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Senecio conrathii N.E.Br. (Asteraceae), a new hyperaccumulator of nickel from serpentinite outcrops of the Barberton Greenstone Belt, South Africa

Abstract Five nickel hyperaccumulators belonging to the Asteraceae are known from ultramafic outcrops in South Africa. Phytoremediation applications of the known hyperaccumulators in the Asteraceae, such as the indigenous *Berkheya coddii* Roessler, are well reported and necessitate further exploration to find additional species with such traits. This study targeted the most frequently occurring species of the Asteraceae on eight randomly selected serpentinite outcrops of the Barberton Greenstone Belt. Twenty species were sampled, including 12 that were tested for nickel accumulation for the first time. Although the majority of the species were excluders, the known hyperaccumulators *Berkheya nivea* N.E.Br. and *B. zeyheri* (Sond. & Harv.) Oliv. & Hiern subsp. *rehmannii* (Thell.) Roessler var. *rogersiana* (Thell.) Roessler hyperaccumulated nickel in the leaves at expected levels. A new hyperaccumulator of nickel was discovered, *Senecio conrathii* N.E.Br., which accumulated the element in its leaves at 1695 ± 637 µg g^{-1} on soil with a total and exchangeable nickel content of 503 mg kg^{-1} and 0.095 µg g^{-1}, respectively. This makes it the third known species in the Senecioneae of South Africa to hyperaccumulate nickel after *Senecio anomalochrous* Hilliard and *Senecio coronatus* (Thunb.) Harv., albeit it being a weak accumulator compared with the latter. Seven tribes in the Asteraceae have now been screened for hyperaccumulation in South Africa, with hyperaccumulators only recorded for the Arctoteae and Senecioneae. This suggests that further exploration for hyperaccumulators should focus on these tribes as they comprise all six species (of 68 Asteraceae taxa screened thus far) to hyperaccumulate nickel.

Introduction

Nickel hyperaccumulation by plants is a worldwide phenomenon spanning many higher plant families and taxa (Severne 1974; Brooks and Radford 1978; Reeves et al. 1999; Mesjasz-Przybylowicz et al. 2001; Reeves and Adigüzel 2004) and is presumably mediated by the primary Fe^{2+} uptake transporter in plant roots (Nishida et al. 2011). Hyperaccumulators have evolved active responses at the molecular level to deal with stressors associated with excessive metal accumulation (Sharma and Dietz 2006; Gall and Rajakaruna 2013). Much uncertainty exists regarding the adaptive significance of metal hyperaccumulation; one hypothesis being that hyperaccumulation, which results in metal toxicity, acts as a defence mechanism against herbivory (Boyd 2004).

Plants will occasionally hyperaccumulate Ni when they occur in environments where this metal is in abundance and available for plant uptake (Shallari et al. 1998). However, most plants will exclude this metal, but it is generally on Ni-rich soils where this ability to accumulate has evolved numerous times world-wide (Van der Ent et al. 2015; Galey et al. 2017). The serpentinites of the Barberton Greenstone Belt in South Africa are no exception (Morrey et al. 1989; Hughes and Noble 1991) where five Ni hyperaccumulator species have been previously discovered (Smith et al. 2001). One of these species, *Berkheya coddii*, has become internationally renowned for its fast growth, high biomass and

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ability to hyperaccumulate, and has been advocated as an ideal subject for phytoremediation and phytomining (Robinson et al. 1997; Chaney et al. 2014).

Although the Ni hyperaccumulation trait is often associated with species from ultramafic regions (Reeves et al. 1999; Jaffré et al. 2013; Galey et al. 2017), and the valuable industrial application of such species in local green economies which encourages their discovery (Ellery and Walker 1986; Robinson et al. 1997; Morgenthal et al. 2004), few taxa with this ability have been identified from South Africa. Smith et al. (2001) tested 56 of 126 Asteraceae species tolerant of serpentinite in the Barberton region and found five taxa to hyperaccumulate Ni. Considering that this family has undergone extensive radiation in South Africa (2481 species; Koekemoer 1996), of which many species have colonized ultramafic soils (Siebert et al. 2002), it would seem that Ni hyperaccumulation is poorly represented, especially considering that hyperacumulation is well represented in the family (Reeves and Baker 2000). Globally 0.2% of the Asteraceae (approximately 50 species) have been identified as Ni hyperaccumulators, contributing to about 10–12% of the 450–470 known Ni hyperaccumulators to date (pers. com. R.D. Reeves).

Cecchi et al. (2010) show that within evolutionary lineages of Ni hyperaccumulating Alysseae (Brassicaceae), accumulation ability has been lost or gained through independent events of microevolutionary adaptation. According to Kruckeberg and Kruckeberg (1990), once an evolving lineage has become metal-tolerant, evolution can continue on metalliferous soils by adaptive radiation and would therefore imply that the hyperaccumulation trait is tribe-, but more probably, genus-specific. This study aims to test the following hypotheses: (1) is hyperaccumulation restricted to the Arctoteae and Senecioneae of the Asteraceae as proposed by Smith et al. (2001), and (2) are certain genera within these tribes more prone to develop the hyperaccumulation trait? In order to test these hypotheses, we determined the Ni concentrations of leaf tissue of taxa from different tribes and genera of the Asteraceae, and their associated soils, collected from serpentinite outcrops of the Barberton greenstone belt (BGB).

Methodology

Field sampling

Eight serpentinite outcrops were randomly chosen for this survey (Fig. 1). During the first survey 2–3 frequently occurring Asteraceae species were sampled from each of these outcrops. In total, 20 species were sampled, which included 12 species tested for Ni hyperaccumulation in South Africa for the first time (not listed by Smith et al. 2001). A second survey was conducted to specifically target the populations of any species that hyperaccumulated Ni at > 1000 μg g^{-1} in leaf tissue.

All plant species were identified and confirmed by the National Herbarium in Pretoria (PRE), and voucher species are housed at PRE and the A.P. Goossens Herbarium (PUC).

Before sampling began, the centre of the outcrop was visually determined and then, in four wind directions away from this point, young leaves from active growth points (five leaves per individual) of five individuals were sampled per species; equating to one plant every 5 m in a single direction and totalling ± 20 individuals sampled per species per outcrop. Leaves were washed in the field with deionized water to remove soil particles and inorganic material. Thereafter, leaves were quickly washed in 0.1 molar HCl solution in the laboratory and rinsed three times with distilled water, before they were stored in paper bags to dry under room temperature. Plant tissue samples were then oven-dried at 70 °C for 48 h and ground to a particle size less than 75 μm in a tungsten carbide milling vessel.

At each site, one soil sample was taken from underneath the sampled plants in the centre of the outcrop and in each of the four sampling directions. Samples were taken up to a depth of 10 cm to coincide with the predominant rooting depth of these species. The five samples were pooled to make a composite sample. Soil was stored in brown paper bags to air dry. Thereafter, samples were slightly pulverized, and put through a 2 mm sieve to break down aggregates and remove any gravel or organic material. Samples were ground into a fine powder (≤ 75 μm) using a tungsten carbide ring mill.

Soil and plant tissue analyses

The pH of each soil sample was estimated via 1:2.5 extract solution. Twenty grams of soil (< 2 mm particle size) was weighed in a plastic beaker to which 50 ml of deionized water was added. The suspension was stirred for five seconds using a glass rod, and left for 4 h. Thereafter the suspension was stirred again and left for 10 min. The pH was then determined by means of a pH meter (Radiometer Copenhagen PHM 80). The electrode was allowed to stabilize for three minutes in solution before the pH was recorded.

The macro- and micro-nutrient content of the samples were determined with a 1:2 extract method. Three drops (1%) of flocculant was added to 200 ml of deionized water in a plastic shaking bottle. A soil solution of 100 ml was transferred systematically to the bottle and shaken for 30 min. The clear supernatant was then decanted to low speed centrifuge tubes, and centrifuged for 10 min at 2000 rpm. The resultant supernatant was then decanted into an Erlenmeyer flask. Two high speed centrifuge tubes per sample was filled from the flask and centrifuged for 12 min at 16,500 rpm. Liquid from the high speed centrifuge tubes were then filtered into two different bottles. Cations (Ca, Mg, K and Na) were determined with Atomic Absorption
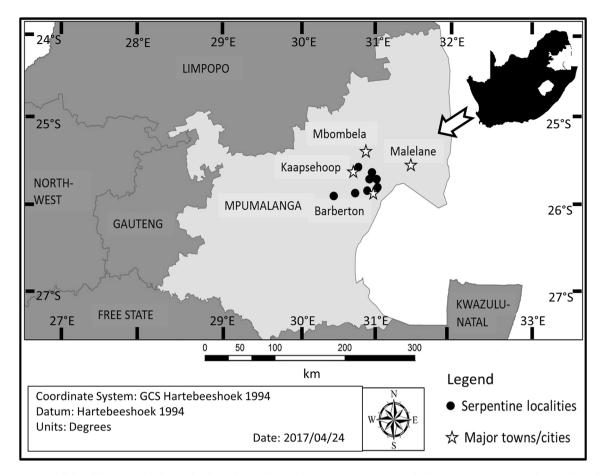
Fig. 1 Localities of the eight serpentinite study sites along the Barberton Greenstone Belt in the Mpumalanga province of South Africa

Spectrometry (Varian SpecttAA.250 Plus). Anions (Cl, NO_3, NO_2, F, SO_4 and PO_4) were determined with ion chromatography (Metrohm 761 Compact IC).

Plant available phosphorus in the soil samples was determined with the P-Bray 1 extraction method. P-Bray 1 solution was prepared by dissolving 2.22 g ammonium fluoride (NH_4F) in deionized water and was transferred to a 2 L volumetric flask. Concentrated hydrochloric acid (5 ml) was then added and the flask filled to volume with deionized water. Ten grams of soil (< 2 mm particle size) was weighed in a Schott-bottle, to which 75 ml of P-Bray 1 (20 °C) solution was added and immediately shaken for 40 s. Two drops of flocculant was added to the solution, which was then gently swirled. Immediately after the suspension settled, the supernatant was filtered into a clean Schott-bottle, and the concentration of phosphate was determined using an Auto-Analyser (Skalar San + +).

The soluble/plant available trace metal concentration of the soil samples was determined by means of the ammonium nitrate (NH_4NO_3) solution method. NH_4NO_3 is known to be chemically less reactive than other extraction methods and, thus, more suitable for the extraction of comparable fractions of mobile heavy metals (Schöning and Brümmer 2008). We acknowledge that more appropriate chelator based methods exist, and

our analyses are therefore limited to the very small amounts of heavy metals in the exchangeable Ni 'pool' (Sabienë et al. 2004). Samples of 20 g each were placed in a 150 ml shaking bottle, to which exactly 50 ml of the NH_4NO_3 solution was added. The mixture was shaken for 2 h at 20 rpm (25 °C). Solid particles were allowed to settle for 15 min before the supernatant solution was filtered with a 0.45 μm filter. The first 5 ml was disposed of, and the solution that remained was collected in a 50 ml bottle for analysis. The samples were analysed with Inductively Coupled Plasma–mass Spectrometry (Agilent 7500) for soluble trace metals.

The total trace metal concentration of the soil and plant tissue samples were conducted by means of acid digestion. Soil samples of 25 g each were placed in a 150 ml beaker, to which 15 ml nitric acid (HNO_3) was added. The samples were immediately covered with watch glasses and placed on a sand stove inside a fume hood, which was set at medium temperature of ± 95 °C. The mixture was left to reflux for an hour during which fumes were generated. When the fumes diminished the samples were fully digested and the watch glasses were removed. The acid was evaporated by heating each sample until the volume was reduced to ± 5 ml. Each sample was then cooled to add 3 ml of 30% hydrogen peroxide (H_2O_2). After cooling 10 ml of

Table 1 Metal concentrations (µg g^{-1}) in leaf tissue of 20 species sampled during the first survey; values in bold are indicative of hyperaccumulation (*species tested for the first time)

Species	Collection no.	Outcrop	Ni	Fe	Al	Mn	Zn	Cu	Cr
*Berkheya echinacea**	Frisby 2	R40 Pass	183	534	61.1	97.9	24.3	3.36	16.75
B. nivea	Siebert and Rajakaruna 16	Mundt's Concession	**3658**	207	10.8	32.1	6.8	3.9	3.16
B. setifera	Siebert and Rajakaruna 4457	Kaapsehoop	2	36	15.7	15.1	4.7	1.17	0.73
B. zeyheri subsp. *rehmannii*	Siebert and Rajakaruna 13	Sassenheim	**1630**	19	7.4	15.4	2.7	1.17	0.39
*Campuloclinium macrocephalum**	Siebert and Rajakaruna 14	Sassenheim	3	134	63.9	18.6	11.4	5.52	1.97
Haplocarpha scaposa	Frisby 10	Sassenheim	26	146	54.5	30.5	7.9	10.88	3.5
*Helichrysum acutatum**	Siebert and Rajakaruna 23	Nelshoogte	29	33	15.7	49.4	10.1	2.27	1.18
*H. aureolum**	Siebert and Rajakaruna 20	Kalkkloof	8	129	19.2	41.7	36.6	7.59	5.9
*H. miconiifolium**	Frisby 4	Nelshoogte	3	716	87.7	53.4	14.7	2.59	1.44
*H. subluteum**	Frisby 1	R40 Pass	14	1230	251.7	70.8	34.8	16.56	15.66
*H. umbraculigerum**	Frisby 3	Nelshoogte	26	206	63.5	27.9	13.1	3.17	2.82
Hilliardiella aristata	Siebert and Rajakaruna 19	Kalkkloof	12	188	34.3	23.2	12.1	7.09	3.74
H. sutherlandii	Siebert and Rajakaruna 4456	Noordkaap Railway	13	33	20.3	17.5	3.6	1.0	0.77
Nidorella auriculata	Frisby 5	Kaapsehoop	5	209	101.2	98.5	46.6	17.4	2.37
*Pegolettia senegalensis**	Frisby 9	Noordkaap	1	167	40.7	23.3	15.9	6.05	5.54
*Psiadia punctulata**	Frisby 8	Noordkaap	1	60	24.7	36.1	13.4	6.05	2.25
*Senecio conrathii**	Siebert 4485	Kaapsehoop	**1558**	64	6.7	7.4	0.9	0.41	11.89
*S. gerrardii**	Frisby 6	Kaapsehoop	22	92	51.9	161.7	22.5	9.34	2.02
S. latifolius	Siebert s.n.	Noordkaap Railway	1	22	5.7	5.7	4.7	1.49	0.63
*S. venosus**	Siebert and Rajakaruna 15	Mundt's Concession	23	29	5.1	18.4	9.6	6.99	2.54
Mean			466	220	47.3	43.5	15.1	5.9	3.8

3 N HCl was added and then covered again with a watch glass. The mixture was placed on the sand bath in the fume hood and was refluxed for about an hour. After the reflux the sample was cooled down to 25 °C. The mixture was filtered through Whatman 40 filter paper into a 50 ml volumetric flask. The filter paper was washed with deionized water. The volumetric flask was filled to volume with deionized water. Total trace metal concentrations in the samples were determined with Inductively Coupled Plasma–mass Spectrometry. The detection limit for Ni is soil samples was 0.0001 ppm.

Results

During the first survey, the leaf tissues of 20 species belonging to the Asteraceae were tested for the accumulation of metals: of these species only three were found to accumulate Ni at concentrations that exceeded 1000 µg g^{-1} (Table 1). This meets the criterion for Ni hyperaccumulation as defined by Van der Ent et al. (2013). Two of these species, *Berkheya nivea* (3658 µg g^{-1}; Table 1) and *B. zeyheri* (1630 µg g^{-1}; Table 1), are known hyperaccumulators reported by Smith et al. (2001), but the third species, *Senecio conrathii* (1558 µg g^{-1}; Table 1, Fig. 2), has not previously been documented as being capable of hyperaccumulating Ni. The remaining 17 species were found to be excluders with much lower concentrations of Ni within the leaf tissue, suggesting tolerance strategies to restrict metal uptake or retain and detoxify the metals within the root tissue (Rascio and Navari-Izzo 2011).

The localities Kaapsehoop, Noordkaap, Noordkaap Railway and R40 Pass had the lowest Ni total and Ni soluble values in the soil (Table 2). *S. conrathii* was sampled at Kaapsehoop (soluble Ni at 0.095 µg g^{-1}; third lowest) and accumulated Ni above 1000 µg g^{-1} (Table 1). Kalkkloof, Mundt's Concession, Nelshoogte and Sassenheim had the highest Ni total and Ni soluble values, with Ni-hyperaccumulating *B. nivea* and *B. zeyheri* sampled from these areas (Table 1). Therefore, at the local scale, it is clear that Ni bioavailability, and not total soil Ni concentration, is the only prerequisite for hyperaccumulation to take place if a species possesses the accumulation trait (of which *S. conrathii* is a good example). Also, soluble Ni is not always a function of total Ni in the soil (Noordkaap Railway is an example thereof; low total and high soluble Ni) or pH (Table 2). The Mg/Ca quotient (Table 2) of the soil from the three localities where hyperaccumulators were found ranged from 11.7 to 13.2, indicating the soil as typically ultramafic (Proctor 1971).

A follow up, more comprehensive sampling process, which focused on *S. conrathii* and *B. zeyheri* (as control), confirmed that the Ni concentrations within the leaf tissue of the two species repeatedly exceeded the 1000 µg g^{-1} criterion set for hyperaccumulation of Ni (Van der Ent et al. 2013), and confirmed that *S. conrathii* is indeed a Ni hyperaccumulator. All five analyses done met the criterion, with an average of 1695 µg g^{-1} for *S. conrathii* compared to the 1793 µg g^{-1} mean recorded for *B. zeyheri* as a known hyperaccumulator (Table 3).

Nickel water soluble fractions from the eight localities (Table 2) were compared to the Ni concentrations in the leaf tissue of the 20 sampled species (Fig. 3). The concentrations of Ni within the plant leaves of the three hyperaccumulator species showed a significant positive relationship with the Ni water soluble fractions of the soil ($R^2 = 0.915$; $P < 0.01$; Fig. 3), but predictably not so for the excluders ($R^2 = 0.013$; $P = 0.961$; Fig. 3).
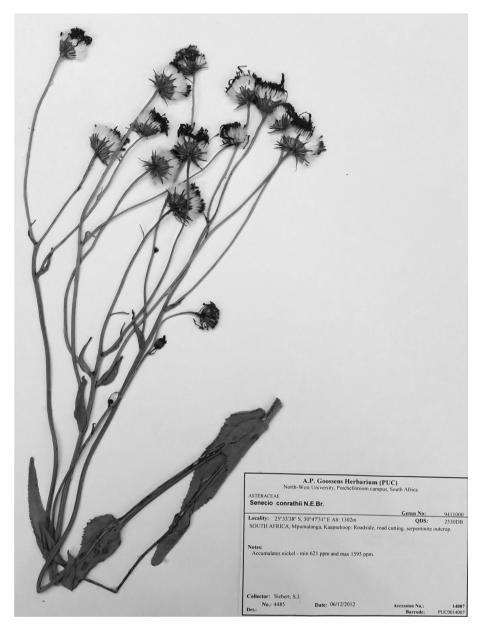
Fig. 2 Voucher specimen of *Senecio conrathii* housed at the AP Goossens Herbarium (PUC). Locality: 25°33′38″ S; 30°47′31″ E, Mpumalanga, Kaapsehoop: roadside, serpentinite outcrop. Collector: Siebert, S.J., no. 4485 (PUC0014007). Date: 2012/12/06

Table 2 Summary of relevant soil analyses from the eight localities along the Barberton Greenstone Belt

Locality	Ni total µg g^{-1}	Ni soluble µg g^{-1}	pH	Mg mg/L	Ca mg/L	Mg:Ca Quotient
Kalkkloof	2530	0.416	6.3	2000	38	51.9
Mundt's Concession	1874	0.817	6.2	957	82	11.7
Nelshoogte	1837	0.368	6.1	1707	173	9.7
Sassenheim	1297	0.220	6.2	1322	106	12.4
Noordkaap	793	0.059	6.2	1013	337	3.1
Noordkaap Railway	722	0.273	6.4	1245	143	8.7
Kaapsehoop	503	0.095	6.1	438	33	13.2
R40 Pass	198	0.039	6.5	1592	1047	1.5

Table 3 Metal concentrations ($\mu g\ g^{-1}$) in leaf tissue of two species sampled during the second survey from an additional four sub-populations per species; values in bold are considered as hyperaccumulation

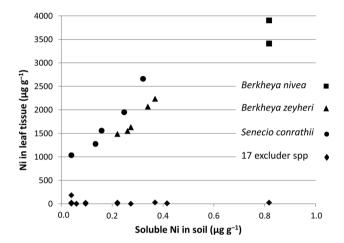| Species	Locality	Ni	Fe	Al	Mn	Zn	Cu	Cr
Senecio conrathii	Kaapsehoop	**1035**	15.1	13.7	5.9	4.4	0.51	0.55
	Kaapsehoop	**2659**	29.3	8.3	4.9	4.9	0.51	5.74
	Kaapsehoop	**1276**	3.7	1.8	0.7	2.4	0.19	1.26
	Kaapsehoop	**1948**	79.8	8.4	9.3	1.2	0.38	14.87
Mean (including survey 1)		**1695**	38.4	7.8	5.6	2.8	0.4	6.86
Berkheya zeyheri	Nelshoogte	**1549**	94.4	17.5	10.9	9.2	5.52	1.23
	Nelshoogte	**1487**	76.1	16.1	10.6	9.7	6.35	1.15
	Sassenheim	**2067**	39.2	44.8	11.3	6.3	3.93	1.63
	Sassenheim	**2234**	35.4	47.2	8.9	7.4	4.72	1.57
Mean (including survey 1)		**1793**	52.8	28.2	9.8	7.1	4.34	1.19

Fig. 3 Relationship between Ni concentration in the leaf tissue and soluble Ni in the soil for the three accumulator species ($R^2 = 0.915$) and the 17 excluder species ($R^2 = 0.013$)

Discussion

The laboratory chemical analyses of the first survey defined two groups within the twenty Asteraceae samples, namely excluders (17 species) and hyperaccumulators (three species) of Ni. Two of the three species that tested positive for hyperaccumulation of Ni were known hyperaccumulators (Smith et al. 2001), whilst the third, *S. conrathii*, had not previously been documented (Table 1). Smith et al. (2001) had an 8% discovery rate of Ni hyperaccumulators during their survey, which is matched by this study (of the 12 Asteraceae species not tested for Ni hyperaccumulation before, one tested positive). The soil analysis of the study sites (Table 2) showed that the two *Berkheya* species known to hyperaccumulate Ni grew on soil that had high total and soluble concentrations, whilst *S. conrathii* grew on soil with lower total and soluble Ni concentrations (Table 2).

One of the most important chemical characteristics of ultramafic soil is that it has a high magnesium to calcium quotient (Proctor 1971), usually > 1 (Rajakaruna et al. 2009). The Mg/Ca quotient of the soil in the locations where the three hyperaccumulators were found ranged between 11 and 13. Robinson et al. (1999) has shown that higher, rather than lower, concentrations of Mg in the soil, inhibits the uptake of Ni, especially when Mg is interacting positively with Ca (Gabbrielli and Pandolfini 1984). Considering the low values of Mg at Kaapsehoop (Table 2), it could therefore be expected that *S. conrathii* should be able to accumulate high values of Ni despite the low soluble concentrations thereof in the soil.

There are only a few studies to date that have used phylogenetic methods to investigate evolutionary trends in Ni hyperaccumulation (Mengoni et al. 2003; Burge and Barker 2010; Cecchi et al. 2010). Nickel hyperaccumulation is generally a rare trait found only in selected species, despite several other related species growing on the same Ni-rich soils (Jaffré et al. 2013; Gall and Rajakaruna 2013). In the phylogeny of angiosperms, the evolution of high metal tolerance is also not homogeneously distributed over taxonomic groups, showing differences not only within a taxonomic group, but even among populations of the same species (Ernst 2006).

Six hyperaccumulators of Ni are now known from South Africa, three in the Arctoteae (*B. coddii*, *B. nivea* and *B. zeyheri* subsp. *rehmannii*) and three in the Senecioneae (*Senecio anomalochrous*, *S. conrathii* and *Senecio coronatus*). This syndrome is well known for the Asteraceae, and especially for the Senecioneae, with 17 species of *Senecio* in the flora of Cuba having been confirmed as Ni-hyperaccumulators (Borhidi 2001). This supports the hypothesis that once a lineage evolves the hyperaccumulation trait it possibly becomes tribe-, and in our case, genus-specific (Kruckeberg and Kruckeberg 1990; Cecchi et al. 2010). This relationship between phylogeny and hyperaccumulation ability is well-known for genera such as *Alyssum* and *Noccaea* in the Brassicaceae (Gall and Rajakaruna 2013). A similar pattern may exist for the Senecioneae and would be worthy of further investigation.

Conclusion

We report that *S. conrathii* from South Africa is a hyperaccumulator of Ni. Results from this and other studies now indicate a high probability of hyperaccu-

mulation in *Senecio* (three of 14 species tested). Similar to other known hyperaccumulators from the study area, the Ni concentrations within plant tissue increased as the amount of water soluble Ni within the soil increased. *S. conrathii* is a hyperaccumulator of Ni on soils with lower levels (total: 500 µg g^{-1}; soluble: 0.1 µg g^{-1}) compared to surrounding ultramafic outcrops.

Our findings further corroborates that the Ni-hyperaccumulation trait is present in the Asteraceae of South Africa, especially in the Senecioneae. The Senecioneae should be considered as an important tribe to screen for Ni hyperaccumulators in South Africa. Further research should evaluate the potential use of *S. conrathii* in phytoremediation programs and as indicators of geological substrates. However, further research is required to find the most suitable trait-bearing genotypes for such applications, as it has been shown that the ability of *S. coronatus* to transport and accumulate Ni is population specific (Mesjasz-Przybyłowicz et al. 2007; Boyd et al. 2008).

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