, and *Typha latifolia*; woody species, most abundantly *Betula papyrifera* and *Picea rubens*; and perennial forbs (*Plantago maritima*, *Solidago rugosa*, and the maritime species *S. sempervirens*). The 'in between' habitat was a patchy matrix of woody vegetation dominated by deciduous trees (*Betula papyrifera*, *Populus tremuloides*); but also supported conifers (*Picea glauca*, *Pinus resinosa*, *Thuja occidentalis*), understory shrubs (*Diervilla lonicera*, *Morella caroliniensis*, *Salix* sp., *Sambucus racemosa*, *Spiraea alba* var. *latifolia*), forbs (the most abundant were *Galium mollugo* and the introduced *Hieracium* spp. and *Vicia cracca* subsp. *cracca*), and several grasses (e.g., *Festuca rubra* subsp. *rubra*, *Poa nemoralis*). The waste rock piles shared some species with the 'in between' and shore habitats, specifically trees (*Betula papyrifera*, *Picea glauca*, *P. rubens*, and *Populus tremuloides*). The waste rock

Table 1. Mean values ± standard errors for two metrics of diversity: species richness and the Shannon diversity index (SDI). Total Richness = the total number of species found in a habitat. Area = the area of the habitat in acres. Habitat codes: TP = tailings pond, WR = waste rock piles, WE = wetland, SH = shore, and IB = in between.

Habitat	Mean Richness (per plot)	Mean SDI (per plot)	Total Richness	Area (acres)
TP	3.7 ± 2.2	0.2 ± 0.2	21	7
WR	2.3 ± 1.1	0.5 ± 0.3	21	2.5
WE	10.6 ± 2.0	1.4 ± 0.3	39	2
SH	16 ± 3.6	1.6 ± 0.2	66	7
IB	15 ± 3.3	1.1 ± 0.3	53	8

piles were also characterized by *Vaccinium angustifolium*, as well as small numbers of other shrubs and woodland herbs. The tailings pond was the most marginally vegetated of the habitats and, in contrast to the waste rock piles, lacked diversity in woody vegetation. Only two tree species were found on the tailings pond (*Betula papyrifera* and *Picea glauca*). Although *Picea rubens* was common on the waste rock piles, it was absent from the tailings pond; likewise, *P. glauca* was abundant on the waste rock piles but sparse on the tailings pond. Aside from the woody vegetation, the tailings pond was dominated by *Festuca rubra*, as well as a few perennial forbs: the introduced legumes *Lotus corniculatus* and *Trifolium repens*, the introduced *Cerastium fontanum*, and the ubiquitous Asteraceae genera *Hieracium* and *Solidago*.

At the species level, the Callahan Mine flora was as similar to the flora of Pine Hill (Jaccard similarity = 0.23) as it was to Settlement Quarry (Jaccard similarity = 0.24), whereas the floras of Pine Hill and Settlement Quarry were relatively more similar to each other (Jaccard similarity = 0.35). When a taxonomic variant of the Jaccard index (Bacaro et al. 2007) was used, the outcome was similar: the higher taxonomy of the flora at Callahan Mine was as similar to Pine Hill (Δ_T = 0.37) as to Settlement Quarry (Δ_T = 0.37), but the higher taxonomies of Pine Hill and Settlement Quarry were relatively more similar (Δ_T = 0.44) to each other. The proportion of species in the Callahan Mine flora shared with Pine Hill (0.27) was marginally larger than the proportion of species in Callahan Mine flora shared with Settlement Quarry (0.21).

Within Callahan Mine, Shannon diversity and species richness were generally correlated across plots (Pearson's ρ = 0.83; Table 1).

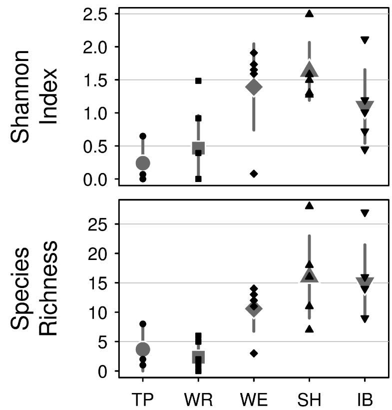

Figure 2. Species richness and Shannon diversity for plots sampled in five different habitats at Callahan Mine. Gray symbols and bracketing lines are means and 95% confidence intervals, respectively. Habitat codes and symbols: TP = tailings pond (circles), WR = waste rock piles (squares), WE = wetland (diamonds), SH = shore (upward triangles), and IB = in between (downward triangles).

Shore, wetland, and 'in between' habitats had greater species richness and Shannon diversity than tailings pond and waste rock piles (species richness, quasi-Poisson GLM: $p < 0.001$, $F_{4,19} = 7.40$; Shannon diversity, normal GLM: $p = 0.012$, $F_{4,19} = 4.25$; Figure 2). The habitats were of varying size: the tailings pond, shore, and 'in between' were the largest, the wetland was the smallest, and the waste rock was intermediate (Table 1). There was no evidence of a correlation between the size of a habitat type and the total species richness in that habitat (Kendall's $\tau = 0.22$, $p = 0.6$). Three groups of habitats separated out clearly along the first nMDS axis (Figure 3): tailings pond and waste rock piles, 'in between' and shore, and wetland. The second nMDS axis described the variation within habitats, and roughly separated tailings from waste rock and shore from 'in between.' Habitat membership (species occupancy) was significantly correlated with nMDS axes ($R^2 = 0.70$, permutation $p < 0.001$), and thus reflected differences in plant community composition. Six plant families had more than three species in the sampled sites at Callahan Mine (in total 48 taxa, Figure 4). Rosaceae spp. were associated with the wetland, shore, and 'in between' habitats, on average, with increasing values of nMDS axis 1. Asteraceae spp. were associated with the waste rock,

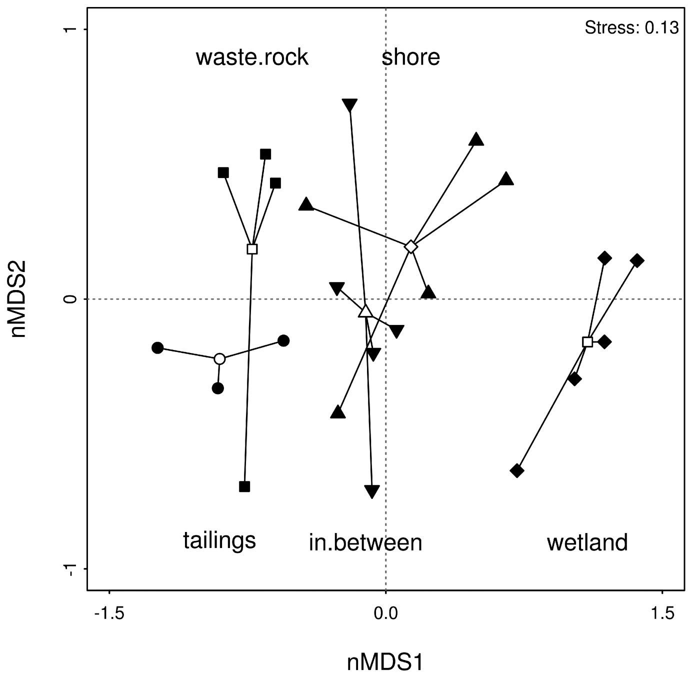

Figure 3. nMDS ordination of the plant community at Callahan Mine, with five habitats indicated by labels and symbols. Circles, squares, diamonds, triangles pointing upward, and triangles pointing downward respectively indicate plots within tailings pond, waste rock piles, wetland, shore, and 'in between' habitats. Unfilled points are centroids for each habitat. Labels are vertically aligned with their respective centroid.

shore, wetland habitats, on average, with increasing values of nMDS axes 1 and 2. Fabaceae spp. were associated with the 'in between', waste rock, and tailings habitats. Pinaceae spp. were associated with the 'in between' habitat. Both Fabaceae and Pinaceae spp. were associated, on average, with decreasing values of nMDS axes 1 and 2. Caryophyllaceae spp. had one member, each, associated with the waste rock, tailings, and shore habitats, and one member associated with three habitats, including 'in-between'; on average, with decreasing values of nMDS axis 1 and increasing values of nMDS axis 2. Poaceae spp. were not clearly associated with any particular habitats or nMDS axes.

Of the six life-form groups considered (all taxa, Figure 5), annual forbs were associated with wetland and shore habitats and, on

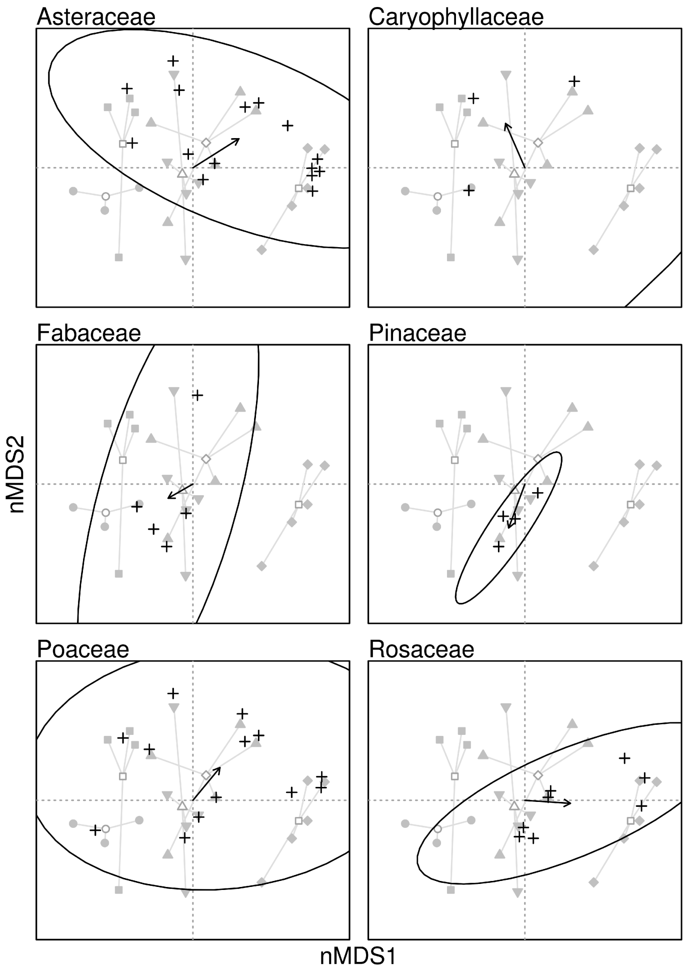

Figure 4. Species scores from the nMDS ordination plotted in the ordination space shown in Figure 3, split by plant family. Only the six families with more than three species found at Callahan Mine are shown. Arrows indicate the centroids for each subset of species scores, and the black circle is a 95% confidence ellipse. Note that the confidence ellipse is for visualization only; no statistical inference is performed using the species scores. The species scores are essentially weighted means of site scores (the position of a given site within each dimension of the ordination), where the weights are the abundance of a species in a given site.

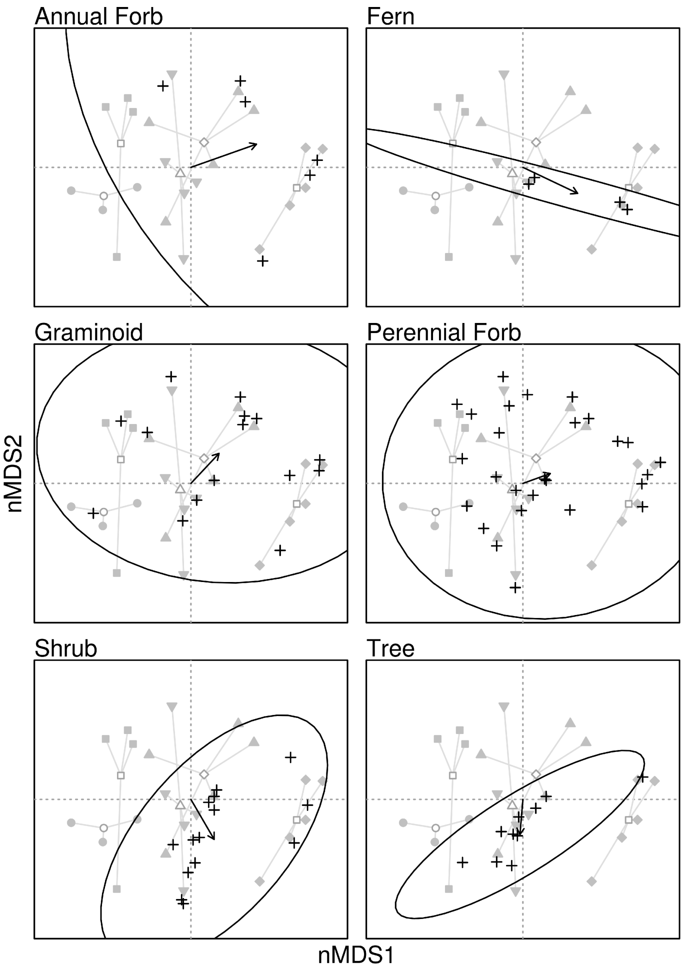

Figure 5. Species scores from the nMDS ordination plotted in the ordination space shown in Figure 3, split by life form. Arrows indicate the centroids for each subset of species scores, and the black circle is a 95% confidence ellipse. Note that the confidence ellipse is for visualization only; no statistical inference is performed using the species scores. The species scores are essentially weighted means of site scores (the position of a given site along each dimension of the ordination), where the weights are the abundance of a species in a given site.

average, with increasing values of nMDS axes 1 and 2. Ferns were associated with the wetland and 'in between' habitats and, on average, with increasing values of nMDS axis 1 and decreasing values of nMDS axis 2. Graminoids and perennial forbs were not clearly associated with any particular habitats or nMDS axes. Shrubs were associated primarily with shore and 'in between' habitats but also with the wetland habitats and, on average, with increasing values of nMDS axis 1 and decreasing values of nMDS axis 2. Trees were also primarily associated with the shore and 'in between' habitats, with one species, each, in wetland and waste rock habitats and, on average, with decreasing values of nMDS axis 2.

The first axis of the soil PCA was positively associated with soils that had high levels of organic matter, Fe, Mg, Mn, N, Na, and K. The second axis of the soil PCA was positively associated with pH, Ca, Cu, P, Mo, and Zn, and negatively associated with Al and Fe. The remaining axes were difficult to interpret, as they explained a relatively small amount of variation in the soil data (Table 2). The PCA axes varied significantly among habitats (MANOVA, approx. $F_{6,15} = 7.96$, $p < 0.001$), indicating that soils of the habitats differed substantially in ionic composition (Table 3). Values of the first PCA axis were significantly predicted by site scores from the nMDS, indicating that plant community type and soil ionic content were associated ($R^2 = 0.65$, permutation $p < 0.001$). PCA axis 1 was associated with increasing values of nMDS axis 1 and thus reflected a soil gradient from waste rock and tailings to wetland soil types (Figure 6). The remaining PCA axes were not significantly predicted by the ordination (Table 4). Table 5 lists the leaf tissue concentrations of macronutrients (Ca, K, Mg, P, N) and Table 6 lists the tissue concentrations of micronutrients, including heavy metals (Al, B, Cu, Fe, Mn, Zn, Ni, Cr, Cu, Cd, Pb, and Mo), for the 20 species collected from Callahan Mine. None of the collected plant species accumulated substantial concentrations of metals in their leaf tissue, except *Salix bebbiana* and *Populus balsamifera*, which accumulated 1070 ppm and 969 ppm Zn in dry leaf tissue, respectively. *Populus balsamifera*, *Spiraea alba* var. *latifolia*, and *S. bebbiana* also accumulated 10.63, 10.47, and 6.73 ppm Cd in dry leaf tissue, respectively.

DISCUSSION

Ours is the first survey of the diversity and tissue metal content of vascular plants of a metal-enriched Superfund site in New England.

Table 2. Principal components loadings for 21 soil variables (log-transformed and centered/scaled prior to PCA). Cum. Var. = the cumulative amount of variance explained by the *n*th axis, %Var. Expl. = the amount of variance explained by the *n*th axis.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
pH	−1.39	3.01	−3.36	1.05	−1.21	1.87
LOI	3.2	−0.15	−0.04	−1.14	−0.01	−4.08
EC	1.87	1.77	0.02	5.14	1.74	1.69
NO_3^-	1.95	−0.07	−4.46	1.24	2	−4.08
NH_4^+	2.98	−0.15	0.52	−2.16	−0.19	3.75
Ca	0.25	3.62	−1.88	−1.67	−0.27	3.62
K	3.09	0.38	−2.45	0.52	−0.9	−2.32
Mg	3.12	0.82	0.29	1.3	−0.21	2.92
Na	2.88	1.16	−0.78	3.25	0.38	−0.61
P	0.59	2.87	−3.57	−1.56	1.46	1.05
S	1.08	0.51	4.56	3.22	3.97	0.17
Al	1.58	−3.35	0.93	−1.05	−1.34	1.86
Cd	0.88	3.66	1.69	−2.39	−0.49	−1.98
Cr	3.27	−0.73	0.18	0.87	0.45	1.87
Cu	−1.34	2.66	2.94	1.5	−1.34	−1.86
Fe	2.63	−2.28	−0.31	−1.3	−2.16	0.35
Mn	2.85	0.01	1.04	0.88	−3.69	0.32
Mo	1.57	3.16	1.33	−2.69	1.13	−0.27
Ni	2.92	0.6	1.81	−2.37	−0.97	−1.48
Pb	−0.79	0.96	−0.29	3.33	−6.83	−0.55
Zn	−0.05	3.91	2.47	−0.8	−1.17	−0.79
Variance						
Cum. Var.	0.39	0.64	0.75	0.83	0.89	0.92
%Var. Expl.	0.39	0.25	0.11	0.08	0.06	0.03

Our results indicate that the various habitats found within Callahan Mine were not equally diverse and harbored distinct plant communities consisting of different plant families and plant habits (life forms). With regard to hypothesis (a): in general, the waste rock piles and the tailings pond were the least species rich, whereas the shore, 'in between,' and wetland habitats were the most species rich (Table 1). This result is not surprising, given that the waste-rock piles and tailings pond had the lowest total N (NO_3^- and NH_4^+) and organic matter content among the five habitats within the Mine (Table 3). The waste-rock piles also had shallow, coarse-textured soils (mostly gravel and rocks) and little water-holding capacity, whereas the tailings pond was made of fine-textured soil particles (mostly silt, clay, and fine sediment) and could be water-logged,

Table 3. Mean values ± standard errors for soil variables; including 17 soil elements (two forms of nitrogen), pH, organic matter content as loss on ignition (LOI), and electrical conductivity (EC). All elements are reported as ppm, LOI as percent (%), and electrical conductivity as mmhos/cm. Codes for individual habitats: TP = tailings pond, WR = waste rock piles, WE = wetland, SH = shore, and IB = in between.

Habitat	pH	LOI	EC	NO_3^-	NH_4^+	Ca	K
IB	5.87 ± 0.15	5.2 ± 1.8	0.66 ± 0.17	6.2 ± 3.1	8.2 ± 5.9	2569 ± 1429	105.7 ± 41.7
SH	6.73 ± 0.38	8.4 ± 3.8	11.84 ± 6.56	49.7 ± 33.2	19.3 ± 16.6	3660 ± 1008	439.4 ± 227.2
TP	7.54 ± 0.07	0.6 ± 0.2	0.79 ± 0.26	1.8 ± 0.4	3.7 ± 0.6	16285 ± 1515	11.7 ± 0.7
WR	5.14 ± 0.61	1.1 ± 0.1	0.75 ± 0.12	1.4 ± 0.3	2 ± 0.1	2411 ± 1292	10.5 ± 1.4
WE	4.39 ± 0.44	25 ± 8	1.82 ± 0.95	48.6 ± 12	37.5 ± 24.5	3264 ± 1512	255.1 ± 14.8
	Mg	Na	P	S	Al	Cd	Cr
IB	102.4 ± 32.3	18.4 ± 6.3	7.3 ± 1.2	164.1 ± 73.2	12.4 ± 11.7	2.7 ± 0.7	0.046 ± 0.019
SH	380.5 ± 146.4	3022.2 ± 1689.9	12.9 ± 4.9	320.5 ± 141.7	1.8 ± 1.2	3.4 ± 1.5	0.142 ± 0.07
TP	100.6 ± 10.7	5.6 ± 1.5	20.1 ± 1.8	89.8 ± 29.2	0.4 ± 0	3 ± 0.9	0.023 ± 0.003
WR	66.7 ± 9.4	5.8 ± 0.7	4.1 ± 1.7	376.7 ± 109.8	9.6 ± 5.8	1.3 ± 0.5	0.032 ± 0.005
WE	378.9 ± 158	285.9 ± 164.4	8.7 ± 1.4	3452.7 ± 3192.7	7.4 ± 3	25.2 ± 22	0.156 ± 0.043
	Cu	Fe	Mn	Mo	Ni	Pb	Zn
IB	131.8 ± 26.3	117.3 ± 62.9	4.2 ± 2	0.38 ± 0.06	1.52 ± 0.58	129.2 ± 78	558 ± 110
SH	56 ± 15.1	139.5 ± 91.8	14.2 ± 4.6	0.39 ± 0.08	1.39 ± 0.52	29.2 ± 5.8	430 ± 146
TP	102.7 ± 7.2	2.5 ± 0.9	0.8 ± 0.2	0.5 ± 0.04	0.49 ± 0.21	9.1 ± 2.7	750 ± 52
WR	107.5 ± 25.7	36 ± 15.6	1.8 ± 0.7	0.27 ± 0.08	0.49 ± 0.06	13.3 ± 5.6	368 ± 119
WE	170.3 ± 146.2	656.9 ± 192.5	13.8 ± 10.8	1.81 ± 1.36	8.68 ± 5.66	1.4 ± 0.4	2934 ± 2632

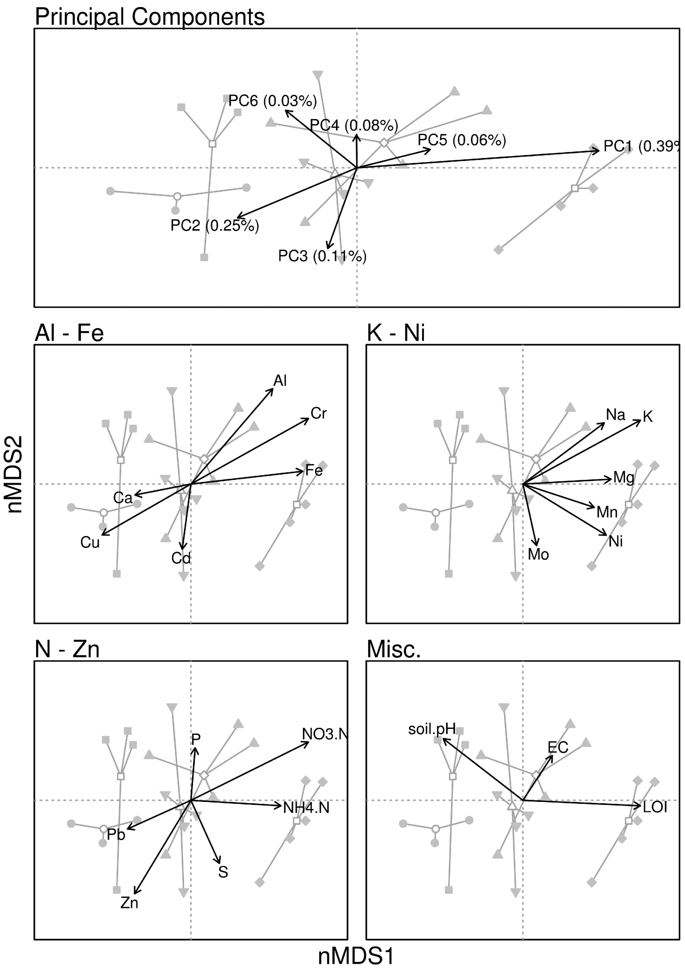

Figure 6. Principal components of (log-transformed) soil variables, and the soil variables themselves, fit to the nMDS ordination and plotted in the ordination space shown in Figure 3. The percentage associated with each PC is the amount of variance that PC explains, in the set of all the soil variables. The fitting process is analogous to multiple regression, where the soil variable(s) is a response and the ordination axes are predictors. The direction of the arrow indicates the nature of the association between the soil variable(s) and the ordination. The lengths of the arrows are proportional to the amount of variance in the soil variable(s) explained by the ordination axes (e.g., the R^2 of the multiple regression).

Table 4. Fit of the variables to the nMDS ordination of the plant community. Note that each variable was fit separately. The p-value was derived from 999 permutations of each variable vector with regard to the site-species matrix; the vector was permuted and R^2 calculated for each permutation. The p-value, therefore, is the fraction of permutations with an R^2 greater than that observed with the original data.

Variable	R^2	p (permutation) $>R^2$
PC1	0.6496	0.001
PC2	0.2218	0.095
PC3	0.1792	0.157
PC4	0.0284	0.785
PC5	0.0676	0.495
Habitats	0.7073	0.001

making both these habitats physically challenging for plant growth. Wetland and shore habitats were fairly nutrient rich, especially in total N, and had the highest organic matter content among the five habitats examined. There was also ample soil development on both these habitats, providing a suitable growth medium for roots.

With regard to hypothesis (b): the ordination of the plant community indicates two distinct floras with little overlap (see Supplementary Table with mean abundances across habitats and NMDS loadings for all plants at http://nrajakaruna.files.wordpress.com/2014/03/supplementary-table.pdf): 1) a wetland flora and 2) a small subset of plants found in the disturbed habitats (waste rock piles and tailings pond). The shore and 'in between' habitats—which fall between the disturbed habitats and the wetland habitat in ordination space—share plant taxa with both the wetland and the disturbed habitats. Woody vegetation was abundant across Callahan Mine but was predominately associated with the shore and 'in between' habitats. The few species of ferns present at the Mine were found in the wetland and in the wooded buffer, and were likely restricted in location by the requirement for a moist rooting zone. Annual forbs were infrequent at the Mine (six species in total), and were associated only with the shore and wetland habitats. The absence of annual forbs from the disturbed habitats—the tailings pond and waste rock piles—is curious. It is unsurprising that Asteraceae (33 taxa; 21%), Poaceae (17 taxa; 11%), and Rosaceae (12 taxa; 8%) were the most species-rich families, as they are also some of the most speciose families in the region.

Of the six families speciose enough to be considered individually, the Rosaceae were associated with the less disturbed habitats

Table 5. Tissue macronutrient concentrations of 20 species found at Callahan Mine. Concentrations were determined by the dry ashing method for all except N, which was estimated by direct combustion analysis. Total nitrogen (TN) is reported as percentage and all other elements are reported as ppm.

Species	Elemental Concentrations (% or ppm)				
	TN (%)	Ca	K	Mg	P
Achillea millefolium subsp. *lanulosa*	1.46	14240.2	26376.95	2266.43	2904.32
Betula papyrifera	1.99	9144.51	11050.3	1245.91	1675.7
Festuca rubra subsp. *rubra*	1.26	5053.65	10281.41	980.97	609.42
Galium mollugo	1.54	18427.37	16191.06	2376.77	2456.64
Hypericum perforatum subsp. *perforatum*	1.74	7890.3	10417.86	1306.47	2126.67
Juncus gerardii	1.15	1424.98	20539.13	1334.07	1174.63
Lotus corniculatus	2.55	14231.27	15718.77	1364.79	813.79
Lupinus polyphyllus var. *polyphyllus*	3.72	16260.96	19001.14	4499.11	1765.87
Morella caroliniensis	1.88	5654.17	6629.28	1724.77	554.05
Onoclea sensibilis	1.82	13506.09	17706.61	2340.29	2456.78
Phragmites australis	2.36	3184.76	17377.79	885.96	1309.96
Populus balsamifera subsp. *balsamifera*	1.77	18956.08	16687.93	1871.71	1459.05
Salix bebbiana	1.55	14515.78	9178.29	1276.71	1621.32
Silene vulgaris subsp. *vulgaris*	1.17	19534.54	40327.08	2224.7	2433.5
Spiraea alba var. *latifolia*	1.56	6754.25	8756.16	1327.15	1387.02
Stellaria graminea	1.09	9272.12	10073.56	1749.45	516.03
Thlaspi arvense	4.82	26132.53	39060.07	2406.16	5118.39
Typha latifolia	2.13	6690.21	21767.28	1038.26	1868.33
Vaccinium angustifolium	1.69	6751.56	3586.61	1681.53	944.7
Vicia villosa subsp. *villosa*	3.07	21476.75	11965.07	1417.51	1523.15

Table 6. Tissue micronutrients, including heavy metal concentrations, of 20 species found at Callahan Mine. Concentrations were determined by the dry ashing method and are reported as ppm.

Species	Al	B	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
Achillea millefolium subsp. *lanulosa*	30.33	43.88	< 5.0	< 5.0	21.83	65.27	64.91	< 5.0	13.54	< 5.0	394.74
Betula papyrifera	20.51	24.15	< 5.0	< 5.0	7.34	57.39	113.22	12.43	< 5.0	< 5.0	620.66
Festuca rubra subsp. *rubra*	162.03	8.14	< 5.0	< 5.0	12.87	255.67	506.76	43.93	< 5.0	5.92	149.75
Galium mollugo	14.89	44.76	< 5.0	< 5.0	6.44	42.46	26.27	< 5.0	< 5.0	< 5.0	610.94
Hypericum perforatum subsp. *perforatum*	< 10.0	35.42	< 5.0	< 5.0	8.16	32.19	61.04	< 5.0	< 5.0	< 5.0	305.02
Juncus gerardii	116.86	11.36	< 5.0	< 5.0	6.34	68.14	54.67	< 5.0	< 5.0	< 5.0	86.23
Lotus corniculatus	< 10.0	19.77	< 5.0	< 5.0	4.73	45.47	34.56	21.12	< 5.0	< 5.0	230.43
Lupinus polyphyllus var. *polyphyllus*	54.86	19.94	< 5.0	< 5.0	13.84	65.13	923.31	< 5.0	< 5.0	< 5.0	137.65
Morella caroliniensis	17.59	25.46	< 5.0	< 5.0	3.82	57.29	98.84	< 5.0	< 5.0	< 5.0	72.69
Onoclea sensibilis	< 10.0	25.4	< 5.0	< 5.0	6.61	53.51	13.74	< 5.0	< 5.0	< 5.0	649.77
Phragmites australis	< 10.0	5.63	< 5.0	< 5.0	1.1	48.06	108.76	< 5.0	< 5.0	< 5.0	47.61
Populus balsamifera subsp. *balsamifera*	< 10.0	33.93	10.63	< 5.0	5.49	36	50.54	< 5.0	< 5.0	< 5.0	969.44
Salix bebbiana	< 10.0	27.84	6.73	< 5.0	4.7	101.29	73.89	< 5.0	< 5.0	< 5.0	1069.58
Silene vulgaris subsp. *vulgaris*	24.9	23.62	5.24	< 5.0	6.87	49.11	25.59	< 5.0	5.73	< 5.0	531.72
Spiraea alba var. *latifolia*	42.46	12.08	10.47	< 5.0	4.78	35.59	120.04	< 5.0	< 5.0	< 5.0	712.26
Stellaria graminea	96.1	13.77	< 5.0	< 5.0	8.9	110.75	63.56	< 5.0	< 5.0	< 5.0	282.26
Thlaspi arvense	76.12	16.06	< 5.0	< 5.0	6.99	145.92	38.1	< 5.0	< 5.0	< 5.0	321.3
Typha latifolia	< 10.0	12.24	< 5.0	< 5.0	4.75	51.6	300.85	7.79	< 5.0	< 5.0	65.13
Vaccinium angustifolium	70.87	26.34	< 5.0	< 5.0	5.25	53.18	683.31	< 5.0	< 5.0	< 5.0	26.34
Vicia villosa subsp. *villosa*	42.17	23.3	< 5.0	< 5.0	7.73	90.57	80.44	10.53	5.59	< 5.0	395.26

(wetland, shore, and 'in between'), whereas the Pinaceae were absent from the wetland habitats. The Asteraceae were associated with all habitats but were a more dominant component of the community in the shore and wetland habitats. The Caryophyllaceae consisted of three introduced species characteristic of disturbed environments (*Cerastium fontanum*, *Silene vulgaris* subsp. *vulgaris*, and *Stellaria graminea*) and a native maritime species (*Spergularia marina*). Collectively, these were found in all habitats except the wetland. Both *Cerastium* and *Silene* consist of metal-tolerant species worldwide and are often dominant perennial forbs on Cu and Zn mine tailings (O'Dell and Rajakaruna 2011). The Poaceae were associated with all habitats, confirming why genera such as *Festuca* and *Agrostis* are often used in the restoration of mine tailings worldwide (O'Dell and Rajakaruna 2011). The Fabaceae were absent from the wetland habitats (unsurprising, as this family is nitrogen fixing and characteristic of marginal soils), and were all introduced perennials characteristic of pastures. The association of various plant families and life forms within different habitats suggests differential tolerance to physical and chemical factors associated with distinct habitats found within Callahan Mine. This is an important result that provides land managers with better guidance to select species of plants that are best suited for the restoration, per Environmental Protection Agency (1996), of the various habitats (tailings pond, waste rock piles, wetland, etc.) found within the Mine.

With regard to hypothesis (c): soil ionic content differed substantially across habitats, and the gradient in the plant community paralleled the primary differences in soils among habitats. Wetland soils contained high levels of organic matter, N, Fe, and the plant nutrients Mn, Mg, Na, and K, whereas the soils of the tailings pond and waste rock piles were marginal in terms of organic matter and N. All habitats were found to have equivalent amounts of Cu (means ranging from 103 to 170 ppm), except the shore (mean 56 ppm). Although it is impossible to infer a causal influence of soil ionic content on the plant community (as opposed to soil physical factors such as depth, texture, and water inundation), our findings are important for management and restoration decisions, especially when suitable species are sought to restore distinct habitats (i.e., tailings pond, waste rock pile, etc.) within Callahan Mine.

Our exploratory tissue analyses also indicate the extent to which metals were accumulated by the plants found at Callahan Mine.

None of the 20 species analyzed were found to accumulate significant concentrations of metals in their leaves (Table 6), except *Salix bebbiana* and *Populus balsamifera*, which accumulated close to a third of the concentration of Zn considered the threshold for hyperaccumulation (Table 6; van der Ent et al. 2012). Thus, although the majority of the species we found at the Mine may not be suitable for phytoextraction of metals, they are likely candidates for restoring (i.e., greening) metal-enriched sites in New England. These species are clearly able to withstand the high concentrations of metals in the soil and to deal with the harsh habitat attributes of mines, including rocky and shallow soils, little shade, water stress, and steep, highly erodible topography. For example, the genus *Thlaspi* (many of which are now in *Noccaea*) consists of many known metal hyperaccumulators (Gall and Rajakaruna 2013). These hyperaccumulating taxa are closely related to *T. arvense*, a non-accumulating species found at the Mine. *Thlaspi arvense* and hyperaccumulating relatives have been used in comparative studies that examined mechanisms of metal tolerance and accumulation (Kramer et al. 2000; Salt et al. 2000). At the Mine, scattered individuals of *T. arvense* were found to the northwest of the tailings pond in an area recently disturbed to build roads for the remediation process. Leaf tissues of *T. arvense* showed no significant accumulation of any of the target elements. Concentrations of Zn were slightly elevated (Table 6) but they were still at levels found to be within the range for 'normal' plants (Kabata-Pendias 2001). Although *T. arvense* does not accumulate significant concentrations of metals in its leaves, it is naturalized at Callahan Mine and thus it may be a good candidate for phytostabilizing the Mine by using plants to physically stabilize contaminated soils (Pilon-Smits 2005).

Hyperaccumulating plants are often slow-growing and low-biomass plants that are not well suited for phytoremediation (Neilson and Rajakaruna 2012). Thus, metal-tolerant species with higher biomass, that grow faster, are often utilized in phytoremediation, particularly in phytostabilization (Pilon-Smits 2005). Two such genera, *Typha* (Pilon-Smits 2005) and *Populus* (Dickinson et al. 2009; Pilon-Smits 2005), are favored for their fast growth and metal tolerance. Fast-growing, metal-tolerant genera such as *Typha* and *Populus* have several advantages over slower growing hyperaccumulators. Their extensive root systems are capable of stabilizing soils, preventing erosion and the spread of contaminated soils, and reducing the bioavailability of metals (Dickinson et al. 2009; Neilson and Rajakaruna 2012). Additionally, high transpiration rates, especially of large trees such as *Populus*, prevent downward leaching of contaminated waters that may otherwise filter into aquifers (Pilon-Smits 2005). *Typha latifolia* is found at Callahan Mine in the tailings pond and in the wetland (Figure 1) and shows slightly elevated concentrations of Mn and Mo in its leaves. *Typha latifolia* has been found to sequester metals in the roots until toxicity is reached, which explains the low concentrations generally found in leaf tissue (Ye et al. 1997). Ye et al. (1997) found populations of *T. latifolia* from both contaminated and non-contaminated soils to be tolerant of certain metals; this suggests constitutional tolerance. There are two species of *Populus* found at the Mine, *P. tremuloides* and *P. balsamifera*. *Populus tremuloides* is found on the shore and the 'in between' habitat. *Populus balsamifera* is found on the edges of waste rock pile 1, around the wetland, and along the northern edges of Goose Pond, and has been shown to accumulate substantial amounts of Zn and a considerable amount of Cd. Lukaszewski et al. (1993) found *Populus* species to accumulate metals in the xylem tissue rather than in the leaves. Similarly, *Salix* taxa are known from metal-polluted sites (Vandecasteele et al. 2002) and have been tested for their potential to extract heavy metals such as Cd and Zn (Pulford and Watson 2002; Vysloužilová et al. 2003a, b). Both *P. balsamifera* and *Salix bebbiana* at the Mine accumulated one third of the concentration of Zn considered as hyperaccumulation for Zn (hyperaccumulation threshold is 3000 ppm; van der Ent et al. 2012) and they, along with *Spiraea alba* var. *latifolia*, accumulated approximately one tenth of the concentration of Cd considered as hyperaccumulation for Cd (hyperaccumulation threshold is 100 ppm; van der Ent et al. 2012). Although none of the species we examined qualified as metal hyperaccumulators (van der Ent et al. 2012), the taxa that accumulated considerable amounts of Zn and Cd are worthy candidates for phytoremediation.

The species we have documented as metal tolerant and metal accumulating, including those in the genera *Populus*, *Salix*, *Spiraea*, *Thlaspi*, and *Typha*, are commonly found at Callahan Mine and in New England. These can be effectively utilized to restore non-vegetated habitats within the Mine, if attention is paid to their tolerance of the specific habitats we have described. For example, *Typha latifolia* can be successfully introduced to regions of the tailings pond and wetland that are currently unvegetated, whereas the two *Populus* taxa and *Salix bebbiana* are ideal for unvegetated settings along the shore and 'in-between' habitats. *Thlaspi arvense*, *Silene vulgaris* subsp. *vulgaris*, and *Achillea millefolium* subsp. *lanulosa* are ideally suited for seeding many of the disturbed settings at the Mine, including regions of the waste-rock piles, shore, and 'in between' habitats. Similarly, the metal-tolerant grass and legume species we have documented (Appendix) are good candidates for phytoremediation practices, as they can stabilize the soil and, in the case of the legumes, also introduce much-needed nitrogen to the soils. Thus, the suite of species we have documented for the Mine can provide a species list from which land managers can choose species that are able to remediate the distinct habitats within the Mine, as well as in other similar disturbed and metal-enriched settings in the region. It is important, however, to 1) pay attention to seed source, as not all populations may be as tolerant of heavy metals due to intraspecific variation for metal tolerance commonly found within a species (O'Dell and Rajakaruna 2011) and 2) select those species that are native or naturalized and are less likely to become invasive.

Degraded, disturbed, and polluted landscapes are often considered as habitats that non-native species readily colonize (Alpert et al. 2000; Decker et al. 2012; Lemke et al. 2013). However, our study confirms that 62% of the taxa we encountered at Callahan Mine are native to North America, including 11 taxa (7%) listed as rare in at least one New England state (Appendix). Only eight taxa (5%) are considered invasive in at least one New England state (New England Wild Flower Society 2012). A similar trend was observed for bryophytes (Briscoe et al. 2009), lichens (Harris et al. 2007), and vascular plants (Pope et al. 2010) at the metal-enriched serpentine quarry at Pine Hill and for vascular plants of a nutrient-enriched guano deposit on an offshore island in the region (Rajakaruna, Pope, and Perez-Orozco 2009). These results suggest that chemically and physically harsh edaphic settings, including those that are disturbed, may contribute to species-rich native plant communities (Hobbs and Humphries 1995). Contrary to our expectation, the vegetation at Callahan Mine was as similar to that of the metal-enriched serpentine quarry at Pine Hill, as it was to that of the granitic outcrop at Settlement Quarry (Pope et al. 2010), both in terms of families and species shared. The proportion of species in the Callahan Mine flora shared with Pine Hill was marginally larger than the proportion of species in Callahan Mine flora shared with

Settlement Quarry. However, this outcome is probably a direct result of the greater diversity in the Pine Hill flora (132 species, relative to 94 at Settlement Quarry), and hence of the greater chance that any given site in the region would share a larger proportion of species with Pine Hill than Settlement Quarry.

Edaphically extreme sites, such as Callahan Mine, hold many potential discoveries in the fields of ecology and evolution (Harrison and Rajakaruna 2011) and green technologies such as phytoremediation and phytostabilization (Pilon-Smits 2005; Whiting et al. 2004). The potential for new discoveries is exciting; however, without prior knowledge of what is growing at sites such as Callahan Mine, these discoveries could not take place. Remediation of the Mine began in 2010 (Environmental Protection Agency 2013) and without this study, there would have been little information on the flora of this unique habitat prior to remediation. The current study of the vascular plants at the Mine provides a baseline to compare vegetation before and after remediation efforts, making more in-depth studies possible in the future. Our study also points to distinct plant-habitat associations within the Mine, and indicates that different plant families and plant forms may be better suited to the restoration of each of the edaphically distinct habitats found within the Mine. Studies such as these, conducted across New England's many contaminated sites, can better inform land managers and conservation authorities on how best to remediate the landscapes degraded by human activities of the past.

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APPENDIX

VASCULAR PLANTS FOUND AT CALLAHAN MINE

Table shows presence/absence within each of the five habitats of Callahan Mine, as well as on the serpentine outcrop, Pine Hill (PH), and the granite outcrop, Settlement Quarry (SQ). Callahan Mine habitats: TP = tailings pond, WR = waste rock piles, WE = wetland, SH = shore, IB = 'in between'. X = species present, – = species absent. Taxa listed, but not found in any of the designated habitats, were collected between plots. Species not native to North America are denoted by an *. Nomenclature and authorities follow Haines (2011). Conservation status in New England (E = endangered; I = invasive; P = prohibited; SC = special concern; T = threatened) is from New England Wild Flower Society (http://gobotany.newenglandwild.org/; most recently accessed Jan 2013).

Family	Species	Callahan Mine Habitats							Conservation
		TP	WR	WE	SH	IB	PH	SQ	Status (State)
ADOXACEAE	*Sambucus racemosa* L.	–	–	X	X	X	X	X	
	Viburnum nudum var. *cassinoides* (L.) Torr. & A. Gray	–	–	–	–	X	–	X	SC (CT)
AMARANTHACEAE	**Chenopodium album* L.	–	–	–	X	–	–	–	
	Salicornia depressa Standl.	–	–	–	X	–	–	–	T (NH)
	**Suaeda maritima* subsp. *maritima* (L.) Dumort.	–	–	–	–	–	–	–	
APIACEAE	**Daucus carota* L.	–	–	–	X	–	–	–	
APOCYNACEAE	*Apocynum androsaemifolium* L.	–	–	–	X	–	–	–	
	Asclepias syriaca L.	–	–	–	X	–	–	–	
AQUIFOLIACEAE	*Ilex mucronata* Torr. & Gray *ex* Gray	–	–	–	–	–	–	X	
	Ilex verticillata (L.) Gray	–	–	–	–	–	–	–	
ARALIACEAE	*Aralia nudicaulis* L.	–	–	–	–	X	X	–	
ASTERACEAE	*Achillea millefolium* subsp. *lanulosa* (Nutt.) Piper	–	–	–	X	X	X	X	
	Antennaria howellii subsp. *petaloidea* (Fernald) Bayer	–	–	–	–	–	–	–	SC (CT)

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats							Conservation Status (State)
		TP	WR	WE	SH	IB	PH	SQ	
	Cirsium muticum Michx.	–	–	X	X	–	–	–	
	**Cirsium vulgare* (Savi) Ten.	–	–	X	–	–	–	X	
	Cirsium sp. Mill.	–	–	X	–	–	–	–	
	Doellingeria umbellata var. *umbellata* (Mill.) Nees	–	–	X	–	–	–	X	
	Erechtites hieraciifolius var. *hieraciifolius* (L.) Raf. *ex* DC.	–	–	X	–	–	–	–	
	Erigeron strigosus Muhl. *ex* Willd.	–	–	–	–	–	X	–	
	Eurybia macrophylla (L.) Cass.	–	–	–	X	–	–	–	
	Euthamia graminifolia (L.) Nutt.	X	–	X	–	–	X	X	
	**Hieracium caespitosum* Dumort.	–	X	–	–	–	X	X	
	Hieracium kalmii L.	–	–	–	–	X	–	–	
	**Hieracium pilosella* L.	X	X	–	–	–	X	X	
	Hieracium sp.	–	–	–	X	X	–	–	
	Lactuca biennis (Moench) Fernald	–	–	X	–	–	–	–	
	Lactuca canadensis L.	–	–	X	–	–	–	–	
	Lactuca sp.	–	–	X	–	–	–	–	
	**Leucanthemum vulgare* Lam.	–	X	–	–	X	X	–	
	Rudbeckia hirta L. var. *pulcherrima* Farw.	–	X	–	–	–	–	–	
	**Scorzoneroides autumnalis* subsp. *autumnalis* (L.) Moench	–	–	–	–	X	–	–	
	Solidago bicolor L.	X	–	X	–	–	X	–	
	Solidago canadensis L. var. *canadensis*	–	–	X	–	–	X	–	

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats TP	WR	WE	SH	IB	PH	SQ	Conservation Status (State)
	Solidago juncea Aiton	X	–	–	–	–	X	X	
	Solidago nemoralis Aiton subsp. *nemoralis*	–	–	–	–	X	X	–	
	Solidago puberula Nutt. var. *puberula*	X	–	–	–	–	–	X	
	Solidago rugosa Mill. subsp. *rugosa*	X	–	X	X	–	X	X	
	Solidago sempervirens L. var. *sempervirens*	–	–	–	X	–	–	–	
	Solidago uliginosa Nutt.	–	–	–	–	–	–	–	
	Solidago sp.	X	–	X	–	X	–	–	
	**Sonchus arvensis* L. var. *arvensis*	–	–	–	–	–	–	–	
	Symphyotrichum novi-belgii (L.) G.L. Nesom var. *novi-belgii*	–	–	X	X	–	X	X	
	Symphyotrichum novi-belgii (L.) G.L. Nesom var. *elodes* (Torr. & Gray) Nesom	X	–	X	–	–	–	–	
	**Taraxacum officinale* Weber *ex* F.H. Wigg.	–	–	–	X	X	X	–	
BALSAMINACEAE	*Impatiens capensis* Meerb.	–	–	X	–	–	X	–	
BETULACEAE	*Alnus viridis* (Vill.) Lam. & DC. subsp. *crispa* (Aiton) Turrill	–	–	–	–	X	X	–	T (MA)
	Betula papyrifera Marshall	X	X	–	X	X	X	X	
BRASSICACEAE	**Brassica nigra* (L.) W.D.J. Koch	–	–	–	X	–	–	–	
	**Thlaspi arvense* L.	–	–	–	–	–	–	–	
CAPRIFOLIACEAE	*Diervilla lonicera* Mill.	–	–	–	–	X	X	X	

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats					PH	SQ	Conservation Status (State)
		TP	WR	WE	SH	IB			
	**Lonicera morrowii* Gray	–	–	–	–	–	–	X	I (CT, MA, NH, RI, VT); P (CT, MA, NH, VT)
CARYOPHYLLACEAE	**Cerastium fontanum* Baumg. subsp. *vulgare* (Hartm.) Greuter & Burdet	X	–	–	–	–	X	–	
	**Silene vulgaris* (Moench) Garcke subsp. *vulgaris*	–	X	–	X	X	–	–	
	Spergularia marina (L.) Griseb.	–	–	–	X	–	–	–	
	**Stellaria graminea* L.	–	X	–	–	–	X	–	
CELASTRACEAE	**Celastrus orbiculatus* Thunb.	–	–	–	–	–	X	–	I (CT, MA, RI, VT); P (CT, MA, NH, VT)
CONVOLVULACEAE	**Cuscuta gronovii* Willd. *ex* J.A. Schult. var. *gronovii*	–	–	X	–	–	–	–	P (MA, VT)
CUPRESSACEAE	*Juniperus communis* L. var. *depressa* Pursh	–	–	–	X	X	X	X	
	Thuja occidentalis L.	–	–	–	X	X	X	–	E (MA); T (CT)
CYPERACEAE	*Carex bebbii* Olney *ex* Fernald	–	–	–	X	–	–	–	
	Carex scoparia Schkuhr *ex* Willd.	X	–	–	X	–	X	X	

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats					PH	SQ	Conservation Status (State)
		TP	WR	WE	SH	IB			
	Eleocharis tenuis (Willd.) J.A. Schult. var. *tenuis*	–	–	–	–	–	–	–	
	Schoenoplectus pungens (Vahl) Palla var. *pungens*	–	–	–	–	–	–	–	
	Scirpus cyperinus (L.) Kunth	–	–	–	–	–	X	–	
DENNSTAEDTIACEAE	*Dennstaedtia punctilobula* (Michx.) T. Moore	–	–	–	X	X	–	–	
DRYOPTERIDACEAE	*Dryopteris carthusiana* (Vill.) H.P. Fuchs	–	–	X	–	X	X	X	
	Dryopteris intermedia (Muhl. *ex* Willd.) Gray	–	–	–	–	X	–	–	
ELAEAGNACEAE	**Elaeagnus umbellata* Thunb. var. *parvifolia* (Royle) Schneid.	–	–	–	X	–	–	–	I (CT, MA, NH, RI); P (MA, NH)
EQUISETACEAE	*Equisetum arvense* L.	–	–	–	–	–	X	–	
ERICACEAE	*Gaultheria procumbens* L.	–	–	–	–	X	–	–	
	Vaccinium angustifolium Aiton	X	–	–	X	X	X	X	
FABACEAE	**Lotus corniculatus* L.	X	–	–	X	–	–	–	
	Lupinus polyphyllus Lindl. var. *polyphyllus*	–	–	–	–	–	–	–	
	**Melilotus albus* Medik.	–	X	–	–	–	–	–	
	**Robinia hispida* L. var. *hispida*	–	–	–	X	–	–	–	
	**Securigera varia* (L.) Lassen	–	–	–	–	–	–	–	

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats					PH	SQ	Conservation Status (State)
		TP	WR	WE	SH	IB			
	**Trifolium arvense* L.	–	–	–	–	–	X	–	
	**Trifolium campestre* Schreb.	–	–	–	–	–	X	–	
	**Trifolium pratense* L.	–	–	–	X	–	X	X	
	**Trifolium repens* L.	X	–	–	–	X	X	–	
	**Vicia cracca* L. subsp. *cracca*	–	–	X	–	X	X	–	
	**Vicia villosa* Roth subsp. *villosa*	–	X	X	–	X	–	–	
GROSSULARIACEAE	*Ribes glandulosum* Grauer	–	–	X	–	–	–	X	SC (CT)
HYPERICACEAE	**Hypericum perforatum* L. subsp. *perforatum*	–	–	–	–	–	X	X	
IRIDACEAE	*Sisyrinchium montanum* Greene var. *montanum*	–	X	–	–	X	–	–	
JUNCACEAE	*Juncus balticus* Willd. subsp. *littoralis* (Engelm.) Snogerup	–	X	–	–	–	–	–	
	Juncus gerardii Loisel.	–	–	–	X	–	–	–	
JUNCAGINACEAE	*Triglochin maritima* L.	–	–	–	X	–	–	–	E (VT)
LAMIACEAE	**Galeopsis bifida* Boenn.	–	–	X	X	–	X	–	
LYCOPODIACEAE	*Diphasiastrum digitatum* (Dill. *ex* A. Braun) Holub	–	–	–	–	–	–	–	
LYTHRACEAE	**Lythrum salicaria* L.	–	–	X	–	–	–	–	I (CT,, ME MA, NH, RI, VT); P (CT, ME, MA, NH VT)

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats							Conservation Status (State)
		TP	WR	WE	SH	IB	PH	SQ	
MYRICACEAE	*Morella caroliniensis* (Mill.) Small	–	–	–	X	–	–	–	
MYRSINACEAE	**Lysimachia borealis* (Raf.) U. Manns & A. Anderb.	–	–	–	–	X	X	X	
ONAGRACEAE	*Chamaenerion angustifolium* (L.) Scop. subsp. *circumvagum* (Mosquin) Moldenke	–	–	X	–	–	–	–	
	Epilobium ciliatum var. *ciliatum* Raf.	–	–	–	–	X	–	–	
	Oenothera parviflora L.	X	X	–	–	–	–	–	
ONOCLEACEAE	*Onoclea sensibilis* L.	–	–	X	X	X	–	–	
ORCHIDACEAE	**Epipactis helleborine* (L.) Crantz	–	–	–	X	X	–	–	
OROBANCHACEAE	*Euphrasia nemorosa* (Pers.) Wallr.	–	–	–	–	X	–	–	
OXALIDACEAE	*Oxalis stricta* L.	–	–	–	–	–	X	–	
PINACEAE	*Abies balsamea* (L.) Mill.	–	–	–	X	–	X	X	E (CT)
	Larix laricina (Du Roi) K. Koch	–	–	–	X	–	–	–	T (RI)
	Picea glauca (Moench) Voss	X	–	–	X	X	X	X	
	Picea rubens Sarg.	–	X	–	X	X	X	X	
	Pinus resinosa Aiton	–	–	–	X	X	–	–	E (CT)
	Pinus strobus L.	–	–	–	X	–	X	–	
	**Pinus sylvestris* L.	–	X	–	–	–	–	–	
PLANTAGINACEAE	**Plantago major* L.	–	–	–	–	–	–	–	
	Plantago maritima L. subsp. *juncoides* (Lam.) Hultén	–	–	–	X	–	–	–	
	**Veronica officinalis* L.	–	–	X	–	–	X	X	

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats					PH	SQ	Conservation Status (State)
		TP	WR	WE	SH	IB			
POACEAE	*Agrostis scabra* Willd.	–	–	–	X	–	–	X	
	**Agrostis stolonifera* L.	X	–	–	X	X	–	–	
	**Elymus repens* (L.) Gould	–	–	X	X	–	X	X	
	Festuca rubra L. subsp. *rubra*	X	X	–	–	X	X	X	
	**Festuca trachyphylla* (Hack.) Krajina	–	–	–	X	–	–	–	
	Glyceria striata (Lam.) A.S. Hitchc.	–	–	X	–	–	X	–	
	Hordeum jubatum L. subsp. *jubatum*	–	–	–	X	–	–	–	
	**Phleum pratense* L.	–	–	–	–	X	–	–	
	Phragmites australis (Cav.) Trin. *ex* Steud.	–	–	–	X	–	–	–	I (CT, MA, NH, VT); P (CT, MA, NH, VT)
	**Poa compressa* L.	–	X	–	X	X	X	X	I (CT); P (CT)
	**Poa nemoralis* L.	–	–	–	–	X	–	–	
	Poa palustris L.	–	–	–	X	–	X	X	
	**Puccinellia maritima* (Huds.) Parl.	–	–	–	X	–	–	–	
	Puccinellia nuttalliana (J.A. Schult.) A.S. Hitchc.	–	–	–	X	–	–	–	
	Spartina alterniflora Loisel.	–	–	–	X	–	–	–	
	Spartina patens (Aiton) Muhl.	–	–	–	X	–	–	–	

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats					PH	SQ	Conservation Status (State)
		TP	WR	WE	SH	IB			
	Torreyochloa pallida (Torr.) Church var. *fernaldii* (A.S. Hitchc.) Dore *ex* Koyama & Kawano	–	–	X	X	–	–	–	
POLYGONACEAE	*Fallopia cilinodis* (Michx.) Holub	–	–	X	–	–	–	–	
	**Fallopia convolvulus* (L.) A. Löve	–	–	–	–	–	–	–	
	**Persicaria maculosa* S.F. Gray	–	–	–	–	–	–	–	SC (MA)
	**Rumex acetosella* L. subsp. *pyrenaicus* (Pourr. *ex* Lapeyr.) Akeroyd	–	X	–	–	–	X	X	I (CT); P (CT)
	**Rumex crispus* L. subsp. *crispus*	–	–	X	X	–	X	–	
RANUNCULACEAE	*Ranunculus abortivus* L.	–	–	X	–	–	–	–	
	**Ranunculus acris* L.	–	–	–	–	X	X	–	
ROSACEAE	*Amelanchier spicata* (Lam.) K. Koch	–	–	–	X	X	–	–	
	Fragaria virginiana Duchesne subsp. *virginiana*	–	X	–	X	X	X	X	
	Malus sp.	–	–	–	X	–	–	–	
	Potentilla norvegica L.	–	–	–	–	–	X	X	
	Potentilla simplex Michx.	–	X	–	–	–	X	X	
	Prunus pensylvanica L. f. var. *pensylvanica*	X	–	–	–	–	X	X	
	Prunus virginiana L. var. *virginiana*	–	–	X	–	X	X	X	
	Prunus sp.	–	–	–	X	X	–	–	
	Rosa palustris Marshall	–	–	X	X	–	–	–	
	Rubus idaeus L. subsp. *idaeus*	–	–	X	X	X	–	–	

APPENDIX. Continued.

Family	Species	Callahan Mine Habitats					PH	SQ	Conservation Status (State)
		TP	WR	WE	SH	IB			
	Rubus idaeus L. subsp. *strigosus* (Michx.) Focke	–	–	X	–	X	X	X	
	Spiraea alba Du Roi var. *latifolia* (Aiton) Dippel	–	–	–	X	X	X	X	
RUBIACEAE	**Galium mollugo* L.	–	X	–	X	X	–	–	
RUSCACEAE	*Maianthemum canadense* Desf.	–	–	–	–	X	–	–	
SALICACEAE	*Populus balsamifera* L. subsp. *balsamifera*	–	X	–	–	–	–	–	
	Populus tremuloides Michx.	–	–	–	X	X	X	X	
	Salix bebbiana Sarg.	X	–	–	–	X	–	–	
	Salix sp.	–	–	–	X	X	–	–	
SAPINDACEAE	*Acer rubrum* L.	–	–	–	–	–	X	X	
SOLANACEAE	**Solanum dulcamara* L. var. *villosissimum* Desv.	–	–	X	X	X	X	–	I (CT); P (CT)
TYPHACEAE	*Typha latifolia* L.	–	–	X	–	–	–	–	