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Phytoextraction of Cd and Zn with *Noccaea caerulescens* for urban soil remediation: influence of nitrogen fertilization and planting density

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ABSTRACT

Phytoextraction field trials with *Noccaea caerulescens* were conducted in Brussels (Belgium) to investigate the benefits of nitrogen fertilization and planting density on phytoextraction efficiency. Both metallicolous (Ganges) and non-metallicolous (NMET, Luxembourg) populations were grown for 6 months in an urban wasteland and in a vegetable garden contaminated with trace metals at two planting densities (50 and 100 plants·m^{-2}, D50 and D100), with and without mineral nitrogen fertilizers. Trials showed that *N. caerulescens* responded positively to nitrogen fertilization through increased biomass production. However due to lower concentrations in Cd and Zn in fertilized plants, the total metal uptake was differently impacted by fertilization: on one site metal uptake was enhanced while on the other it was reduced. Moreover fertilization had collateral effects as enhancing fungal pathogen development. The effect of planting density of *N. caerulescens* assessed in this work for the first time highlighted a clear competition between individuals at higher density – proved by lower individual biomass – but the highest metal uptake was nevertheless achieved at the highest density of 100 plants·m^{-2} because of higher total biomass. Six months after harvest soil exchangeable concentrations were reduced by about 25% for Cd with Ganges population and by 9% for Zn with the NMET population on the best treatment (N fertilized and D100). The feasibility of using *N. caerulescens* for bioavailable contaminant stripping of moderately contaminated soils was confirmed for Cd and to a lesser extent for Zn.

1. Introduction

Among the different phytoremediation approaches, phytoextraction of metals and metalloids is suggested as a promising eco-friendly technique to remediate soils moderately contaminated with trace elements (Vangronsveld et al. 2009; Li et al. 2012). The main approach that has so far been investigated relies on the use of crops or trees tolerant to above-threshold soil metal concentrations which produce high biomass of low/moderate metal content with a valorization potential in bioenergy (Evangelou et al., 2012). Successive croppings slightly decrease soil metal concentrations through metal accumulation in plants and most importantly allow the economic valuation of polluted soils (Dickinson et al. 2009; Mench et al. 2010). The second approach relies on the use of hyperaccumulator plants which are undomesticated wild species accumulating about 100 times more metals in shoots compared to normal plants, at concentrations above defined thresholds when grown in their natural habitats, *i.e.* 100 µg·g^{-1} for Cd, 1000 µg·g^{-1} for Ni or 3000 µg·g^{-1} for Zn, on a dry weight basis (Krämer, 2010; van der Ent et al., 2013). In this second approach, the aim is to decrease soil metal concentrations to allow future re-use of the soil; this can only address substrates with moderate total and highly available trace metal concentrations (Hammer and Keller, 2003; Zhao et al., 2003), *e.g.* less than 10^3 and 10 mg·kg^{-1} of soil total Zn and Cd, respectively. The related concept of bioavailable contaminant stripping – which aims at removing the bioavailable metal pool – is however thought to be a more realistic alternative (Hamon and McLaughlin 1999) specifically in the case of conversion of wastelands for urban agriculture and on agricultural lands marginally contaminated (Dickinson et al. 2009). Despite over 20 years of research on phytoextraction using hyperaccumulators, large-scale field trials which test basic cultural practices such as fertilization, length of growth or irrigation are however still lacking (Dickinson et al. 2009; Mench et al. 2010; Robinson et al., 2015).

Noccaea caerulescens (Brassicaceae, formerly *Thlaspi caerulescens*), is a well-known Zn, Cd, Ni hyperaccumulator (Reeves et al. 2001). Three ecotypes have been described: metallicolous plants naturally grow on soils enriched in Cd-Zn-Pb (calamine, CAL) and on serpentine sites (enriched in Ni), whereas non-metallicolous (NMET) grow on uncontaminated soils (Reeves et al. 2001). These ecotypes differ in many ways, both for metal-related traits like tolerance and accumulation

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(Meerts and Van Isacker, 1997; Escarré et al., 2000) and for life cycle strategies (Dechamps et al. 2011; Sterckeman et al. 2017) or breeding systems (Mousset et al., 2016). The relative advantage of southern France CAL populations for Cd extraction has been demonstrated early on (Lombi et al., 2000; Schwartz et al. 2003; Zhao et al., 2003). Zinc and Ni uptake are higher in NMET in both pot experiments (Meerts and Van Isacker, 1997; Sterckeman et al. 2017) and field conditions (Jacobs et al., 2017). Moreover NMET have the advantage of being more resistant to herbivory than CAL (Noret et al., 2007), resulting in lower mortality rates and higher individual biomass in field conditions (Jacobs et al., 2017).

The lack of field trials assessing the best cultural practices for *N. caerulescens* is still the major limit for large scale application of the technique and assessment of its real remediation potential (McGrath et al., 2006; Li et al., 2012) contrarily to the use of high biomass and domesticated plant species (Mench et al., 2010). We have shown in a previous study that biomass production as high as 5 t·ha^{-1} was achievable in the field but was highly dependent on growth conditions, metal content and nutrient supply (Jacobs et al., 2017). Previous field trials also had variable yields ranging from 1 to 3 t·ha^{-1} without obvious explanations on the large variations in biomass (Hammer and Keller, 2003; McGrath et al., 2006; Maxted et al., 2007; Simmons et al., 2015; Tlustoš et al., 2016; Sterckeman and Puschenreiter, 2018).

One unknown variable is the planting density required for optimal phytoextraction with *N. caerulescens*. In most field studies an arbitrary density of either 50 (McGrath et al., 2006; Maxted et al., 2007) or 100 plants·m^{-2} is used (Hammer and Keller, 2003). The interest for comparing planting densities is justified by their effect on competition between plants for light, water, and uptake of nutrients and trace metals. The latter is particularly interesting as the species is known to have a metal-foraging behaviour, *i.e.* enhanced root allocation in metal-enriched soil patches (Whiting et al., 2000; Dechamps et al., 2008). Increased soil exploration through increased root proliferation due to competition (Hodge, 2004) might enhance metal uptake of closely interacting individuals. To the best of our knowledge, this hypothesis has however never been tested. Another factor linked to planting density is the strong effect of weed competition, especially in the spring when seedlings do not cover the surrounding soil. On the other hand, for practical implementation of phytoextraction and upscaling, there is an interest of transplanting fewer seedlings for limiting costs of production and transplantation.

How nitrogen fertilization influences growth and metal accumulation of *N. caerulescens* has so far been tested in both hydroponics and pot experiments, but never in field conditions. Most studies showed a positive effect of N addition on *N. caerulescens* growth (Bennett et al., 1998; Schwartz et al., 2003; Sirguey et al., 2006; Monsant et al., 2008; Xie et al., 2009) (see Table 1). The increase in biomass can be as high as +120–140% with high nitrogen inputs (86–150 mg N kg^{-1} dry soil (DS)) (Monsant et al., 2008; Xie et al., 2009), while with 31 mg N kg^{-1} DS (considered by the authors as equivalent to 110 kg N ha^{-1}) the increase is more tenuous (+15–30%) (Sirguey et al., 2006). It is worth noting that in all experiments N treatments resulted in lower Cd and/or Zn shoot concentrations, with a stronger reduction obtained with ammonium (NH_4^+) compared to nitrate (NO_3^-) (Schwartz et al., 2003; Sirguey et al., 2006; Monsant et al., 2008; Xie et al., 2009; Monsant et al., 2010). This difference of uptake between nitrogen forms is neither linked to pH nor to organic acids in the rhizosphere (Monsant et al., 2010; White-Monsant and Tang, 2013). It is hypothesized that NO_3^- could increase cation uptake through root cell membrane polarization and enhance shoot translocation of metals compared to NH_4^+ (Xie et al., 2009; Monsant et al., 2010). This effect partly compensates for the reduction in metal uptake. Interestingly, this pattern seems to be species-specific as for Cd accumulators *Carpobrotus rossii* and *Solanum nigrum* shoot Cd accumulation is greater with NH_4^+ than with NO_3^- supply (Cheng et al., 2016). On the whole, high nitrogen input has a strong effect on *N. caerulescens* biomass and therefore increases metal uptake (Xie et al., 2009; Monsant et al., 2010), while lower doses of nitrogen have no effect on metal uptake (Sirguey et al., 2006). Fertilizing *N. caerulescens* with nitrogen has however never been directly tested in the field where possible negative effects could occur such as pathogens development or excessive weed growth. N fertilization with excessive doses can also alter water quality and ecosystem functioning. It is thus of high interest to test the effect of environmentally sensible inputs of nitrogen to a crop of *N. caerulescens*.

Table 1

Summary of experiments testing the effect of nitrogen fertilization on *N. caerulescens* growth and metal accumulation in pot or rhizobox trials with contaminated soil. The effect of fertilization on shoot biomass production and metal concentrations (μg·g^{-1}) are expressed as the percentage of increase (+) or decrease (−) obtained with N treatment compared to the control (no N addition). Cd concentrations were not measured in some studies.

	Length of growth (days)	N dose (mg·kg^{-1})	N form	Biomass effect	Concentration effect	
					Cd	Zn
Bennett et al. (1998)	140	25	NH_4NO_3	+146%	/	ns
		50	NH_4NO_3	+116%	/	ns
		100	NH_4NO_3	+196%	/	−43%
Schwartz et al. (2001, 2003)	70	80	NH_4^+	+85%	/	−34%
		80	NO_3^-	+118%	/	−26%
		200	NH_4^+	+108%	/	−50%
		200	NO_3^-	+180%	/	−28%
Sirguey et al. (2006)	150	31	NH_4NO_3	+31%	−36%	−25%
Monsant et al. (2008)	86	86	NH_4^+	+100%	/	−27%
		86	NO_3^-	+125%	/	ns
Xie et al. (2009)	100	150	NH_4^+	+72%	−73%	−80%
		150	NO_3^-	+141%	−50%	−66%

Contaminated agricultural land as well as urban and peri-urban wastelands could be adequate candidates for remediation by phytoextraction. Their frequently moderate contamination (Hough et al., 2004; Joimel et al., 2016), the need of non-destructive methods and the relatively low pressure on such areas could favour the use of ecological methods of remediation (Sterckeman and Puschenreiter, 2018). Moreover the small size, quite similar to vegetable crops such as corn salad, and short life cycle of hyperaccumulating plants are advantages for the remediation of small and enclosed urban surfaces where the use of larger perennial plants or trees is more difficult.

In order to improve phytoextraction efficiency with *Noccaea caerulescens* through increased biomass production and metal uptake, the objectives of this study were: (i) to test the effect of two planting densities (50 and 100 plants·m^{-2}) and (ii) to investigate the benefits of nitrogen fertilization in field trials.

2. Material and methods

2.1. Site description

Trials were conducted on field plots in two sites located in Brussels, Belgium. *Masui* (MAS) (50°52′31″N, 4°21′40″E), is a wasteland lying on the old bed of the river Senne, which has been diverted and filled in the middle of the 20th century. Navez (NAV) (50°52′21″N 4°22′19″E), is a community vegetable garden located along a railway. Both sites are contaminated with trace metals most probably due to the poor quality of the embankment material (MAS), and to the railway activity (NAV). Topsoils of both sites are characterised by a neutral to slightly alkaline pH (NAV: 7.5 ± 0.2, MAS: 7.8 ± 0.1, mean ± SD) and a coarse texture (sandy loam). Climatic conditions during the 25 weeks of