South African Journal of Botany 158 (2023) 80–83

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Testing the suitability of portable X-Ray Fluorescence (pXRF) analysis of dried herbarium specimens to detect Ni hyperaccumulators in South Africa

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ARTICLE INFO

Article History:
Received 13 April 2023
Revised 28 April 2023
Accepted 2 May 2023
Available online 5 May 2023

Edited by Dr J. Gruz

Keywords:
Agromining
Geobotany
Metal hyperaccumulation
Phytomining
Ultramafic

ABSTRACT

Metal hyperaccumulators accumulate particular metals or metalloids in their leaves to concentrations hundreds or thousands of times greater than is normal for most plants. Globally, Ni is the most often hyperaccumulated metal, with 532 hyperaccumulator species documented to date. Hyperaccumulators have attracted much attention as potential candidates for green technologies, including phytoremediation and agromining. In South Africa, six serpentinite-associated plants in the genera *Berkheya* and *Senecio* hyperaccumulate Ni (to > 0.1% of leaf tissue dry weight). It is surprising that only six of about 70 *Berkheya* and 290 *Senecio* species native to South Africa hyperaccumulate Ni, given about ~10–20% of taxa from each genus occur on serpentinite. While it is costly and time consuming to field collect and chemically analyze leaves of all species in these genera, a novel method (portable X-Ray Fluorescence or pXRF analysis of herbarium specimens) allows for rapid (100 s of specimens/day) and non-destructive measurement of Ni in dry herbarium specimens. We tested the accuracy of this approach on known Ni hyperaccumulators vouchered at two South African herbaria (C.E. Moss Herbarium (J) of the University of the Witwatersrand and A.P. Goossens Herbarium (PUC) from North-West University). While the absolute concentrations of Ni determined by ICP-MS and pXRF were not always directly in agreement, we had 100% success in confirming those that were known to hyperaccumulate Ni with those that did not. We propose pXRF as a cheap, effective, and efficient approach to rapidly screen herbarium specimens across South Africa to discover additional metal hyperaccumulators for much-needed remediation purposes.

1. Introduction

Ultramafic soils are found along continental margins, faults, and shear zones on almost all continents and island arcs on Earth (Moores, 2011). They are generally deficient in essential plant nutrients such as Ca, N, and P and have elevated concentrations of toxic metals, including Ni, Cr, and Co (O'Dell and Rajakaruna, 2011). Due to intense selective pressures generated by elemental and other physical stressors, ultramafic soils promote speciation and evolution of edaphic endemism, contributing to unique floras globally (Garnica-Díaz et al., 2022). Some plants of ultramafic areas take up exceedingly high concentrations of metals and translocate them into their shoots (Ferrero et al., 2020). These so-called metal hyperaccumulators accumulate these potentially toxic elements in their leaves, phloem tissue, or latex to concentrations hundreds or thousands of times greater than normal for most plants (Van der Ent et al., 2015). Hyperaccumulators have attracted much attention in the disciplines of phytoremediation and phytomining (Ferrero et al., 2020), green technologies used to restore metal-contaminated sites or extract valuable metals from metal-enriched soils (Van der Ent et al., 2015), respectively. Nickel is the metal most often hyperaccumulated, with 532 known hyperaccumulator species globally (Reeves et al., 2018).

Major ultramafic outcrops occur along the Greenstone Belt of the Barberton Mountains (Mpumalanga) and from Potgietersrus to Duiwelskloof in Limpopo, with smaller outcrops in Gauteng, KwaZulu-Natal and North-West (Garnica-Díaz et al., 2022). Six plants growing within the Barberton serpentinite outcrops hyperaccumulate Ni [to > 0.1% of leaf tissue dry weight (Van der Ent et al., 2013)]. These are a small subset of *Berkheya* and *Senecio* spp. in the Asteraceae (Siebert et al., 2018). It is surprising that only three each of about 70 *Berkheya* and 290 *Senecio* native species hyperaccumulate Ni, given about ~10–20% of taxa from each genus occur on serpentinite soils in South Africa (Smith et al., 2001). The six Ni hyperaccumulators

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https://doi.org/10.1016/j.sajb.2023.05.005

currently known (Table 1) were discovered by screening 68 species of Asteraceae (Smith et al., 2001; Siebert et al., 2018); therefore, it is likely others are still to be discovered. While it is costly and time consuming to field collect and laboratory analyze leaves of all species in these genera, a novel method (portable X-Ray Fluorescence (pXRF) analysis of herbarium specimens) allows rapid (100 s of specimens/day, with a specimen scan per minute) and non-destructive measurement of Ni and other metals in dry specimens. The method is cheap, effective, extremely efficient and has been utilized with great success elsewhere (van der Ent et al., 2019). It could be a useful tool to rapidly assess the flora of South Africa to detect Ni-hyperaccumulators. Therefore, the aim was to test the effectiveness of the pXRF instrument to positively identify Ni-hyperaccumulators by scanning known herbarium specimens that have already been chemically analysed upon collection and tested positive (>1000 μg/g Ni in plant sap) for high Ni concentrations with the colorimetric reagent, Dimethylglyoxime (DMG) (Smith et al., 2001).

2. Material and methods

2.1. Calibration of XRF unit

pXRF technology is typically used to sample higher-density materials such as soil and rock. Therefore, to gain accurate readings of plant tissue element content, a linear correction factor had to be generated. A set of 25 leaf tissue standards was utilized. Samples of these standards were previously analyzed using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), a very precise analytical technique to determine Ni concentration of leaf tissue. Each of the standards was scanned using a Niton XL3t GOLDD+ (ThermoFisher) pXRF unit and raw readings were recorded. Then, by comparing the pXRF readings with the ICP-MS results, a correction factor was generated which can be multiplied by all downstream pXRF results to improve the device's accuracy. This is the same calibration method used in the original van der Ent et al. paper (2019). The specific correction factor of the device was XRF value = 1.18*ICP value.

2.2. Herbarium specimens

Hyperaccumulation of Ni has only been reported in two genera of the Asteraceae in South Africa and to test the pXRF device it was important to focus our effort on known hyperaccumulators (Table 1). Specimens were sourced from the C.E. Moss Herbarium (J) of the University of the Witwatersrand and A.P. Goossens Herbarium (PUC) from North-West University as they keep large collections of metallophytes collected on ultramafic soil. Voucher specimens were selected for scanning if it was indicated on the herbarium sheets whether fresh material had a positive or negative reaction to DMG paper [saturated with 5 g DMG dissolved in 500 mL acetone], with positive meaning that the chemical reaction turned paper pink/red if there was more than 1000 μg/g of Ni in plant sap (van der Ent et al., 2015). These marked specimens provided a reference to test the calibrated pXRF device.

Table 1
Known Ni hyperaccumulators from South Africa and accumulation concentrations based on published literature.

Species	Ni (μg/g)	Reference
Berkeya coddii Roessler*	17,900	Mesjasz-Przybyłowicz et al. (2004)
B. nivea N.E.Br.	3658	Siebert et al. (2018)
B. zeyheri (Sond. & Harv.) Oliv. & Hiern subsp. *rehmannii* (Thell.) Roessler var. *rogersiana* (Thell.) Roessler	5849	Mesjasz-Przybylowicz et al. (1996)
Senecio anomalochrous Hilliard	4600	Mesjasz-Przybylowicz et al. (2001)
S. conrathii N.E.Br.	2659	Siebert et al. (2018)
S. coronatus (Thunb.) Harv.	12,100	Boyd et al. (2002)

* Mesjasz-Przybyłowicz et al. (2004) also reports a value of 55 000 μg/g for this species, but this is much higher than any other record and not included here.

2.3. XRF scanning of specimens

Before each round of scanning, the Niton XL3t GOLDD+ device was first checked for accuracy by scanning a few known soil standards and a quartz blank. The pXRF results were compared to the known values for these standards to ensure the device was functioning properly. Each herbarium specimen was scanned for 60 s using the device's Soils Mode, which is most accurate for lower-density material. To ensure that only the elements in the leaf tissue were detected, a 2 mm thick plate of pure titanium was placed underneath the portion of the specimen being scanned, which stops the x-rays from penetrating into the table below. For each specimen, 2–3 of the leaves with the largest surface area were scanned. Each leaf was brushed gently to remove any soil prior to scanning. Results were viewed and exported using Thermo Scientific™ Niton Data Transfer software.

3. Results and discussion

Scanning results reported >1000 μg/g of Ni for all leaf material of herbarium specimens previously marked as having had a positive reaction to DMG on day of collection (Table 2). This was a 100% success rate for the pXRF in detecting those voucher specimens that hyperaccumulated Ni, and those that did not. This suggests that a calibrated pXRF is an accurate device to indicate Ni hyperaccumulation in dried plant material. It did, however, record different

Table 2
pXRF results for herbarium specimens at the A.P. Goossens Herbarium and C.E. Moss Herbarium that tested positive or negative for Ni with the Dimethylglyoxime (DMG) test. All specimens were collected from ultramafic soils. All species with Ni concentrations of >1000 μg/g in dry leaf tissue are known hyperaccumulators.

Species	Barcode	pXRF Ni (μg/g)	DMG test
Berkheya coddii	J76378	4223	Positive
	J76378A	4794	Positive
B. echinacea (Harv.) O.Hoffm. ex Burtt Davy	J86363	215	Negative
	J94366	111	Negative
	PUC0014512	514	Negative
B. insignis (Harv.) Thell.	J76592	Not detected	Negative
	J79704	Not detected	Negative
	J86581	482	Negative
B. nivea	PUC0014524	3249	Positive
B. setifera DC.	J86637	Not detected	Negative
	J94331	Not detected	Negative
	PUC0015460	Not detected	Negative
B. species nova	J97010	Not detected	Negative
B. zeyheri subsp. *rehmannii* var. *rogersiana*	PRE0991317-0	6507	Positive
	PUC0014527	5754	Positive
Gazania krebsiana Less.	J87790	61	Negative
	J89333	56	Negative
Haplocarpha scaposa Harv.	J86853	142	Negative
	J86904	Not detected	Negative
Senecio conrathii	PUC0014007	2150	Positive
*S. coronatus**	J78982	Not detected	Negative
	J86560	148	Negative
	J86575	7604	Positive
S. erubescens Aiton	J100281	198	Negative
	J99907	64	Negative
S. gerrardii Harv.	J94397	61	Negative
S. latifolius DC.	J81410	104	Negative
S. lydenburgensis Hutch. & Burtt Davy	J91392	75	Negative

* Facultative hyperaccumulator (Pollard et al., 2014).

concentrations of Ni compared to standard ICP-MS analysis. Siebert et al. (2018) conducted ICP-MS analysis of the leaves of specimens belonging to *B. nivea, B. zeyheri* and *S. conrathii* and recorded 3658, 1630 and 1588 and μg/g of Ni, respectively, compared to the 3249, 5754 and 2150 μg/g with the pXRF device recorded from the same specimens in this study (Table 2). This could be due to localised concentration of metals in the leaf tissue, as hyperaccumulator plants sometimes have heterogenous distributions of metals within leaf organs and multiple scans can yield different results (Purwadi et al., 2021). The ICP-MS analysed 3–5 whole digested leaves in a composite sample, whereas, with the pXRF, only an area of 1 cm^2 (up to 5 mm tissue thickness) of three leaves was scanned. So, the same value from ICP-MS and pXRF will rarely be obtained on the same specimen. This is why it is important to scan multiple leaves of each plant.

Certain pitfalls are associated with the use of the instrument on herbarium sheets. Initial readings produced high concentrations for Sc and V above 1000 μg/g. These erroneous concentration values arise for Sc and V in the Thermo software as an artefact of the high Ti peak that results from using a Ti plate background since Sc and V are adjacent to Ti on the periodic table (Purwadi et al., 2021). Thus, pXRF is not suitable for assessing all metals in plant leaves. Additionally, contamination of specimen leaf surfaces with soil particles can generate inflated values because of the ultramafic substrate's elevated metal concentrations. This is why it is important to thoroughly brush each leaf surface to remove soil and dust particles. However, this is not always effective, as could be seen for the elevated values recorded for *B. zeyheri* in this study, probably as the leaves are hairy and/or sparsely arachnose above, and white-felted beneath.

4. Conclusion

This study shows that the use of DMG paper in the past has provided an accurate indication of Ni hyperaccumulation in South African plants. While it is possible to use DMG paper on moistened and cut leaves of herbarium specimens, the process of detection is still time consuming as well as destructive. pXRF has shown that it is a more efficient method to detect hyperaccumulators, with the added advantage of giving Ni concentrations of plant material without having to remove leaves from the specimen (i.e., non-destructive sampling). Although concentrations are not as accurate as ICP-MS, rapid scanning (three repeats maximum per specimen), is sufficient to indicate hyperaccumulation. pXRF devices are available at most South Africa universities, and herbaria with collections from ultramafic areas are easily accessible, making it time and cost effective to scan large numbers of dried specimens. It is therefore valuable to rapidly assess large collections to identify potential hyperaccumulators, which can then be followed by field collections of plant material and subsequent ICP-MS analyses to get more accurate measurements of Ni concentrations.

Given large areas of serpentinite and other ultramafic rocks in Africa have undergone mining activities, the discovery of native metal hyperaccumulating plants with the pXRF method can aid in more effective restoration efforts with the use of African native plants, including the recovery of metals from plant tissue via agromining operations (van der Ent et al., 2015). Additionally, the discovery of plants with unusual metal hyperaccumulation can also provide model organisms for basic research across southern Africa, particularly in ecophysiology, genetics and biotechnology (Ferrero et al., 2020; Roebuck et al., 2022). Future scanning efforts should consider other families, such Euphorbiaceae, Phyllanthaceae, Rubiaceae and Violaceae, which are also known to hyperaccumulate Ni elsewhere (Reeves et al., 2018). Further, pXRF can be calibrated to measure other heavy metals including Cd, Co, Cr and Mn, also known to be high in ultramafic soils and hyperaccumulated by plants of ultramafic areas elsewhere (van der Ent et al., 2015). Additionally, it will be important to do such screening in all herbaria in South Africa with known collections of plants from ultramafic soils. Discovering new metal hyperaccumulating plants remains a priority for the remediation of vast areas of the country contaminated with Ni and other heavy metals. Plant species from ultramafic-associated areas with high levels of endemism (Siebert et al., 2001; Williamson and Balkwill, 2015)] should be prioritized during field and herbarium surveys.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Our appreciation to the Geology Subject Group at the North-West University, specifically Ricart Boneschans and Hancko Ostmann, for training and making the handheld XRF available for this study. Charl Clark is thanked for assistance on trips to herbaria. Reneé Reddy kindly provided access to and assistance at the C.E. Moss Herbarium, University of the Witwatersrand. Funding for this study was provided by California Polytechnic State University, North-West University (Potchefstroom), and the Sub-Saharan Africa Fulbright US Scholar Program.

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