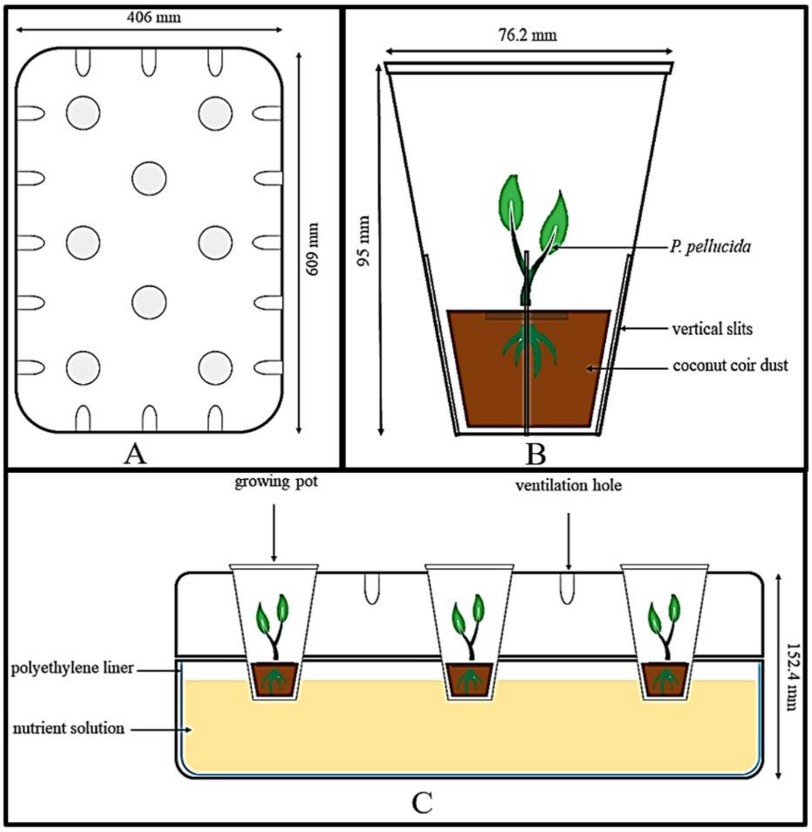

Figure 1. Schematic diagram of SNAP hydroponic system; (A) top view of the hydroponic culture box; (B) growing pots; (C) cross-section view of the hydroponic system

2.3 Monitoring of growth response

Growth response to lead contamination was determined by measuring the observable physical changes in the test plants, such as height, leaf area and number of leaves. Measurement was done periodically before and after Pb contamination. These factors are the indications that the tested plant species had undergone lead bioaccumulation in terms of physical changes. Dry weight of the plants was calculated using the formula:

$$\text{Dry Weight (g)} = \frac{\text{Total dry weight of plants in treatment (g)}}{\text{Total number of plants in treatment}}$$

2.4. Plant harvesting

At the end of the 28-day growth period, the *P. pellucida* plants were gently removed from each growing pot while carefully separating the coconut coir dust from roots. After which, they were rinsed with distilled water to remove adherent debris, taking extra caution to preserve as much root as possible. The roots of the plant were then gently separated from shoots (stems and leaves). Separately, the above ground and below ground parts were cut into smaller pieces and blot-dried. Root and shoot samples were separately placed in microwavable containers, labelled and subjected to oven-drying at 80^{o}C for 72 h. After which, the dried plant samples were weighed and acid-digested.

2.5 Sample preparation and analysis

The USEPA (2007) Method 7000B as modified by Atayese et al. (2009) was used in the preparation of plant tissues for lead-content analysis. Once the drying process is completed, the tissue was removed from the containers and ground using a mortar and pestle. Approximately, 0.5 g of homogenized powder (shoot or root) was transferred into a 100 mL conical flask and 5 mL of concentrated H_2SO_4 was added, followed by the addition of 25 mL of concentrated HNO_3. Then, the contents of the conical flask were heated on a heating plate at 100°C until a clear solution is obtained. After cooling, distilled water was added to the falcon tube to obtain a final volume of 25 mL. The filtered and rinsed solution was collected in a sterile, 25 mL capacity graduated falcon tube. Finally, the falcon tube was left to settle down for 24 h. Each tube was labelled and prepared for Flame Atomic Absorption Spectroscopy (Flame AAS) analysis.

2.6 Computation of absorptive capacity and tolerance parameters

In response to Pb exposure, the following heavy metal absorptive capacity and tolerance parameters were determined: survival rate (SR), tolerance index (TI), bioconcentration factor (BCF), translocation factor (TF) and lead metal uptake. These were calculated following the equations of Meeinkuirt et al. (2012), Zhivotovsky et al. (2011), Yaowakhan et al. (2005), Tanhan et al. (2007), Niu et al. (2007), and Vamerali et al. (2010):

1) Survival rate (SR): This is percentage of plants still alive after the experimentation period computed as:

$$\mathrm{SR} = \frac{\text{Final number of plants}}{\text{Initial number of plants}} \times 100$$

2) Tolerance index (TI): This is the ratio of dry weight in plants grown on contaminated solution and control plants grown on uncontaminated solution, and is expressed as a percentage using the formula:

$$\mathrm{TI}\ (\%) = \frac{\text{Dry weight of plant in Pb treatment (g)}}{\text{Dry weight of plant in control treatment (g)}} \times 100$$

3) Bioconcentration factor (BCF): This was determined by the ratio of lead concentration in plant tissues to the total metal initial concentration expressed in mg/kg. This was obtained using the following equation (Wilson and Pyatt, 2007):

$$\mathrm{BCF} = \frac{\text{Pb concentration in whole plant (mg/kg)}}{\text{Initial Pb concentration in solution(mg/L)}}$$

4) Translocation factor: This was used to evaluate the efficiency of *P. pellucida* in translocating the accumulated metal from its roots to shoots. The value was obtained by measuring the ratio in concentration in the aerial tissues and that in the roots, respectively, with the heavy metal content expressed as mg/kg. The following formula was used (Padmavathiamma and Li, 2007):

$$\mathrm{TF} = \frac{\text{Heavy metal concentration in shoot (mg/kg)}}{\text{Pb concentration in root (mg/kg)}}$$

5) Pb concentration: This is the ratio of the product of AAS reading and dilution factor to the weight of sample used for acid digestion. This was calculated using the formula:

$$\text{Pb accumulation} = \frac{\text{AAS Reading (μg/mL)} \times \text{Dilution Factor (mL/g)}}{\text{Weight of sample (g)}}$$

6) Pb uptake: It is the ratio of the lead concentration in the tissues of *P. pellucida* and the dry biomass of the plant after experimentation. This is determined by the following formula:

$$\text{Pb uptake (μg/plant)} = \text{Pb accumulation (μg/g)} \times \text{dry weight (g/plant)}$$

2.7 Statistical analysis

Paired Student's t-test was used to evaluate the growth response of the plant on Pb exposure. The dry weight was also compared using the said test. For heavy metal absorptive capacity and tolerance parameters, the values were computed based on given formula.

3. RESULTS AND DISCUSSION

3.1 Growth response of *P. pellucida* on lead (Pb) contamination

The growth response and performance of *P. pellucida* in the presence of Pb can determine its viability as a phytoremediator. The physiological parameters and growth response of *P. pellucida* upon lead-exposure is presented in Figure 2-4.

Throughout the experimental growth period, *P. pellucida* plants grown exclusively on nutrient solution were constantly growing, as noticed in the gradual and continuous increase in plant height, leaf number and leaf area (Figure 2-4). On the other hand, *P. pellucida* plants exposed to 500 mg/L of Pb were growing relatively at the same rate as the unexposed plants during the first two weeks of experimentation; however, manifestation of lead phytotoxicity appeared during the third week, where biomass production was affected as implied in the reduction of plant height, leaf number and leaf area of lead-exposed plants.

Relative growth of uncontaminated and contaminated plants became divergent during the third week of experimentation. Plants grown solely on nutrient solution depicted an upward slope of relative growth, exhibiting constant linear increment in height, and leaf number and area; whereas lead-contaminated plants showed a curb in the figures of plant height, leaf number and leaf area during the third week of exposure (Figure 2-4), mainly due to the slowed growth and death of some plants. Symptoms of lead phytotoxicity such as drooping, necrosis and leaf bleaching also started to manifest as early as the second week, while the indications became more apparent during the third and fourth week where lead-exposed plants started to wilt, lose turgidity and die.

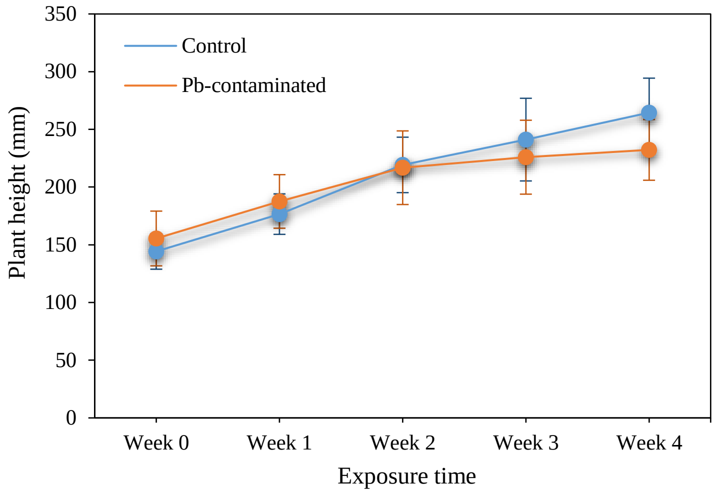

Figure 2. Effect of 500 mg/L Pb on the plant height of *P. pellucida.*

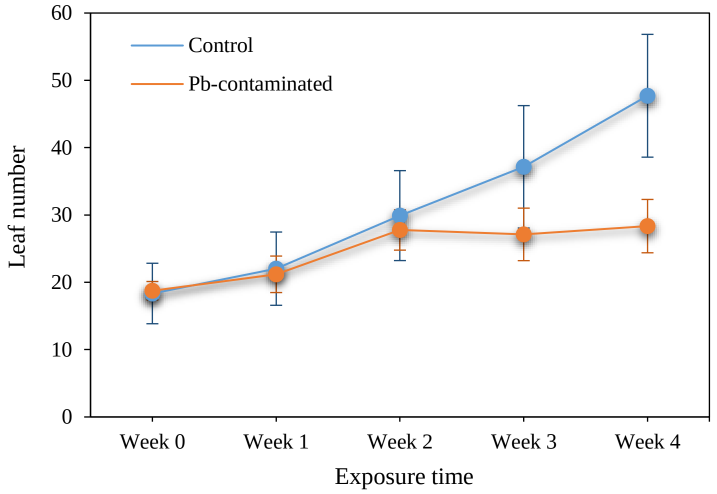

Figure 3. Effect of 500 mg/L Pb on the leaf number of *P. pellucida.*

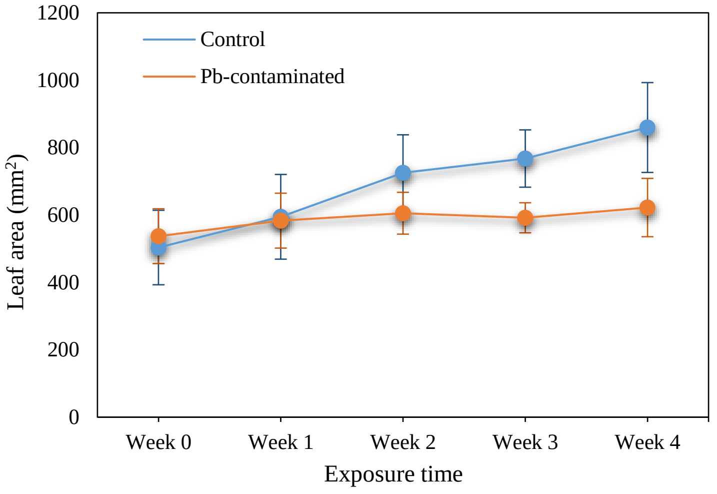

Figure 4. Effect of 500 mg/L Pb on the leaf area of *P. pellucida.*

Throughout the first two weeks of experimentation, the plant height, leaf number and leaf area of the plants were not significantly affected ($p>0.05$) by the presence of 500 mg/L of Pb concentration. This is because of the consistent increment of biomass produced in both treatments during the first two weeks. However, at the conclusion of the experimental period, final mean values for plant height, leaf number and leaf area were determined to be significantly different from each other ($p>0.05$) with respect to the presence or absence of 500 mg/L of Pb.

As plants grow in a contaminated environment, they tend to continue to absorb Pb and sequester the metal within their tissues, causing the toxicity symptoms in the plant to increase. In hydroponics experiment, *P. pellucida* grew well without the presence of Pb concentrations, and little to no morphological symptoms such as chlorosis and necrosis were observed. However, lead-exposed plants had reduced total biomass which is a result of decreased number of leaves, fresh and dry weight, and length of root and shoot. According to Belonias (2009), *P. pellucida* can tolerate lead levels of up to 400 ppm, with Pb-treated plants and the control showing comparably uniform vigorous growth during a 3-week experimental period. However, in this study, the 500 mg/L concentration had significant effect on the growth response of the plant. Same results were obtained by some other studies at the calculated lead concentration on different plants; root, shoot and leaf growth, fresh and dry biomass were critically reduced in *Pisum sativum*, *Zea mays*, *Paspalum distichum*, *Cynodon dactylon*, *Lycopersicon esculentum*, *Ipomoea aquatica*, *Phaseolus vulgaris*, and *Lens culinaris* (Nas and Ali, 2018; Jaja and Odoemena, 2004; Gothberg et al., 2004).

The result of the study shows that manifestation of Pb effect on plant is evident after two to three weeks. Manifestations of toxicity became apparent as the plants were increasingly exposed to the Pb. Visual examination of the plants exposed to Pb also shows signs of toxicity such as necrosis of leaf tip and darkened shoot base, pale-colored leaves, drooped and shrunken shoots and short and dark roots, with heavily shrunken shoot base (Figure 5).

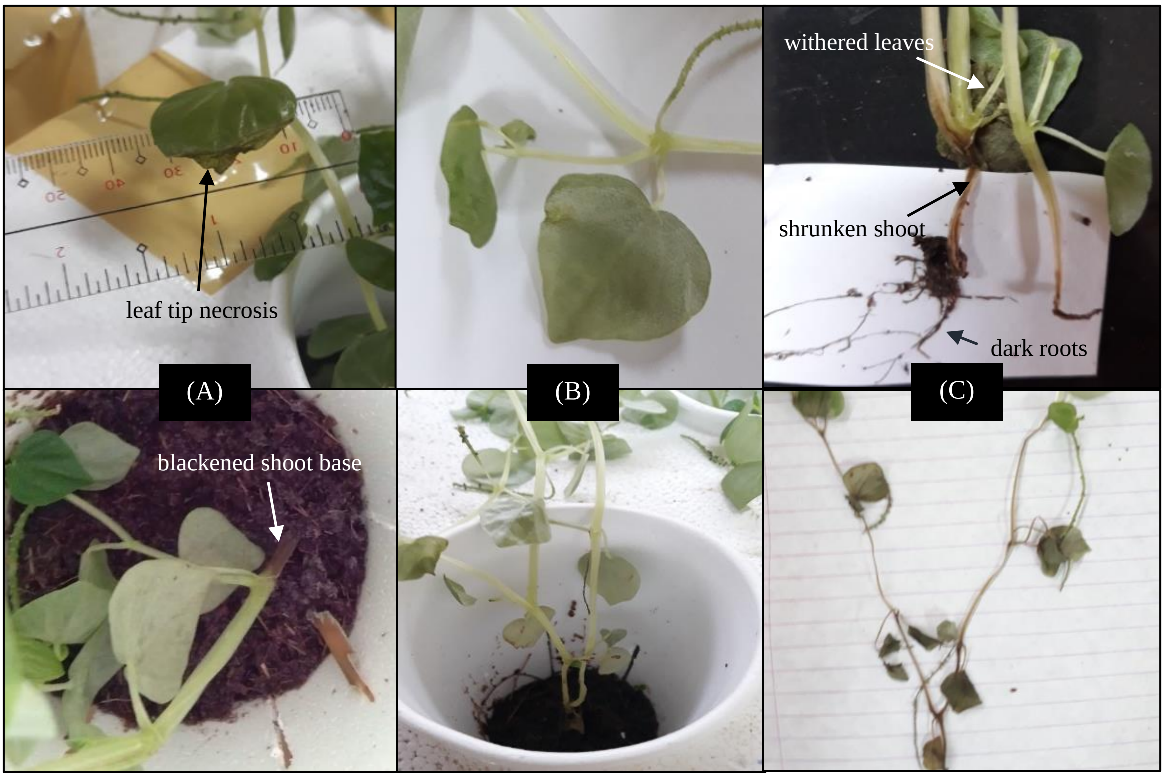

Figure 5. Lead-phytotoxicity symptoms during the four-week exposure period; (A) second week plants showing necrosis of leaf tip and darkened shoot base; (B) third week plants with pale-colored leaves and drooped and shrunken shoots; (C) final week plants showing short and dark roots, with heavily shrunken shoots base (top) and deceased plant (bottom).

These results and observations corroborate with the findings of by Sharma and Dubey (2005) and Nas and Ali (2018). According to them, the visual non-specific symptoms of Pb toxicity are rapid inhibition of root growth, stunted growth of the plant and chlorosis. This claim is also supported. They stated that Pb toxicity is manifested outwardly in the plant such as stunted growth, chlorosis and blackening of the root system. In addition, Pb inhibits photosynthesis, upsets mineral nutrition and balance, changes hormonal status and affects membrane structure and permeability. These disorders upset normal physiological activities of the plants (Nas and Ali, 2018).

It can also be noted in the result that symptoms of Pb toxicity were manifested by the plants in the later part of the experimental period. This may mean that the amount of Pb absorbed in the earlier part of the experiment is not yet considerable. According to Putra et al. (2016), the amount of Pb accumulation must be in considerable amount to inhibit plant metabolism before showing a visible phytotoxic and oxidative damage effect.

3.2 Lead tolerance of *P. pellucida*

Tolerance is an organism's ability to cope with heavy metals that are excessively accumulated within its body. The effect of lead on biomass production and the parameters for lead tolerance are presented in Table 1.

Results showed that plants exposed to Pb have a lower biomass compared to those plants that were not contaminated by Pb. The DW of *P. pellucida* was significantly affected ($p>0.05$) by 500 mg/L of lead. The low biomass may be attributed to the relatively lower plant height, number of leaves and leaf area. In addition, the dry weight reduction relates to high Pb concentration, since plants may have to use energy to cope with the high Pb concentration in their tissues (Karimi et al., 2012).

Table 1. Lead tolerance of *P. pellucida* after 28 days of exposure.

Treatments	Dry weight (g)			Lead tolerance parameters	
	Roots	Shoots	Total	Survival rate (%)	Tolerance index (%)
Control (0 mg/L)	0.43 ± 0.20^{a}	2.65 ± 0.95^{a}	3.08 ± 1.08^{a}	93.70	-
Pb-contaminated (500 mg/L)	0.21 ± 0.10^{b}	1.15 ± 0.37^{b}	1.35 ± 0.46^{b}	71.80	43.40

Note: Each value is mean of four replications ± standard deviation. Mean of each column indexed with different small letters denote a significant difference of relative leaf number between absence and presence of Pb concentration (500 mg/L) as determined by paired t-test at $p\leq0.05$.

Higher survival rate was also observed in the control group with 93.70%, while only 71.80% survival rate was observed in Pb contaminated plants. Result also shows that plants exposed to 500 mg/L Pb had a tolerance index (TI) of 43.40% which is relatively low. Herlina et al. (2020) suggested that plants with TI values greater than 100% reflect a net increase in biomass and tolerance-acquisition of the plant, whereas, TI values lower than 100% indicate a net decrease in biomass and a stressed plant condition. However, it was suggested by Zhivotovsky et al. (2011) and Wang et al. (2014) that a 60% TI criterion value indicates ability of plants to tolerate heavy metals. The recorded TI value for *P. pellucida* (43.40%) was far below 100%, indicating that the plants have become stressed throughout the experiment as evident in the retardation of growth of the plants.

Different plant species develop different mechanisms to tolerate excess levels of metals (Aini Syuhaida et al., 2014). The earliest mechanism is a synthesis of polysaccharide such as callose (β-1, 3 glucan) deposited on the outside of the cell membrane, thereby reducing the diffusion of heavy metal ions into the plant cell. In *in situ* applications and in soil experiments, plant roots secrete exudates into the soil matrix to chelate metals and to prevent their uptake inside the cells (Furini, 2012; Małachowska-Jutsz and Gnida, 2015). However, in a hydroponics experiment, this mechanism is hardly employed due to the roots being suspended in a liquid environment, thus preventing the roots from releasing the exudates and thereby reducing the resistance from lead uptake.

3.3 Lead accumulation and lead absorptive capacity of *P. pellucida*

Table 2 presents the accumulation and absorptive capacity of *P. pellucida* after 28 days of exposure to 500 mg/L to Pb. This lead absorptive capacity was measured in terms of Pb uptake (µg/plant), bio-accumulation factor (BCF) and translocation factor (TF).

Table 2. Lead absorptive capacity of *P. pellucida* after 28 days of exposure.

	Pb-accumulation (µg/g)		Lead absorptive capacity parameters		
	Roots	Shoots	Lead uptake (µg/plant)	BCF	TF
Pb-contaminated (500 mg/L)	158.60	43.20	272.43	0.40	0.27

Results showed that there is an accumulation of Pb in the tissues of P. *pellucida* plants. The amount of Pb accumulated in the roots (158.6 µg/g) is greater than the amount accumulated in the shoots (43.2 µg/g). This result implies that the roots system of the *P. pellucida* has the ability to absorb Pb; however, translocation into the shoot system might have been restricted. According to Mleczek et al. (2019), mechanisms of metal uptake and accumulation in plants have demonstrated that plants have the ability to translocate selected elements in its tissues, with the roots being the most capable organ in taking up significant quantities of Pb whilst simultaneously restricting translocation to hypogeal parts.

The Pb concentrations (158.6 µg/kg in shoots, and 43.2 µg/kg in roots) from this study were lower than Pb concentrations reported in shrubs; for example, *Chromolaena odorata* (L.) with Pb in shoots 1,721 mg/kg, and in roots 51,493 mg/kg (Niu et al., 2007). The results follow a similar trend as some other studies. The other common phenomenon is Pb accumulation in roots more than that in shoots and several previous studies, including this study, show the same pattern. Zhivotovsky et al. (2011) found that at the highest Pb concentration of 241 µM, *Salix lucida*, *Salix nigra*, and *Salix serissima* had higher Pb concentration in roots than in aerial tissue, such as wood, in shoot and in leaves. Liu et al. (2015) found that *Phyllostachys pubescens* grown in nutrient solution supplemented with 200 µM Pb contained higher Pb in the root (1,221 mg/kg) as compared to that in the stem (351 mg/kg) and in leaf (165 mg/kg).

The Pb uptake of *P. pellucida* plants was calculated to be 272.43 µg/plant. Meanwhile, BCF and TF values were recorded at 0.40 and 0.27, respectively. Ramana et al. (2021) stated that (BCF) and Translocation Factor (TF) are the defining parameter in phytoremediation by providing insight on metal uptake, mobilization and storage. Since, BCF and TF values are used to evaluate a plant's ability to accumulate and translocate heavy metals, and identify the suitability of plants for phytoextraction and phytostabilization (Niu et al., 2007; Wang et al., 2014), values >1 indicate that the plant has the potential for phytoextraction (Ali et al., 2013). With BCF criterion, *P. pellucida* shows relatively low potential for bioaccumulation.

According to Napoli et al. (2020), a high value of TF (TF>1) signifies promising ability of a plant to translocate heavy metals from roots to aerial tissues. On the other hand, a low value (TF<1) indicates a limited capacity of a plant to translocate the metal to aerial tissues. In this study, *P. pelludica* recorded TF value <1. This is indicative of *P. pellucida*'s low capacity to uptake high quantity of Pb, potentially classifying the plant as an excluder of Pb.

The uptake and translocation of a pollutant in plants depends on many factors: (1) the pollutant's concentration in the solution, (2) its efficiency to enter the root system, and (3) the rate of transpiration in the plants (Glime, 2017). Pepper elder plants are very reliant to the turgor pressure within their systems, and any disruption in this system might compromise the integrity of their structures and the plant in general.

In this study, *P. pellucida* was determined to have the capacity to accumulate and uptake lead in its tissues. On the other hand, the results showed values of phytoremediation parameters (SR, TI, BCF, TF) that are below the criterion for each respective parameter. This implies that, the plants were affected by the spiking of 500 mg/L of Pb. Despite this, the plants showed the capacity to uptake lead and still survive, which is promising considering that the concentration of lead used to spike the nutrient solution was relatively high. Nevertheless, it must still be taken into account that the experiment was conducted *ex situ* in a hydroponic setting with a single treatment. The present conditions might have affected the capacity of *P. pellucida* to survive, uptake and accumulate lead in its tissues, and different results may be obtained in a pot experiment.

4. CONCLUSION

Manifestations of toxicity became apparent as the plants were exposed longer to Pb. Prolonged exposure to Pb induced phytotoxicity symptoms and reduced biomass production, affecting plant height, leaf number and leaf area as implied in the curb of the respective growth parameters during the third week of exposure, which consequently caused retardation of growth. Lead-absorptive capacity parameters imply that the plant may be classified as a heavy metal excluder. Despite the manifestations of phytotoxicity, *P. pellucida* absorbed considerable amounts of lead within its tissues, especially its roots. This indicates that the plant may be further exploited for their capacity to absorb heavy metals. Only a limited number of studies and reports on bioaccumulation, phytoremediation potential and heavy metal uptake capacity of *P. pellucida* are available. Hence, this study may establish a framework for future studies to improve efficiency and ability of the plant in heavy metal accumulation.

ACKNOWLEDGEMENTS

The author would like to give special thanks to the faculty of the Department of Biological Sciences of Isabela State University for their assistance in carrying out this study.

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