

Effect of Soil Metals on Pollination of Subalpine Wildflowers

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Abstract

Metals-rich soils can occur naturally or through human activity, such as mining. Plants growing in metal-rich soils often incorporate metals into their tissues; the concentration of these metals varies, and plants which concentrate the metals most strongly are termed hyperaccumulators. Accumulation of metals in plant tissues has mainly been studied in the context of phytoremediation. We examined the effects of metal-rich soils on natural systems surrounding mines in Gunnison County, Colorado. Some native plants growing on mine soils accumulated metals in their tissues compared to those growing on uncontaminated soils. In addition, plant communities growing on uncontaminated sites were more diverse than those growing on mine sites. Plants growing on mines were generally shorter and had fewer flowers compared to those from uncontaminated sites, including *Lupinus argenteus*, *Thlaspi montanum*, and *Draba aurea*. For *D. aurea*, pollination rates were lower at mine sites, which could be a result of changes in plant or pollinator community composition or of the shorter, less showy plants. This study provides evidence to suggest that mine sites may affect native plant pollination; however, more investigation must be done to understand the mechanisms of how soil metals affect pollination and subsequent plant fitness.

Introduction

Ecologists are fundamentally interested in how variation in abiotic factors affects the survival, performance, and abundance of plants and animals. One factor that might be strongly associated with the success of plants is metals. Metals occur in trace amounts in all soils, and in some cases may occur in high concentrations naturally, for example in serpentine soils (Brady et al 2005). In other cases, high metal concentrations may be human-induced. There are 23,000 abandoned mines in the state of Colorado alone (“Abandoned Mines”, 2008), encompassing hundreds of acres of land contaminated by waste mining material. While acid mine drainage and its effects on aquatic life are well-studied by ecologists, metal contamination in terrestrial systems is less so. However, mine tailings (rocks and particles remaining from the ore milling process) and the surrounding soil have elevated levels of many metals (Fey and Church, 1999). Their small particle size and high surface area to volume ratio make them very reactive so that metals can easily enter the soil-water system (Nash, 2002). Water flowing from the bottoms of tailings piles has been shown to have far higher concentrations of metals than water entering these areas (Manning et al., 2008).

Plants rely on soil water for hydration, nutrients, and minerals (Brady and Weil, 2002), and plants growing in metal-rich soils often incorporate metals into their tissues (Boyd and Martens, 1998). In general, metal uptake can occur in three ways: by roots from the soil and its pore water, through leaves from the atmosphere, or onto leaves from wet and dry deposition (Witte et al., 2004). The rate of each of these processes may change with contamination when more metals are present in soil and air.

For some plants, metals are toxic; others can grow in metal-rich soils, but growth may be reduced compared to normal soils, and only a few species thrive (Foy et al., 1978). Metal tolerance can be accomplished either by excluding metals from tissues or accumulating them (Chaney, 1994). Plants incorporating high concentrations of metals into their tissues are termed hyperaccumulators (Baker and Brooks, 1989). The ability to take up metals can be constitutive (always present in a species) or facultative (present to varying degrees depending on the environment) depending on species, or even for different metals within the same species (Remon et al., 2007). There are no known hyperaccumulators in the Rocky Mountains of Colorado (Pilon-Smits, personal communication), but there is evidence of accumulation of metals in close relatives of native species in the Colorado Rockies (Table 1).

Table 1. Congeners of Gothic plants species which have been shown to accumulate metals.

Species	Accumulator of	Gothic congener	Source
<i>Draba japonica</i>	Nickel	<i>Draba aurea</i>	Horie et al., 2000
<i>Thlaspi caerulescens</i>	Nickel	<i>Thlaspi montanum</i>	Seregin and Kozhevnikova, 2008
<i>Lupinus albus</i>	Zinc	<i>Lupinus argenteus</i>	Pastor et al., 2003
<i>Linaria spartea</i>	Nickel, Chromium	<i>Linaria vulgaris</i>	Frietas et al, 2003
<i>Salix</i> species	Cadmium	<i>Salix</i> species	Larison et al., 2000
<i>Fragaria anassa</i>	Cadmium	<i>Fragaria virginiana</i>	Cieslinski et al., 1996

In contaminated areas, metal-accumulating plants may provide a mechanism for allowing metals to enter food webs. For instance, ptarmigan (*Lagopus leucurus*) feeding on willow in contaminated environments accumulate high levels of cadmium over their lifetimes (Larison et al., 2000). Arthropods feeding on the leaves of nickel hyperaccumulators have also been shown to have elevated body metal loads (Boyd et al. 2006). However, no studies to my knowledge have tested whether metals accumulate in pollination webs. For example, no studies have tested pollen for metal content from contaminated sites, although pollen in areas with high levels of air pollution has been shown to have elevated metal levels (Kalbande et al. 2008). Due to some functional similarities between herbivory and pollination (e.g., Williams, 2003), it is reasonable to hypothesize that the consumption of pollen and nectar by pollinators could also mobilize metals into the food web. Bodies of honeybees (*Apis mellifera*) foraging on hyperaccumulators have been shown to have elevated levels of nickel (Boyd et al. 2006), supporting this hypothesis. In addition, metal concentrations below hyperaccumulator levels have been shown to reduce herbivore performance (Coleman et al., 2005), so even plants with metals in their tissues below levels deemed hyperaccumulation could affect herbivores and pollinators.

Because some plants can better tolerate metals in the soil, whether by exclusion or assimilation, differences in plant diversity are common between contaminated and non-contaminated sites. Differences in plant community composition have been noted on metal-rich soils in Tuscany since the 19th century (Chiarucci, 2003). In Spain, plant diversity was significantly lower around a barium mine than in an uncontaminated pasture (Hernandez and Pastor, 2008). Similarly, grassland community composition

changes over a gradient of increasing soil copper concentration (Strandberg et. al. 2005).

Not only can soil metals affect plant community composition, but they can affect the morphology and success of plants at contaminated sites. Plants growing in extreme environments, which include those contaminated with heavy metals, may partition resources away from floral growth towards survival (Grundt et. al., 1999). For instance, plants growing in high-copper environments are often shorter than those in uncontaminated environments (Strandberg et. al., 2005), potentially affecting their visibility to pollinators. *Mimulus* growing in metal-rich soils often flower and set seed earlier than their counterparts in uncontaminated soils, as well as having different floral morphologies (Gardner and McNair 1999). These changes in morphology may lead to reproductive isolation of these populations (Brady et. al., 2005) and may affect pollinator behavior and plant reproductive success. While floral characteristics are under selection pressure from many factors, including predation and resource partitioning, pollination can also be an important selective agent. Plants with more flowers are often more successful at attracting pollinators, which can confer reproductive benefits (Brody and Mitchell, 1997). Flower morphology, including shape and size, also has an important role in attracting pollinators (Galen and Cuba, 2001). Changes in these characteristics due to resource portioning could lead to changes in pollinator visitation rates. In addition, seed set is often lower on metal-rich soils (Grundt et. al., 1999), which could be due either to reduced pollinator visitation or to partitioning of resources away from reproduction and towards survival.

I investigated four research questions related to the effects of soil metals on pollination systems. *First, do metals from the soil accumulate in flowers and pollen? Are*

there differences in plant diversity between metal-rich and uncontaminated sites? Do plants growing on metal-rich soils express different floral traits than their counterparts on uncontaminated soils? How does the presence of soil metals affect pollination rates?

Methods and Materials

Study sites

Study sites were selected as three matched pairs, with one contaminated and one control site per pair. Two contaminated sites were located at the Peanut Mine outside of Crested Butte, Colorado. The Peanut Mine was a coal mine with on-site hard-rock ore processing from 1904 to the 1970s (USEPA, 2005) and then housed a silver mill in the 1970s (Renner, 2006). It was acquired in 2001 by the Crested Butte Land Trust and, with an EPA grant, reclamation was completed in 2005 (Crested Butte Land Trust, 2008). Tailings and refuse were excavated and covered with a layer of topsoil, which was seeded with native grasses and forbs (Renner, 2006). Although remediation is deemed complete, there are small areas of the mine that were not remediated and remain contaminated. The control plots for the Peanut Mine site were on Slate River Road in Crested Butte, where the hillsides have similar vegetation patterns but no mining has occurred.

The third pair of study sites was located above the Elk Creek Trail off of Kebler Pass, Colorado. The contaminated site was located in the upper basin of the Standard Mine, while the control site was below the mine on the Elk Creek Trail. The Standard Mine was an active silver mine from approximately 1874 to 1974. It is one of the most contaminated mines in the area and was designated a Superfund site in 2005. The site has

arsenic, barium, lead, zinc, cadmium, copper, and chromium contamination in the surface and ground water as well as the soil (USEPA, 2008).

In addition to these three major pairs of study sites, additional pairs were selected for collection of plant tissues and for one-day plant diversity surveys. Some of these sites were located in other parts of the Standard Mine, with control sites at different locations along the Elk Creek Trail. Other supplemental site pairs were located in the Paradise Basin, with control sites in Elko Park, and Virginia Mine, with control sites in apparently unaffected areas below the mines.

Metal content of pollen and flowers

Tissue samples (leaves, flowers, and pollen) were collected from plants growing in each of the site pairs. Samples were taken from several species, including *Thlaspi montanum* (Brassicaceae), *Draba aurea* (Brassicaceae), *Salix* species (Salicaceae), *Lupinus argenteus* (Fabaceae), *Potentilla pulcherrima* (Rosaceae), and *Fragaria virginiana* (Rosaceae). These species were chosen because of their presence across all sites and because many have congeners which have been shown to accumulate metals. Soil samples were also collected to help identify the pool of metals available to these plants. Samples were collected using nitrile gloves. Tissue samples were placed in plastic vials and air-dried for several days. Soil samples were placed in paper bags, dried in an oven at 60° C, and then sieved using a 2 mm sieve. Samples were digested using ~1 mL of trace elements-grade nitric acid in screw-top teflon vials which could sustain heat and pressure buildup. The vials were placed on a hot-plate overnight to aid digestion.

Samples were then removed from the vials, diluted with filtered, deionized water to ~10 mL, and centrifuged to remove any remaining solids.

Samples were processed using Inductively Coupled Plasma-Atomic Emission Spectra (ICP-AES) at the Colorado School of Mines in Golden, Colorado. The ICP-AES was calibrated with 5 standards before runs, as well as with metals and non-metals standards before and after the samples as well as every fifteen samples during the run to ensure that the instrument remained calibrated. Each sample was measured three times and the average and standard deviation were displayed to ensure consistency. The ICP-AES detected concentrations of 32 elements: Ar, Ag, Al, As, B, Ba, Be, Ca, Cd, Cr, Co, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, V, Zn, and U.

Using the dry mass of each tissue sample, solution concentrations were converted to tissue concentrations. Samples that were below detection limits for a given metal were assigned the concentration of the detection limit, which was then converted to tissue concentration to account for differing sample masses. For each element, I computed the logarithmic proportion of the tissue concentrations of the mine and control sample in each pair.

$$\text{Log (mine concentration / control concentration)}$$

A positive mean to the distribution indicated that the mine site statistic was greater than the control statistic; a negative mean indicated that the mine site statistic was less than the control statistic; and a mean of zero indicated no difference between sites. For each element, the set of all proportions was tested against a mean of zero using a one-sided, right-tailed t-test.

Plant diversity and density

Two 20 m transects were set up at each site, 3-5 m apart running parallel to the areas of highest contamination. Every two weeks, plant diversity was measured in one square meter every five meters on each transect. Numbers of plants and flowers of each species were counted in each quadrat and percent ground cover was visually estimated for each species.

Species richness was calculated as a count of all species present (flowering and non-flowering) in the two transects combined on a given day, and averaged across all days for each site. All grasses were counted as one species due to the difficulty of identifying grasses to the species level in the field. Richness was calculated for total species number, as well as for flowering and non-flowering plants separately. In the future, these data will be rarefied using EcoSim, but there was not time to complete this analysis this summer. Richness was compared by computing the logarithmic proportion between the mine and control site of each pair, as was performed for the metals data. For each variable, the set of all proportions was tested against a mean of zero using a one-sided, left-tailed t-test.

Floral density was calculated for each site by dividing the total number of open flowers in all quadrats of a site by the area of the quadrats. This statistic was also compared between sites in a site pair using the log proportion and a one-sided, left-tailed t-test.

Plant and floral traits

Ten plants of three focal species (*Draba aurea*, *Lupinus argenteus*, and *Thlaspi montanum*) were tagged at each site where they were present. For approximately five consecutive days during the flowering season, each plant was examined for flower number, plant height (to nearest half cm), and number of stalks. These data were averaged across all observation days for each plant. The distributions of these variables for mine and control sites were then compared within each species using a left-tailed t-test. Levene's test was used to determine whether variances were equal. While the t-test examined differences for each species, averages for species at a site were compared within the site pairs using the log proportion, and the distribution of these proportions was tested against a hypothesized mean of zero using a one-sided, left-tailed t-test to track general trends across all species for each variable.

The distance from each focal plant to the place of most obvious contamination was recorded for *Draba* and *Lupinus* at the Peanut Mine sites. At the upper site, this area was a stream, and at the lower site, this area was a pond. I used correlation test to test whether there was a relationship between distance to potentially contaminated water and the plant and floral traits.

Visitation rates

When the 10 tagged plants of *Draba aurea* and *Thlaspi montanum* began flowering, pollinator visits were observed. I observed each focal plant for ten minutes each day, with all pollinator activity being recorded. Pollinators were identified to functional group as solitary bee, bumble bee (to species), fly, butterfly, or unknown. Pollinators were followed throughout a visit, and the time spent on each flower, the

number of flowers visited, and the time spent on the plant were recorded. Numbers of other visitors such as ants, beetles, true bugs, and caterpillars, which do not typically act as pollinators, were also noted. Data were standardized by the total amount of time the plant was observed. Number of nonpollinators per minute, percent time with pollinating visitors, number of pollinating visitors per minute, average visit length to plant, number of flowers visited per minute, and average visit length per flower were compared between mine and control sites for each species using t-tests.

Reproductive success

All of the fruits from each focal plant were collected when they matured. Fruit production was compared between mine and control sites for each species using a t-test. A correlation test was used to determine whether there was a relationship between per-plant fruit production at the Peanut Mine sites and the distance to the nearest contaminated water source.

Seeds from the fruits will be counted in the lab at Dartmouth College in the fall, and similar analysis will be performed for seeds per fruit and seeds per plant.

Results

Metal content of plant tissue (leaves, pollen, and flowers) and soil

To date, only soil samples from mine sites and tissue samples of *Draba aurea*, *Thlaspi montanum*, and *Salix* samples have been analyzed in the ICP-AES. While soil metals concentrations cannot be compared between mine and uncontaminated sites, mean

element concentrations at mine sites (Table 2) can be compared to literature values and used as a reference to define the pool of metals available to plants growing on these sites.

Table 2. Mean and standard error of element concentrations in soil samples from 7 mine sites.

element	Mean ug/g	Std Err ug/g	element	Mean ug/g	Std Err ug/g
Ar	11.7395	0.5541	Li	3.7934	0.4123
Ag	0.1977	0.0895	Mg	1200.2744	155.2628
Al	3560.6327	298.5859	Mn	743.9900	253.8259
As	16.0578	7.6554	Mo	0.0461	0.0020
B	2.4213	0.8765	Na	32.6609	9.8238
Ba	66.2239	9.9639	Ni	3.6700	0.5733
Be	0.2503	0.0461	P	348.8872	92.3494
Ca	1795.7041	547.2875	Pb	707.4849	537.1293
Cd	6.6139	4.0337	S	485.6246	122.4156
Co	4.5278	0.4712	Si	16.4326	5.8611
Cr	2.4324	0.3663	Sr	26.1632	11.6400
Cu	51.9864	13.4543	Ti	3.3558	1.9360
Fe	8459.1150	715.3097	U	19.7657	7.3391
K	380.9143	70.1049	V	3.2645	0.6320
			Zn	517.5122	254.1145

Sample size was too small for tissue samples to compare concentrations between species and tissue types (leaf, flower, and pollen). This analysis will be completed when the rest of the tissue samples have been run in the ICP-AES. In the meantime, concentrations can be compared between mine and control sites for all species and tissue types compined (Table 3).

Table 3. Mean and standard error of metal and other element concentrations in plant tissues on (n=22) and off (n=12) of mine sites. Averages combine flower, leaf, and pollen samples from *Draba aurea*, *Thlaspi montanum*, and *Salix* species.

	Control		Mine	
	Mean	Std Err	Mean	Std Err
Ar ug/g	590.4420	304.6862	404.3689	114.1146
Ag ug/g	1.4737	0.7715	1.2150	0.2958
Al ug/g	290.8841	142.2277	274.6139	90.5808
As ug/g	19.6492	10.2865	13.4628	3.8890
B ug/g	95.8599	71.5613	35.2555	5.6924
Ba ug/g	312.2666	294.1571	32.7030	14.2690
Be ug/g	0.2095	0.0960	0.4617	0.3526
Ca ug/g	12447.4986	1700.7888	10483.3721	1247.2562
Cd ug/g	1.1625	0.3718	2.5032	0.6557
Co ug/g	3.3849	1.5884	2.0798	0.6093
Cr ug/g	1.7716	0.9745	1.2288	0.3688
Cu ug/g	2.0798	0.7858	11.8892	3.6393
Fe ug/g	273.1236	90.4676	228.5369	67.3478
K ug/g	28881.8657	4548.5453	23053.8834	1583.1953
Li ug/g	1.6166	0.6446	1.4501	0.3738
Mg ug/g	2967.5087	362.5843	2340.3042	173.6415
Mn ug/g	41.5154	10.6962	86.1238	12.1518
Mo ug/g	3.7063	1.1811	3.4944	0.9290
Na ug/g	1062.6356	985.8570	101.4333	46.2454
Ni ug/g	22.1692	11.3465	2.6133	0.5592
P ug/g	6364.6760	1648.1907	6164.7605	672.8725
Pb ug/g	12.5043	6.4029	11.4314	3.4153
S ug/g	8818.3612	1287.0044	7929.0286	811.6725
Si ug/g	16.9426	9.7280	74.9107	28.9605
Sr ug/g	158.1630	73.4491	124.7373	29.0020
Ti ug/g	5.7943	2.3018	3.0596	0.9603
U ug/g	58.4966	32.2520	36.3908	10.5063
V ug/g	0.7724	0.3813	0.5519	0.1453
Zn ug/g	355.4832	138.9757	541.3882	100.3066

Out of the samples processed so far, eleven pairs with the same species and tissue type could be compared. I found that copper concentrations were significantly higher at mine than at control sites ($t_{11} = 3.09$, $p = 0.0057$; Figure 1). Silicon ($t_{11} = 1.47$, $p=0.086$) and manganese ($t_{11} = 1.41$ $p=0.095$) had marginally statistically significant results.

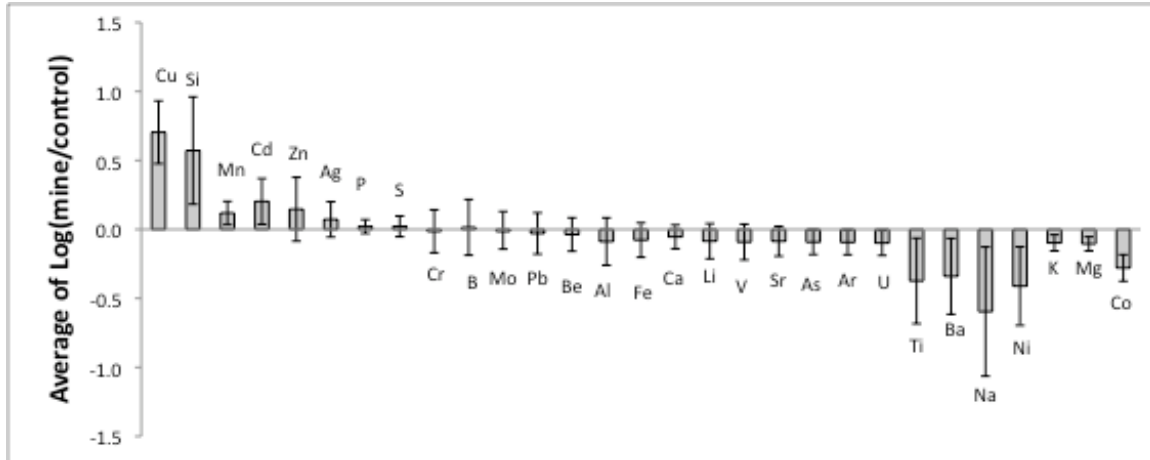


Figure 1. Averages of log proportions comparing metal and other elements concentrations in plant tissues at mine and uncontaminated sites. Error bars represent one standard error; n=11.

Interestingly, concentrations appear to be significantly lower in plants growing on mine sites for some metals, such as cobalt and magnesium.

Plant diversity and density

A total of 130 species, excluding grasses, were observed across all sites. Average species richness was higher at the control than at the mine site for every site pair (Figure 2). Mine sites had significantly lower total species richness than control sites ($t_6 = -3.95$, $p=0.006$). This corresponded to a significantly lower richness of both flowering species ($t_6 = -2.19$, $p=0.04$) and non-flowering species ($t_6 = -4.86$, $p=0.002$). Some families with species much less common at mine sites included Asteraceae (*Achillea lanulosa*, *Erigeron flagellaris*, *Heliomeris multiflora*, *Senecio cana*), Fabaceae (*Lathyrus leucanthus*, *Vicia americana*), Violaceae (*Viola adunca*, *Viola praemorsa*), Ranunculaceae (*Delphinium nuttalianum*, *Ranunculus inaemoeus*), Chenopodiaceae (*Chenopodium album*), Onagraceae (*Epilobium brachycarpum*), Rubiaceae (*Galium*

boreale), and Polygonaceae (*Polygonum douglasii*). Conversely, the invasive chamomile *Matricaria perforata* (Asteraceae) was much more prevalent at mine sites.

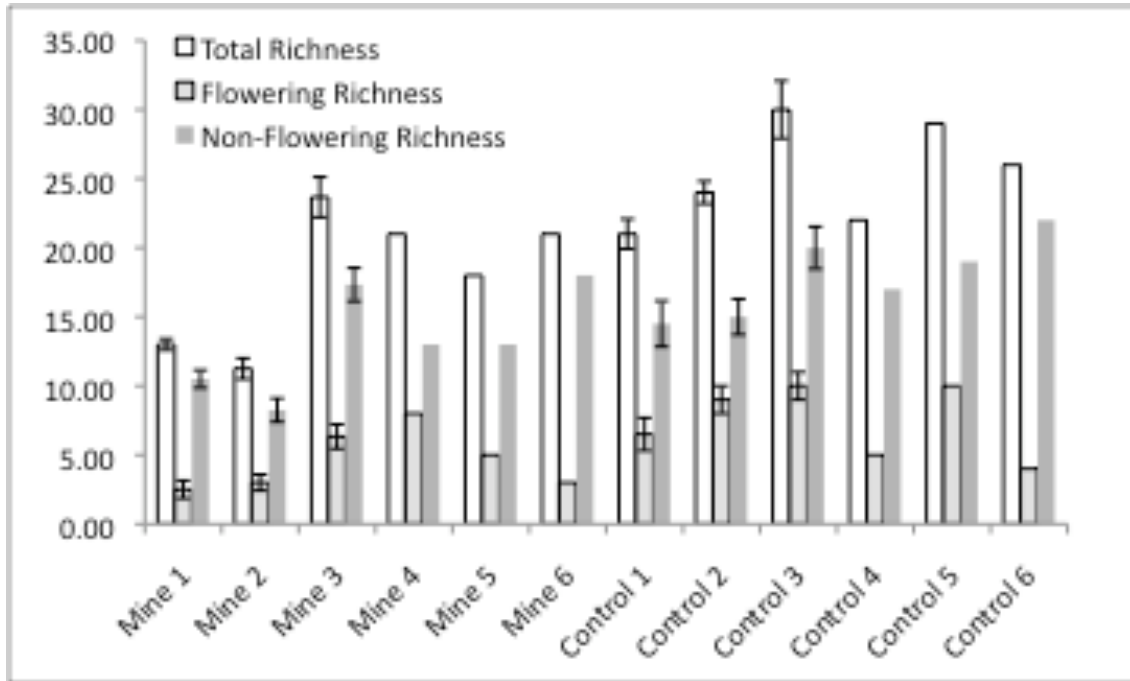


Figure 2. Average species richness for all species, flowering species, and non-flowering species at mine and control sites over the course of the summer. Error bars represent $\pm$ one standard error for sites which were surveyed multiple times; $n=4$ for pairs 1 and 2, $n=3$ for pair 3, and $n=1$ for pairs 4, 5 and 6.

Average number of open flowers per 10 m^2 was also higher at control than mine sites, with an average of 171.28 flowers at mine sites across all dates and 226 flowers at control sites (Figure 3). There were marginally significantly fewer open flowers at mine sites within a site pair ($t_6=-1.72$, $p=0.07$).

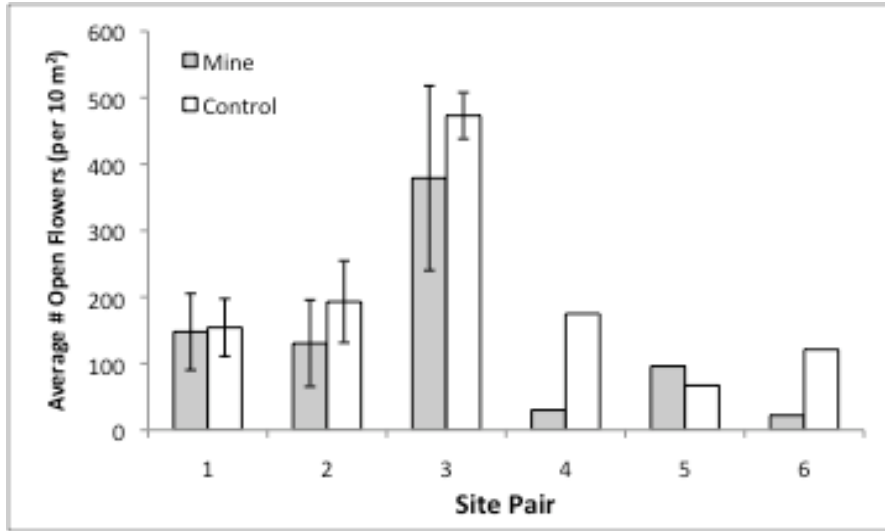


Figure 3. Average number of open flowers across observation days at mine and control sites within a site pair. Bars represent one standard error for sites measured more than once; n=4 for pairs 1 and 2, n=3 for pair 3, and n=1 for pairs 4, 5, and 6.

Plant and floral traits

Plant and floral data were taken for *Draba* and *Lupinus* at two site pairs and for *Thlaspi* at one site pair (Table 3).

Table 3. Average plant heights, number of stalks, and number of open flowers on *Draba aurea*, *Lupinus argenteus*, and *Thlaspi montanum* plants growing on and off mines. T-tests are left-tailed and an integer value for degrees of freedom indicates equal variances and a pooled t-test.

	Species	control		mine		t statistic	D.F.	p value
		Mean	Std Err	Mean	Std Err			
plant height (cm)	Draba	39.16	2.28	23.29	1.91	-5.34	38	<0.001
	Lupine	58.07	1.89	30.56	1.94	-10.15	38	<0.001
	Thlaspi	13.05	0.88	10.78	1.00	-1.71	18	0.052
#stalks	Draba	4.03	0.72	2.94	0.51	-1.24	34.36	0.112
	Lupine	14.46	2.60	10.54	1.44	-1.32	38	0.097
	Thlaspi	1.12	0.12	2.23	0.61	1.78	9.68	0.947
# flowers	Draba	99.35	21.10	34.77	6.57	-2.92	22.65	<0.001
	Lupine	74.44	11.48	11.38	4.67	-5.08	25.11	<0.001
	Thlaspi	5.15	0.69	12.63	2.78	2.61	10.09	0.987

Levene's test was used to determine whether pooled or unequal variances should be used for all t-tests; results varied by species and variable. *Draba* and *Lupinus* plants were

significantly shorter at mine than at control sites, with *Thlaspi* plants showing marginal significance. No species showed significant decreases in number of stalks at mine sites compared to control sites. *Draba* and *Lupinus* plants growing on mines had significantly fewer open flowers than those at uncontaminated sites, but open *Thlaspi* flowers did not decrease. Across all species, there were significantly shorter plants at mine sites within a site pair ($t_5 = -4.99$, $p = 0.004$). Mine site plants did not have significantly fewer stalks ($t_5 = -0.40$, $n = 5$, $p = 0.35$), but had marginally significantly fewer flowers ($t_5 = -1.86$, $n = 5$, $p = 0.069$).

Within mine sites for *Draba* and *Lupinus*, the averages for each plant showed no significant correlation to the distance of the plant from the nearest contaminated water source for all three response variables.

Visitation rates

For *Draba aurea*, number of non-pollinators per minute, percent time with pollinating visitors, number of pollinating visitors per minute, average visit length to plant, number of flowers visited per minute, and average visit length per flower were all significantly lower at mine sites than at control sites (Table 4). None of these metrics showed significant differences between site type for *Thlaspi montanum* (Table 4). While some of these differences could be related to the number of open flowers on a plant, average time spent per flower likely is not, so it is important to note that this variable was significantly lower at mine sites for *Draba aurea* than it was at control sites. For *Draba aurea*, combining mine and control distributions, visitors per minute ($r^2 = 0.30$, $p < 0.001$), time per visit ($r^2 = 0.21$, $p = 0.004$) and flowers visited per minute ($r^2 = 0.61$, $p < 0.001$)

showed a significant positive relationship to the average number of open flowers. For *Thlaspi montanum*, none of the pollination rate response variables showed significant relationships to the number of open flowers. Pollination rates for *Draba aurea* were not significantly correlated with the distance to the nearest visually contaminated water source.

Table 4. Pollinator visitation to *Draba aurea* and *Thlaspi montanum* plants growing on and off mine sites. Statistics are from left-tailed t-tests, and integer values for degrees of freedom indicate equal variances and a pooled t-test.

		control		mine				
	Species	Mean	Std Err	Mean	Std Err	t statistic	D.F.	p value
nonpollinators per minute	Draba	11.9	1.76	1.11	0.42	-5.97	21.14	<0.001
	Thlaspi	0	0	0.24	0.16	1.50	9.00	0.916
% time with visitors	Draba	14.49	3.56	3.27	1.13	-3.00	22.79	0.003
	Thlaspi	0.21	0.11	0.05	0.02	-1.47	18	0.079
visitors per minute	Draba	6.1	1.46	2.48	0.54	-2.32	24.08	0.014
	Thlaspi	1.44	0.48	1.47	0.6	0.04	18	0.515
time per visit (s)	Draba	34.53	8.78	5.81	1.89	-3.20	20.74	0.002
	Thlaspi	1.3	0.81	0.4	0.15	-1.09	18	0.145
# flowers visited per minute	Draba	29.4	7.63	9.75	3.97	-2.29	28.35	0.015
	Thlaspi	2.74	0.92	1.62	0.62	-1.01	18	0.162
avg time per flr (s)	Draba	4.37	0.94	2.02	0.98	-1.73	38	0.046
	Thlaspi	0.47	0.23	0.35	0.13	-0.47	18	0.323

Reproductive Success

Using Levene's test, I found that variances were unequal for fruit set at mine and control sites for all three species. While fruit set for *Draba* was significantly lower at mine sites compared to control sites, there were no significant differences for *Lupinus* or *Thlaspi* (Table 5).

Table 5. Average fruit set for *Draba aurea*, *Lupinus argenteus*, and *Thlaspi montanum* plants growing on and off mine sites. Statistics are from unequal-variance, left-tailed t-tests.

	control		mine				
Species	Mean	Std Err	Mean	Std Err	t statistic	D.F.	p value
Draba	227.7	52.34	117.6	34.02	-2.56	32.62	0.008
Lupine	36.65	6.24	30.1	30.1	-0.50	29.31	0.311
Thlaspi	15	1.85	23.5	23.5	1.25	10.43	0.881

Within mine sites, fruit set of *Draba* showed a weak significant negative correlation ($r=-0.44$, $p=0.048$) to distance to nearest contaminated water source, but there was no significant correlation for *Lupinus* (Figure 4).

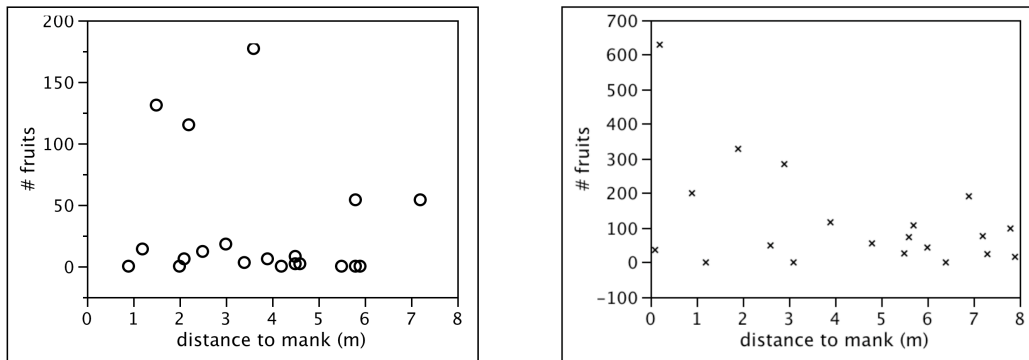


Figure 4. Scatterplots of fruit production and distance to nearest contaminated water source for *Lupinus argenteus* (left) and *Draba aurea* (right) growing on mine sites.

Discussion

While interesting trends were observed in the metals data, the small sample size and large error limited the power to statistically detect differences. As a result, the statistical significance and biological relevance of results were mismatched. While copper was present in significantly higher concentrations at mine sites, it had an average concentration at those sites of only $11.89 \mu\text{g/g}$ plant dry mass, which is far below the

level of hyperaccumulation of 1,000 $\mu\text{g/g}$, established in the literature (Baker and Brooks, 1989). However, aphids feeding on wheat plants with zinc levels lower than those found in this study have been shown to accumulate zinc in their bodies (Merrington et al., 1996), and cadmium concentrations less than one-twentieth of hyperaccumulation levels have been passed on to aphids feeding on broad beans (Crawford et al., 1995). This indicates that the concentrations of metals may be relevant even if they do not reach levels deemed hyperaccumulation. When the rest of the samples are processed, sample size will triple or quadruple, which should increase the power to detect these differences, and further analysis and comparison to levels in literature will be performed. In addition, with a larger sample size, concentrations will be able to be compared between tissue types to determine whether there are differences in accumulation in leaf and floral tissues.

While some metals seemed to be accumulated by plants growing on mines, others seemed to be present in lower levels in these plants than those from control sites. One possible explanation for this pattern is that accumulation of one metal can lead to accumulation of associated metals as well as lower levels of nutrient uptake (Leon et al., 2006). Even if plants can incorporate increased soil metals into their tissues, this may come with a cost. Inhibition of nutrient uptake may lead to the decreased height, for example, seen in plants growing on mine sites compared to those growing in uncontaminated soil.

Plant and floral traits and pollinator visitation support the hypothesized connection between soil metals and pollination. If costs of metal accumulation lead to shorter plants and fewer flowers, then those plants may be less visible or attractive to pollinators as suggested by Brody and Mitchell (1997), leading to lower pollination rates.

Another potential mechanism is that increased metals in floral tissue and pollen may deter pollinators based on taste or toxicity. I plan to further investigate this possibility with greenhouse experiments and feeding trials, since this mechanism could not feasibly be tested in the field. In either case, the decrease in pollination services may have led to the decrease in fruit production for *Draba*, depending on whether the species can self-pollinate and what level of pollen transfer it requires. As such, the increased soil metals could affect the entire life history of plants.

While it is possible that soil metals affect a plant's ability to attract pollinators on an individual level, it is also possible that soil metals affect plants' abilities to attract pollinators on a population or community level. A combination of these two effects could lead to the dramatic decline in pollination rates observed for *Draba aurea*, for example. Changes in plant and floral density within a population can significantly affect pollinator behavior and visitation choice (Smithson and MacNair, 1997). Meanwhile, the diversity of a plant community, or of congeners within that community, can attract more or fewer pollinators (Moeller, 2004). Thus the disappearance of some species within a family from uncontaminated to mine sites could lead to fewer pollinator visits. For example, seven *Senecio* (Asteraceae) species were observed at all of the sites combined; however, the diversity of *Senecio* species and density of plants in each population may affect pollinator visits to individual plants within a site.

More work is needed to confirm whether this is indeed a likely mechanism. For example, it is possible that mine sites have a decreased density of pollinators because they are less suitable nesting habitat, and that lower pollination rates are due to a density effect rather than any trait of the plant in question. Given the apparent effect of soil

metals on plant densities, it is possible that the toxicity of metals to arthropods could limit pollinator distributions around mine sites. Completing the metals data and comparing it to literature values will determine whether soil, plant tissues or pollen could be toxic or deterrent to pollinators and may help answer this question.

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