The physiological responses of two aquatic plants, Alternanthera philoxeroides (Alligator Weed) and Nasturtium officinale (Watercress) to heavy metal exposure

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Research Article

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Abstract

Alternanthera philoxeroides is a nonnative invasive plant species that has spread throughout the wetland ecosystem in the southern part of the United States. Its invasion is facilitated by its ability to thrive in a wide range of abiotic conditions. *Nasturtium officinale* is an aquatic plant that is sensitive to relatively low amounts of pollution and is most commonly found in springs and shallow bodies of water. While *A. philoxeroides* tolerates organic pollution and heavy metals, *N. officinale* exhibits stress at low levels of pollution. We examined the effects of copper and lead on the antioxidant enzyme response of *A. philoxeroides* and *N. officinale*, which was measured using a benchtop luminometer. *Alternanthera philoxeroides* antioxidant enzyme production was unaffected by increasing concentrations of both copper and lead. The antioxidant enzyme response of *N. officinale* showed a significant increase when plants were exposed to 10 and 25 ppm lead. Endogenous peroxidase concentrations of the control plants were also compared showing that *A. philoxeroides* possessed a significantly higher concentration of peroxidases than *N. officinale*. We hypothesize that a higher endogenous peroxidase concentration may be a mechanism that hyperaccumulator plants use to tolerate inhospitable environmental conditions.

Introduction

One of the biggest concerns for wetland ecosystems is the increasing levels of anthropogenic contamination, including organic and heavy metal pollutants (Keskinkan et al. 2002). Aquatic copper contamination results from mining, agricultural practices (Yruela 2005), and wastewater effluent (Yeh et al. 2009) while lead contamination results from historic leaded gasoline combustion, leachate from lead pipes, industry, and mining activities (Keskinkan et al. 2003). Soil copper concentrations resulting from agriculture can range between 5–20 ppm with heavily contaminated soils exhibiting copper concentrations as high as 100 ppm (Panagos et al. 2018), and though copper applications occur in the surface horizon, significant amounts of copper contaminate groundwater (Mirlean et al. 2008). Concentrations of lead, even in soils contaminated by mining, rarely exceed 1 ppm with most concentrations less than 0.5 ppm, but bioaccumulation and magnification of lead concentrations in crop products and human consumption can expose people to concentrations up to 4.245 mg/day (Dike et al. 2020).

Heavy metals, such as copper and lead, can harm native species, cause a decrease in plant and insect reproduction, and cause a decrease in overall biodiversity (Pylon-Smits 2005). Physiologically, excess metal concentrations in plant tissues inhibit the photosystem electron transport pathway, biochemistry, and plant growth (Ferenandes and Henriques 1991; Yruela 2005), but some plants, such as *Alternanthera philoxeroides*, have evolved a tolerance to these toxicants (Ansari and Sharma 2017) and can be used for phytoremediation of heavy metals from contaminated sites.

Using plants to remediate heavy metal contaminated systems has been a successful and cost-effective strategy (Fritioff and Greger 2005; Alvarado et al. 2008; Yeh et al. 2009; Ansari and Sharma 2017). Aquatic plants, such as *A. philoxeroides* and *Nasturtium officinale*, are known to bioaccumulate heavy metals including copper, lead, and chromium (Naqvi and Rizvi 2000, Kara 2005). *Alternanthera philoxeroides* is native to the temperate regions of South America and can survive diverse environmental conditions within terrestrial and aquatic ecosystems (Zuo et al. 2012a). However, In the United States, *A. philoxeroides* is a nonnative invasive plant species that has spread rapidly throughout wetland ecosystems where it has been known to displace native plants, clog waterways and industrial pipes, and alter community dynamics (Reddy and Raju 2008) by negatively impacting algae (Zuo et al. 2012b). *N. officinale* is native to Eurasia but is considered a mildly invasive plant that is restricted to relatively clean springs and shallow bodies of water (Howard and Lyon 1952) and is considered a bioindicator of stream health. In this study, plant physiological effects of copper and lead exposure were compared using *A. philoxeroides*, a plant tolerant to aquatic pollution (Pal and Kundu 2014), and *N. officinale*, a plant that is sensitive to low concentrations of aquatic pollution (Zurayk 2001).

Both *N. officinale* and *A. philoxeroides* were exposed to 1, 5, 10 ppm concentrations of copper with *A. philoxeroides*, because of its tolerance to copper, also exposed to 100 ppm copper. Concentrations of 10, 25, 50 ppm of lead were used for both plant species with all exposures occurring over a 72 hr period. Increased peroxidase production, resulting from stress, was measured using a benchtop luminometer. *N. officinale* showed an increase in peroxidase enzyme production with the lowest concentration of lead while *A. philoxeroides* showed no significant increases in peroxidase concentration with either metal or metal concentration. A comparison of the endogenous peroxidase concentration of both species of plants demonstrated that *A. philoxeroides* possessed a significantly higher enzyme concentration than *N. officinale*. We hypothesize that an increased antioxidant enzyme system may be one way that hyperaccumulator plants tolerate adverse conditions.

Materials And Methods

Littoral *A. philoxeroides* plants were collected from Lake Baldwin on the Abraham Baldwin Agricultural College campus in Tifton, GA and rinsed in tap water in the lab. They were placed in beakers containing 500 mL of dechlorinated water in order to acclimate to laboratory conditions. *Nasturtium officinale* plants were grown in the laboratory from seeds in a hydroponic setup. All plants were fertilized using hydroponic fertilizer (MaxiGro™) and placed under grow lights on a 12 hr photoperiod. For heavy metal exposure experiments, cuttings of *A. philoxeroides* and *N. officinale* were used and allowed a week to establish adventitious root growth.

In root exposure experiments, six replicates of *A. philoxeroides* plants were exposed to 1, 5, 10, 100 ppm $CuSO_4$ or 10, 25, 50 ppm $Pb(NO_3)_2$, and six replicates of *N. officinale* plants were exposed to in 1, 5, and 10 ppm $CuSO_4$ or 10, 25, 50 ppm $Pb(NO_3)_2$ for a 72 hr exposure period. Copper concentrations exceeding 100 ppm for *A. philoxeroides* and 10 ppm for *N. officinale* caused macroscopic necrosis (e.g. wilting, chlorosis) while concentrations of lead exceeding 50 ppm were lethal to both plants. Results were compared to six replicates of control plants in water without $CuSO_4$ or $Pb(NO_3)_2$ added. A pH between 6.5–7 was maintained using $NaHCO_3$. After the plants had been exposed to metal solutions for 72 hrs, whole plants were crushed using a manual food processor and mortar and pestle in order to liberate antioxidant enzymes. 1 ml of distilled water was added to the plant slurry, and 1 g of the crude slurry was placed in a benchtop luminometer for measurements. The luminometer was programmed to inject 100 µL of 3% hydrogen peroxide into the slurry with each measurement. After the slurry was placed in the luminometer, a one-minute waiting period occurred before recording luminescent measurements in order to discount residual photosystem-produced photons. Ten luminescent measurements were taken for each sample with averages of measurements reported. The luminometer measures the light produced when peroxidases from the plant slurry react with hydrogen peroxide injected by the luminometer. Only peroxidases, endogenous or produced as a result of plant stress, cause a luminescent reaction when hydrogen peroxide is broken down into water and an oxyradical (Beals and Byl 2014).

Statistics

IBM SPSS statistics software was used to generate a one-way ANOVA with a Tukey-Cramer post hoc test for statistical analyses of the control compared to heavy metal treatments for both *N. officinale* and *A. philoxeroides* at $\alpha = 0.05$. A Student's t-test was used to compare reference level antioxidant enzyme concentrations of *N. officinale* and *A. philoxeroides* at $\alpha = 0.05$ prior to heavy metal exposure.

Results

Though peroxidase production appears to increase with increasing concentrations of copper in both *N. officinale* and *A. philoxeroides*, no significant differences were found comparing treatments to the control (Fig. 1). *Nasturtium officinale* demonstrated a significant increase in peroxidase enzymes in response to lead concentrations of 10 ppm ($p < 0.001$) and 25 ppm ($p = 0.047$) compared to the control (Fig. 2) while *A. philoxeroides* showed no significant increases in peroxidase production. A comparison of endogenous peroxidase production of *N. officinale* and *A. philoxeroides* prior to metal exposure demonstrated that *A. philoxeroides* possessed a significantly higher peroxidase concentration than *N. officinale* ($p = 0.02$) (Fig. 3). Percent change in peroxidase enzyme production due to toxic exposure was calculated by comparing the enzyme response of unexposed control plants to the average enzyme response of all metal concentrations of the treatment. *Alternanthera philoxeroides* demonstrated a 17% change in peroxidase production when exposed to copper and a 5% change when exposed to lead. *Nasturtium officinale* exhibited a 29% change in peroxidase production when exposed to copper and a 54% change when exposed to lead.

Discussion

To prevent tissue damage from toxic metal exposure, hyperaccumulator plants, such as *A. philoxeroides*, possess metal-chelating proteins and transporters that enable these plants to sequester heavy metals within vacuoles as well as an antioxidant enzyme system to help mitigate damage from oxidants (Ansari and Sharma 2017; Usman et al. 2020). An assortment of antioxidant enzymes including catalase, superoxide dismutase, and peroxidase are produced as a result of increased oxidants (Usman et al. 2020) due to toxic exposures. This study focuses on peroxidases produced by plants exposed to lead or copper. The method used here exploits the chemiluminescent reaction produced when liberated peroxidases react with hydrogen peroxide with increased luminescent intensity indicating that increased peroxidases were produced during the 72 hr toxic exposure.

Lead, in concentrations exceeding 30 ppm, is generally toxic to most plants (Usman et al. 2020), but this study has shown that *A. philoxeroides* can tolerate lead concentrations up to 50 ppm while exhibiting no antioxidant peroxidase response. In comparison to *N. officinale*, which displayed macroscopic evidence of stress including wilting and chlorosis with copper concentrations exceeding 10 ppm and lead concentrations greater than 50 ppm, *A. philoxeroides* showed no visible evidence of stress.

Both plants showed an increase in peroxidase production when they were exposed to copper though these results were not significantly different from the unexposed control. With lead exposures, however, *N. officinale* showed a significant increase in peroxidase concentrations following exposure to 10 ppm lead while *A. philoxeroides* demonstrated no increase in peroxidase activity at any concentration of lead. The percent change in peroxidase production of all copper treatments compared to the control for *A. philoxeroides* was 17%, and only 5% for lead concentrations. *Nasturtium officinale* exhibited an overall 29% change in peroxidase production for copper exposures and 54% change in peroxidase production in lead exposures compared to the control. This demonstrates that both copper and lead caused more of an antioxidant enzyme response in *N. officinale* compared to *A. philoxeroides*.

A comparison of the endogenous peroxidase concentration in control plants showed that *A. philoxeroides* possesses a significantly higher peroxidase concentration than *N. officinale* prior to exposure treatments. We hypothesize that in addition to previously described protective mechanisms, hyperaccumulators may also have a higher concentration of endogenous antioxidant enzymes in comparison to other plants. A comparison of the antioxidant enzyme systems of a broad range of hyperaccumulators would help to develop a comprehensive understanding of the protective mechanisms these plants use to live in relatively inhospitable environments.

Declarations

Ethics approval and consent to participate: Not applicable

Consent to Participate: Not applicable

Consent to Publish: Not applicable

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All authors contributed to the study, conception, and design. Material preparation, data collection, and analysis were performed by Christopher Beals, Hannah King, and Ginger Bailey. The first draft of the manuscript was written by Christopher Beals, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

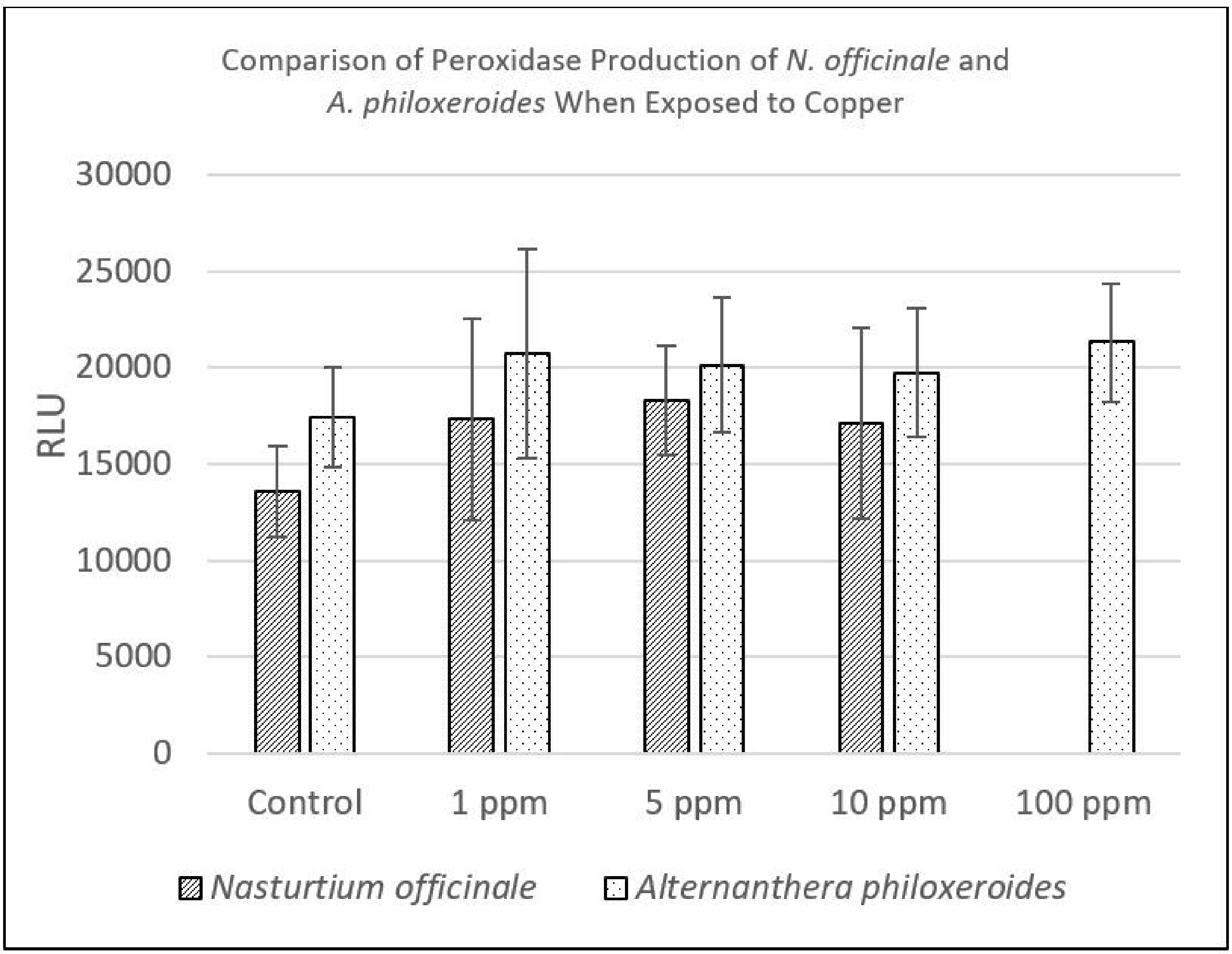

Figure 1

Peroxidase activity, reported in Relative Luminescent Units (RLU), of plants following exposure to four concentrations of copper (Control, 1, 5, 10, 100 ppm) over a 72 hr period. Ten luminescent measurements were taken with each plant (n = 6) with averages of readings and replicates shown. The error bars represent standard deviation. A one-way ANOVA comparing peroxidase activity of plants to the unexposed control was used $\alpha = 0.05$. No significant differences occurred, though both plants show elevated responses due to copper exposure.

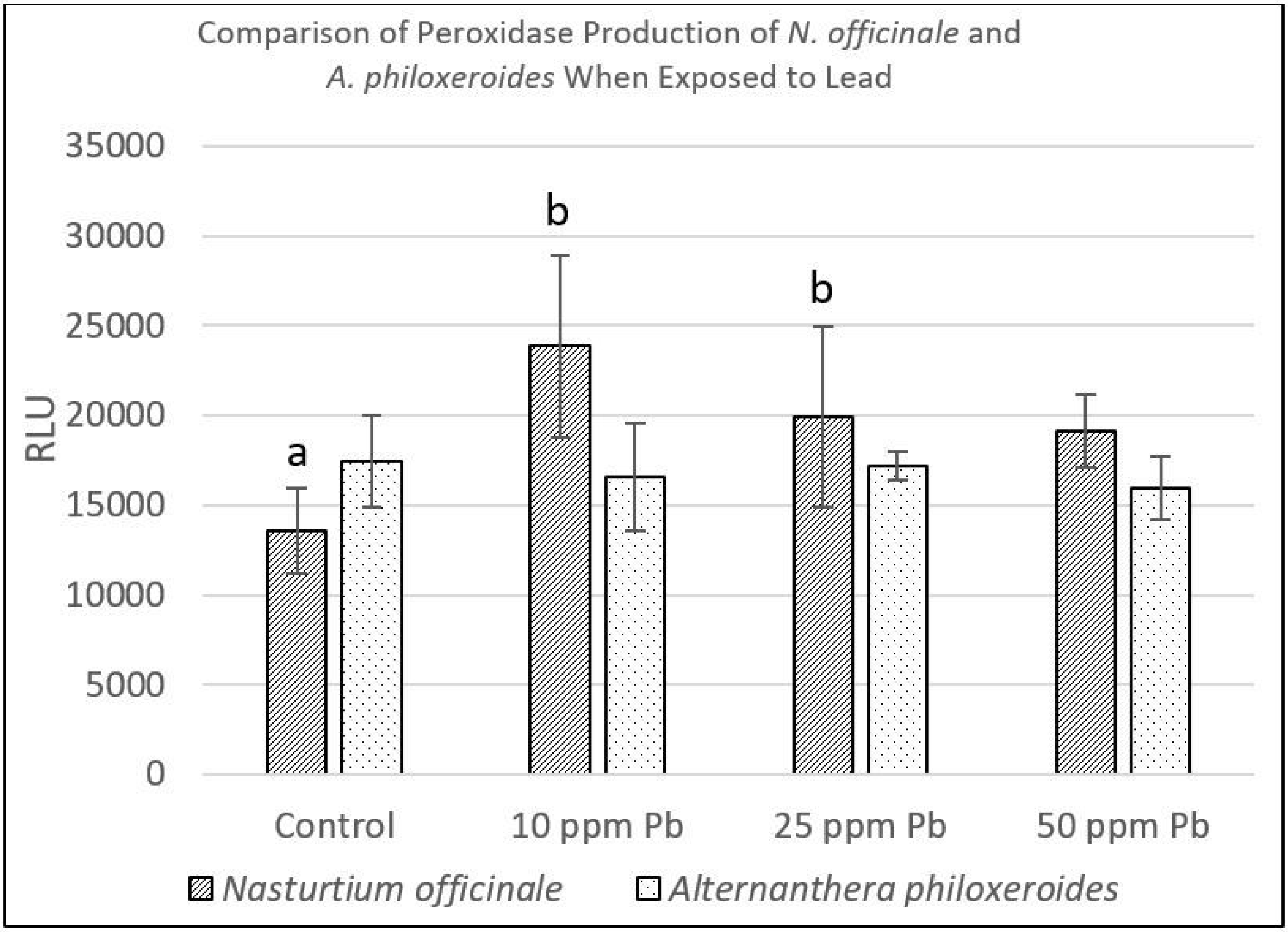

Figure 2

Peroxidase activity, reported in Relative Luminescent Units (RLU), of plants following exposure to three concentrations of lead (Control, 10, 25, and 50 ppm) over a 72 hr period. Ten luminescent measurements were taken with each plant (n = 6) with averages of readings and replicates shown. The error bars represent standard deviation. Different letters indicate statistically significant differences determined by a one-way ANOVA comparing peroxidase activity of plants to the unexposed control and Tukey-Kramer multiple comparisons test at $\alpha = 0.05$. *Nasturtium officinale* showed a significant increase in peroxidase activity following exposure to 10 ppm ($p < 0.001$) and 25 ppm ($p = 0.047$) lead compared to the control plants.

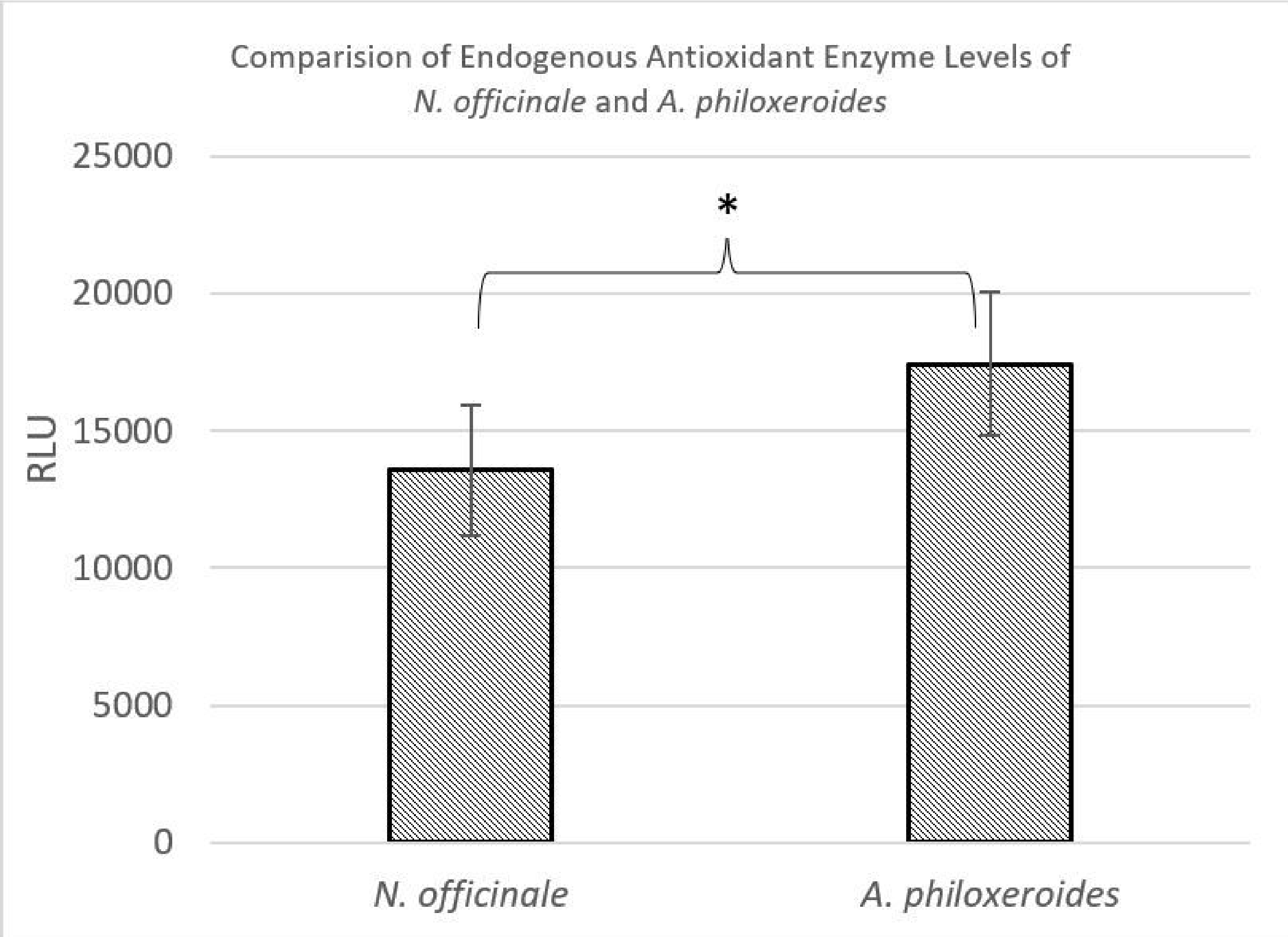

Figure 3

Peroxidase activity, reported in Relative Luminescent Units (RLU), of unexposed control plants. Ten luminescent measurements were taken with each plant ($n = 6$) with averages of readings and replicates shown. The error bars represent standard deviation. The asterisk is used to indicate a statistically significant difference determined by a Student's T-test at $\alpha = 0.05$. *Alternanthera philoxeroides* possessed a significantly higher endogenous peroxidase concentration than *N. officinale* ($p = 0.02$).