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# Sex and heavy metals: Study of sexual dimorphism in response to soil pollution

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ABSTRACT

In dioecious plants, males and females often show distinct morphological, physiological and life history traits as result of their different demands for reproduction. Such sexual dimorphism is likely to be accentuated under stressful conditions, such as that imposed by exposure to heavy metals. However little is known about the response of dioecious plants to stress by heavy metals. Here we use the dioecious herb *Silene latifolia* to investigate the growth and reproduction of males and females growing in soil polluted with either Cu or Cd. We also examined whether the sexes differed in the patterns of metal accumulation in their tissues. Patterns of biomass allocation to reproduction, roots, leaves and shoots (stem and leaves) were compared in male and female *S. latifolia* plants that were harvested after growing for 14 weeks in different soil conditions (non-polluted soil, Cu-polluted soil and Cd-polluted soil). In addition, patterns of metal accumulation between the sexes were also compared post-harvest by analysing the metal content in their tissues. Overall, metals decreased plants' total dry mass allocated to leaves and to roots-particularly in males. Females accumulated more Cu in their tissues. However, this did not seem to correspond with females performing worse (in terms of growth and reproduction) than males when growing in soil polluted with this metal. Despite males and females having similar levels of Cd in their tissues, males seemed to have lower tolerance to this metal, as indicated by a lower total and leaf dry mass than females, and also by a lower number of flowers when growing in Cd-polluted soil. We also found contrasting differences in female seed production due to heavy metals, with number of seeds (but not mass) decreasing with Cu and increasing with Cd. Our results indicate the presence of sexual dimorphism in response to heavy metals, with the sexes differing in both patterns of accumulation and tolerance.



## 1. Introduction

Dioecy, where male and female functions are housed in different individuals, is a rare sexual system in flowering plants (~6–7%; Renner and Ricklefs 2008). Despite its rarity, it has evolved repeatedly among flowering plants, occurring in almost half of all angiosperm families (Heilbuth, 2000) probably in response to selection either for inbreeding avoidance (Charlesworth and Charlesworth, 1978; Freeman et al., 1997) or sexual specialization (Charnov et al., 1976; Freeman et al., 1997). Once unisexuality has evolved, selection may favour further divergence between the sexes as consequence of their different reproductive roles (production of seeds vs. pollen). Sexual dimorphism in plants is quite common (Barrett and Hough, 2013 Willson, 1991) and males and females usually differ in their vegetative morphology (Dawson and Geber, Dawson and Geber, 1999; Obeso, 2002; Sánchez-Vilas and Retuerto, 2009; Sánchez-Vilas et al., 2012), phenology (Bullock and Bawa, 1981; Delph, 1990), physiology (Case and Ashman, 2005; Dawson and Geber, 1999; Sánchez-Vilas and Retuerto, 2011, 2009), life history (Geber et al., 1999), competitive abilities and allocation to defence (Cornelissen and Stiling, 2005; Sánchez-Vilas et al., 2011).

Sexual dimorphism has been commonly attributed to the different cost of reproduction in males vs. females, namely, as the result of trade-offs between allocation to reproduction and to other functions (e.g., to growth and/or defence). Such trade-offs are likely to be accentuated under more stressful conditions, such as under nutrient-deficient soil, strong competition from other plants, or herbivory. In fact, several studies have found that the differences between males and females are affected by the environmental context, particularly by resource availability (Dawson and Ehleringer, 1993; Eppley, 2001; Geber et al., 1999). Female function is

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usually associated with a greater demand for resources, needed to produce both flowers and fruits (Agren, 1988; Korpelainen, 1992), and male-biased sex ratios are commonly found in more stressful environments (see review in Field et al., 2013). However, there is not yet a clear pattern in the responses of the sexes to environmental stress, and their differences in tolerance seem to vary depending on the species considered and type of stress, demanding a greater range of studies (Juvany and Munné-Bosch, 2015).

Soil pollution by heavy metals, as consequence of human activities (e.g., mining, waste disposal, industrial activities), represents a dramatic case of environmental stress for living organisms. High concentration of heavy metals are considered environmental pollutants due to their strong toxic effects; in plants, they may interfere with growth by disturbing nutrient uptake, altering photosynthesis and other metabolic processes (Flemming and Trevors 1989; Pahlsson, 1989; Tyler et al., 1989; Fernandes and Henriques 1991). Since they interfere with resource uptake, patterns of allocation to growth and reproduction may be also expected to change. However, despite the widespread contamination of soils by heavy metals, few studies have considered how they affect to the cost of reproduction (but see, Saikkonen et al., 1998; Koivunen et al., 2004) and we are only aware of a handful of studies that have examined how males and females of the same species (in particular, trees of the genus *Populus*) respond to their presence in the soil (Chen et al., 2013a, 2013b, 2011; Han et al., 2013; Jiang et al., 2013).

Here, we aim to increase our knowledge of responses to stress in dioecious plants, by investigating the effect of heavy metals in growth and reproductive traits of males and females. Specifically, we explore how soil contaminated with two different heavy metals, Cu and Cd, affects the extent of sexual dimorphism in males and females of the dioecious herb *Silene latifolia* Poir. Considering that female function demands a greater amount of resources and that heavy metals may interfere with resource uptake, we expect a poorer performance of females relative to males in terms of growth and reproduction in heavy metal contaminated soils.

## 2. Materials and methods

### 2.1. Study species

*S. latifolia* (Caryophyllaceae), the white campion, is a short-lived perennial and dioecious plant native to most of Europe, Western Asia and Northern Africa. It is commonly found growing in disturbed or cultivated ground (Tutin et al., 1993) and has also been found growing in heavily contaminated mine sites in Southern France (Escarré et al., 2010). Sexual dimorphism in *S. latifolia* has been extensively studied (Bernasconi et al., 2009), with males having less allocation of biomass to vegetative growth and reproduction, but also producing more flowers (smaller) than females (see Delph et al., 2005; and references therein).

### 2.2. Experimental design

In summer 2011, seeds were collected from five natural populations of *S. latifolia* growing in Galicia, Northwest Spain (see map in Fig. 1) by sampling approx. 20 individuals per population (8–10 capsules per individual). Seeds were separated from capsules, discarding the damaged ones, and stored at room temperature.

In March 2012, seeds from these five populations were randomly sown in 40-cell germination trays in a greenhouse at the Faculty of Biology, University of Santiago de Compostela (Spain). We transplanted a total of 240 seedlings (evenly and randomly chosen across populations) to individual 1.6 L pots, filled with garden soil, when the second pair of true leaves started to grow (approx. 2 months after germination). Pots were distributed spatially on the greenhouse benches and their position re-arranged weekly in order to avoid position effects. One week after transplanting, seedlings were randomly assigned to one of three treatments: Control (no metal), Cadmium contaminated soil (10 ppm Cd) and Copper contaminated soil (125 ppm Cu); 80 seedlings per treatment. Cu was added as sulphate (copper sulphate 5-hydrate: $CuSO_4·5H_2O$ Panreac, Barcelona, Spain) and Cd

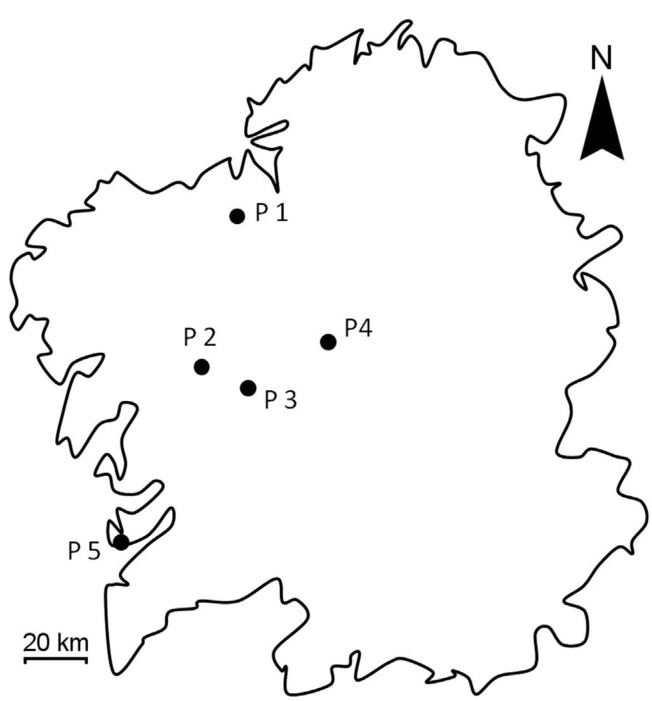


**Fig. 1.** Map showing the location of the five populations (P1–P5) of *S. latifolia* sampled in Galicia (NW Spain) and used in this study.