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Copper Uptake Studies on *Erica andevalensis*, a Metal-Tolerant Plant from Southwestern Spain

Alfredo Asensi,[a] Frances Bennett,[b] Robert Brooks,[b,1]
Brett Robinson,[c] and Robert Stewart[b]

[a]*Departamento de Biologia Vegetal, Universidad de Málaga, Spain*
[b]*Soil Science, Institute of Natural Resources, Massey University, Palmerston North, New Zealand*
[c]*Horticultural and Food Research Institute of New Zealand Ltd, Palmerston North, New Zealand*

ABSTRACT

Pot trials and chemical analyses were carried out on wild specimens of *Erica andevalensis* Cabezudo et Rivera (Ericaceae) and their associated soils. Natural soils hosting *E. andevalensis* all had elevated copper concentrations. Pot trials showed that the *Erica* would grow in sulphide-ore substrates containing up to 12,500 µg g^{-1} (1.25%) copper, with or without amendment with EDTA. The addition of EDTA to these substrates increased the solubility of the copper to levels that killed other metal-tolerant species (*Biscutella laevigata* and *Brassica juncea*), but not *E. andevalensis*. The copper concentrations in wild plants were only 1 to 24 µg g^{-1} (dry weight) despite the high levels of copper (up to 3,676 µg g^{-1}) in the supporting soils. In the pot trials, the plant was able partially to exclude total copper up to 1,000 µg g^{-1} in

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[1] Corresponding author.

the soil. Thereafter, there was an exponential increase of copper in plant until the limit of tolerance was reached. Conclusions of the study were: (a) tolerance to copper by the *Erica* is much higher compared with other metal-tolerant plants tested, (b) the plant does not have hyperaccumulator status (>1,000 μg g^{-1} dry weight) for copper, (c) copper uptake by the plant is governed by the extractable and not total copper content of the soil, hence limiting its use in biogeochemical prospecting, (d) uptake of copper by the plant follows the typical pattern of a metal-excluding plant, (e) *Erica andevalensis* is a good geobotanical indicator of copper because of its faithful association with sulphide mineralization in the Huelva area of Spain.

INTRODUCTION

The ecology of *Erica andevalensis* Cabezudo and Rivera (Ericacaeae) has been described by Aparicio (1995). This plant was first described by Cabezudo and Rivera (1980). It was previously mistaken for *E. tetralix* by Wilkomm (1893), but can be distinguished from it by the glabrous ovary of *E. andevalensis*. There is a non-overlapping geographic range between the two species.

Erica andevalensis (Figure 1) is an edaphic endemic of the Andévalo, a geographically defined section of Huelva Province in southwestern Spain with an area of about 3,500 km^2 of which this species is found sporadically in about 1,500 km^2 (Figure 2). The region is known for its ancient mining activities where pyrite was mined for copper, gold, and silver as far back as Roman times.

The severe environmental impact of mining activities in the Andévalo is evidenced by soil acidification (pH close to 3 in some places) and high concentrations of heavy metals such as copper, zinc, and iron (Soldevilla et al., 1992).

Erica andevalensis is restricted to mine tailings and the vicinity of mines as well as along the banks of rivers where the acidic (H_2SO_4) water is a dark red due to metal contamination (Lopez Archilla et al., 1994). These damp mineralized sites are said to be the natural habitat of this species (Nelson et al., 1985).

At present, little is known about the ecology and biogeochemistry of *E. andevalensis*, although Aparicio (1995) studied the germination of its seeds, Nelson et al. (1985) studied its ecology, and Arroyo and Herrera (1988) reported on its pollination. The *Erica andevalensis* community (*Junco rugosi - Ericetum andevalensis*) is a somewhat hygrophilous, fairly thick heather scrub developing on silt-rich soils. It can also occupy secondary, more xeric stations on wasteland in the *Ericetum australi - andevalensis* plant community.

Nelson et al. (1985) proposed that this species cannot be indigenous since the substrate is directly derived from mining activities of past centuries. The *Erica* is remarkable in being able to colonize the highly toxic soils of the spoil heaps, but does not grow in the newer mine tips because it may take several hundred years for the exposed pyrites to decompose and generate an acidified soil that is toxic enough to exclude other competing species. Nelson et al. (1985) analyzed the

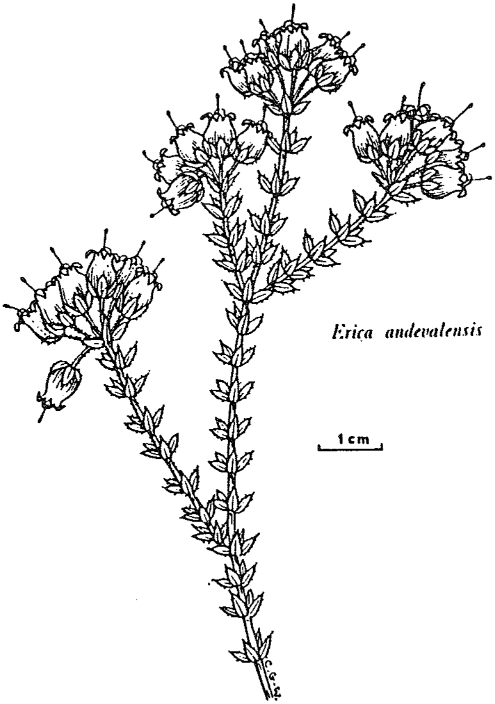

FIGURE 1. *Erica andevalensis*. Source: Nelson et al. (1985).

substrate of this plant at three localities and reported 4 to 67 µg g^{-1} copper in mine tailings, 11 to 60 µg g^{-1} in the riverine soils, and an astonishing 1,476 µg g^{-1} in evaporates of a lake in the Odiel Valley around which the plant was growing.

A parallel can be drawn with the related *Calluna vulgaris* L. Hull. that grows over highly contaminated copper waste at the abandoned Parys Mountain copper mine in Anglesey, England, and is the only plant capable of colonizing this toxic environment. *Erica andevalensis* fills this niche in the Huelva area because although *Calluna* and other *Erica* species are found here, none grows with *E. andevalensis* on the most toxic habitats.

The edaphic-endemic nature of this plant as well as its scarcity have led to the Andalusian Regional Government to include this plant in the register of endangered species in Spain.

The degree to which *E. andevalensis* accumulates copper is unknown, and the species could be classified as a hyperaccumulator, here defined as containing >1,000 µg g^{-1} copper in its dry tissue. If this latter character would eventuate as being true, this region of southwest Spain would rank as one of only three

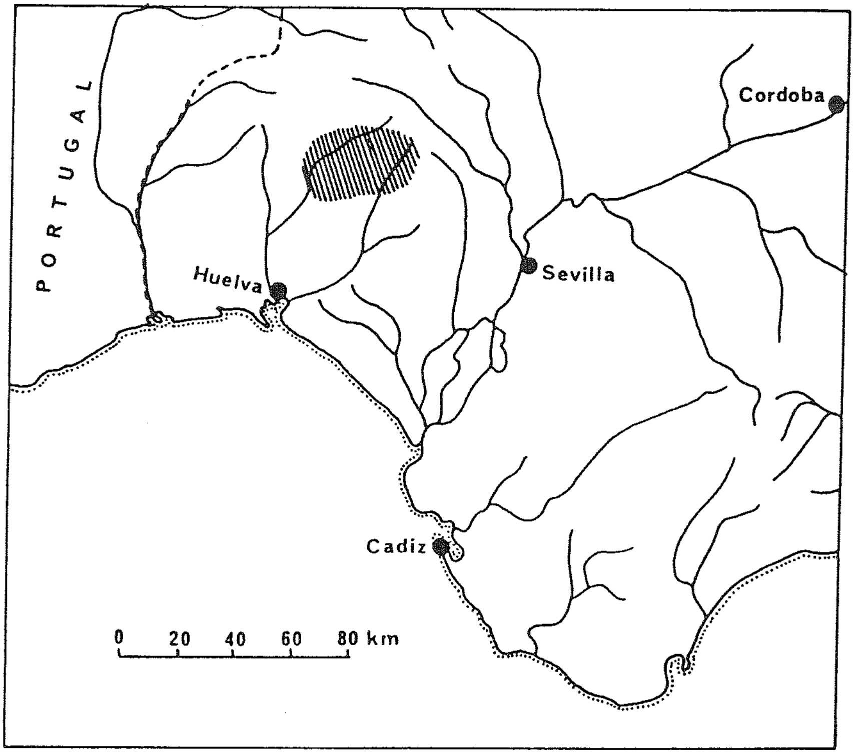

FIGURE 2. Map of the Huelva region of southwestern Spain showing the distribution (hatched area) of *Erica andevalensis*. Source: Nelson et al. (1985).

worldwide that contains plants of hyperaccumulator status for copper. The other two are Democratic Republic of Congo (formerly Zaïre) described by Brooks et al. (1992), and Hubei Province, China (Se and Sjuj, 1953).

The aims of our research were as follows: 1) to establish the copper-tolerance of *Erica andevalensis* and compare this species with non-tolerant plants; 2) to establish whether this *Erica* has hyperaccumulator status; and 3) to determine the relationship between the copper uptake by the plant and the concentration of total as well as soluble copper in the soil. A relationship could indicate the usefulness of the plant for biogeochemical prospecting.

MATERIALS AND METHODS

Seed and dried plants of *Erica andevalensis* together with their associated soils were collected from the Andévalo in southwestern Spain by the senior author and were germinated in a 1:1 mixture of crushed bark and copper ore from the Champion Mine, Nelson, New Zealand. This ore contained 2.5% copper. Commercial potting mixture was also used and was composed of a 1:1 peat/pumice mixture with added slow-release fertilizers.

Aparicio (1995) has shown that seed of this species requires a chilling period before sowing. The seeds were therefore chilled at 5°C for 90 days before sowing in the copper ore. Germination was quite satisfactory, and when the plants were about 3 cm tall, they were transferred to a series of pots (125 mL capacity) containing the mixtures described below:

(a) Commercial potting mixture: ore, in the weight ratios 1:1, 4:1, 8:1, 16:1, and 32:1. The non-mineralized control was the potting mix without addition of ore. The nominal copper content (dry weight) of the mixtures was: 12,500, 6,250, 3,125, 1,562, and 781 μg g^{-1} with 10 μg g^{-1} for the control.

(b) Commercial potting mixtures to which were added amounts of copper (as the sulphate) to provide two sets of pots containing nominally 0 to 3,250 μg g^{-1} (dry weight) as shown later in Table 2. After plants had become well established in these plots, one set of six in duplicate was treated with EDTA at 2 g kg^{-1} of substrate, and the other set of six was left untreated. The effect of the EDTA was to increase the solubility of copper in the substrate, and hence, to increase its availability to the plant. Extractable copper in these substrates was determined by shaking 1-g samples with ammonium acetate (pH 7.0) for 24 hours. This method gives some indication of the bioavailability of heavy metals in soils (Ernst, 1996).

Erica plants were grown as replicates of five specimens for (a) and in duplicate for (b). In addition, plants of *Brassica juncea* (L.) Czern. (Indian mustard) and *Biscutella laevigata* L. were transferred to the pots of mixture (b) above and with the same replicate pattern. These two species are metal-tolerant and were included to serve as a comparison with *Erica andevalensis*.

New shoots of *Erica* plants from the Andévalo were dried, and 0.2-g subsamples were placed in borosilicate test tubes (20 mL capacity) and ashed at 500°C overnight. The ash was redissolved in 2 *M* hydrochloric acid. The same treatment was used for all other plants studied.

Soil samples (0.5 g) from the Andévalo were digested with 10 mL of a 1:1 mixture of nitric and hydrofluoric acids contained in 50 mL Griffin-type polypropylene beakers immersed in a water bath. After taking to dryness in the water bath, the residues were redissolved in 10 mL of 2 *M* hydrochloric acid.

All solutions of plant and soil samples were analyzed for copper and iron by standard flame atomic absorption spectrometry using a GBC 904 instrument. The reason for determining iron was that this element (along with chromium and titanium) can serve as an indication of the probable contamination of plant samples by soils and other substrates. Vascular plants usually do not accumulate more

TABLE 1. Copper and iron concentrations (μg g^{-1} dry mass) in *Erica andevalensis* and its corresponding metal-rich soils (pH 3.5-5.0) from the Andévalo, Huelva Province, Spain.

	Concentration in soil (μg g^{-1})			Concentration in plant (μg g^{-1})		
#	Cu	Fe	Cu/Fe	Cu	Fe	Cu*
1	375	5,854	0.064	30	331	28
2	547	5,883	0.093	42	588	15
3	515	5,960	0.086	43	703	8
4	3,676	5,936	0.62	24	214	24
5	2,034	6,320	0.32	21	239	21
6	1,668	6,464	0.25	28	327	21
7	23	6,094	0.0038	13	436	12
8	216	5,854	0.036	16	94	16
9	177	5,638	0.031	10	643	1
10	191	5,854	0.032	12	119	12

*Corrected values calculated by assuming that any iron concentration >300 μg g^{-1} in plant material is due to wind-borne contamination from soil. The copper concentration due to contamination as indicated from this excess iron, is calculated from column 4 and removed from the original total copper in the plant.

than 300 μg Fe g^{-1} iron in their leaves via the root systems. Any value higher than this is usually an indication of wind-borne contamination from the soil.

RESULTS AND DISCUSSION

Copper Tolerance in *Erica andevalensis*

Copper concentrations in ten Andévalo soils are shown in Table 1. They range from 23 to 3,676 μg g^{-1}, a wide fluctuation. The high levels found in some of the soils indicate that *E. andevalensis* is able to tolerate elevated levels of copper in its natural range.

The results of pot trials with *Erica andevalensis* grown in copper ore (Champion Mine, New Zealand) diluted with standard commercial seed-raising mixture in various proportions are shown in Table 2. Some of the data are also shown in Figure 3. The plants would not grow in 100% Champion ore (2.5% Cu), but tolerated copper concentrations half of this level (12,500 μg g^{-1}). This degree of copper tolerance is almost unequaled in the plant kingdom and is paralleled only in the specialized copper-tolerant flora of the Democratic Republic of Congo (Brooks et al., 1992).

The results of trials in which *Erica andevalensis* and two other species were

TABLE 2. Mean (n=2) copper concentrations ($\mu g\ g^{-1}$ dry mass) in soils and plants before and after addition of EDTA (2 g kg^{-1}) to the soil.

A[a]	B	C	D	E	F	G	H	I
0.2	0.2	78	4	6	7	13	13	13
5.0	78	504	6	15	10	43	8	24
5.9	339	691	8	21	7	80	14	133
13.9	376	1,918	6	225	19	173	6	81
86	474	1,926	8	*	*	*	11	472
264	1,439	3,175	28	*	*	*	14	*

[a] A=extractable Cu in soil without EDTA, B=as A but after addition of EDTA, C=total Cu in soil, D=*Biscutella laevigata* without addition of EDTA to the soil, E=as D but with addition of EDTA, F=*Brassica juncea* without addition of EDTA to the soil, G=as F but with addition of EDTA, H=*Erica andevalensis* without addition of EDTA to the soil, I=as H but with addition of EDTA.

*Plants did not survive addition of EDTA. Plant parts were new whole shoots.

Clearly, the tolerance of the *Erica* to copper is much greater than that of the other two metal-tolerant plants.

Copper Uptake by *Erica andevalensis*

Copper concentrations in *E. andevalensis* are shown in Tables 1 and 2 and Figure 3. Plants with very high iron concentrations were assumed to have been contaminated by wind-borne dust, and corrected copper values have been calculated. Plants taken from natural populations (Table 1) had copper concentrations below 25 $\mu g\ g^{-1}$, well below the threshold for hyperaccumulation at 1,000 $\mu g\ g^{-1}$. Copper accumulation by *E. andevalensis* was enhanced by the addition of EDTA to plants grown in artificial substrates (Table 2), but to a lesser degree than the other metal-tolerant species tested, and was well below the threshold of 1,000 $\mu g\ g^{-1}$. At a level of 1,918 μg Cu g^{-1} in soil, above which the other species would not grow, the *Erica* contained less than half of the copper of the others, thus demonstrating its ability to limit copper accumulation, no doubt as a survival mechanism.

The Relationship Between the Copper Uptake by *Erica andevalensis* and the Concentration of Copper in the Substrate

No correlation occurred between the total copper content of plants and soils from Andévalo (Table 1). This result is not unexpected because elements such as

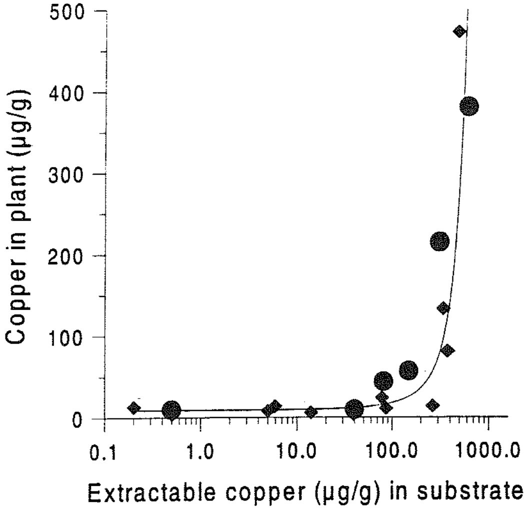

FIGURE 3. Mean concentrations of copper in fresh shoots of *Erica andevalensis* as a function of extractable copper in the substrate. Solid diamonds: commercial potting mixture with copper initially added as sulphate and with subsequent addition of EDTA at a rate of 2 g kg^{-1} of dry substrate. This plot includes 5 duplicates with EDTA in soil and 5 without addition. Solid circles: mixtures of copper ore (pH 6.0) and commercial ('Liddle Wonder') seed-raising compost composed of 1:1 peat/pumice with added slow-release fertilizers.

copper, that are essential to plant nutrition, tend to have constant concentrations within the plant because of internal control mechanisms, whereas non-essential elements are externally controlled and taken up passively by plants to an extent proportional to the concentration in the substrate (Timperley et al. 1970). *Eriva andevalensis* is nevertheless a good geobotanical indicator of copper because its distribution appears to be confined to this type of mineralization.

A highly significant correlation (r=0.68, P<0.01) occurred between the concentration of copper in the plants and the extractable copper concentration in the soil (Figure 3). The shape of the curve in Figure 3 is illustrative of the behavior of non-accumulator plants to elements essential in plant nutrition. As elemental concentrations in the substrate increase, the internal control system of the plant is able to restrict uptake of the metal in question until a threshold is reached where the control mechanism starts to break down and unrestricted uptake occurs and is terminated by necrosis of the plant at the limit of tolerance.

A strong behavioral correlation occurred between copper accumulation by *Erica* in two very different substrates, one natural and the other synthetic, when such uptake is based on extractable copper values (Figure 3). This correlation clearly emphasizes the importance of assessing tolerance to heavy metals on the basis of the plant-available fraction rather than on the total heavy metal concentration. It is probable that in the Andévalo, the copper is tightly bound into organic matter because the plant will tolerate apparently very high levels of heavy metals in the damp humus-rich environments in which they grow. For example, Nelson et al. (1985) found it growing in evaporates with as much as 1,500 μg Cu g^{-1}.

CONCLUSIONS

The following conclusions can be derived from our studies on *Erica andevalensis*:

(1) The tolerance of the plant to copper is extremely high compared with other metal-tolerant species.
(2) The plant is clearly not a hyperaccumulator (>1,000 μg g^{-1}) of copper.
(3) The copper accumulation by the plant is a function of the extractable (rather than the total) copper concentration in the substrate.
(4) Uptake of copper follows the pattern of metal-excluding plants: i.e., restricted uptake until the threshold of metal tolerance is exceeded. Thereafter, a rapid exponential increase occurs over a short range until the plant will no longer grow in the copper-rich substrate. The same pattern was obvious even after EDTA was added to the soil to complex copper.
(5) *Erica andevalensis* has no use for biogeochemical prospecting because its copper content does not reflect the total copper content of the substrate until the tolerance threshold is exceeded. This unsuitability is mainly because there is little correlation between total and extractable copper in the Andévalo soils.
(6) The plant is an excellent geobotanical indicator of copper mineralization because it appears to be restricted to copper-rich substrates. Further work on this interesting species should be devoted to tolerance studies and its possible role in revegetation of mine tips and other mineralized waste material.

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