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Nutrient and metal concentrations in *Nepenthes macfarlanei* Hemsl. (Nepenthaceae) from a Malaysian montane forest

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Abstract

Palaeotropical pitcher plants (*Nepenthes*) are a fascinating evolutionary case but their nutrient relationships are poorly known. To determine nutrient and metal concentrations in *Nepenthes macfarlanei* from Peninsular Malaysia, and contribute to our understanding of nutrient relationships in this genus, plants were sampled from the Genting Highlands – a disturbed montane forest location. Whilst many foliar nutrients showed typical concentrations, the foliar N concentration was, surprisingly, greater than most lowland species although the N:P ratio (*c.* 24) indicated nitrogen limitation in line with other studies on *Nepenthes*. Of particular note was the variable but high (< 240 µg g⁻¹) lead concentrations that have not been reported in carnivorous plants before. This data adds to our understanding of the nutrient relationships of carnivorous plants and shows that they may accumulate high concentrations of certain metals.

Keywords: carnivorous plants; lead; nutrient limitation; plant-soil relationships; pollution

Introduction

The montane forests of Peninsular Malaysia are a rich source of plant diversity (Saw, 2010). One example is the Genting Highlands, well-studied due to its proximity to the capital city Kuala Lumpur (Piggot, 1977; Stone, 1981; Chua and Saw, 2001; Bedawi *et al.*, 2009; Ng *et al.*, 2012). Whilst much of the area has been converted to buildings to service the tourist industry, there are still fragments of disturbed and natural vegetation. Of the 14 species of *Nepenthes* found in Peninsular Malaysia (McPherson and Robinson, 2012; Ghazalli *et al.*, 2020; Tamizi *et al.*, 2020), three species, *viz.* *Nepenthes macfarlanei* Hemsl., *Nepenthes ramispina* Ridl. and *Nepenthes sanguinea* Lindl. (along with natural hybrids between them) are found here (Shivas, 1983; Chua and Saw, 2001; Bourke, 2003). These pitcher plants present a fascinating case of plant evolution whereby they have developed a container ('pitcher') at the end of their leaf blades to catch insects and other nutritious materials to supplement their soil-derived nutrients (Thorogood *et al.*, 2018). Through examination of nutrient concentrations and nutrient ratios, it appears this genus of pitcher plants are nitrogen limited (Brearley and Mansur, 2012; Mansur *et al.*, in revision). However, there is little data from montane *Nepenthes* species to further support this suggestion. Additionally, there is little data on metal concentrations in *Nepenthes* species (but see van der Ent *et al.*, 2015) and the current development in this area may lead us to use this genus for biomonitoring of pollution in the future. The objective of this study was to determine

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nutrient and metal concentrations in *Nepenthes macfarlanei* and contribute to our understanding of nutrient relationships in this genus.

Materials and Methods

Description of the study site

The Genting Highlands are found at 3°26'N, 101°47'E in Peninsular Malaysia on the Selangor-Pahang border at around 1750 m a.s.l. and based on a granitic geology (Whitmore and Burnham, 1969). Tropical montane forest is the dominant vegetation found in the area although around the summit has been heavily disturbed by roads and high-rise buildings for the development of the well-known tourist resort.

Sampling design

Three plants of *Nepenthes macfarlanei* (Figure 1) were sampled from around the summit of Gunung Ulu Kali (the Telecom Tower) in February 2011. A single leaf (all leaves were estimated to be less than one year old) was collected from each plant, dried in a forced air oven and stored dry until analysis.



Figure 1. *Nepenthes macfarlanei* at the Genting Highlands, Malaysia

Experimental procedures

About 650 mg of leaf material was digested in 2.5 ml concentrated sulphuric acid with a lithium sulphate/selenium (100:1) catalyst at 375 °C for 4 hours, diluted with deionized water, and analysed for

nutrient concentrations on a Dionex ICS-5000 Ion Chromatography System (for N) or a Thermo iCAP 6300 Duo Inductively Coupled Plasma Optical Emission Spectrometer (for all other elements).

Results and Discussion

The results for the macronutrients showed typical concentrations (Figure 2) although nitrogen was high when compared with other studies (Osunkoya *et al.*, 2007; Brearley and Mansur, 2012), which is surprising given that nitrogen availability generally decreases with elevation in tropical regions (Tanner *et al.*, 1998). High nitrogen concentrations at this location could be due to localised deposition from traffic and other sources. However, the other macronutrients fell within the range for other *Nepenthes* species from Borneo (Osunkoya *et al.*, 2007; Brearley and Mansur, 2012; van der Ent *et al.*, 2015; Mansur *et al.*, in revision). The nitrogen-to-phosphorus ratio was around 24 suggestive of nitrogen limitation (using the criteria of Ellison, 2006) although was wider than other studies from lowland (Osunkoya *et al.*, 2007; Brearley and Mansur, 2012) or submontane (Mansur *et al.*, in revision) elevations – this was due to the high nitrogen rather than low phosphorus. There is very little data on micronutrients in *Nepenthes* species but, again, our data (Table 1) were all within the range for typical ‘healthy’ plants (Pugnaire, 2001) across a range of habitats and were similar to previous studies from peat swamp forest (Brearley and Mansur, 2012) but were lower than concentrations from plants growing in ultramafic soils where greater foliar nickel, chromium and cobalt were shown (van der Ent *et al.*, 2015). Granitic soils do not present high concentrations of these metals, but the acidic montane soils can increase the availability to plants of many metals. However, *Nepenthes* seems to exclude these metals to a certain extent, possibly due to their small root systems that limits nutrient uptake (van der Ent *et al.*, 2015). Metal concentrations within the *Nepenthes macfarlanei* plants were variable and, interestingly, lead was much higher than other studies with c. 240 $\mu\text{g g}^{-1}$ as a maximum concentration which is particularly high and could be due to the density of traffic at this location. Highly variable concentrations of these micronutrients suggest that uptake via roots may not be the main pathway of uptake and direct deposition via leaf surfaces or via the pitchers may be more important (Rotkittikhun *et al.*, 2006). The high lead concentration from this study site contrasts markedly with a site in central Borneo where lead was below detection limits in the majority of samples but there was a single plant of *Nepenthes albomarginata* with 19.6 $\mu\text{g g}^{-1}$ foliar lead (Brearley, unpubl. data). Adlassnig *et al.* (2009) have shown that pitcher plants other than *Nepenthes* take up the metallic elements iron and manganese, so the uptake from insects highly enriched in such metals could be another possibility but would need additional data on elemental concentration in insects to test this.

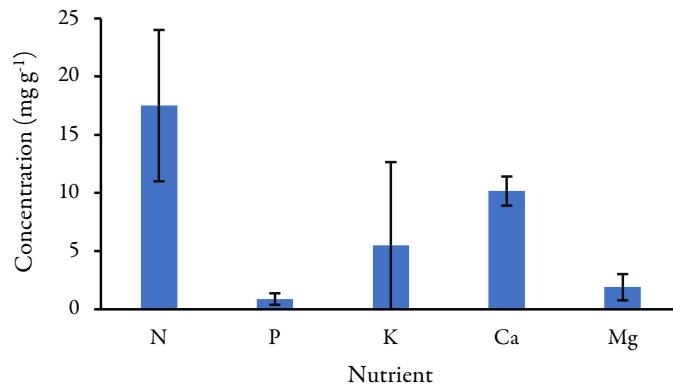


Figure 2. Macronutrient concentrations (mean \pm standard deviation) in *Nepenthes macfarlanei* at the Genting Highlands, Malaysia

Table 1. Metal concentrations (mean \pm standard deviation) in *Nepenthes macfarlanei* at the Genting Highlands, Malaysia; metals are ordered by their concentration in the leaf tissue

Metal	Concentration ($\mu\text{g g}^{-1}$)
Mn	240 \pm 139
Al	98.0 \pm 85.0
Pb	80.6 \pm 139.7
Zn	60.3 \pm 68.5
Cu	4.94 \pm 5.35
Ni	1.84 \pm 0.65
As	1.78 \pm 1.11
Co	0.10 \pm 0.18

Although there is considerable amount of protected forest in the Genting Highlands, *Nepenthes* species are threatened by informal plant collection as well as for construction activities. However, there is increased interest in *Nepenthes* conservation in the area (*e.g.* The Star, 2016) so we hope for positive conservation outcomes for this genus into the future.

Conclusions

This paper adds another data point to our understanding of *Nepenthes* pitcher plant nutrient relationships and further suggests primary nitrogen limitation of this genus whilst noting additional micronutrient composition of the plants and the potential to take up metallic elements to unexpectedly high concentrations.

Authors' Contributions

Conceptualization (FQB); Data curation (FQB); Formal analysis (FQB); Investigation (FQB); Methodology (FQB); Project administration (FQB); Resources (FQB); Validation (FQB); Visualization (FQB); Writing - original draft (FQB); Writing - review and editing (FQB). The author read and approved the final manuscript.

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Conflict of Interests

The author declares that there are no conflicts of interest related to this article.

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