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Phytoremediation potential of Dilang-aso (*Pseudelephantopus spicatus* (Juss.) Rohr) in lead-contaminated soil

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Abstract

This study investigated *Pseudelephantopus spicatus*, a member of the family Asteraceae that has not yet been explored in terms of its phytoremediation potential. This study utilized two pot experiments: (1) plant tolerance to lead (Pb)-contaminated soil, and (2) plant mechanism for Pb uptake. For the plant tolerance experiment, plants were exposed to different lead concentrations, and the shoot length, width of the largest leaf, and number of leaves were recorded. For the Pb uptake experiment, *P. spicatus* plants were transplanted in polyethylene bags containing 1 kg of 500 ppm Pb-treated soil, and were observed every five days for 30 days. Results showed that *P. spicatus* plants in Pb-treated soil had significantly shorter shoot length, smaller width of largest leaf, and reduced number of leaves, as compared to the control treatment. The Pb uptake of *P. spicatus* also increased with longer exposure time. However higher Pb concentration was still observed in the soil (218.57 ppm) compared to the Pb concentration in roots (29.49 ppm) and shoot (17.07 ppm). Thus, the *P. spicatus* plant demonstrated tolerance as a Pb excluder, and may not be a good candidate for phytoremediation. However, other studies may investigate whether the phytoremediation potential of *P. spicatus* can be improved by observing the effects of different Pb concentrations, higher time intervals, or the use of chelating agents and fertilizers.

Keywords - Asteraceae, bioconcentration factor, lead excluder, phytoremediation, phytostabilizer, translocation factor

Introduction

Heavy metals are one of the main pollutants in the environment which can be present in air, water or soil. These heavy metals include cadmium, copper, nickel and lead. Lead (Pb) is one of the most toxic metals that pose a danger to all living organisms, including humans (Sahu & Elumalai, 2017). Lead ions are toxic metal ions that may accumulate in the brain, liver, kidney and bones, and can hamper the normal functions of cells in the body leading to devastating consequences in health. A high level of Pb exposure may cause coma, convulsions and even death. Lead exposure may also cause anemia, hypertension, renal impairment, immunotoxicity and toxicity to the reproductive organs (WHO, 2018). Studies have also shown that associated lead overexposure will result to decreased intelligence, reduced short-term memory, reading disabilities, and deficits in vocabulary (Juberg et al., 1997; Mason et al., 2014). Despite its harmful effect to humans, Pb is still widely used globally due to its versatility arising from its physico-chemical properties. It is used for batteries, pigments, cable sheathing, radiation shielding, insecticides, and as an anti-knock agent (Royal Society of Chemistry, 2020). However, its continuous use and non-biodegradable nature cause Pb to accumulate in the environment with increasing hazards. This heavy metal accumulation poses risks to both living organisms and the ecosystem, particularly in water and soil. Remediation of heavy metal contamination is costly when done

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artificially. For this reason, the tendency is to leave the contaminated area untreated, thereby putting the ecosystem at risk, and eventually threatening human health. Thus, it is important to find ways to lessen the concentration of Pb in the soil through a low-cost and sustainable technology (Rigoletto et al., 2020).

One such technology is through phyto-remediation, a bioremediation process that uses plants to transform, immobilize and extract contaminants from the soil or groundwater (Rigoletto et al., 2020). It has been established that bioremediation, particularly phytoremediation and phytoextraction, is a good alternative for treating heavy metal contamination through the use of plants as a translocating medium (Tangahu et al., 2011). Several studies have reported the phytoremediation of heavy metal contaminated sites. Zhang et al. (2020) used *Paspalum conjugatum* for the phytoremediation of metal-contaminated rare-earth mining sites, and reported that the plant extracted the metal contaminants from the soil and significantly decreased Pb and Cd concentrations. Likewise, Lago-Vila et al. (2019) reported *Cytisus scoparius* as a Zn accumulator, Pb phytostabilizer, and Cd excluder species, making it able to restore soils from a Pb/Zn mine.

In the Philippines, phytoremediation studies have validated the efficiency of plants to accumulate nickel (Quimado et al., 2015), copper (Chua et al., 2019; Dahilan & Dalagan, 2017), gold (Alcantara et al., 2020), and mercury (Alcantara et al., 2020; Bretaña et al., 2019; Puzon et al., 2015). In the case of Pb, Napaldet and Buot (2020) found that the Pb uptake of aquatic plants *Pennisetum purpureum* and *Eleusine indicia* was only a small fraction of the Pb concentration in the water and soil medium. However, much of this Pb was translocated to the stem or leaves. This result is significant because most plants tend to localize Pb in roots rather than in their aerial parts (Mitra et al., 2020; Pourrut et al., 2011).

Species of the family Asteraceae had been investigated for their ability to accumulate heavy metals. Alirzayeva et al. (2006) recorded *Artemisia fragrans*, *A. scoparia*, and *A. caucasica* growing in contaminated sites that accumulated heavy metals in their biomass without toxicity symptoms. Another Asteraceae species, *Helianthus annuus* or sunflower, had been shown to be capable of Pb uptake, but the Pb concentration in the soil medium remained higher than that in the shoots and roots (Alaboudi et al., 2018; Forte & Mutiti, 2017). This present study was conducted to investigate the phytoremediation potential of another member of the Asteraceae plant family that had not yet been explored in terms of its phytoremediation potential. In particular, this study determined the suitability of *Pseudelephantopus spicatus* (*P. spicatus*), locally known as Dilang-aso, as a translocating medium. The plant is commonly found in many areas in the Philippines (Dichoso, 2012; Stuart, 2020).

The main objective of the study was to evaluate the *P. spicatus* as a potential phytoextractor for lead-contaminated soil. Specifically, the study aimed to (1) assess the tolerance of *P. spicatus* in contaminated soil with different lead concentrations; (2) determine Pb uptake of the *P. spicatus* within 30 days of observation; (3) measure the Pb concentration in plant roots and shoots, and soil; and (4) evaluate the phytoextraction potential of the plant after 30 days of propagation using bioconcentration and translocation factor.

Materials and Methods

Plant Identification and Propagation

P. spicatus seeds were collected from a netted experimental field set-up in Ugalingan, Carmen, Cotabato. The location was not adjacent to any agricultural area. The seeds were germinated for 10 days in a seed bed and grown for 75 days. Matured plants were sent to the Department of Biological Sciences and the Agronomy Department, University of Southern Mindanao, Kabacan, North Cotabato for identification and confirmation of the species.

Soil Sample Collection and Preparation

Top soil (0- to 30-cm depth) was collected from the grassland area of Purok 3, Ugalingan, Carmen, Cotabato. The collected soil samples were analyzed for its physical and chemical characteristics. Testing included moisture content, Pb analysis, pH determination and soil water holding capacity (WHC). The collected soil was air-dried, pulverized and passed through a 2-mm mesh size standard (mm) sieve to remove non-soil particulates.

Soil Treatment

The study was composed of two pot experiments: (1) plant tolerance to lead-contaminated soil, and (2)

plant mechanism for lead uptake. Analytical grade lead (II) nitrate ($Pb(NO_3)_2$) pellet was used to prepare all solutions for soil treatments.

For the plant tolerance experiment, the amount of 1.44, 4.32, 7.19, and 11.51 g $Pb(NO_3)_2$ was dissolved in 1L distilled deionized (DD) water. The solutions were mixed with 9 kg moisture-free soil batches in polyethylene bags that corresponded to Pb concentrations of 100, 300, 500, and 800 ppm in soil. For 0 ppm Pb 1L DD water was mixed with 9 kg soil. Treated soils were mixed with 100 mL distilled water every day for three days to homogenize the mixture. The soils were sun-dried for five days to obtain moisture-free soil mixtures. For plant lead uptake, an amount of 31.50 g $Pb(NO_3)_2$ was dissolved in 5 L DD water, which was mixed with 63 kg moisture-free soil to obtain 500 ppm Pb in soil. Soil conditioning was conducted in the same way as with the previous preparations.

Pot Experiments

Plant Tolerance Experiment

Three (3) 85-day old matured *P. spicatus*, of approximately the same height, were transplanted in 1 kg treated soil (Figure 1) in a polyethylene bag. Five (5) treatments (0, 100, 300, 500, and 800 ppm) of lead in soil were replicated thrice. Each replication was distributed in three blocks (Figure 2). Within each block, plants were arranged by increasing lead concentration, with pots randomly assigned for each day of exposure. The shoot length, width of the largest leaf, and number of leaves for each of the three plants in each replicate were determined.

Lead Uptake Experiment

Two (2) *P. spicatus* matured plants were transplanted in 1 kg of either untreated or 500 ppm Pb-treated soil in a polyethylene bag. For each of the two treatments (untreated soil, 500 ppm Pb-treated soil), and for each of the seven observation periods (Day 0, 5, 10, 15, 20, 25, 30), three replicates were prepared, for a total of 42 set-ups (Figure 3). After each observation period, soil and plant samples were collected and prepared for Pb content analysis.

Housing and Maintenance

The pot experiments were performed in a net-covered plant house in natural light. The set-up was watered twice daily with 50 mL of DD water. To ensure no water leakage, the set-up was placed in a catch basin. In case of fallen leaves during the growing period, the leaves were transferred into a paper bag dedicated to each treatment. The weeds that grew inside the pots were removed from the set-up.

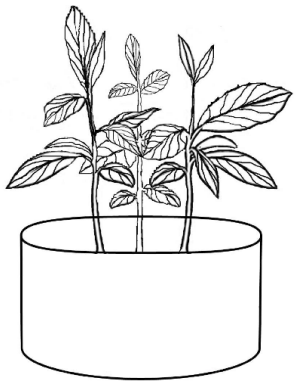

Figure 1. Three (3) matured *P. spicatus* plants in a polyethylene bag in 1 kg treated soil (illustrated by R. Cabantug).

Bioconcentration Factor (BCF) and Translocation Factor (TF)

From the Pb uptake experiment setup, the plant and soil samples from the 30th day exposure of *P. spicatus* in 500 ppm Pb-treated soil were used to determine the BCF and TF. The shoots and roots of the plants were segregated and prepared for Pb content analysis. The BCF and TF were computed as follows:

$$BCF = \frac{\text{concentration of lead in the plant roots}}{\text{concentration of lead in soil media}}$$

$$TF = \frac{\text{concentration of lead in the plant shoot}}{\text{concentration of lead in the plant roots}}$$

The BCF and TF values indicate the capacity of a plant species to remediate metals in metal-contaminated soils. If BCF < 1, the plant can only absorb, but not accumulate metals (Embrandiri et al., 2017). These plants can be categorized as an excluder, and survive through avoidance or restriction of uptake of heavy metals (Adriano, 2001; Rigoletto et al., 2020). If BCF > 1, the plant can be considered an accumulator (Embrandiri et al., 2017). Some of these accumulators are hyperaccumulators, capable of taking in metal ions in the thousands of ppm; for lead, these are plants that can accumulate more than 1000 ppm (Baker & Brooks, 1989).

Plants with BCF > 1 can further be classified,

depending on whether the TF is greater or less than 1. Plant species with both BCF > 1 and TF > 1 have a phytoextraction potential. That is, metals are accumulated in the shoots or the aerial part of the plant which can be eliminated by harvesting (Lago-Villa et al., 2019). Plant species with BCF > 1 and TF < 1 indicate a phytostabilization potential. This means that the metals accumulated by the plant tend to be immobilized in the roots and reduce the bioavailability of lead in the soil media (Rigoletto et al., 2020).

Characterization of Soil Sample

Soil pH Determination

Twenty grams of the soil samples were placed in a beaker and weighed using an analytical balance. Forty mL deionized water were added and the mixture was stirred thoroughly for 5 to 10 sec and allowed to equilibrate for 10 min. The solution was stirred again and then a pH reading was taken using a pH meter (Barrett et al., 2009).

Soil Water Holding Capacity

A 50 g moisture-free soil sample was placed on filter paper fixed into a funnel. The funnel was gently tapped to compact the soil sample and 100 mL DD water was added into the soil. The set-up was covered with a damp cloth to minimize evaporation and left overnight to drain the excess water. A 10 g saturated soil sample was accurately weighed in a beaker and oven dried to reach constant weight. The set-up was replicated thrice. The soil water holding capacity (WHC) was computed as follows:

$$\%WHC = \frac{FC - ODW}{ODW} \times 100$$

where: WHC = water holding capacity, FC = weight of saturated soil (g), and ODW = weight of oven-dried soil (g).

Preparation of Plant Samples for Flame Atomic Absorption Spectrophotometry (FAAS)

The plants were uprooted and washed using tap water and rinsed with DD water. The samples were soaked in 0.2 N hydrochloric acid (HCl) for 10 min. A whole plant was considered as one sample for lead uptake and BCF experiment, while the

Block 1					Block 2					Block 3				
T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
D3	D2	D1	D1	D2	D1	D3	D2	D3	D1	D3	D2	D2	D3	D1
D2	D1	D2	D2	D1	D3	D2	D3	D1	D2	D1	D3	D3	D2	D2
D1	D3	D3	D3	D3	D2	D1	D1	D2	D3	D2	D1	D1	D1	D3

Figure 2. Set-up for plant tolerance experiment. Soil treatments (T1=0, T2=100, T3=300, T4=500, T5=800 ppm) with three replications (block). Each treatment has assigned day (D1=0, D2=15, D3=30 days) of exposure.

Block 1		Block 2		Block 3	
T1	T2	T1	T2	T1	T2
D7	D1	D2	D6	D5	D3
D5	D3	D3	D4	D3	D7
D3	D5	D6	D2	D2	D4
D1	D7	D7	D1	D7	D5
D2	D6	D5	D3	D6	D1
D4	D4	D4	D5	D1	D3
D6	D2	D1	D7	D4	D6

Figure 3. Set-up for plant uptake experiment. Soil treatments (T1= negative control, T2= 500 ppm Pb-treated soil) with three replications (block). Each treatment has assigned day (D1=0, D2=5, D3=10, D4=15, D5=20, D6=25, D7=30 days) of exposure.

shoots and roots were separated as samples for translocation factor. Samples were dried in an oven at 70°C until constant weights were obtained. Dried samples were cooled and pulverized using mortar and pestle. The accurate mass of 0.5 g test samples were placed in porcelain crucibles and ignited in a furnace at 550°C for 5 hours. The crucibles were allowed to cool at room temperature. The crucibles were added with 3 mL of 5N nitric acid (HNO_3) to dissolve the ash and the solutions were evaporated to dryness using hotplate inside the fume hood. The dried crucibles were cooled, moistened with DD water, added with 3 mL of concentrated HCl, and evaporated to dryness. The crucibles were cooled, added with 2 mL of 2N HNO_3, and stirred using a rubber policeman to dissolve the residue salts. The mixtures were filtered through Whatman No. 42. The filtrates were collected into a 50 mL volumetric flasks and diluted to volume using DD water. The test solutions were analyzed in Flame-AAS for Pb concentration (Motsara & Roy, 2008).

Preparation of Soil Samples for FAAS

Ten grams of dried samples were accurately weighed and placed in centrifuge tubes. The samples were added with 20 mL diethylenetriaminepentaacetic acid (DTPA) extracting solution. The soil samples were mixed for 2 hours in a mechanical shaker and filtered through Whatman No. 42 filter paper. The filtrate was collected for the determination of total Pb concentration using Flame AAS (Motsara & Roy, 2008).

Analysis of Total Pb using FAAS

Shimadzu A-7000 FAAS with air-acetylene fuel (2.0 L min^{-1} flow rate) was used for the analysis of total Pb. A deuterium lamp was used as continuous source and the detector was set with the wavelength of 283.3 nm. Reference standard solution (1000 ppm) was used to prepare 100 ppm working standard solution that was used to prepare the calibration standard solutions 10.0, 5.0, 2.0, 1.0, 0.5, and 0 ppm. The calibration curve was established using calibration standards. Background correction was conducted before the analysis. Blank samples were run before, between, and after sample batches were read as standard check. Soil and plant samples were prepared in triplicate and all samples were read thrice with an acceptable reading of <1.5% RSD. The final concentration of Pb in plants and soil samples was calculated using the corrected concentration of the sample using the blank concentration, final volume of the solution, and the mass of the sample, using the formula

$$\text{Pb (mg kg}^{-1}) = \frac{[\text{Csoil/plant} - \text{Cblank}] \times \text{Vsol'n}}{\text{Msample}},$$

where:

Csoil/plant = Concentration (mg L^{-1}) of soil or plant obtained from FAAS;
Cblank = concentration (mg L^{-1}) of blank sample
Vsol'n = Volume (L) of solution
Msample = Mass (kg) of sample

Statistical Analysis

Plant measurements (length of shoot, width of the largest leaf, number of leaves) and plant Pb uptake were based on the average of all plants (nine plants in the plant tolerance experiment, and six plants in the Pb uptake experiment). Significant differences across the varying Pb concentrations were determined using a one-way ANOVA. Lead uptake of plants in treated and untreated soil media, and the lead concentration of treated and untreated soil were compared using a t-test. The relationship between lead uptake concentration of plant samples and the days of exposure to lead contamination was analyzed using linear regression. In the same manner, regression analysis was performed to investigate the relationship between lead concentration of the soil media after harvest and the number of days of exposure to lead contamination.

Results

The pH and water holding capacity of the soil samples before Pb contamination, as well as the soil pH after Pb contamination are shown in Table 1. The WHC of the soil material used in this study was 65.12%. The soil pH before and after treatment were slightly acidic.

Plant Growth Response to Lead Concentrations

Table 2 shows the average shoot length, width of the largest leaves, and number of leaves of *P. spicatus* at different Pb concentrations. Across all concentrations, the length of the shoot and the width of the largest leaf increased from Day 0 to Day 30, but the number of leaves decreased at the higher Pb concentrations. It can be observed that the various concentrations of Pb resulted to morphological

variations. At Day 0, there was no significant difference in shoot length (p = 0.35), width of largest leaf (p = 0.57), and number of leaves (p = 0.11) across plants exposed to varying Pb concentrations. However, after 30 days, there was a significant difference in shoot length (p < 0.001), maximum leaf width (p = 0.02), and number of leaves (p < 0.001) across different concentrations. The Tukey HSD test revealed that plants of the control had the longest shoot length compared to plants in all other treatments. For width of the largest leaf, the only significant pairwise difference was observed between the control treatment and the plants exposed to 800 ppm Pb. For number of leaves, there was a significant difference between the control and the plants exposed to 300, 500, or 800 ppm Pb, and

Table 1. Soil water holding capacity (n = 12), lead concentration (n = 3), and pH (n = 9) before lead contamination (Day 0) and after lead contamination (Day 30).

	Before lead contamination	After lead contamination
Water holding capacity (%)	65.12	--
Soil pH before treatment	6.88	6.45

--not measured

Table 2. Average plant growth parameters of *P. spicatus* in different concentrations of lead at different time intervals (n=9).

	Treatment	Days of exposure to lead contamination		
		0	15	30
Shoot length (cm)	0 ppm	6.97	9.610	12.35
	100 ppm	6.70	8.24	9.50
	300 ppm	6.97	8.65	9.59
	500 ppm	7.72	7.95	9.00
	800 ppm	7.60	7.40	8.26
Width of largest leaf (cm)	0 ppm	3.05	3.87	4.70
	100 ppm	2.95	3.48	4.16
	300 ppm	2.92	3.55	4.26
	500 ppm	2.82	3.35	4.22
	800 ppm	2.88	3.19	3.79
Number of leaves	0 ppm	7.11	7.56	7.78
	100 ppm	6.78	6.44	7.00
	300 ppm	6.67	6.22	5.89
	500 ppm	5.78	6.00	5.78
	800 ppm	6.22	6.21	5.22

between the plants exposed to 100 ppm and plants exposed to 800 ppm Pb.

Lead Uptake of *P. spicatus*

The mean Pb uptake of *P. spicatus* grown in the untreated (0 ppm) and treated soil (500 ppm) at different days of exposure are shown in Table 3. Plants grown in untreated soil showed variability in Pb uptake on different days of exposure. However, plants grown in high level of Pb treated soil (500 ppm Pb) increased in Pb uptake in longer exposure days.

Figure 4 shows the increasing trend of the absorbed Pb in the plants as the number of exposure days increased. The Pb uptake of plants grown in treated soil showed a positive linear trend as the number of days of exposure increased (r = 0.97). The regression model also shows a regression formula of Pb concentration uptake = 0.51 (days) – 0.74. This suggests that the Pb concentration of a *P. spicatus* increases by 0.51 ppm for every day of

Table 3. Average lead uptake (ppm) of *P. spicatus* grown in untreated and treated (500ppm) soil media at different periods of exposure.

Days	Lead concentration (ppm)	
	Untreated	Treated (500 ppm)
0	1.26	1.26
5	0.91	1.49
10	1.83	2.79
15	2.37	5.23
20	3.96	8.65
25	5.65	13.48
30	3.66	14.92

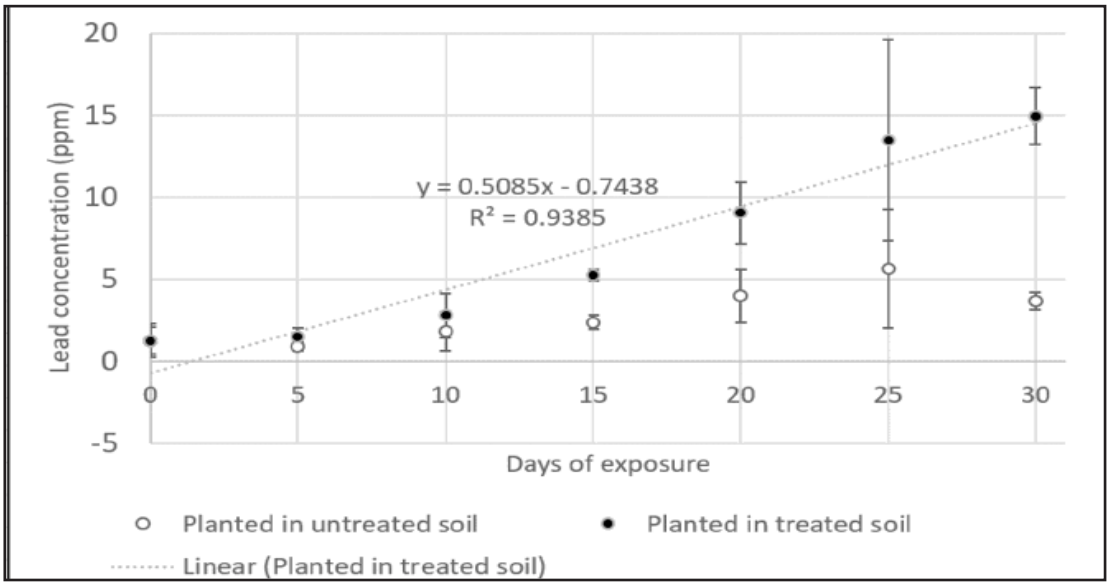

Figure 4. Regression of the absorbed lead of *P. spicatus* as a function of the days of exposure to contamination.

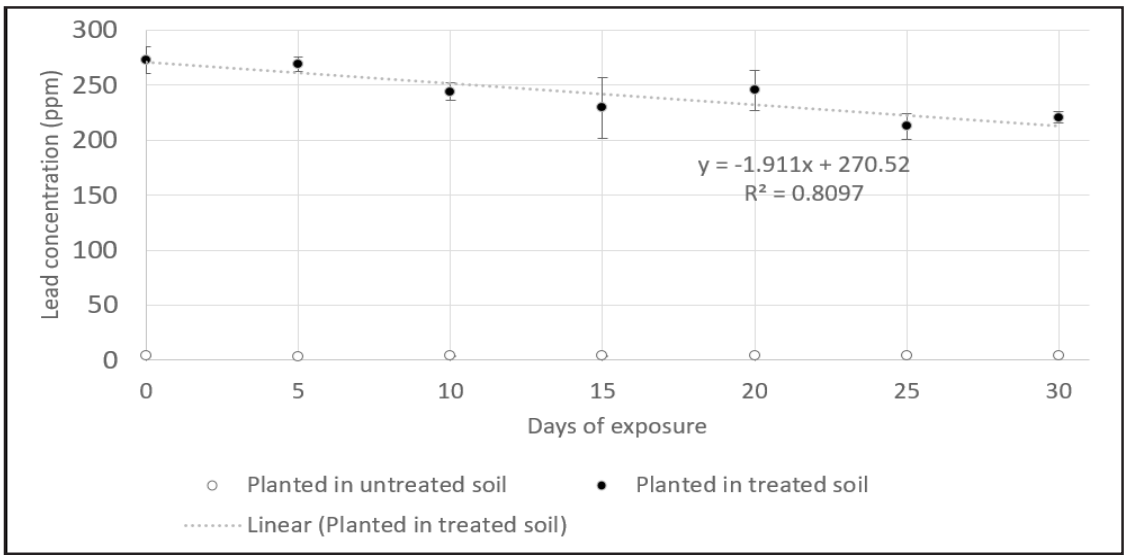

Figure 5. Regression of the absorbed lead in the soil media as a function of the number of days.

exposure within the 30-day observation period.

Lead Concentration of Soil Media

The Pb content of the soil samples is shown in Figure 5. The Pb concentration of the untreated soil was 3.67 ppm at Day 0, and ranged from 3 to 5 ppm across all plant samples analyzed for each day of observation. By contrast, the Pb concentration of the treated soil ranged from 210 to 275 ppm and the highest detected Pb concentration was at Day 0 with 272.65 ppm. The difference in the detected Pb concentration compared to 500 ppm soil treatment can be attributed to losses during the soil equilibration and conditioning stage, and the reduced Pb sequestration by DTPA due to strong Pb-soil and Pb-organic matter interactions (Ahmed et al., 2015; Blaylock et al., 1997), among others.

The untreated soil media planted with *P. spicatus* showed no detectable downward trend in the Pb concentration while there was a detectable downward trend in the Pb concentration in the lead-treated soil media. The downward trend in the Pb concentration is clear from how close the data points are to the negatively sloped regression line (r = 0.90). The net lead concentration reduction (y) of the treated soil as days (x) progressed from 0 to 30 days followed the equation: $y = -1.91x + 270.52$. The Pb concentration in the soil decreased by 1.911 ppm every day for 30 days. However, the downward trend was not consistent, as shown by the Pb concentration of the soil media measured at Days 15, 20, and 25, possibly due to some non-homogeneity in the soil.

There was significantly higher Pb content in treated soils in Day 0 as compared to that in Day 30 (p = 0.03). In other words, for the treated soil, there was a significant decrease in Pb content over the 30 days. For untreated soils, the Pb content of the soil media varied across the observed samples.

Bioconcentration Factor (BCF) and Translocation Factor (TF)

The mean Pb concentration in the shoot and the root of the plants grown in the untreated and treated soil after 30 days of exposure, as well as the Pb concentration of the soil media are shown in Table 4. The Pb concentration in the roots is higher than that in the shoot, which indicates that lead was more stabilized in the root system of the plant. Plants grown in treated soil had significantly higher Pb content in shoots ($p < 0.001$) and in roots ($p < 0.001$), as compared to the Pb content in shoots and roots in the untreated soil. Table 5 shows the BCF and TF of the *P. spicatus* grown in treated and untreated soil. In treated and untreated soil media, both the BCF and TF of the treated *P. spicatus* were less than 1.

Discussion

This study investigated the phytoremediation potential for Pb accumulation of *P. spicatus*, due to its abundance and availability. The WHC of the soil

Table 4. Lead uptake of shoot and root of *Psedelephantopus spicatus* after 30 days of exposure to 500 ppm Pb-treated soil.

	Lead concetration (ppm)		
	Shoot	Root	Soil media
Untreated	1.64	3.27	4.03
Treated	17.07	29.49	218.57

Table 5. Bioconcentration factor and translocation factor of *Psedelephantopus spicatus* after 30 days of exposure to 500 ppm Pb-treated soil.

	Treatment	
	Untreated	Treated
Bioconcentration Factor (BCF)	0.82	0.14
Translocation Factor (TF)	0.50	0.58

sample in this study is within the ideal range (60-80%) for nutrient absorption of plants. The soil sample had an initial Pb concentration of 3.67 ppm which is well within the natural levels for Pb (< 50 ppm) (Pourrut et al., 2011; United States Environmental Protection Agency (USEPA), 2001).

This study has shown that when *P. spicatus* was subjected to lead-contaminated soil, it developed significantly shorter length of shoot, smaller width of largest leaf, and reduced number of leaves compared to the control treatment. Elevated Pb concentration exposure has a defoliating effect in agreement with the observations reported Mitra et al. (2020) and Pourrut et al. (2011). Despite inhibited growth, the shoot length and width of the largest leaf still increased over the 30-day duration. This indicates some tolerance of *P. spicatus* to Pb concentrations higher than 100 ppm. As a reference point, the US Environmental Protection Agency generally recommends a screening level of 400 ppm for residential soils (USEPA, 2014).

The mechanisms for plant tolerance of *P. spicatus* may be deduced from the BCF value of 0.14 for plants in treated soil media (29.49 ppm in roots and 17.07 ppm in shoots on soil containing 218.57 ppm Pb). These values suggest that the plant excludes lead effectively and prevents significant uptake into the roots. This is one mechanism by which plants tolerate heavy metals (Adriano, 2001). The BCF for plants in untreated soil media is even higher, indicating that higher levels of Pb concentration does not result to proportionately higher values of Pb uptake. The plant can therefore be classified as an excluder, where metal concentrations remain low over a wide range of soil concentrations (Baker, 1981); they survive through avoidance and are not good candidates for phytoremediation (Adriano, 2001).

The results of this study reflect those of previous studies on the phytoremediation potential of other species of the family Asteraceae. The BCF of *H. annuus* ranged from 0.2 to 0.4 in lead concentrations of 10, 20, 40, 80, 100, and 200 ppm (Alaboudi et al., 2018). In soils with higher Pb concentration (10,000 ppm) the BCF was found to be 0.03, with an average uptake of 62 ppm in the stems and 37 ppm in the leaves (Forte & Mutiti, 2017). Despite this low BCF, the Pb concentration in the soil decreased from 10,000 ppm to 2,912 ppm.

There are plants in the Asteraceae family with BCF > 1. Alizaryeva et al. (2006) studied plants growing on contaminated sites and found three plant species with BCF > 1. In their research, the highest value for BCF was 12.1, for the *Artemisia scoparia* plant that was collected along a steel plant in Azerbaijan. Plants outside the Asteraceae family

have also been shown to be good accumulators of Pb under certain conditions. These include *Glycine max* L. (BCF (whole plant) = 3.13 in 10 ppm Pb soil) (Aransiola et al., 2013) and *Sida acuta* (BCF (whole plant) = 2.1 in 1000 ppm Pb soil, with fertilizer) (Oseni et al., 2016). Plants growing in contaminated sites have also been shown to accumulate lead, with BCF values greater than 50 (Zhang et al., 2020).

The TF was found to be 0.579, indicating that more of the Pb uptake was found in the roots than in the shoots. This is consistent with findings from other lead phytoremediation studies where majority of the absorbed lead is accumulated in the roots (Lim et al., 2004; Oseni et al., 2016; Pourrut et al., 2011; Yoon et al., 2006). Lead can be precipitated as an insoluble phosphate in the rhizosphere, which minimizes its translocation to the stem and leaves (Baker & Brooks, 1989).

The soil in this study was tested for pH and WHC, but not for organic matter or other properties. The soil pH before and after contamination was within the range of 5.5-7.5. For this pH range, very little lead is available to plants even if the plant is genetically capable of lead accumulation (Blaylock et al., 1997). Dube et al. (2001) explained that the soil particles contain hydroxyl groups (^{-}OH) that exist on the surface area especially when the soil pH is less than 6. The hydrogen (H) is replaced by a Pb^{2+} ion that can bond (not tightly held) to oxygen and thus the release the H^{+} ion. This process was observed in the study of Chen et al. (2018) when the concentrations of available Pb^{2+} ion in the soils were much higher in acid soils (pH < 6.5) than in alkaline soils (pH > 7.5). Reddy and Patrick (1977) reported that the Pb uptake of rice plants within the roots and the shoots decreased with an increase in soil pH, which is due to the decrease of water-soluble Pb^{2+} ions.

Some soil properties that were not tested may be deduced from WHC. There are indications that the WHC of soil could be related to soil texture, structure, and organic matter, and contributes to the ability of plants to absorb minerals from the soil (Olorunfemi et al., 2016). Very high or very low WHC may not be favorable for plant growth. Inubushi et al. (1996) reported that in soils with more than 80% WHC, some minerals tend to undergo redox reaction and are converted to a form unavailable for plants to absorb. Further, in soils with less than 60% WHC, minerals tend to settle below the soil profile, making them inaccessible to the plant root system. Nath (2014) reported that WHC is positively correlated to clay and organic matter content, and negatively correlated to sand content. Based on the soil description set by Leonard (1980), the soil texture used in this study can be described as silt loam that has 65-69% WHC; and the mold was brittle when dry. Future studies must report factors that affect lead availability to plants, including soil properties and composition (texture, type, particle size, organic material, cation exchange capacity (CEC), iron oxides, heavy metals) and the plant's root structure, root mycorrhiza, exudates, and transpiration rate (Mitra et al., 2020).

Another limitation in this study was that the experiment for Pb uptake was restricted to only one Pb concentration (500 ppm) which was chosen because it was higher than the screening level for soils intended for food production (USEPA, 2014). However, varying soil Pb concentrations may result to differences in Pb uptake. For example, in Oseni et al.'s (2018) research, the BCF of *Sida acuta* exceeded 1 only in soils with Pb concentration of 1000 ppm, and not in soils with concentrations 0, 200, 400, and 800 ppm. Also, Sikka et al. (2010) observed that a higher Pb concentration in the soil led to higher Pb uptake by Indian mustard (*Brassica juncea* (L.) Czern). Future studies can consider the effect of soil Pb concentration on lead uptake to determine whether the lead uptake of *P. spicatus* can be improved.

Conclusion and Future Directions

This study has shown that *P. spicatus* grown in lead-contaminated soil had inhibited growth in comparison to the control treatment. The Pb uptake of plants grown in lead-contaminated soil increased as the days progressed. However, even after *P. spicatus* plants were exposed to lead-treated soil for 30 days, the Pb uptake in the roots and in the shoots were considerably low. The results imply that *P. spicatus* is an excluder at 500 ppm Pb soil concentration. Nevertheless, further studies on the phytoremediation potential of the *P. spicatus* plant, which is common in many regions of the Philippines (Dichoso, 2012), are warranted. Lead accumulation is exceedingly rare (Baker & Brooks, 1989) so additional research on improving Pb uptake would be relevant. Some strategies for increasing lead accumulation is through the use of chelating agents (Zhao et al., 2011), fertilizers (Oseni et al., 2016), or exposure to higher Pb concentrations (Aranisola

et al. 2013; Oseni et al. 2016). Future studies may also investigate *P. spicatus* plants growing in or near contaminated sites because such plants may demonstrate more tolerance and accumulate metals more readily as compared to plants sourced from non-contaminated areas (Islam et al., 2008).

Disclosure Statement

No potential conflict of interest was reported by the authors.

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