

## Research review

# Copper and cobalt accumulation in plants: a critical assessment of the current state of knowledge

Author for correspondence:

Bastien Lange

Tel: +33 0 6 89 83 73 17

Email: bastien.lange2@lasalle-beauvais.fr

Received: 21 July 2016

Accepted: 27 July 2016

**Bastien Lange<sup>1,2</sup>, Antony van der Ent<sup>3,4</sup>, Alan John Martin Baker<sup>3,4,5</sup>, Guillaume Echevarria<sup>4</sup>, Grégory Mahy<sup>6</sup>, François Malaisse<sup>6</sup>, Pierre Meerts<sup>2</sup>, Olivier Pourret<sup>1</sup>, Nathalie Verbruggen<sup>7\*</sup> and Michel-Pierre Faucon<sup>1\*</sup>**

<sup>1</sup>Hydrogeochemistry and Soil–Environment Interactions (HydRISE), UP.2012.10.102, Institut Polytechnique LaSalle Beauvais,

Beauvais 60026, France; <sup>2</sup>Laboratory of Plant Ecology and Biogeochemistry, Université Libre de Bruxelles, Brussels 1050, Belgium;

<sup>3</sup>Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, Brisbane, Qld 4072, Australia;

<sup>4</sup>Laboratoire Sols et Environnement, UMR 1120, Université de Lorraine – INRA, Vandoeuvre-les-Nancy 54518, France; <sup>5</sup>School of

BioSciences, The University of Melbourne, Parkville, Vic. 3010, Australia; <sup>6</sup>Department of Forest, Nature and Landscape, Biodiversity

and Landscape Unit, Gembloux Agro-Bio Tech, University of Liège, Gembloux 5030, Belgium; <sup>7</sup>Laboratory of Plant Physiology and

Molecular Genetics, Université Libre de Bruxelles, Brussels 1050, Belgium

## Summary

This review synthesizes contemporary understanding of copper–cobalt (Cu–Co) tolerance and accumulation in plants. Accumulation of foliar Cu and Co to  $> 300 \mu\text{g g}^{-1}$  is exceptionally rare globally, and known principally from the Copperbelt of Central Africa. Cobalt accumulation is also observed in a limited number of nickel (Ni) hyperaccumulator plants occurring on ultramafic soils around the world. None of the putative Cu or Co hyperaccumulator plants appears to comply with the fundamental principle of hyperaccumulation, as foliar Cu–Co accumulation is strongly dose-dependent. Abnormally high plant tissue Cu concentrations occur only when plants are exposed to high soil Cu with a low root to shoot translocation factor. Most Cu-tolerant plants are Excluders *sensu* Baker and therefore setting nominal threshold values for Cu hyperaccumulation is not informative. Abnormal accumulation of Co occurs under similar circumstances in the Copperbelt of Central Africa as well as sporadically in Ni hyperaccumulator plants on ultramafic soils; however, Co-tolerant plants behave physiologically as Indicators *sensu* Baker. Practical application of Cu–Co accumulator plants in phytomining is limited due to their dose-dependent accumulation characteristics, although for Co field trials may be warranted on highly Co-contaminated mineral wastes because of its relatively high metal value.

*New Phytologist* (2017) **213**: 537–551

doi: 10.1111/nph.14175

**Key words:** accumulation, cobalt (Co), copper (Cu), hyperaccumulation, metal tolerance, metallophyte, phytomining, toxicity.

## Introduction

Plant species that grow and reproduce on soils strongly enriched in trace elements, i.e. metalliferous soils of natural or anthropogenic origin, have proved to be powerful model systems for studying the ecophysiology and evolution of adaptation to toxicity stresses (Baker, 1987). Metallophytes are plants able to tolerate high concentrations of such trace elements (e.g. nickel, Ni; copper, Cu; or cobalt, Co) in soil that are toxic for most other species (Baker, 1981). These metallophytes can be restricted to metalliferous soils

(obligate metallophytes) or also occur on ‘normal’ soils (facultative metallophytes) (Pollard *et al.*, 2014). Among these, some have the ability to actively accumulate certain trace elements in leaves, from either low or high available concentrations in soil, without toxicity symptoms or growth inhibition and are known as hyperaccumulator plants (Jaffré *et al.*, 1976; van der Ent *et al.*, 2013).

By far the greatest number of hyperaccumulator plants are known for Ni ( $> 1000 \mu\text{g g}^{-1}$  foliar Ni) and occur on ultramafic soils (Reeves, 2003). (Hyper)accumulation of Cu and Co was first defined as  $> 1000 \mu\text{g g}^{-1}$  foliar Cu/Co (Malaisse *et al.*, 1978), but later revised downwards to  $> 300 \mu\text{g g}^{-1}$  (van der Ent *et al.*, 2013). More than 95% of the putative Cu–Co hyperaccumulator plant

\*These authors contributed equally to this work.

species are found in the southeastern Democratic Republic of Congo (D. R. Congo) (see Brooks *et al.*, 1980), where there are a large number of natural Cu–Co outcrops; the so-called ‘Copper Hills’ of the Katangan Copperbelt. However, Co accumulation has also been reported in plants from other countries growing on ultramafic soils enriched in Co, for example in some individuals of two Ni-hyperaccumulating *Rinorea* species (Violaceae) (Brooks *et al.*, 1977b), and also in the curious case of *Nyssa sylvatica* (Cornaceae) (Brooks *et al.*, 1977a; McLeod & Ciravolo, 2007). A few cases of putative Cu hyperaccumulation have also been reported on Cu-enriched ultramafic soils in Sri Lanka, Brazil and Malaysia (Rajakaruna & Bohm, 2002; van der Ent & Reeves, 2015). Summarizing, putative Cu and/or Co hyperaccumulation may occur in: Cu–Co outcrops (principally the Katangan Copperbelt), Cu–Co-enriched ultramafic soils (e.g. Goiás state, Brazil) and Co-enriched ultramafic soils (e.g. Barberton, South Africa). An overview of Cu- and/or Co-accumulator plants is provided in Fig. 1.

High inter- and intraspecific variations in foliar/shoot Cu and/or Co have been reported among Cu–Co accumulator plants (Faucon *et al.*, 2009; Lange *et al.*, 2014). Some of the early reports of Cu–Co (hyper)accumulation have been based upon analysis of herbarium materials (see Baker & Brooks, 1989; Reeves & Baker, 2000) and there is now substantial experimental data to suggest that many of these samples were contaminated with soil dust, so giving spurious high values in foliar dry matter analyses (Faucon *et al.*, 2007).

This review synthesizes recent advances and contemporary understanding of Cu–Co tolerance and (hyper)accumulation, by: (1) reviewing the current state of knowledge about Cu–Co tolerance and (hyper)accumulation by physiological, ecological and biogeochemical approaches; (2) presenting an updated global record of unusually high Cu–Co accumulation in plant species; and (3) evaluating possible applications for Cu–Co accumulating plants.

## Ecophysiology for tolerance and accumulation of Cu and Co

### Strategies of tolerance

Plants that can grow and reproduce on metalliferous soils can be classified based upon their trace element uptake response when exposed to increasing soil concentrations of the element under consideration (Antonovics *et al.*, 1971). The three main strategies of tolerance are: ‘Excluder’, ‘Bioindicator’ and ‘Hyperaccumulator’ (Baker, 1981). Tolerance to certain trace elements and their accumulation patterns have been widely studied over the past decades, especially for zinc (Zn), Ni and cadmium (Cd) (Verbruggen *et al.*, 2009). Copper- and Co-tolerant populations have been reported in the literature for a large number of species collected from nature, but the capacity to grow under elevated Cu and/or Co concentrations without toxicity symptoms or growth inhibition has rarely been demonstrated experimentally (Table 1).

Most Cu-tolerant plants respond ecophysologically as Excluders *sensu* Baker with very limited Cu translocation from the roots to

the shoots (Macnair, 1981; Schat *et al.*, 1993; Song *et al.*, 2004; Chipeng *et al.*, 2010; Lange *et al.*, 2016). *Crassula helmsii* (Crassulaceae) was found to accumulate  $> 9000 \mu\text{g g}^{-1}$  in its shoots at low Cu concentration in the nutrient solution and so is an exception (Küpper *et al.*, 2009). Cobalt accumulation can occur in *Anisopappus chinensis* (Asteraceae), as foliar Co concentration increases in relation to Co concentration in the nutrient solution without a decrease in the biomass or toxicity symptoms (Lange, 2016). None of the putative Cu or Co hyperaccumulator plants, however, appear to comply with the fundamental principle of hyperaccumulation that high shoot concentrations are maintained over a wide concentration range in the soil or growth medium (van der Ent *et al.*, 2013).

### Physiology of Cu tolerance and accumulation

Copper is an essential transition metal with two oxidation states under physiologically relevant conditions. Due to its ability to cycle between the oxidized Cu(II) and reduced Cu(I) states, it is involved in biological processes such as photosynthesis, respiration, oxygen superoxide scavenging, ethylene sensing, cell wall metabolism and lignification (Burkhead *et al.*, 2009). For the very reason that it is essential, Cu can also be highly toxic (Fernandes & Henriques, 1991). Free Cu catalyzes Fenton reactions that generate hydroxyl radicals causing damage to lipids, proteins and DNA (Cohu & Pilon, 2010). Copper also has been reported to interfere with iron homeostasis (Bernal *et al.*, 2012). An overall reduction of plant biomass, inhibition of root growth, chlorosis, bronzing and necrosis are the usual reported symptoms of a Cu excess due to increased production of reactive oxygen species and harmful interactions at the cellular level.

Copper tolerance in metallophytes is thought to arise through adaptation of components of basic metallic homeostasis. Cellular Cu concentrations are controlled by interplay between the ATPase exporters and the Ctr family of Cu permeases (Leary & Winge, 2007). Most Cu-ions in cells are either compartmentalized or bound to proteins or metabolites. Copper is delivered to enzymes by specific chaperones, whereas excess can be chelated by thiol-rich compounds such as metallothioneins which are low molecular weight cysteine-rich proteins, or phytochelatins, which are synthesized from glutathione (Yruea, 2009). Long-distance transport of Cu apparently involves nicotianamine, an ubiquitous metal chelator in higher plants, synthesized from three molecules of S-adenosyl-methionine (Zheng *et al.*, 2012).

As previously stated, most Cu-tolerant plants behave as Excluders. The genetic control of Cu tolerance in *Silene vulgaris* (Caryophyllaceae) and *Mimulus guttatus* (Phrymaceae) appears to be governed by a single major gene whereas tolerance level appears to be controlled by a number of modifiers (Macnair, 1983; Schat & Ten Bookum, 1992). Major determinants of Cu tolerance in Cu-tolerant plants are not known or genetically confirmed. In *S. vulgaris*, Cu tolerance is associated with efficient ATP-dependent Cu efflux through the root plasma membrane (van Hoof *et al.*, 2001b). In *Arabidopsis thaliana* (Brassicaceae), which is sensitive to Cu, *HMA5* (HEAVY METAL ATPase 5) was shown to co-localize with a major quantitative trait locus (QTL) explaining 52% of the





**Fig. 1** Visual appearance of selected copper (Cu)- and/or cobalt (Co)-accumulator plants. (a) *Haumaniastrum robertii* (Lamiaceae) (D. R. Congo). Photo credit: copperflora.org; (b) *Hibiscus rhodanthus* (Malvaceae) (D. R. Congo). Photo credit: copperflora.org; (c) *Anisopappus chinensis* (Asteraceae) (D. R. Congo). Photo credit: B. Lange; (d) *Rinorea bengalensis* (Violaceae) (S-E Asia). Photo credit: A. van der Ent; (e) *Crepidorhopalon tenuis* (Linderniaceae) (D. R. Congo). Photo credit: B. Lange; (f) *Berkheya coddii* (Asteraceae) (South Africa). Photo credit: A. van der Ent; (g) *Alyssum murale* (Brassicaceae) (Albania). Photo credit: A. van der Ent.

difference in Cu tolerance between two ecotypes (Kobayashi *et al.*, 2008). *HMA5* is a Cu ATPase with a role in Cu compartmentation and detoxification in roots by moving Cu from the symplasm to apoplasm (Andrés-Colás *et al.*, 2006; del Pozo *et al.*, 2010). *HMA5* activity could correspond to the ATP-dependent Cu efflux highlighted in the study of van Hoof *et al.* (2001b) with

*S. vulgaris*. Some authors have suggested a role for *HMA5* in xylem loading (Kobayashi *et al.*, 2008), which has been debated because this transport was mostly maintained in the *HMA5* loss-of-function *Arabidopsis* mutant (Andrés-Colás *et al.*, 2006). Enhanced tolerance in *S. vulgaris* also has been associated with increased transcript levels of metallothionein 2B (van Hoof *et al.*, 2001a).

**Table 1** Plant species for which tolerance to copper (Cu) and/or cobalt (Co), or for which the capacity to grow under elevated concentrations of Cu and/or Co has been demonstrated experimentally; the reported concentrations correspond to the highest experimental treatment, or to the concentration above which plant growth was inhibited

	Origin	Experimental demonstration of Cu tolerance, or of the capacity to grow under elevated concentration of Cu	Experimental demonstration of Co tolerance or of the capacity to grow under elevated concentration of Co	Growth medium	References
<b>Asteraceae</b>					
<i>Anisopappus chinensis</i> Hook. f. & Arn.	Katangan Copperbelt (D. R. Congo)	Up to 500 $\mu\text{g g}^{-1}$ (total Cu)	Up to 50 $\mu\text{g g}^{-1}$ (total Cu)	Soil	Lange <i>et al.</i> (2014)
<i>Berkheya coddii</i> Roessler	Barberton (South Africa)	–	333 $\mu\text{g g}^{-1}$	Soil	Keeling <i>et al.</i> (2003)
Caryophyllaceae					
<i>Minuartia hirsuta</i> (M.Bieb.) Hand. -Mazz.	Kilkis (Greece)	Up to 5 $\mu\text{g g}^{-1}$ [80 $\mu\text{M}$ ]	–	Nutrient solution	Ouzounidou <i>et al.</i> (1994)
<i>Silene burchelli</i> Othth ex DC. (Ecotype)	Luitia (D. R. Congo)	> 1000 $\mu\text{g g}^{-1}$ (total Cu)	Up to 800 $\mu\text{g g}^{-1}$ (total Co)	Soil	Baker <i>et al.</i> (1983)
<i>Silene burchelli</i> var. <i>angustifolia</i> Sond.	Kundelungu Plateau (D. R. Congo)	Up to 500 $\mu\text{g g}^{-1}$ (total Cu)	Up to 100 $\mu\text{g g}^{-1}$ (total Co)	Soil	Baker <i>et al.</i> (1983)
<i>Silene cobalticola</i> P. A. Du vign. & Plancke	Katangan Copperbelt (D.R. Congo)	> 1000 $\mu\text{g g}^{-1}$ (total Cu)	> 1000 $\mu\text{g g}^{-1}$ (total Co)	Soil	Baker <i>et al.</i> (1983)
<i>Silene paradoxa</i> L.	Tuscany (Italy)	3 $\mu\text{g g}^{-1}$	–	Nutrient solution	Gonnelli <i>et al.</i> (2001)
<i>Silene vulgaris</i> (Moench) Garcke	Ontario (Canada)	–	Up to 5 $\mu\text{g g}^{-1}$ [5 $\text{mg l}^{-1}$ ]	Nutrient solution	Paliouris & Hutchinson (1991)
	Marsberg – Imsbach – Harlingerode (Germany)	Up to 0.5 $\mu\text{g g}^{-1}$ [8 $\mu\text{M}$ ]	–	Nutrient solution	Schat & Ten Bookum (1992)
	Imsbach (Germany)	19–8645 $\mu\text{g g}^{-1}$ (total Cu)	–	Soil	Song <i>et al.</i> (2004)
<b>Cornaceae</b>					
<i>Nyssa sylvatica</i> var. <i>biflora</i> (Walt.) Sarg.	Taylor County (Florida, USA)	–	Up to 58.9 $\mu\text{g g}^{-1}$	Soil	Malik <i>et al.</i> (2000)
<i>Nyssa sylvatica</i> var. <i>sylvatica</i> Marsh.	Cobb County (Georgia, USA)	–	Up to 58.9 $\mu\text{g g}^{-1}$	Soil	Malik <i>et al.</i> (2000)
<b>Crassulaceae</b>					
<i>Crassula helmsii</i> (Kirk) Cockayne	Oer-Erkenschwick (Germany)	Up to 1.3 $\mu\text{g g}^{-1}$ [20 $\mu\text{M}$ ]	–	Nutrient solution	Küpper <i>et al.</i> (1996)
Lamiaceae					
<i>Aeolanthus subcaulis</i> (Baker) Hua & Briq. var. <i>linearis</i> (Burkill) Ryding	Katangan Copperbelt (D.R. Congo)	Up to 9000 $\mu\text{g g}^{-1}$ (total Cu)	Up to 670 $\mu\text{g g}^{-1}$ (total Co)	Soil	Morrison <i>et al.</i> (1979)
<i>Eisholtzia haichowensis</i> Sun.	Yangtze River (China)	0.02–32 $\mu\text{g g}^{-1}$ [0.32–500 $\mu\text{M}$ ]	–	Nutrient solution	Lou <i>et al.</i> (2004)
<i>Eisholtzia splendens</i> Nakai ex F. Maek.	Zhejiang (China)	Up to 1000 $\mu\text{g g}^{-1}$ (total Cu)	–	Soil	Jiang <i>et al.</i> (2004)
<i>Haumanistrum katangense</i> (S. Moore) Du vign. & Plancke	Zhejiang (China)	Up to 5146 $\mu\text{g g}^{-1}$ (total Cu)	–	Soil	Song <i>et al.</i> (2004)
	Katangan Copperbelt (D.R. Congo)	Up to 6.3 $\mu\text{g g}^{-1}$ [100 $\mu\text{M}$ ]	–	Nutrient solution	Chipeng <i>et al.</i> (2010) and Peng <i>et al.</i> (2012)
<i>Haumanistrum robertii</i> (Robyns) P. A. Du vign. & Plancke	Katangan Copperbelt (D.R. Congo)	Up to 1400 $\mu\text{g g}^{-1}$ (total Cu)	Up to 600 $\mu\text{g g}^{-1}$ (total Co)	Soil	Morrison <i>et al.</i> (1979)
		Up to 8500 $\mu\text{g g}^{-1}$ (total Cu)	Up to 4000 $\mu\text{g g}^{-1}$ (total Co)	Soil	Morrison <i>et al.</i> (1979)
<b>Linderniaceae</b>					
<i>Crepidodhrolon tenuis</i> (S. Moore) Eb. Fisch.	Katangan Copperbelt (D.R. Congo)	Up to 3.8 $\mu\text{g g}^{-1}$ [60 $\mu\text{M}$ ]	–	MS medium	Faucon <i>et al.</i> (2012a)
<i>Crepidodhrolon perennis</i> (P. A. Du vign.) Eb. Fisch.	Katangan Copperbelt (D.R. Congo)	Up to 3.8 $\mu\text{g g}^{-1}$ [60 $\mu\text{M}$ ]	–	MS medium	Faucon <i>et al.</i> (2012a)



Table 1 (Continued)

	Origin	Experimental demonstration of Cu tolerance, or of the capacity to grow under elevated concentration of Cu	Experimental demonstration of Co tolerance or of the capacity to grow under elevated concentration of Co	Growth medium	References
Phrymaceae					
<i>Mimulus guttatus</i> Fischer ex DC.	California (USA)	2700 $\mu\text{g g}^{-1}$ (total Cu)	–	Soil	Allen & Sheppard (1971)
		1 $\mu\text{g g}^{-1}$	–	Nutrient solution	Allen & Sheppard (1971)
Poaceae					
<i>Agrostis capillaris</i> L.	Parys Mountain, Anglesey (UK) Black Forest (Germany)	Up to 1 $\mu\text{g g}^{-1}$ [15 $\mu\text{M}$ ]	–	Nutrient solution	Jowett (1958)
<i>Agrostis gigantea</i> Roth. (one clone)	Ontario (Canada)	Up to 1271 $\mu\text{g g}^{-1}$ [20 mM]	Up to 10 000 $\mu\text{g g}^{-1}$ [170 mM]	Nutrient solution	Hogan & Rauser (1979)
<i>Agrostis stolonifera</i> L.	Plesky (Slovakia)	Up to 1 $\mu\text{g g}^{-1}$ [15 $\mu\text{M}$ ]	–	Nutrient solution	Jowett (1958)
<i>Calamagrostis epigeios</i> (L.) Roth	Legnica (Poland)	Up to 127 $\mu\text{g g}^{-1}$ [2 mM]	–	Nutrient solution	Lehmann & Rebele (2004)
<i>Cynodon dactylon</i> (L.) Pers.	Guangdong (China)	Up to 0.5 $\mu\text{g g}^{-1}$ [0.5 $\text{mg l}^{-1}$ ]	–	Nutrient solution	Shu <i>et al.</i> (2002)
<i>Deschampsia cespitosa</i> (L.) Beauv.	Coniston smelter population, Ontario (Canada)	0.3 $\mu\text{g g}^{-1}$ [0.3 $\mu\text{g cm}^{-3}$ ]	–	Nutrient solution	Cox & Hutchinson (1980)
<i>Paspalum distichum</i> L.	Guangdong (China)	Up to 1 $\mu\text{g g}^{-1}$ [1 $\text{mg l}^{-1}$ ]	–	Nutrient solution	Shu <i>et al.</i> (2002)

Furthermore, Cu tolerance was found to be inversely correlated with long-term  $\text{K}^+$  efflux in several species, sensitive or tolerant, including *M. guttatus* (Strange & Macnair, 1991; Murphy *et al.*, 1999).  $\text{K}^+$  serves as a counter-ion for citrate that is released upon exposure to Cu excess, thus reducing Cu availability in the medium (Murphy *et al.*, 1999).

No molecular mechanism of (hyper)accumulation has been described yet in any of the putative Cu hyperaccumulator plants. Similar to the hyperaccumulation of other trace elements, root sequestration is limited to allow mobility of Cu to the shoot. Due to its toxicity, Cu excess is released in the shoot and immobilized by efficient chelation and sequestration mechanisms. In the aquatic plant *C. helmsii*, Cu was found to be almost exclusively bound by oxygen ligands, likely organic acids, and not sulphur ligands (Küpper *et al.*, 2009). The concentration of organic acids in Crassulacean Acid Metabolism (CAM) plants may be so high that it is not limiting for Cu complexation. These authors suggested that main storage sites of Cu were vacuoles and cell walls. Similar investigations have not yet been undertaken on land C3 Cu accumulator plants.

### Physiology of Co tolerance and accumulation

Cobalt is a transition metal not essential for plants with seven possible oxidation states. In physiological conditions, the oxidation states of Co are mainly II and III, which makes Co a possible catalyzer of Fenton reactions. Beneficial effects of Co supply have been associated with symbiotic rhizobia that inhabit in the nodules of leguminous plants, and need vitamin  $\text{B}_{12}$  (cobalamin) for the activity of several enzymes involved in nitrogen fixation. Other reported beneficial effects include retardation of leaf senescence through inhibition of ethylene biosynthesis, and increased drought resistance in seeds (reviewed by Pilon-Smits *et al.*, 2009). Toxicity of Co excess is linked to oxidative stress, inhibition of photosynthesis and iron deficiency (Palit *et al.*, 1994; Morrissey *et al.*, 2009). Cobalt has been reported to disrupt iron homeostasis and compete with iron for access to transporters in many organisms including plants (Barras & Fontecave, 2011). Cobalt can be transported into root epidermal cells of *A. thaliana* by IRT1 (Korshunova *et al.*, 1999). Once inside cells, ferroportins IRON REGULATED1 (IREG1/FPN1) and IREG2/FPN2 play a role in Co detoxification. IREG2/FPN2 can transport  $\text{Co}^{2+}$  inside vacuoles of root epidermal and cortical cells, thereby sequestering Co in the outer most layers of roots. Mobile Co can be loaded into the xylem by FPN1 and translocated to the shoot (Morrissey *et al.*, 2009). Additionally, it is believed that *HMA3*, which is expressed mainly in roots, also transports  $\text{Co}^{2+}$  inside vacuoles in addition to other metallic ions ( $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ) (Morel *et al.*, 2009).

Mechanisms of Co tolerance and accumulation are still poorly understood. Because some Co accumulators seem also to accumulate Cu (Faucon *et al.*, 2007), some tolerance and/or accumulation mechanisms are believed to be shared. Considering the targets of Co toxicity, plants that (hyper)accumulate Co have most probably evolved adjustments of Fe homeostatic mechanisms. Such adaptation has already been observed in Zn and Cd hyperaccumulators showing modified regulation of genes involved in iron homeostasis (Krämer, 2010; Hanikenne & Nouet, 2011; Shanmugam *et al.*,

2011). Another critical aspect of Co (hyper)accumulation is expected to be an efficient Co detoxification system to avoid the accumulation of free ions that can induce oxidative stress. Cellular Co tolerance of *C. cobalticola* was associated with an increase in citric acid as well as in cysteine, without any further increase in phytochelatins (Oven *et al.*, 2002).

## Functional and evolutionary ecology of Cu and Co accumulation

### Trace element (hyper)accumulation as a functional trait

Functional traits are defined as 'morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival' (Violle *et al.*, 2007). Under that definition, trace element concentrations in leaf tissues qualify as functional traits. Hypotheses related to the ecological significance of trace element accumulation should be reformulated in the broader framework of functional plant ecology (Delhay *et al.*, 2016). Due to trade-offs between different traits, some trait combinations are more likely to occur than others. The range of possible traits has become known as the 'Leaf Economics Spectrum' (Wright *et al.*, 2004). How (hyper)accumulation relates to the Leaf Economics Spectrum is still an open question, but the concept is being extended to elements other than carbon and nitrogen (Reich, 2014). Is (hyper)accumulation more likely to evolve in association with traits favouring fast resource capture or with traits favouring nutrient conservation? Trade-offs between the capacity to accumulate trace elements and the uptake of major nutrients might strongly constrain the evolution of trace element accumulation. Recent findings confirm that trace element accumulation is correlated to the capture/use of major nutrients (Lambers *et al.*, 2015), thus supporting the so-called hypothesis of 'inadvertent uptake' (Boyd & Martens, 1992). However, this hypothesis should be confirmed contrary to the hypothesis that trace element (hyper)accumulation may serve as a defence against herbivores and pathogens (demonstrated for Ni, Zn and Cd hyperaccumulation) (Boyd, 2013; Cappa & Pilon-Smits, 2014). Functional leaf and root traits involved in nutrient acquisition and conservation should be studied to understand Cu–Co accumulation.

### Evolution of trace element accumulation

Does evolution of increased trace element accumulation capacity involve changes in the niche parameters? Circumstantial evidence suggests that trace element exclusion is more frequently observed in broad-niched metallophytes compared with narrow-niched (Faucon *et al.*, 2012b; Delhay *et al.*, 2016). This observation is thought to be the consequence of the cost of tolerance and/or (hyper)accumulation (Maestri *et al.*, 2010). Future work should test if evolutionary transitions to (hyper)accumulation are linked to changes in competitive ability and niche metrics. Another hypothesis posits that lower occurrence of pathogenic fungi and herbivores in Cu-enriched soils may conceivably relax selection pressure of defences in Cu-tolerant populations (Chipeng *et al.*, 2010). Copper-tolerant

populations would then be more susceptible to pathogenic fungi compared with nontolerant populations.

## Inter- and intraspecific variations of shoot/foliar Cu and Co concentrations

### Phylogeny of variations in Cu and Co accumulation

The absence of a suitable field test for Cu–Co in plant materials as exists for Ni (in the form of dimethylglyoxime impregnated paper) has so far limited the widespread screening for significant accumulation of these elements. Furthermore, foliar Cu–Co concentrations are not routinely measured in many ecological studies around the world. Many of the records for abnormal Cu–Co concentrations in plants, therefore, result from targeted testing of plants from the Katangan Copperbelt, and for most part by atomic absorption spectrometry (and hence not obtaining multi-element data – but see Brooks *et al.*, 1986). As a result, systematic analysis of the Cu–Co concentrations in plant species is limited. Only recently has systematic multi-element mass-screening of herbarium collections using nondestructive techniques (X-ray fluorescence spectroscopy or XRF) commenced (van der Ent, 2016). Therefore, at present it is difficult to objectively observe phylogenetic patterns of Cu and Co accumulation. Nevertheless, in Central Africa, substantial Cu–Co accumulation is mostly recorded in the Lamiaceae (Table 2). On ultramafic soils, accumulation of Co is a feature in a number of Ni hyperaccumulators, especially in the Brassicaceae, Phyllanthaceae and Violaceae.

### Genetic variability of Cu and Co accumulation

The substantial phenotypic variation of Cu–Co accumulation recorded in Cu–Co metallophytes is expressed between species, populations and individuals within a population (Faucon *et al.*, 2007). For Cu, these variations can be of genetic origin. Peng *et al.* (2012) demonstrated the foliar Cu variability for six distinct metallicolous populations of *Haumaniastrum katangense* (Lamiaceae). Six times as much Cu was obtained in nonmetallicolous compared with metallicolous plants of *Crepidiorhopalon tenuis* (Linderniaceae) (*c.* 115 µg g<sup>-1</sup>) (Faucon *et al.*, 2012a). For a given concentration in the growth medium, Cu accumulation variation could be related to variations in the degree of tolerance, with the less Cu-accumulating populations the most tolerant (Ouzounidou *et al.*, 1994; Gonnelli *et al.*, 2001; Weng *et al.*, 2005).

In the case of Co, accumulation variation has been demonstrated between three closely related *Silene* taxa (Baker *et al.*, 1983). The most tolerant taxon was the lowest accumulating for a given soil Co concentration. The intraspecific variation of Co accumulation by metallophytes from natural Co-enriched soils has not yet been tested. For the facultative metallophyte *Anisopappus chinensis*, the strong variation in the degree of Co tolerance between populations suggests intraspecific variation of accumulation (Lange, 2016). It may be hypothesized that a lower accumulation variation within metallicolous populations than within nonmetallicolous populations exists, as a result of a long-term directional selection in metallicolous populations (Dechamps *et al.*, 2007).

**Table 2** Updated list of plant species recorded with at least one specimen with a foliar concentration of copper (Cu) and/or cobalt (Co) exceeding 300 mg kg<sup>-1</sup> DW

	Foliar Cu ( $\mu\text{g g}^{-1}$ DW)	Foliar Co ( $\mu\text{g g}^{-1}$ DW)	Origin of samples	Site	Restriction to metalliferous soils	Lifecycle	References
<b>Magnoliopsida</b>							
<b>Amaranthaceae</b>							
<i>Celosia trigyna</i> L.	345–603	75–501	<i>In situ</i>	KC (D. R. Congo)	Facultative	Annual	Faucon <i>et al.</i> (2007)
<i>Pandaka carsonii</i> Baker (C.B. Clarke)	273–445	6–7	<i>In situ</i>	KC (D. R. Congo)	Facultative	Perennial	C. Lefebvre <i>et al.</i> (unpublished)
	740	na	<i>In situ</i>	KC (D. R. Congo)			Duvigneaud & Denaeys-De Smet (1963)
	1870	na	<i>In situ</i>	KC (D. R. Congo)			Delhay <i>et al.</i> (2016)
<b>Apocynaceae</b>							
<i>Carissa spinarum</i> L.	702 (leaf + stem)	1.6 (leaf + stem)	<i>In situ</i>	Ginigalpelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm (2002)
<b>Asclepiadaceae</b>							
<i>Calotropis gigantea</i> L. R. Br.	582 (leaf + stem)	0.8 (leaf + stem)	<i>In situ</i>	Ginigalpelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm (2002)
<b>Asteraceae</b>							
<i>Anisopappus chinensis</i> Hook.f. & Am.	4–2800	3–1300	<i>In situ</i>	KC (D. R. Congo)	Facultative	Perennial	Lange <i>et al.</i> (2014)
<i>Berkheya coddii</i> Roesler	na	40–2116	<i>Ex situ</i>	South Africa	Obligate	Perennial	Keeling <i>et al.</i> (2003)
<i>Vernoniastrum latifolium</i> (Steetz) H. Rob.	269–1942	82–549	<i>In situ</i>	KC (D. R. Congo)	Facultative	Annual	Faucon <i>et al.</i> (2007)
<b>Boraginaceae</b>							
<i>Onosma stenophyllum</i> Boiss.	300–657	na	<i>In situ</i>	Sarcheshmeh (Iran)	Facultative	Biennial to perennial	Ghaderian & Ravandi (2012)
<b>Brassicaceae</b>							
<i>Alyssum corsicum</i> Duby	na	1080 $\pm$ 260 (shoot)	<i>Ex situ</i>	Greece	Facultative	Perennial	Malik <i>et al.</i> (2000)
<i>Alyssum heldreichii</i> Hausskn.	na	<5 $\rightarrow$ 1000	<i>Ex situ</i>	na	Obligate	Perennial	Homer <i>et al.</i> (1991)
<i>Alyssum murale</i> Waldst. & Kit.	na	2070 $\pm$ 252 (shoot)	<i>Ex situ</i>	na	Facultative	Perennial	Tappero <i>et al.</i> (2007)
	na	1320 $\pm$ 300 (shoot)	<i>Ex situ</i>	Greece			Malik <i>et al.</i> (2000)
<i>Alyssum pintosilvae</i> T.R. Dudley	na	<5 $\rightarrow$ 1000	<i>Ex situ</i>	na	Facultative	Perennial	Homer <i>et al.</i> (1991)
<i>Alyssum tenium</i> Halácsy	na	<5 $\rightarrow$ 1000	<i>Ex situ</i>	na	Obligate	Perennial	Homer <i>et al.</i> (1991)
<i>Alyssum troodii</i> Boiss.	71	10–2325	<i>Ex situ</i>	Republic of Cyprus	Obligate	Perennial	Homer <i>et al.</i> (1991)
<b>Caryophyllaceae</b>							
<i>Silene cobalticola</i> P.A. Duvign. & Plandke	1660	na	<i>In situ</i>	KC (D. R. Congo)	Obligate	Perennial	Duvigneaud & Denaeys-De Smet (1963)
<b>Cornaceae</b>							
<i>Nyssa sylvatica</i> var. <i>biflora</i> (Walt.) Sarg.	na	0–438	<i>Ex situ</i>	Southeastern USA	Facultative	Perennial	McLeod & Ciravolo (2007)
	na	300	<i>Ex situ</i>	Florida (USA)	Facultative	Perennial	Malik <i>et al.</i> (2000)
	na	800	<i>Ex situ</i>	Florida (USA)	Facultative	Perennial	Malik <i>et al.</i> (2000)
<i>Nyssa sylvatica</i> var. <i>sylvatica</i> Marsh.							
<b>Crassulaceae</b>							
<i>Crassula helmsii</i> (Kirk) Cockayne	9200 $\pm$ 1500	na	<i>Ex situ</i>	Oer-Erkenschwick (Germany)	Facultative	Perennial	Küpper <i>et al.</i> (2009)
<b>Euphorbiaceae</b>							
<i>Acalypha cupricola</i> Robyns	390–2890	207–904	<i>In situ</i>	KC (D. R. Congo)	Obligate	Perennial	Faucon <i>et al.</i> (2007)
<i>Croton bonplandianus</i> Baill.	2163 (leaf + stem)	2 (leaf + stem)	<i>In situ</i>	Ginigalpelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm (2002)
<b>Fabaceae</b>							
<i>Senna auriculata</i> L.	885 (leaf + stem)	1 (leaf + stem)	<i>In situ</i>	Ussangoda (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm (2002)
<i>Tephrosia villosa</i> Pers.	1858 (shoot)	0.2	<i>In situ</i>	Indikolapelessa (Sri Lanka)	Facultative	Annual to perennial	Rajakaruna & Bohm (2002)
<i>Vigna dolomitica</i> R. Wilczek	222–1000	241–540	<i>In situ</i>	KC (D. R. Congo)	Obligate	Perennial	Faucon <i>et al.</i> (2007)

Table 2 (Continued)

	Foliar Cu ( $\mu\text{g g}^{-1}$ DW)	Foliar Co ( $\mu\text{g g}^{-1}$ DW)	Origin of samples	Site	Restriction to metalliferous soils	Lifecycle	References
<b>Lamiaceae</b>							
<i>Aeolanthus subcaulis</i> (Baker)	0–78	0–1900	Ex situ	KC (D. R. Congo)	Facultative	Perennial	Morrison <i>et al.</i> (1979)
Hua & Briq. var. <i>linearis</i> (Burkill)							
Ryding							
<i>Elsholtzia haichowensis</i> Sun.	NA	17–391	In situ	Yangtze River (China)	Obligate	Annual	Tang <i>et al.</i> (1999)
<i>Elsholtzia splendens</i> Nakai ex F. Maek.	< 10–> 500	na	Ex situ	Zhuji (China)	Facultative	Annual	Yang <i>et al.</i> (2002)
<i>Ocimum tenuiflorum</i> L.	2265 (leaf + stem)	11 (leaf + stem)	In situ	Yodhagannawa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm (2002)
<i>Haumaniastrum katangense</i>	187–317	224–772	In situ	KC (D. R. Congo)	Facultative	Annual	Faucon <i>et al.</i> (2007)
(S. Moore) Duvern. & Plancke							
<i>Haumaniastrum robertii</i> (Robyns)	62–6159	62–326	In situ	KC (D. R. Congo)	Obligate	Annual	Duvigneaud & Denaeys-De Smet (1963)
P. A. Duvern. & Plancke	28	> 7200	Ex situ	KC (D. R. Congo)			Morrison <i>et al.</i> (1979)
<b>Linderniaceae</b>							
<i>Crepidorhopalon tenuis</i>	0–2524	8–605	In situ	KC (D. R. Congo)	Facultative	Annual	Faucon <i>et al.</i> (2007)
(S. Moore) Eb. Fisch.							
<i>Crepidorhopalon perennis</i>	80–1384	61–1105	In situ	KC (D. R. Congo)	Obligate	Short-lived perennial	Faucon <i>et al.</i> (2007)
(P. A. Duvern.) Eb. Fisch.							
<b>Malvaceae</b>							
<i>Abutilon indicum</i> Sweet	915 (leaf + stem)	1 (leaf + stem)	In situ	Indikolapelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm (2002)
<i>Hibiscus rhodanthus</i> Gürke	102–515	42–274	In situ	KC (D. R. Congo)	Facultative	Perennial	Faucon <i>et al.</i> (2007)
<i>Triumfetta welwitschii</i> Mast.	111–704	162–1971	In situ	KC (D. R. Congo)	Facultative	Perennial	Faucon <i>et al.</i> (2007)
<b>Onagraceae</b>							
<i>Epilobium hirsutum</i> L.	1300–1581	na	In situ	Sarcheshmeh (Iran)	Facultative	Perennial	Ghaderian & Ravandi (2012)
<b>Orobanchaceae</b>							
<i>Alectra sessiliflora</i> (Vahl) O. Ktze.	45–769	21–866	In situ	KC (D. R. Congo)	Facultative	Annual	Faucon <i>et al.</i> (2007)
<i>Buchnera henriquesii</i> Engl.	142–242	404–930	In situ	KC (D. R. Congo)	Facultative	Annual	Faucon <i>et al.</i> (2007)
<b>Phymaceae</b>							
<i>Mimulus guttatus</i> Fischer ex DC.	120–780	na	Ex situ	USA	Facultative	Annual and perennial	Tilstone & Macnair (1997)
<b>Phyllanthaceae</b>							
<i>Aporosa chalarocarpa</i> Airy Shaw	na	468	In situ	Sabah (Malaysia)	Facultative	Perennial	van der Ent <i>et al.</i> (2015b)
<i>Glochidion cf. sericeum</i> (Blume)	na	442–1310	In situ	Sabah (Malaysia)	Facultative	Perennial	van der Ent <i>et al.</i> (2015b)
Zoll. & Moritz							
<b>Poaceae</b>							
<i>Agrostis stolonifera</i> L.	100–1000	na	In situ	Prescot (UK)	Facultative	Perennial	Wu <i>et al.</i> (1975)
<i>Polygonum fugax</i> Nees ex Steud.	1550–4012	na	In situ	Sarcheshmeh (Iran)	Facultative	Annual	Ghaderian & Ravandi (2012)
<b>Polygonaceae</b>							
<i>Rumex acetosa</i> Linn.	340–1102	na	In situ	Yangtze River (China)	Obligate	Perennial	Tang <i>et al.</i> (1999)
<b>Solanaceae</b>							
<i>Hyoscyamus senecionis</i> Willd.	87–331	na	In situ	Sarcheshmeh (Iran)	Facultative	Perennial	Ghaderian & Ravandi (2012)
<b>Sterculiaceae</b>							
<i>Waltheria indica</i> L.	1504 (leaf + stem)	1.3 (leaf + stem)	In situ	Ginigalpelessa (Sri Lanka)	Facultative	Short-lived perennial	Rajakaruna & Bohm (2002)
<b>Verbenaceae</b>							
<i>Clerodendrum infortunatum</i> L.	2280 (leaf + stem)	0.6 (leaf + stem)	In situ	Ginigalpelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm (2002)
<b>Violaceae</b>							
<i>Rinorea bengalensis</i> (Wall.) O. Ktze.	na	0.5–545	In situ	S. E. Asia	Facultative	Perennial	Brooks <i>et al.</i> (1977b)
<i>Rinorea javanica</i> (Bl.) O. Ktze.	na	3–670	In situ	S. E. Asia	Facultative	Perennial	Brooks <i>et al.</i> (1977b)



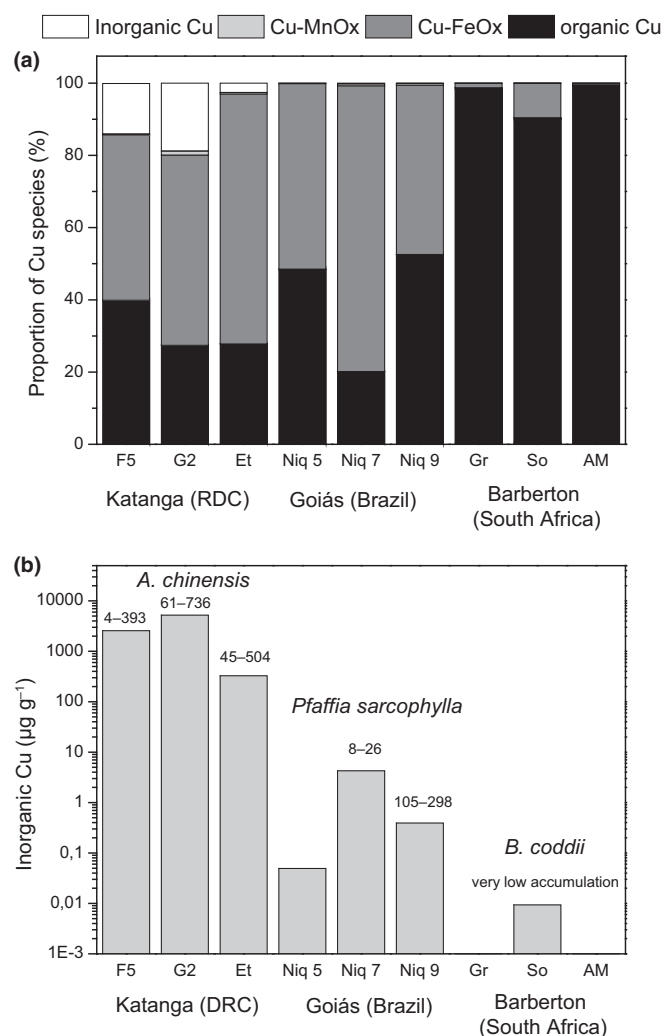
Table 2 (Continued)

	Foliar Cu ( $\mu\text{g g}^{-1}$ DW)	Foliar Co ( $\mu\text{g g}^{-1}$ DW)	Origin of samples	Site	Restriction to metalliferous soils	Lifecycle	References
Liliopsida							
Commelinaceae							
<i>Commelina communis</i> Linn.	19–587	na	<i>In situ</i>	Yangtze River (China)	Facultative	Annual	Tang <i>et al.</i> (1999)
<i>Commelina zigzag</i> P. A. DuVign. & Dewit	1210	na	<i>In situ</i>	KC (D. R. Congo)	Obligate	Perennial	Duvigneaud & Denaeuer-De Smet (1963)
<i>Cyanotis longifolia</i> Benth. var. <i>longifolia</i>	7.5–286	2.3–1697	<i>In situ</i>	KC (D. R. Congo)	Facultative	Perennial	G. Delhay <i>et al.</i> (unpublished)
Cyperaceae							
<i>Ascolepis metallorum</i>	1200	na	<i>In situ</i>	KC (D. R. Congo)	Obligate	Perennial	Duvigneaud & Denaeuer-De Smet (1963)
P. A. DuVign. & G. Léonard							

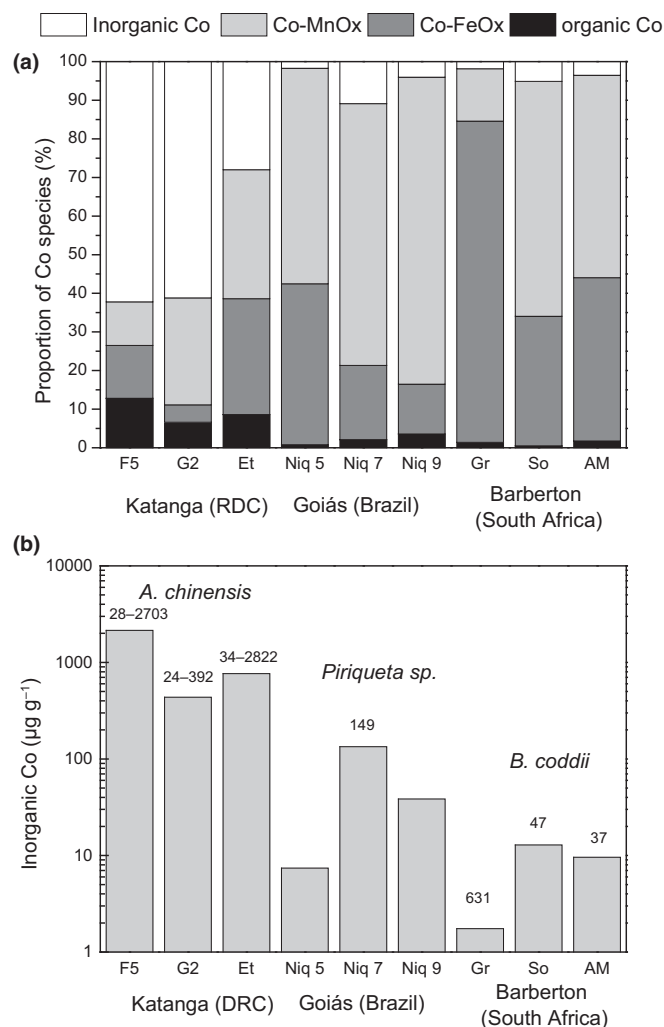
The plant species listed in this table have received a washing protocol (clearly mentioned in the publication). When available, the range of variation is given. Otherwise, values are single measurements, or mean values with SD. na, data not available. *Ex situ*, measurement on plants growing under experimental conditions. KC, Katangan Copperbelt.

## Copper and Co chemical speciation in metalliferous soils and accumulation by plants

The Cu–Co outcrops from Katanga have high total Cu–Co soil concentrations (up to  $27\,793\,\mu\text{g g}^{-1}$  and  $3451\,\mu\text{g g}^{-1}$ , respectively; Pourret *et al.*, 2016) from which a fraction is mobile ( $1145$ – $5225\,\mu\text{g g}^{-1}$  and  $39$ – $2146\,\mu\text{g g}^{-1}$ , respectively; Figs 2a, 3a). This permits high Cu–Co accumulation by *A. chinensis* (Figs 2b, 3b; up to  $736\,\mu\text{g g}^{-1}$  foliar Cu and  $2822\,\mu\text{g g}^{-1}$  foliar Co). Moreover, variations in Cu accumulation (by *C. tenuis* and *A. chinensis*) were mostly controlled by Cu adsorbed onto manganese (Mn) and iron (Fe) oxides (Lange *et al.*, 2014). Mobility is higher in soils impacted by mining. Overall, the strong affinity of Mn oxides for Co may explain the lower Co mobility in Mn-rich soils (Collins & Kinsela, 2011). Cobalt accumulation (in *C. tenuis* and *A. chinensis*) was strongly influenced by free (inorganic Co on Fig. 3) Co and by Co adsorbed onto the OM and Fe oxide fractions (Lange *et al.*, 2014).



**Fig. 2** Copper (Cu) speciation in soils (modelled after the method used by Pourret *et al.*, 2015) and accumulation in metallophytes from various origins. (a) Proportion of Cu chemical species in considered soils; (b) inorganic Cu concentrations (mobile concentrations considered as available) in soils. Accumulated concentrations in plants are added when available. F5, Fungurume 5; G2, Goma 2; Et, Etoile; Niq, Niquelândia; Gr, Groenvaly; So, Songimvelo; AM, Agnes Mine.



**Fig. 3** Cobalt (Co) speciation in soils (modelled after the method used by Pourret *et al.*, 2015) and accumulation in metallophytes from various origins. (a) Proportion of Co chemical species in considered soils; (b) inorganic Co concentrations in soils (mobile concentrations considered as available). Accumulated concentrations in plants are added when available. F5, Fungurume 5; G2, Goma 2; Et, Etoile; Niq, Niquelândia; Gr, Groenvaly; So, Songimvelo; AM, Agnes Mine.

Copper and Co concentrations in soils from Goiás (513–2015  $\mu\text{g g}^{-1}$  and 430–1230  $\mu\text{g g}^{-1}$ , respectively) only have a small mobile fraction (0.05–4.25  $\mu\text{g g}^{-1}$  and 7–134  $\mu\text{g g}^{-1}$ , respectively; Figs 2a, 3a). The Ni-hyperaccumulator *Pfaffia sarcophylla* (Amaranthaceae) has high foliar Cu concentrations (105–298  $\mu\text{g g}^{-1}$ ) on Niquelândia 7 soil but far lower concentrations (8–26  $\mu\text{g g}^{-1}$ ) on Niquelândia 5 soil (van der Ent & Reeves, 2015; Fig. 2b). *Piriqueta* sp. (Turneraceae) can accumulate up to 149  $\mu\text{g g}^{-1}$  foliar Co (van der Ent & Reeves, 2015; Fig. 3b).

Soils from Barberton have lower Cu–Co concentrations (50–150  $\mu\text{g g}^{-1}$  and 95–272  $\mu\text{g g}^{-1}$ , respectively) with a very low mobile fraction (0.01–1.86  $\mu\text{g g}^{-1}$  and 2–13  $\mu\text{g g}^{-1}$ , respectively; Figs 2a, 3a). No Cu accumulation has been found. When soils are waterlogged (i.e. as at Groenvaly; Fig. 3), Co is associated mainly with amorphous Fe oxides. Soluble Co : Ni ratio becomes then

higher (i.e. 1 : 5) than in typical ultramafic soils (1 : 20–1 : 100) and *Berkheya coddii* (Asteraceae) may thus accumulate > 600  $\mu\text{g g}^{-1}$ . Cobalt is preferred over Ni by *B. coddii* when both are supplied at isomolar ratios as for *Alyssum*-species (Brassicaceae) (Homer *et al.*, 1991). Cobalt (hyper)accumulation in ultramafic soils is triggered by the available Co : Ni ratio.

### Influence of the rhizosphere biota on trace element uptake by plants

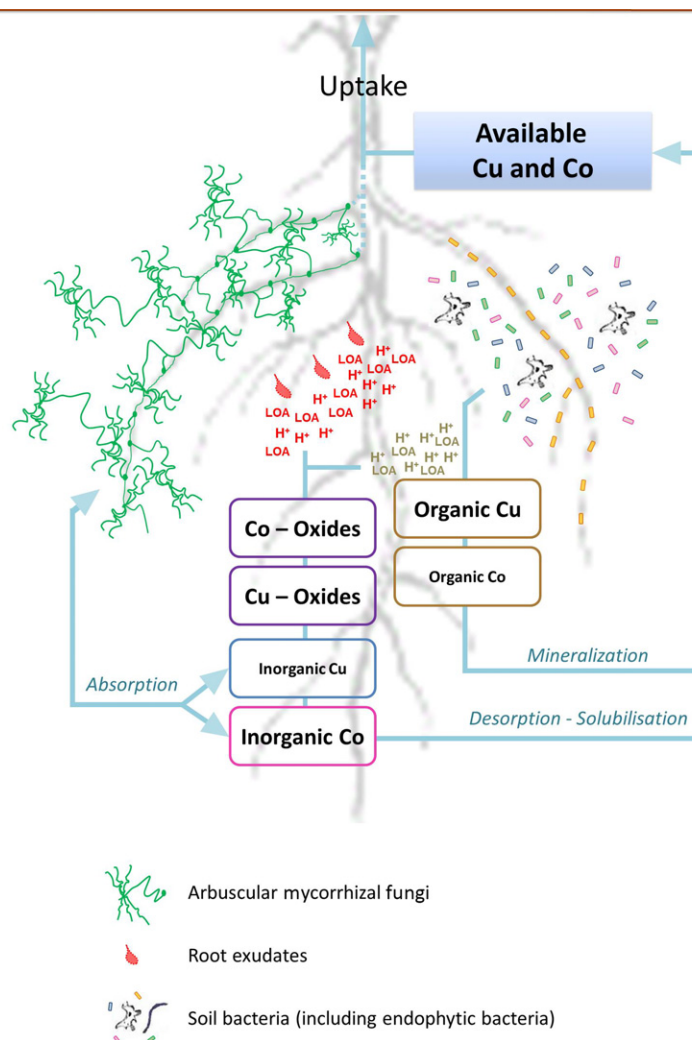
Soil–plant–microbe interactions are widely investigated in the study of soil–plant processes (Huang *et al.*, 2014; Dessaux *et al.*, 2016). The soil microbial community has the capacity to mobilize/immobilize trace elements, and thus, to affect the soil trace element speciation (reviewed by Gadd, 2004) and availability (Cuypers *et al.*, 2013). However, the rhizosphere biota influence on Cu–Co accumulation remains poorly understood. It should be emphasized that natural soil properties are difficult to replicate synthetically and directly influence accumulation patterns in plants. The most appropriate method to study strategies of tolerance, therefore, is using natural soils from the habitat of the species under investigation (van der Ent *et al.*, 2015c).

A diversity of metal-tolerant bacteria and fungi are naturally present in metalliferous soils (including plant growth-promoting bacteria, endophytic bacteria and arbuscular mycorrhizal fungi, AMF), having in some cases a beneficial effect on plant establishment, growth and trace element tolerance and accumulation (Sessitsch *et al.*, 2013; Lenoir *et al.*, 2016; Ma *et al.*, 2016). However, the majority of studies have focused on Ni- and Zn-accumulating plants. For Cu, Chen *et al.* (2005) demonstrated that Cu-tolerant bacteria strains from the rhizosphere of *Elsholtzia splendens* (Lamiaceae) had a positive effect on both the Cu mobility in soil and uptake by the plant. In the same species, the presence and diversity of AMF had a positive effect on the uptake of Cu from a Cu-contaminated soil ( $\pm 15\%$ ) (Wang *et al.*, 2005, 2007).

Endophytic bacteria associated with *H. katangense* and *C. tenuis* (Katangan Copperbelt) have been characterized (Kabagale *et al.*, 2010). However, no studies have yet investigated the relationships between Cu–Co accumulation by these plants and their rhizosphere biota diversity/activity. Hypothetical influence of rhizosphere biotic processes on Cu–Co speciation, availability and uptake is displayed in Fig. 4. Further studies should be performed, especially *in situ* characterization of the rhizosphere microbial community from a Cu–Co metallophyte in relationship to Cu–Co uptake and accumulation.

### Phytomining technology using Cu–Co (hyper) accumulator plants

Phytomining involves extracting target elements from the soil by accumulation in the plant biomass, which is then harvested and processed for recovery of the target elements from the ashed 'bio-ore' (Chaney *et al.*, 1998; van der Ent *et al.*, 2015a). The feasibility of phytomining has been demonstrated at field scale (Brooks & Robinson, 1998; Angle *et al.*, 2001; Chaney *et al.*, 2007; Bani *et al.*, 2015; van der Ent *et al.*, 2015a), but is limited principally to Ni.



**Fig. 4** Hypothetical rhizosphere processes influencing copper (Cu) and cobalt (Co) availability and uptake by metallophytes originating from naturally Cu- and Co-enriched soils. LOA, low molecular weight organic acids. The proportions of the different Cu and Co fractions in the soil are related to the font size (Lange *et al.*, 2014; Pourret *et al.*, 2015).

Copper extraction yield was tested experimentally using *A. chinensis* from southeastern D. R. Congo, and some individuals could accumulate up to 6.8 mg Cu per plant – that is, a yield of 1 kg Cu ha<sup>-1</sup> yr<sup>-1</sup> (Clavé *et al.*, 2016). The amount of Cu phytoextracted would be of the same magnitude as other species such as *E. splendens* (Yang *et al.*, 2005). Given the relatively low economic value of Cu, combined with very limited achievable yields, it is unlikely that Cu phytomining will ever be commercially viable.

Cobalt phytomining was first proposed in the early 1990s as a possibility using Ni-hyperaccumulator species on ultramafic soils (Homer *et al.*, 1991), but it was noted that Ni limits the uptake of Co in most Ni-hyperaccumulator plants (Malik *et al.*, 2000). *Alyssum* species can grow with shoot concentrations > 1000 µg g<sup>-1</sup> in Co-contaminated soils (Malik *et al.*, 2000) and *B. coddii* can have foliar Co concentrations of > 2000 µg g<sup>-1</sup> (Keeling *et al.*, 2003). In the Copperbelt flora, *Haumaniastrum robertii*, can achieve leaf concentrations of > 4000 µg g<sup>-1</sup> (Morrison *et al.*, 1981), and might be a good candidate for Co phytomining. *B. coddii* could also be a candidate in Co-enriched tailings due to its high biomass and

foliar Co (up to 5000 µg g<sup>-1</sup>; unpublished results). Cobalt phytomining may, therefore, be considered for Co-contaminated lands because of the possible attainable yields and the high metal product value (LME was USD \$25 000 per tonne in August 2016).

## Conclusions and outlook

Accumulation of Cu and/or Co in plant leaves is exceptionally rare globally and known principally from the Cu–Co outcrops of the Copperbelt of Central Africa. None of the putative Cu or Co hyperaccumulator plants appears to comply with the fundamental principle of hyperaccumulation that uptake and accumulation characteristics are not dose-dependent. In the case of plants growing on ultramafic soils, we postulate that Co accumulation coincides with Ni accumulation operating on similar physiological pathways. True Cu hyperaccumulation does not appear to exist because Cu-tolerant plants are essentially Excluders *sensu* Baker and there is no physiological mechanism of Cu hyperaccumulation known in these species. The occasional observations of high foliar Cu concentrations can be explained by stress and the breakdown of



tolerance mechanisms reducing Cu storage capacity in the roots, leading to a release of Cu in the plant shoot, which highly Cu-tolerant genotypes can survive up to a certain concentration.

The ecophysiology of Co tolerance and accumulation is still poorly understood compared with the state of knowledge on Cu regulation in plants, and investigations of the interactions with simultaneous tolerance/accumulation of Co and Cu makes a strong science case. Application of next-generation sequencing technologies to study nonmodel organisms such as Cu- and Co-tolerant and Co-accumulating plants is expected to uncover novel adaptation mechanisms (Verbruggen *et al.*, 2013). The ecological significance of Cu–Co accumulation should be investigated in relation to the ‘Leaf Economics Spectrum’, and in relation to the adaptive significance of Cu–Co accumulation. Adaptations to local soil conditions, by characterizing the soil microbial communities and comparing the response of Cu–Co-tolerant vs nontolerant populations to the pathogen pressure, should also be investigated.

The potential of Co accumulator plants in future phytomining applications is limited because their accumulation characteristics mean that only ‘ore-grade soils’ may yield sufficient accumulation in the shoots to develop this technology. Cobalt phytomining may be considered, however, for strongly Co-contaminated soils and tailings, because of the high metal value of this element.

## Acknowledgements

The Belgian Fund for Scientific Research (FRS-FNRS, Belgium) is acknowledged for financial support to B.L. as research fellow of the ‘Fonds pour la Recherche dans l’Industrie et l’Agriculture’. A.V.D.E. was the recipient of a post-doctoral scholarship from the French National Research Agency through the national ‘Investissements d’avenir’ program (ANR-10-LABX-21 – LABEX RESSOURCES21), and currently the recipient of an Australian Research Council Discovery Early Career Researcher Award (DE160100429). This work was also supported by the French National Research Agency through the ANR-14-CE04-0005 project ‘Agromine’.

## Author contributions

B.L., A.V.D.E., N.V. and M-P.F. conceived the manuscript; B.L., A.V.D.E., A.J.M.B., G.E., G.M., F.M., P.M., O.P., N.V. and M-P.F. wrote the manuscript with B.L. coordinating the writing process.

## References

- Allen WR, Sheppard PM. 1971. Copper tolerance in some Californian populations of the monkey flower *Mimulus guttatus*. *Proceedings of the Royal Society of London B: Biological Sciences* 177: 177–196.
- Andrés-Colás N, Sancenón V, Rodríguez-Navarro S, Mayo S, Thiele DJ, Ecker JR, Puig S, Peñarrubia L. 2006. The *Arabidopsis* heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots. *Plant Journal* 45: 225–236.
- Angle JS, Chaney RL, Baker AJM, Li YM, Reeves RD, Volk V, Rosenberg R, Brewer E, Burke S, Nelkin J. 2001. Developing commercial phytoextraction technologies: practical considerations. *South African Journal of Science* 97: 619–623.
- Antonovics J, Bradshaw AD, Turner RG. 1971. Heavy metal tolerance in plants. *Advances in Ecological Research* 7: 2–85.
- Baker AJM. 1981. Accumulators and excluders—strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* 3: 643–654.
- Baker AJM. 1987. Metal tolerance. *New Phytologist* 106: 93–111.
- Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. *Biorecovery* 1: 81–126.
- Baker AJM, Brooks RR, Pease AJ, Malaisse F. 1983. Studies on copper and cobalt tolerance in three closely-related taxa within the genus *Silene* L. (Caryophyllaceae) from Zaire. *Plant and Soil* 73: 377–385.
- Bani A, Echevarria G, Sulçe S, Morel JL. 2015. Improving the agronomy of *Alyssum murale* for extensive phytomining: a five-year field study. *International Journal of Phytoremediation* 17: 117–127.
- Barras F, Fontecave M. 2011. Cobalt stress in *Escherichia coli* and *Salmonella enterica*: molecular bases for toxicity and resistance. *Metallomics* 3: 1130–1134.
- Bernal M, Casero D, Singh V, Wilson GT, Grande A, Yang H, Dodani SC, Pellegrini M, Huijser P, Connolly EL *et al.* 2012. Transcriptome sequencing identifies SPL7-regulated copper acquisition genes FRO4/FRO5 and the copper dependence of iron homeostasis in *Arabidopsis*. *The Plant Cell* 24: 738–761.
- Boyd RS. 2013. Exploring tradeoffs in hyperaccumulator ecology and evolution. *New Phytologist* 199: 871–872.
- Boyd RS, Martens SN. 1992. The raison d’être for metal hyperaccumulation by plants. In: Baker AJM, Proctor J, Reeves RD, eds. *The vegetation of ultramafic (serpentine) soils*. Andover, UK: Intercept, 279–289.
- Brooks RR, Naidu SM, Malaisse F, Lee J. 1986. The elemental content of metallophytes from the copper/cobalt deposits of Central Africa. *Bulletin de la Société Royale de Botanique de Belgique* 119: 179–191.
- Brooks RR, Reeves RD, Morrison RS, Malaisse F. 1980. Hyperaccumulation of copper and cobalt: a review. *Bulletin de la Société Royale de Botanique de Belgique* 13: 166–172.
- Brooks RR, Robinson BH. 1998. The potential use of hyperaccumulators and other plants for phytomining. In: Brooks RR, ed. *Plants that hyperaccumulate heavy metals – their role in phytoremediation, microbiology, archeology, mineral exploration, and phytomining*. Cambridge, UK: CAB International, 327–356.
- Brooks RR, McCleave JA, Schofield EK. 1977a. Cobalt and nickel uptake by the Nyssaceae. *Taxon* 26: 197–201.
- Brooks RR, Wither ED, Zepernick B. 1977b. Cobalt and nickel in *Rinorea* species. *Plant and Soil* 47: 707–712.
- Burkhead JL, Gogolin Reynolds KA, Abdel-Ghany SE, Cohu CM, Pilon M. 2009. Copper homeostasis. *New Phytologist* 182: 799–816.
- Cappa JJ, Pilon-Smits EA. 2014. Evolutionary aspects of elemental hyperaccumulation. *Planta* 239: 267–275.
- Chaney RL, Angle JS, Baker AJ, Li YM. 1998. Method for phytomining of nickel, cobalt and other metals from soil. *United States Patent* 5: 711–784.
- Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL. 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. *Journal of Environmental Quality* 36: 1429–1433.
- Chen YX, Wang YP, Lin Q, Luo YM. 2005. Effect of copper-tolerant rhizosphere bacteria on mobility of copper in soil and copper accumulation by *Elsholtzia splendens*. *Environment International* 31: 861–866.
- Chipeng KF, Hermans C, Colinet G, Faucon MP, Ngongo Luhembwe M, Meerts P, Verbruggen N. 2010. Copper tolerance in the cuprophyte *Haumaniastrum katangense* (S. Moore) P.A. Duvign. and Plancke. *Plant and Soil* 328: 235–244.
- Clavé G, Garel C, Poullain C, Renard BL, Olszewski TK, Lange B, Shutcha M, Faucon M-P, Grison C. 2016. Ullmann reaction through ecocatalysis: insights from bioresource and synthetic potential. *RSC Advances* 6: 59 550–59 564.
- Cohu CM, Pilon M. 2010. Cell biology of copper. In: Hell R, Mendel RR, eds. *Cell biology of metals and nutrients. Plant cell monographs, vol. 17*. Berlin, Germany: Springer, 55–74.

- Collins RN, Kinsela AS. 2011. Pedogenic factors and measurements of the plant uptake of cobalt. *Plant and Soil* 339: 499–512.
- Cox RM, Hutchinson TC. 1980. Multiple metal tolerances in the grass *Deschampsia cespitosa* (L.) Beauv. from the Sudbury smelting area. *New Phytologist* 84: 631–647.
- Cuyper A, Remans T, Weyens N, Colpaert J, Vassilev A, Vangronsveld J. 2013. Soil–plant relationships of heavy metals and metalloids. In: Alloway BJ, ed. *Heavy metals in soils*. Dordrecht, the Netherlands: Springer, 161–193.
- Dechamps C, Noret N, Mozek R, Escarré J, Lefebvre C, Gruber W, Meerts P. 2007. Cost of adaptation to a metalliferous environment for *Thlaspi caerulescens*: a field reciprocal transplantation approach. *New Phytologist* 177: 167–177.
- Delhaye G, Violle C, Séleck M, Ilunga wa Ilunga E, Daubie I, Mahy G, Meerts P. 2016. Community variation in plant traits along copper and cobalt gradients. *Journal of Vegetation Science* 27: 854–864.
- Dessaux Y, Grandclément C, Faure D. 2016. Engineering the rhizosphere. Special issue: unravelling the secrets of the rhizosphere. *Trends in Plant Science* 21: 266–278.
- Duvigneaud P, Denaeyer-De Smet S. 1963. Cuivre et végétation au Katanga. *Bulletin de la Société Royale de Botanique de Belgique* 96: 92–231.
- Faucon MP, Chipeng F, Verbruggen N, Mahy G, Colinet G, Shutcha M, Pourret O, Meerts P. 2012a. Copper tolerance and accumulation in two cuprophytes of South Central Africa: *Crepidodhapon perennis* and *C. tenuis* (Linderniaceae). *Environmental and Experimental Botany* 84: 11–16.
- Faucon MP, Colinet G, Mahy G, Ngongo Luhembwe M, Verbruggen N, Meerts P. 2009. Soil influence on Cu and Co uptake and plant size in the cuprophytes *Crepidodhapon perennis* and *C. tenuis* (Scrophulariaceae) in SC Africa. *Plant and Soil* 317: 201–212.
- Faucon MP, Shutcha MN, Meerts P. 2007. Revisiting copper and cobalt concentrations in supposed hyperaccumulators from SC Africa: influence of washing and metal concentrations in soil. *Plant and Soil* 301: 29–36.
- Faucon MP, Tshilong BM, Van Rossum F, Meerts P, Decocq G, Mahy G. 2012b. Ecology and hybridization potential of two sympatric metallophytes, the narrow endemic *Crepidodhapon perennis* (Linderniaceae) and its more widespread congener *C. tenuis*. *Biotropica* 44: 454–462.
- Fernandes JC, Henriques FS. 1991. Biochemical, physiological, and structural effects of excess copper in plants. *The Botanical Review* 57: 246–273.
- Gadd GM. 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma* 122: 109–119.
- Ghaderian SM, Ravandi AG. 2012. Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. *Journal of Geochemical Exploration* 123: 25–32.
- Gonnelli C, Galardi F, Gabrielli R. 2001. Nickel and copper tolerance and toxicity in three Tuscan populations of *Silene paradoxa*. *Physiologia Plantarum* 113: 507–514.
- Hanikenne M, Nouet C. 2011. Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. *Current Opinion in Plant Biology* 14: 252–259.
- Hogan GD, Rauser WE. 1979. Tolerance and toxicity of cobalt, copper, nickel and zinc in clones of *Agrostis gigantea*. *New Phytologist* 83: 665–670.
- Homer FA, Morrison RS, Brooks RR, Clemens J, Reeves RD. 1991. Comparative studies of nickel, cobalt, and copper uptake by some nickel hyperaccumulators of the genus *Alyssum*. *Plant and Soil* 138: 195–205.
- van Hoof NA, Hassinen VH, Hakvoort HW, Ballintijn KF, Schat H, Verkleij JA, Ernst WH, Karenlampi SO, Tervahauta AI. 2001a. Enhanced copper tolerance in *Silene vulgaris* (Moench) Garcke populations from copper mines is associated with increased transcript levels of a 2b-type metallothionein gene. *Plant Physiology* 126: 1519–1526.
- van Hoof NA, Koevoets PLM, Hakvoort HWJ, Ten Bookum WM, Schat H, Verkleij JA, Ernst WH. 2001b. Enhanced ATP-dependent copper efflux across the root cell plasma membrane in copper-tolerant *Silene vulgaris*. *Physiologia Plantarum* 113: 225–232.
- Huang XF, Chaparro JM, Reardon KF, Zhang R, Shen Q, Vivanco JM. 2014. Rhizosphere interactions: root exudates, microbes, and microbial communities. *Botany-Botanique* 92: 267–275.
- Jaffré T, Brooks RR, Lee J, Reeves RD. 1976. *Sebertia acuminata*: a hyperaccumulator of nickel from New Caledonia. *Science* 193: 579–580.
- Jiang LY, Yang XE, He ZL. 2004. Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. *Chemosphere* 55: 1179–1187.
- Jowett D. 1958. Populations of *Agrostis* spp. tolerant of heavy metals. *Nature* 182: 816–817.
- Kabagale AC, Cornu B, van Vliet F, Meyer CL, Mergeay M, Lumbu Simbi JB, Droogmans L, Vander Wauven C, Verbruggen N. 2010. Diversity of endophytic bacteria from the cuprophytes *Haumaniastrum katangense* and *Crepidodhapon tenuis*. *Plant and Soil* 334: 461–474.
- Keeling SM, Stewart RB, Anderson CWN, Robinson BH. 2003. Nickel and cobalt phytoextraction by the hyperaccumulator *Berkheya coddii*: implications for polymetallic phytomining and phytoremediation. *International Journal of Phytoremediation* 5: 235–244.
- Kobayashi Y, Kuroda K, Kimura K, Southron-Francis JL, Furuzawa A, Kimura K, Iuchi S, Kobayashi M, Taylor GJ, Koyama H. 2008. Amino acid polymorphisms in strictly conserved domains of a P-type ATPase HMA5 are involved in the mechanism of copper tolerance variation in *Arabidopsis*. *Plant Physiology* 148: 969–980.
- Korshunova YO, Eide D, Clark WG, Guerinot ML, Pakrasi HB. 1999. The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. *Plant Molecular Biology* 40: 37–44.
- Krämer U. 2010. Metal hyperaccumulation in plants. *Annual Review of Plant Biology* 61: 517–534.
- Küpper H, Gotz B, Mijovilovich A, Küpper FC, Meyer-Klaucke W. 2009. Complexation and toxicity of copper in higher plants. I. Characterization of copper accumulation, speciation, and toxicity in *Crassula helmsii* as a new copper accumulator. *Plant Physiology* 151: 702–714.
- Küpper H, Küpper FC, Spiller M. 1996. Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. *Journal of Experimental Botany* 47: 259–266.
- Lambers H, Hayes PE, Laliberté E, Oliveira RS, Turner BL. 2015. Leaf manganese accumulation and phosphorus-acquisition efficiency. *Trends in Plant Science* 20: 83–90.
- Lange B. 2016. *Tolérance et accumulation du cuivre et du cobalt chez les métallobytes facultatives d'Afrique tropicale*. PhD thesis, Université Libre de Bruxelles, Brussels, Belgium & Université Picardie Jules Verne, Amiens, France.
- Lange B, Faucon MP, Meerts P, Shutcha M, Mahy G, Pourret O. 2014. Prediction of the edaphic factors influence upon the copper and cobalt accumulation in two metallophytes using copper and cobalt speciation in soils. *Plant and Soil* 379: 275–287.
- Lange B, Pourret O, Meerts P, Jitaru P, Cancès B, Grison C, Faucon MP. 2016. Copper and cobalt mobility in soil and accumulation in a metallophyte as influenced by experimental manipulation of soil chemical factors. *Chemosphere* 146: 75–84.
- Leary SC, Winge DR. 2007. The Janus face of copper: its expanding roles in biology and the pathophysiology of disease. *EMBO Reports* 8: 224–227.
- Lehmann C, Rebele F. 2004. Evaluation of heavy metal tolerance in *Calamagrostis epigejos* and *Elymus repens* revealed copper tolerance in a copper smelter population of *C. epigejos*. *Environmental and Experimental Botany* 51: 199–213.
- Lenoir I, Fontaine J, Sahraoui ALH. 2016. Arbuscular mycorrhizal fungal responses to abiotic stresses: a review. *Phytochemistry* 123: 4–15.
- Lou LQ, Shen ZG, Li XD. 2004. The copper tolerance mechanism of *Elsholtzia haichowensis*, a plant from copper-enriched soils. *Environmental and Experimental Botany* 51: 111–120.
- Ma Y, Rajkumar M, Zhang C, Freitas H. 2016. Beneficial role of bacterial endophytes in heavy metal phytoremediation. *Journal of Environmental Management* 174: 14–25.
- Macnair MR. 1981. The uptake of copper by plants of *Mimulus guttatus* differing in genotype primarily at a single major copper tolerance locus. *New Phytologist* 88: 723–730.
- Macnair MR. 1983. The genetic control of copper tolerance in the yellow monkey flower *Mimulus guttatus*. *Heredity* 50: 283–293.
- Maestri E, Marmiroli M, Visioli G, Marmiroli N. 2010. Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. *Environmental and Experimental Botany* 68: 1–13.

- Malaisse F, Grégoire J, Brooks RR, Morrison RS, Reeves RD. 1978. *Aelanthus biformifolius*: a hyperaccumulator of copper from Zaïre. *Science* 199: 887–888.
- Malik M, Chaney RL, Brewer EP, Li YM, Angle JS. 2000. Phytoextraction of soil cobalt using hyperaccumulator plants. *International Journal of Phytoremediation* 2: 319–329.
- McLeod KW, Ciravolo TG. 2007. Cobalt uptake by *Nyssa aquatica*, *N. sylvatica* var. *biflora*, and *Taxodium distichum* seedlings. *Wetlands* 27: 40–43.
- Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasour A, Richaud P. 2009. AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. *Plant Physiology* 149: 894–904.
- Morrison RS, Brooks RR, Reeves RD, Malaisse F. 1979. Copper and cobalt uptake by metallophytes from Zaïre. *Plant and Soil* 53: 535–539.
- Morrison RS, Brooks RR, Reeves RD, Malaisse F, Horowitz P, Aronson M, Merriam GR. 1981. The diverse chemical forms of heavy metals in tissue extracts of some metallophytes from Shaba province, Zaïre. *Phytochemistry* 20: 455–458.
- Morrissey J, Baxter IR, Lee J, Li L, Lahner B, Grotz N, Kaplan J, Salt DE, Guerinet ML. 2009. The ferroportin metal efflux proteins function in iron and cobalt homeostasis in *Arabidopsis*. *Plant Cell* 21: 3326–3338.
- Murphy AS, Eisinger WR, Shaff JE, Kochian LV, Taiz L. 1999. Early copper-induced leakage of K<sup>+</sup> from *Arabidopsis* seedlings is mediated by ion channels and coupled to citrate efflux. *Plant Physiology* 121: 1375–1382.
- Ouzounidou G, Symeonidis L, Babalonas D, Karataglis S. 1994. Comparative responses of a copper-tolerant and a copper-sensitive population of *Minuartia hirsuta* to copper toxicity. *Journal of Plant Physiology* 144: 109–115.
- Oven M, Grill E, Golan-Goldhirsh A, Kutchan TM, Zenk MH. 2002. Increase of free cysteine and citric acid in plant cells exposed to cobalt ions. *Phytochemistry* 60: 467–474.
- Paliouris G, Hutchinson TC. 1991. Arsenic, cobalt and nickel tolerances in two populations of *Silene vulgaris* (Moench) Garcke from Ontario, Canada. *New Phytologist* 117: 449–459.
- Palit S, Sharma A, Talukder G. 1994. Effects of cobalt on plants. *The Botanical Review* 60: 149–181.
- Peng H, Wang-Müller Q, Witt T, Malaisse F, Küpper H. 2012. Differences in copper accumulation and copper stress between eight populations of *Haumanistrum katangense*. *Environmental and Experimental Botany* 79: 58–65.
- Pilon-Smits EA, Quinn CF, Tapken W, Malagoli M, Schiavon M. 2009. Physiological functions of beneficial elements. *Current Opinion in Plant Biology* 12: 267–274.
- Pollard AJ, Reeves RD, Baker AJM. 2014. Facultative hyperaccumulation of heavy metals and metalloids. *Plant Science* 217: 8–17.
- Pourret O, Lange B, Bonhoure J, Colinet G, Decrée S, Mahy G, Séleck M, Shutcha M, Faucon M-P. 2016. Assessment of soil metal distribution and environmental impact of mining in Katanga (Democratic Republic of Congo). *Applied Geochemistry* 64: 43–55.
- Pourret O, Lange B, Houben D, Colinet G, Shutcha M, Faucon MP. 2015. Modeling of cobalt and copper speciation in metalliferous soils from Katanga (Democratic Republic of Congo). *Journal of Geochemical Exploration* 149: 87–96.
- del Pozo T, Cambiazo V, González M. 2010. Gene expression profiling analysis of copper homeostasis in *Arabidopsis thaliana*. *Biochemical and Biophysical Research Communication* 393: 248–252.
- Rajakaruna N, Bohm BA. 2002. Serpentine and its vegetation: a preliminary study from Sri Lanka. *Journal of Applied Botany* 76: 20–28.
- Reeves RD. 2003. Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant and Soil* 249: 57–65.
- Reeves RD, Baker AJM. 2000. Metal hyperaccumulating plants. In: Raskin I, Ensley BD, eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. New York, NY, USA: Wiley, 193–229.
- Schat H, Kuiper E, Ten Bookum WM, Vooijs R. 1993. A general model for the genetic control of copper tolerance in *Silene vulgaris*: evidence from crosses between plants from different tolerant populations. *Heredity* 70: 142–147.
- Schat H, Ten Bookum WM. 1992. Genetic control of copper in *Silene vulgaris*. *Heredity* 68: 219–229.
- Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, Puschenreiter M. 2013. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology and Biogeochemistry* 60: 182–194.
- Shanmugam V, Lo JC, Wu CL, Wang SL, Lai CC, Connolly EL, Huang JL, Yeh KC. 2011. Differential expression and regulation of iron-regulated metal transporters in *Arabidopsis halleri* and *Arabidopsis thaliana* – the role in zinc tolerance. *New Phytologist* 190: 125–137.
- Shu WS, Ye ZH, Lan CY, Zhang ZQ, Wong MH. 2002. Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*. *Environmental Pollution* 120: 445–453.
- Song J, Zhao FJ, Luo YM, McGrath SP, Zhang H. 2004. Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils. *Environmental Pollution* 128: 307–315.
- Strange J, Macnair MR. 1991. Evidence for a role for the cell membrane in copper tolerance of *Mimulus guttatus* Fischer ex DC. *New Phytologist* 119: 383–388.
- Tang S, Wilke B, Huang C. 1999. The uptake of copper by plants dominantly growing on copper mining spoils along the Yangtze River, the People's Republic of China. *Plant and Soil* 209: 225–232.
- Tappero R, Peletier E, Gräfe M, Heidel K, Ginder-Vogel M, Livi KJT, Rivers ML, Marcus MA, Chaney RL, Sparks DL. 2007. Hyperaccumulator *Alyssum murale* relies on a different metal storage mechanism for cobalt than for nickel. *New Phytologist* 175: 641–654.
- Tilstone GH, Macnair MR. 1997. The consequence of selection for copper tolerance on the uptake and accumulation of copper in *Mimulus guttatus*. *Annals of Botany* 80: 747–751.
- van der Ent A. 2016. Analysis of the content of copper, cobalt and other elements in plant leaves. In: Malaisse F, Schaijes M, D'Outreligne C, eds. *Copper–cobalt flora of Upper Katanga and Copperbelt – field guide*. Gembloux, Belgium: Presses agronomiques de Gembloux, 27–37.
- van der Ent A, Baker AJM, Reeves RD, Chaney RL, Anderson C, Meech J, Erskine PD, Simonnot MO, Vaughan J, Morel JL *et al.* 2015a. 'Agromining': farming for metals in the future? *Environmental Science and Technology* 49: 4773–4780.
- van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H. 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant and Soil* 362: 319–333.
- van der Ent A, Erskine PD, Sumail S. 2015b. Ecology of nickel hyperaccumulator plants from ultramafic soils in Sabah (Malaysia). *Chemoecology* 25: 243–259.
- van der Ent A, Reeves RD. 2015. Foliar metal accumulation in plants from copper-rich ultramafic outcrops: case studies from Malaysia and Brazil. *Plant and Soil* 389: 401–418.
- van der Ent A, Reeves RD, Baker AJM, Pollard J, Schat H. 2015c. A Commentary on "Toward a more physiologically and evolutionarily relevant definition of metal hyperaccumulation in plants". *Frontiers in Plant Science* 6: 554.
- Verbruggen N, Hanikenne M, Clemens S. 2013. A more complete picture of metal hyperaccumulation through next-generation sequencing technologies. *Frontiers in Plant Science* 4: 388.
- Verbruggen N, Hermans C, Schat H. 2009. Molecular mechanisms of metal hyperaccumulation in plants. *New Phytologist* 181: 759–776.
- Vielle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. 2007. Let the concept of trait be functional! *Oikos* 116: 882–892.
- Wang F, Lin X, Yin R. 2005. Heavy metal uptake by arbuscular mycorrhizas of *Elsholtzia splendens* and the potential for phytoremediation of contaminated soil. *Plant and Soil* 269: 225–232.
- Wang F, Lin X, Yin R. 2007. Role of microbial inoculation and chitosan in phytoextraction of Cu, Zn, Pb and Cd by *Elsholtzia splendens* – a field case. *Environmental Pollution* 147: 248–255.
- Weng G, Wu L, Wang Z, Luo Y, Christie P. 2005. Copper uptake by four *Elsholtzia* ecotypes supplied with varying levels of copper in solution culture. *Environment International* 31: 880–884.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M *et al.* 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.
- Wu L, Bradshaw AD, Thurman DA. 1975. The potential for evolution of heavy metal tolerance in plants. III. The rapid evolution of copper tolerance in *Agrostis stolonifera*. *Heredity* 34: 165–187.



Yang MJ, Yang XE, Römhild V. 2002. Growth and nutrient composition of *Elsholtzia splendens* Nakai under copper toxicity. *Journal of Plant Nutrition* 25: 1359–1375.

Yang XE, Peng HY, Jiang LY, He ZL. 2005. Phytoextraction of copper from contaminated soil by *Elsholtzia splendens* as affected by EDTA, citric acid, and compost. *International Journal of Phytoremediation* 7: 69–83.

Yruea I. 2009. Copper in plants: acquisition, transport and interactions. *Functional Plant Biology* 36: 409–430.

Zheng L, Yamaji N, Yokosho K, Ma JF. 2012. YSL16 is a phloem-localized transporter of the copper-nicotianamine complex that is responsible for copper distribution in rice. *The Plant Cell* 24: 3767–3782.



## About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <28 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**