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# Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes

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ABSTRACT

In southern Québec, supplement roadside ground covers (i.e. *Trifolium* spp.) struggle to establish near edges of major roads and thus fail to assist turf recruitment. It creates empty niches vulnerable to weed establishment such as common ragweed (*Ambrosia artemisiifolia*). We hypothesized that heavy metal stresses may drive such species shifts along roadside edges. A growth chamber experiment was conducted to assess effects of metals (Zn, Pb, Ni, Cu, and Cd) on germination and seedling behaviors of roadside weed (*A. artemisiifolia*) and ground cover legumes (*Coronilla varia*, *Lotus corniculatus*, and *Trifolium arvense*). All metals inhibited *T. arvense* germination, but the effect was least on *A. artemisiifolia*. Low levels of Pb and Ni promoted germination initiation of *A. artemisiifolia*. Germination of *L. corniculatus* was not affected by Zn, Pb, and Ni, but inhibited by Cu and Cd. Germination of *C. varia* was decreased by Ni, Cu, and Cd and delayed by Zn and Pb. Metal additions hindered seedling growth of all test species, and the inhibitory effect on the belowground growth was greater than on the aboveground growth. Seedling mortality was lowest in *A. artemisiifolia* but highest in *T. arvense* when exposed to the metal treatments. *L. corniculatus* and *C. varia* seedlings survived when subjected to high levels of Zn, Pb, and Cd. In conclusion, the successful establishment of *A. artemisiifolia* along roadside edges can be associated with its greater tolerance of heavy metals. The findings also revealed that *L. corniculatus* is a potential candidate for supplement ground cover in metal-contaminated roadside edges in southern Québec, especially sites contaminated with Zn and Pb.



## 1. Introduction

Roadsides along major roads are a hostile environment for turfgrass establishment (Brown and Gorres, 2011). To help roadside turf establishment, certain species of perennial legumes are commonly utilized as supplemental ground cover (NCDOT, 1998; Sincik and Acikgoz, 2007). Roadsides along primary roads and highways in southern Québec, Canada have been planted with perennial legumes, usually clover (*Trifolium* spp.) cultivars, together with a cold-season salt-tolerant turf grass mix (GTCVC, 2006). However, the clovers often struggle to establish near pavement edges and fail to support permanent turf-recruitment. This results in creating vacant niches vulnerable to weed establishment along roadside edges. *Ambrosia artemisiifolia* (common ragweed) has frequently exploited the empty niches and colonized along roadside edges in southern Québec (DiTommaso, 2004). Roadside mowing does not control *A. artemisiifolia* as mowed plants develop secondary branches below cutting height (Simard and Benoit, 2011).

*A. artemisiifolia* is not only a noxious agricultural weed, but also one of the most prevalent allergenic weeds. In Québec, *A. artemisiifolia* pollens cause seasonal rhino-conjunctivitis and dermatitis, which affects 1 in 7 people (over million people) in the province (MSSS, 2012). Up to 10% of the overall US population is sensitive to ragweed pollen (Wilken et al., 2002). Additionally, the species is spreading across many parts of Europe and Asia, where it poses threats to public health and native ecosystems (Bullock et al., 2010; Fang and Wan, 2009). Roadways contributed to the spread of *A. artemisiifolia* in both native and invaded regions (Brandes and Nitzsche, 2006; Joly et al., 2011; Lavoie et al., 2007; Milakovic et al., 2014; Vitalos and Karrer, 2009). Therefore, management of roadside populations of *A. artemisiifolia* is necessary to prevent its further spread.

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Soil heavy metal concentrations in the vicinity of major roads in the metropolitan area of Montréal, Québec often exceed environmental thresholds (Cloutier-Hurteau et al., 2014; Ge et al., 2000). Surface soils (pH 7.5–7.8) of roadside edges along heavy-traffic highways ($\geq$30,000 vehicles per day) in southern Québec contain Zn, Pb, and Cu at bioavailable levels phytotoxic to sensitive species (Bae et al., 2015). Metals generally cause a concentration-dependent inhibition of seed germination and seedling growth, but the degree of inhibition varies depending on plant species and metal types (Kranner and Colville, 2011). A response to metal stress during the plant's early life cycle can be one of the potential drivers underlying establishment success near heavy-traffic roadside edges. We found that *A. artemisiifolia* emergence was positively correlated with Zn, Pb and Cu bioavailability, while *Trifolium arvense* (rabbitfoot clover) emergence was negatively correlated (Bae et al., 2015). We hypothesized that *A. artemisiifolia* establishment along roadside edges may arise from its tolerance to metal stresses, which provides a competitive advantage over the current roadside cover legumes (i.e. *Trifolium* spp.). Therefore, a growth chamber experiment was conducted to evaluate seed germination and seedling growth of *A. artemisiifolia* (common ragweed), *T. arvense* (rabbitfoot clover), *Lotus corniculatus* (birdsfoot trefoil) and *Coronilla varia* (crown vetch) in response to heavy metals (Zn, Pb, Ni, Cu and Cd).

## 2. Materials and methods

### *2.1. Seed collection and storage*

In November 2011, mature plants (13–15 plants per site) of *A. artemisiifolia* were randomly collected from six roadside sites along Québec Route 104 in Saint-Jean-sur-Richelieu (45.19°N, 73.16°W), Québec, Canada. They were dried for one week at room temperature and then seeds were extracted. Seeds of *L. corniculatus*, *C. varia* and *T. arvense* were purchased from Richters (Goodwood, ON), Pickseed Eastern Canada (Saint-Hyacinthe, QC), and Indigo (Ulverton, QC) in December 2011. All seeds were stored in the dark at 4 °C in a cold storage room with 40% relative humidity.

### *2.2. Metal treatments*

Zinc (Zn), lead (Pb), nickel (Ni), copper (Cu), and cadmium (Cd) were used because they are the most common metals encountered in roadside topsoil in southern Québec (Cloutier-Hurteau et al., 2014). For germination trials, Zn, Pb, Ni, and Cu treatments were 0, 50, 100, and 200 mg/kg and Cd treatments were 0, 5, and 10 mg/kg. For seedling growth tests, Zn, Pb, Ni and Cu treatments were 0, 25, 50, 75, and 100 mg/kg and Cd treatments were 0, 2.5, 5, 7.5, and 10 mg/kg. The concentrations were based on the background metal levels in soils of southern Québec (50 mg Pb/kg; 100 mg Zn/kg; 50 mg Ni/kg; 50 mg Cu/kg; and 1.5 mg Cd/kg) (MDEFP, 2003). Metal solutions were prepared by diluting 10,000 mg/kg (Zn, Pb, Ni, and Cu) and 1000 mg/kg (Cd) of ICP standard solution of each metal (99.9%, SCP Science, Canada) with deionized water. The control was deionized water.

### *2.3. Seed germination experiment*

#### *2.3.1. Seed preparation*

Six hundred seeds of *A. artemisiifolia* and *C. varia* were soaked in 6% sodium hypochloride solution for 5 min and rinsed by running deionized water for 10 min. The seeds were placed on two layers of filter paper (Whatman No. 1) moistened with 5 ml of deionized water in ten petri dishes (60 seeds per dish) and the dishes were stored in the dark at 4 °C for six weeks. Six hundred seeds of *L. corniculatus* and *T. arvense* were scarified by 120-grit sandpaper and wet-cold stratified (60 seeds per dish) for 24 h.

#### *2.3.2. Germination substrate and condition*

Metal treatment solutions were solidified with 0.8% (w/v) Bacto agar (DIFCO) and adjusted to pH 5.8 prior to autoclaving because metal phytotoxicity assessment on agar media is more sensitive than filter papers (Di Salvatore et al., 2008). The metals were applied as ICP standard metal solutions dissolved in 4% nitric acid. Since agar solidification is pH dependent (an optimal range between 5.4 and 5.8) and most metal ions usually begin to precipitate above pH 6, potassium hydroxide (2 M KOH) was added to adjust to pH 5.8. We found no effect of the additives (i.e. nitric acid, potassium hydroxide, nitrate, and potassium) on germination of the test species (Bae et al., 2014).

Micro centrifuge tubes (1.5 ml) were used to avoid statistical issues in the petri-dish germination test (Bae et al., 2014). A small hole was made in the tube cap to allow airflow. After autoclaving and cooling at room temperature, 1.3 ml of treatment media was added to each micro centrifuge tube. The stratified seeds were examined under a dissecting microscope and pressure-tested with a forceps. Badly deteriorated seeds and those that did not resist the slight pressure were discarded. For each treatment, 25 tubes with each test species were assigned as a replicate and each replicate was randomly assigned on tube racks. The tubes were transferred to a growth chamber with alternating temperatures of 10 °C (10 h dark cycle) and 25 °C (14 h light cycle), 60% relative humidity and 6100 lu/$m^2$ light intensity. The alternating temperatures and photoperiods were similar to the field conditions during late spring or early summer in southern Québec. Due to the chamber capacity, each germination trial was conducted for each metal type with three replicates for each treatment and was repeated one more time.

#### *2.3.3. Germination experiment*

The number of germinated seeds was recorded every 24 h for two weeks. A seed was considered to have germinated when an emerging radicle was longer than 2 mm. Tubes with germinated seed were removed. Germination response variables were germination rate ($T_{50}$) and final germination percentage (TG). Germination rate ($T_{50}$) referred to the time (hours) to reach 50% of final germination over the two-week trial and was calculated by the following formula (Farooq et al., 2005):

$$T_{50} = \frac{\left\{\left(\frac{N}{2}\right) - n_i\right\}(t_i - t_j)}{(n_i - n_j)}$$

where N is the final number of seed germination at two weeks and ni, nj cumulative number of seeds germinated by consecutive counts at times ti and tj measured in hours when $ni < N/2 < nj$. Final germination percentage (TG) at the end of the experiment period was calculated by the following formula:

$$TG = \frac{\text{Number of germinated seeds at 2 weeks}}{\text{Total number of seeds}} \times 100$$

### *2.4. Seedling growth experiment*

#### *2.4.1. Seed preparation*

The seeds were prepared in the same manner as in the germination experiment. The prepared seeds of each test species were