Uptake of lead by *Nepenthes* via their pitchers

Elizabeth M. Cusick • Department of Natural Sciences • Manchester Metropolitan University • Chester Street • Manchester • M1 5GD • UK • cusicklizzie67@gmail.com

Francis Q. Brearley • Department of Natural Sciences • Manchester Metropolitan University • Chester Street • Manchester • M1 5GD • UK • f.q.brearley@mmu.ac.uk

Keywords: assimilation, contamination, metals, pitcher plants
Received: 5 September 2025
https://doi.org/10.55360/cpn551.ec025

Abstract: To assess whether *Nepenthes* can take up contaminant metals via their pitchers, and if this is more efficient than root uptake, we conducted an experiment in which we added lead (Pb)-contaminated maggots or Pb solution to pitchers, or Pb solution to the soil. We found that adding Pb to the pitchers increased pitcher Pb concentrations but there was no indication that Pb added to the soil was taken up by the plants, nor was Pb transferred from the pitchers to the leaves or roots. This study indicates that *Nepenthes* can, indeed, take up non-essential pollutant metals via their pitchers.

Introduction

Carnivorous plants are dependent upon their insect trapping and digestion structure for at least part of their nutrition, but particularly the key plant nutrients nitrogen (N) and phosphorus (P) (Adamec & Pavlovič 2018). However, insects and other prey items will also contain a range of other elements: many of these will be one of the 15 essential plant nutrients, but others may be non-essential or contaminant metals accumulated by the prey items. Most studies of metals in plant-soil-arthropod systems have examined metal transfer from plants to arthropods (Tibbett *et al*. 2021; Joubert *et al*. 2024), but the volant nature of many insects makes it possible for them to transfer metals back to carnivorous plants. One of the first studies to show this was that of Moody & Green (2010) who found that *Sarracenia* was able to take up cadmium (Cd) and copper (Cu) from contaminated insects in an experimental feeding study. More recently, in a similar vein, Dennett *et al*. (2023) showed how *Sarracenia* plants growing in the wild were able to accumulate elements from contaminant dust in Canada, and Brearley (2021) showed high lead (Pb) uptake in *Nepenthes macfarlanei*, although it was difficult to assign the source of the contaminant metal in this latter study. Other studies on *Nepenthes* have also shown high concentrations of certain non-essential metals (Mansur & Brearley 2024; Brearley *et al*. 2026) indicating that they can take these metals up in some cases.

It has been put forward that carnivorous plants have a limited root system (Adlassning *et al*. 2005) so *Nepenthes* pitchers might serve a very similar function in terms of nutrient uptake; if pitcher plants rely primarily on certain nutrients obtained via their pitchers, we might consider that the roots' necessary function would be the uptake of water and essential nutrients that are available from the soil at sufficient amounts. This hypothesis matches the structure of *Nepenthes* roots as they are fine hair roots. However, the question remains if pitcher plants can take up elements other than essential nutrients through their pitchers. To answer this question, we examined if *Nepenthes* can take up the non-essential metal Pb and assessed if the pitcher uptake pathway is more efficient at this than the root uptake pathway.

Methods

To assess the different possible uptake pathways of Pb into the plants we grew *Nepenthes* × *ventrata* and gave them Pb via three pathways: i) Pb-contaminated maggots, ii) Pb solution into the pitchers and iii) Pb solution to the roots. To create Pb-contaminated maggots we injected 10 µl of a 60 mg l^{-1} $Pb(NO_3)_2$ solution into commercially-obtained green bottle (*Lucilia sericata*) maggots: one maggot (each containing 0.6 µg of Pb) was then fed to one pitcher per plant in this treatment. To add Pb solution via the pitchers, 10 µl of the solution noted above was added directly into one pitcher per plant, and to add Pb solution via the soil, 10 µl was added to three points on the soil before being watered in with 10 ml deionised water. Plants were grown in a temperature-controlled room (25°C) by an easterly-facing window with a 12-hour day/night cycle and supplemented by a light with a 60 W bulb as the experiment was conducted during the northern hemisphere winter. The plants were misted with deionised water every two to three days ensuring that the pitchers contained enough liquid for digestion. After one month of growth, plants were harvested, divided into pitchers, leaves, and roots, washed free of contaminating material and dried at 105°C for 24 hours. Samples of c. 80 mg were then digested in 10 ml of nitric acid in a CEM Mars Xpress microwave, diluted to 50 ml with deionised water and analysed on a Thermo iCAP 6300 Duo inductively coupled plasma optical emission spectrometer. LGC7162 Strawberry Leaves were used as a certified reference material from which the mean recovery of Pb was 94%.

Results

We found significant differences in pitcher Pb concentrations among the three treatments (Fig. 1; $F = 5.72$, $p = 0.013$). Plants that had been fed with Pb-contaminated maggots took up the greatest amount of Pb: in fact, on average, all of the Pb added was taken up by the plants when it was added via this pathway (Fig. 1). When Pb was added via a solution, approximately half of the added Pb was taken up (although this value was not significantly different from either the maggot-to-pitcher pathway or the control; Fig. 1). When the Pb was added to the soil, the pitcher Pb concentrations were not significantly greater than the control (Fig. 1). We did not find significant differences in leaf or root Pb concentrations among the treatments ($F = 0.26$, $p = 0.85$) although mean root Pb was greater than leaf Pb (2.92 ± s.e. 0.20 versus 0.99 ± s.e. 0.18 mg kg^{-1}; $F = 46.7$, $p < 0.001$). There were no indications of Pb toxicity with additions of Pb-containing maggots or solutions.

Discussion

We show here that *Nepenthes* × *ventrata* can take up the non-essential element Pb via its pitchers. The greatest Pb uptake occurred when it was added within a maggot (although this was not significantly greater than when added via a solution) with all of the added Pb taken up. It, therefore, appears that *Nepenthes* has an uptake pathway for Pb via the pitchers as has also been reported for the metals iron (Fe) and manganese (Mn) (Adlassning *et al*. 2009). Although Pb is toxic to plants at high concentrations, it seems that *Nepenthes* does not regulate its uptake at the low amounts added in this study. That the greatest uptake of Pb was found when maggots were added (rather than Pb being added as a solution) suggests assimilation from the maggot in a co-transport with its digestion products rather than Pb contamination from the Pb-containing solution (which was not present in this treatment). If Pb were taken up independently, we would most likely expect metals in a more soluble form to be taken up more readily in contrast to what we found here, and our findings also contrast with the work of Butler *et al*. (2008) who found greater uptake of isotopically-labelled N when added to *Sarracenia purpurea* via a solution rather than via isotopically-enriched prey. However, it is known that the addition of An invertebrate food source may trigger production of digestive enzymes and digestion products that may make the plant able to take up additional nutrients. (Saganová *et al*. 2018).

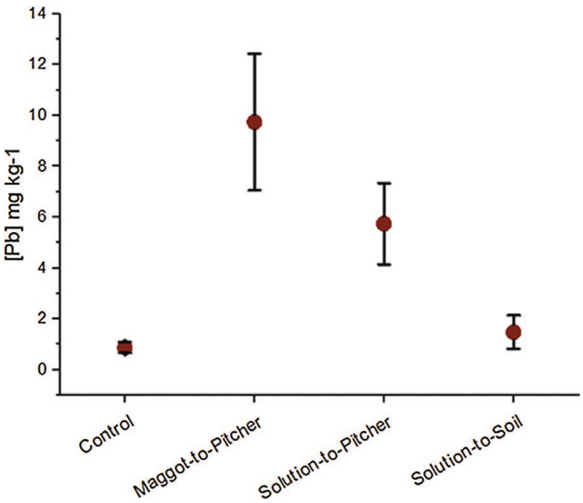

Figure 1. Pitcher lead concentrations in *Nepenthes* × *ventrata* after addition of a lead-contaminated maggot (Maggot-to-Pitcher) or a solution containing the same amount of lead to the pitcher (Solution-to-Pitcher) or soil (Solution-to-Soil). Values are mean ± standard error.

In this study, Pb was not transferred from the pitcher to the leaves or roots although an earlier study (Brearley 2021) found notably high Pb (240 mg kg^{-1}) in *N. macfarlanei* leaves in the field in Peninsular Malaysia (although possibly from dust as the leaves were not washed). There was also Cu transfer to the roots of *Sarracenia leucophylla* in a study where Cu was added as a solution to the pitchers (Moody & Green 2010). In that study, Moody & Green (2010) found that concentrations of Cu and Cd were actually greater in the roots than in the shoots when these metals were added to the pitchers of *S. leucophylla* although there were more consistent correlations between the dose of metal applied and the shoot, rather than root, concentrations.

There was no indication of Pb uptake via roots. This may have been due to *Nepenthes* having roots that are inefficient in the uptake of this metal or, probably more likely, was due to adsorption of the small volume of Pb-containing solution onto soil particles thus preventing easy uptake from this soil. Other field-based studies have shown uptake of metals by *Nepenthes* (Brearley 2021; Mansur & Brearley 2024; Brearley *et al*. 2026) and *Sarracenia* (Gotelli *et al*. 2008; Dennett *et al*. 2023) but it was not possible to attribute this to either pitcher or root uptake pathways in any of those studies.

In conclusion, we clearly show here that Pb can be assimilated by *Nepenthes* via pitchers and this further ‘expands the menu’ to additional non-essential metals for this fascinating plant genus.

Acknowledgements: Thanks to Natalie Devine, Kane Norton, and Graham Tinsley for assistance with technical aspects of this study.

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