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ORIGINAL ARTICLE **OPEN ACCESS**

A study of potential lead metal hyperaccumulator in tissue cultured plants of *Tradescantia spathacea* Sw. and *Chlorophytum orchidastrum* grown in hydroponics

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ABSTRACT
Prolific plants of Tradescantia spathacea Sw. and Chlorophytum orchidastrum were tissue cultured and grown in hydroponics. The plants were investigated for their capacity to accumulate lead metal in greenhouse conditions. The results of this study revealed that the Pb-treated plants of Tradescantia spathacea Sw. and Chlorophytum orchidastrum showed hyperaccumulating capacity with values in their shoots of 99.9897 mgg^{-1} dry weight (10% Pb) and 44.7950 mgg^{-1} dry weight (4.48 % Pb), respectively. These values exceed the threshold value of >1mgg^{-1} (0.1%) dry weight of Pb in the shoots as a good criteria for a plant to be hyperaccumulator. Most of the absorbed Pb were retained in the roots with 215.66811mgg^{-}1 for Tradescantia spathacea Sw. and 43.0504 mgg^{-1} for Chlorophytum orchidastrum. Lead analysis was done by atomic absorption spectrophotometric method. The results of the study suggest that the two plants investigated were suitable for phytoremediation of lead metal due to its high tolerance and hyperaccumulation of the Pb metal in their roots with subsequent uptake to their shoots.
Keywords: phytoremediation, metal hyperaccumulators, *Tradescantia spathacea* Sw., *Chlorophytum orchidastrum*

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INTRODUCTION

Heavy metal contamination in soil, water, sediments and air as a result of industrial revolution has dramatically increase [1-3]. Industrial activities such as the energy and fuel production, mining and smelting of metalliferous ores [1,4], and other human activities such as the use of sewage sludge or urban composts, pesticides and fertilizers, emissions from municipal waste incinerators and car exhausts [1,4, 5, 6] are primary sources of heavy metal contamination in the environment**.** Among the heavy metals, lead (Pb) is one of the major contaminants found in soil, sediments, water and air [4, 7]. Lead is in fact one of the most persistent metals with a soil retention time of about 150-5000 years in the environment [7], and it usually accumulates in the surface of soils [8]. Pb input and contamination in the environment continues to be one of the most serious global environmental and human hazards despite regulatory measures and policies adopted in many countries.

Pb is a non-essential toxic heavy metals that do not have biological functions, but affects plants and are able to enter plant tissues through transport systems of plant metabolism for the essential micronutrients like Zn, Ni, Cu and Mn [9]. Lead is easily absorbed and accumulated [4, 10] and alters the plant's metabolism [11]. Some plants respond to Pb overload and caused a number of toxicity symptoms that includes chlorosis [12], stunded growth [13], and blackening of the root system [4]. While other plants do not exhibit deleterious effects and these plants are said to be metal-hyperaccumulators. These metal-hyperaccumulators have the potential to address metal-pollution by phytoremediation [14-15], a viable alternative to traditional approaches for the remediation of toxic heavy metal. Phytoremediation uses the ability of plants to concentrate elements and compounds from the environment and metabolize these molecules in their tissues [16].

Intensive investigation has been conducted on the ability of plants in removing Pb from soil using *Polygonum hydropiper L.* [17], *Rumex acetosa L.* [18], *Brassica juncea* [19], *Tithonia diversifolia* and *Helianthus annuus,* sunflower species [20], *Brassica napus L.* [21], *Catharanthus roseus L.* [22], *Sebertia acuminata*, *Armeria maritima*, *Aeollanthus biformifolius* and *Eichhornia crassipes* [1]. The current research was concerned on using locally-prolific plants growing along the roadsides and highways in Iligan City, Lanao del Norte, Philippines such as *Tradescantia spathacea* Sw. and *Chlorophytum orchidastrum*. The research's objective was to tissue culture the said plants to evaluate their ability to accumulate Pb upon treatment with 1000 ppm concentration of Pb^{2+} in hydroponics media in the greenhouse.

Tradescantia spathacea Sw. (Figure 1A) is also known as *Rhoeo discolor* (L. Her.) Hance whose common name is Moses of the Cradle or as "Bangka-bangkaan" in the local dialect of the Philippines. It is a short-stemmed tender foliage plant having small, dense, spreading clumps. It forms a solid groundcover of upright leaves. The six to eight- inch long, sword-shaped leaves are green above and purplish below. The plant is known to have an antioxidant and chemoprotective antimutagen [23], antimicrobial properties [24] and antitumoral property [25].

Chlorophytum orchidastrum Lindl (Figure 1B) is one of the 215 species of Chlorophytum, an important genus of the family Liliaceae [26], is found mainly in tropical and subtropical countries and is cultivated for their ornamental flowers. The plant is about 30-40 cm high and 60-80 cm in diameter having green leaves with an orange petiole and midrib. Among all the species of Chlorophytum genus, *Chlorophytum comosum* was the only species reported to be a potential Pb hyperaccumulator [27] and a potential Cd hyperaccumulator [28] having known to exhibit stress tolerance and phytoremediation capacity [29].

Figure 1. (A) *Tradescantia spathacea* Sw. and (B) *Chlorophytum orchidastrum* L. plants

MATERIALS AND METHODS

Plant materials

Plant samples were botanically authenticated by Dr. Victor B. Amoroso of the Center of Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University, University Town, Musuan, Bukidnon, Philippines. Voucher specimens of the plant samples were deposited at Central Mindanao University Herbarium (CMUH).

***In vitro* micropropagation of the test plants.** *In vitro* micropropagation of the plant was conducted at the Tissue Culture Laboratory, Department of Horticulture, College of Agriculture, Central Mindanao University, University Town, Musuan, Bukidnon, Philippines.

***In vitro* micropropagation of *Tradescantia spathacea* Sw.** Actively growing shoots of *Tradescantia spathacea Sw.* bearing seven to eight nodes, were collected from 1.0-yr-old plants. These shoots were used as explants source removing shoot tips and leaves. The node segments were rinsed thoroughly under running tap water to remove any soil attached to the surfaces and finally rinsed with distilled water. Aseptically, the stalks containing the nodal segments were surface-sterilized in enough volume of commercial bleach solution of 4.5%–5.0% (w/v) sodium hypochlorite with 2 drops of Tween 20 (Sigma-Aldrich) for 20 min, and subsequently rinsed five times for 10 min with sterilized distilled water. After sterilization, explants were excised as single node segments (~1.5 cm), and were aseptically dissected to remove the fleshy pulp covering to expose the node using sharp surgical blades and tweezers. Then, the nodal segments were placed in sterilized paper prior to culture. The segments were then cultured on a nutrient medium consisted of modified MS medium [30] solidified with 6 g/L agar (Vetec®). The pH of the MS medium was adjusted to 5.8, before autoclaving for 15 min at 120 °C.

***In vitro* micropropagation of *Chlorophytum orchidastrum*.** Seeds of *Chlorophytum orchidastrum* were surface sterilized with 4.5%–5.0% (w/v) sodium hypochlorite with 2 drops of Tween 20 (Sigma-Aldrich)

for 20 min and subsequently rinsed five times for 10 min with sterilized distilled water. After rinsing in sterilized water, the seeds were then transferred aseptically nutrient medium consisted of modified MS medium [30] solidified with 6 g/L agar (Vetec®). The pH of the MS medium was adjusted to 5.8, before autoclaving for 15 min at 120 °C.

Culture initiation, multiplication and elongation of *Tradescantia spathacea* Sw. and *Chlorophytum orchidastrum*. The cultured plants with shoot clusters were transferred on growth medium supplemented with 1 ppm of N-naphthylacetic acid (rooting hormone). After rooting was established, the test plants were further transferred to MS medium supplemented with 5 ppm benzylaminopurine (BAP) for shoot elongation [31].

Rooted shoots from different culture media were transferred in the greenhouse with the following growth conditions: oxygenized hydroponic solution with pH adjusted to 5.7, 16 h: 8 h photoperiod regime at 25 °C during the night and 28 °C during the day. The test plants were grown until carrying about 5-7 leaves.

Heavy Metal (Pb) Treatment. Twelve-weeks old hydroponics grown *Tradescantia spathacea* Sw. and *Chlorophytum orchidastrum* with 5-7 leaves were randomly selected. The test plants were group into two: the first group was taken as control while the other group was treated with 1000 ppm Pb^{2+} solution. Treatment commenced with a single application of 1000 ppm Pb^{2+} in the hydroponics media, and the volume was maintained by irrigation with oxygenated hydroponic solution weekly. After 30 days of exposure, 10 biological replicates with 3 technical replicates each were taken (total 30 samples). For each of the control and treated plants, roots, stems and leaves were harvested separately. Stems and leaves were washed thoroughly with deionized water while root samples were immersed in 10 mM $CaCl_2$ in an ultrasonic bath for 15 min and then rinsed with deionized water to remove surface-bound Pb [32]. The plant tissues were then freeze dried for 3 days followed by oven drying for 10 days at 45 °C to constant weight. All dried samples were weighed and ground for Pb analysis.

Pb Metal Analysis. The analytical method for the determination of Pb employed the sub-sampling process of coning and quartering a representative sample from the freeze-dried plant samples. Approximately 100 mg samples in triplicate were digested with a mixture of 5 mL of 65 % conc. HNO_3 and 2 mL of 30 % H_2O_2 in a pressurized oven using an automatic microwave digester (Ethos 1 Microwave Digester, Milestone, USA). The digestion process was set with a temperature of 200 °C for 45 minutes, applying a pressure of 45 bar, while a 1500 W microwave power supply was used for heating. Pb levels of the different plant parts were analyzed using Flame Atomic Absorption Spectrophotometry (Spectra Flame Atomic Absorption Spectrophotometer, Agilent, USA), according to published protocols [33].

RESULTS and DISCUSSION

After 30 days of growth following heavy metal exposure, the plants *Tradescantia spathacea* Sw. exhibited tolerance and resistance with minimal toxicity symptoms as manifested by wilting and yellowing of the basal leaves and very minimal browning of the roots as assessed by the naked eye. Similarly, there was yellowing of the basal leaves and browning of the roots of *Chlorophytum orchidastrum* that was observed after 20 days of metal exposure, in which slight toxicity symtoms were exhibited earlier than the *Tradescantia spathacea* Sw.

Results of the study revealed that the roots of Pb-treated plant *Tradescantia spathacea* Sw. has the highest Pb content of 215.6681 mgg^{-1} dry weight Pb followed by the stem with 93.8937 mgg^{-1} dry weight and only 6.0960 mgg^{-1} Pb dry weight was translocated to the leaves as shown Table 1. *Tradescantia spathacea* Sw. has Pb accumulation on the shoots of 10.00% (i.e. stem of 9.39 % and 0.61% Pb was accumulated in the leaves) (Table 2).

Higher levels of lead were determined on the roots of *Chlorophytum orchidastrum* with the value of 43.0504 mgg^{-1} dry weight then followed by the stem of 34.8325 mgg^{-1} dry weight and 9.9624 mgg^{-1} dry weight was determined on the leaves as shown in Table 1. *Chlorophytum orchidastrum* has 4.48% Pb accumulated on the shoots (Table 2).

Table 1. Mean Pb concentrations of the plant parts of *Tradescantia spathacea* Sw. *and Chlorophytum orchidastrum*, in mgg^{-1} dry weight (mean ± SD).

Samples	Mean Pb content in the roots, mgg^{-1} DW	Mean Pb content in the stems, mgg^{-1} DW	Mean Pb content in the leaves, mgg^{-1} DW	Mean Pb content in the shoots, mgg^{-1} DW
TTC	0.0271 ± 0.0010	0.0029 ± 0.0000	0.0101 ± 0.0020	0.0130 ± 0.0020
TTPb	215.6681 ± 12.3880	93.8937 ± 2.9500	6.0960 ± 0.2430	99.9897 ± 3.1520
COC	0.0448 ± 0.0026	0.0021 ± 0.0020	0.0008± 0.000	0.0027 ± 0.0017
COPb	43.0504 ± 0.4288	34.8325 ± 3.9745	9.9624 ± 0.7188	44.7950 ± 3.5750

TTC= *Tradescantia spathacea* Sw.*;* control, TTPb= Pb-treated *Tradescantia spathacea* Sw.; COC= *Chlorophytum orchidastrum;* control; COPb= Pb-treated *Chlorophytum orchidastrum; DW – dry weight*

Table 2. % Pb levels on the plant parts of *Tradescantia spathacea* Sw. and *Chlorophytum orchidastrum*.

Samples	Mean Pb content in the roots, mgg^{-1} DW	Mean Pb content in the stems, mgg^{-1} DW	Mean Pb content in the leaves, mgg^{-1} DW	Mean Pb content in the shoots, mgg^{-1} DW
TTC	0.00	0.00	0.00	0.00
TTPb	21.57	9.39	0.61	10.00
COC	0.00	0.00	0.00	0.00
COPb	4.31	3.48	1.00	4.48

TTC= *Tradescantia spathacea* Sw.*;* control, TTPb= Pb-treated *Tradescantia spathacea* Sw.; COC= *Chlorophytum orchidastrum;* control; COPb= Pb-treated *Chlorophytum orchidastrum*.; *DW – dry weight*

A lead metal -hyperaccumulator plant is one that can concentrate the metals to a level >1 mgg^{-1} (0.1%) of the shoots dry weight for Pb [35, 36, 9]. *Tradescantia spathacea* Sw. and *Chlorophytum orchidastrum,* have values of 10.00 % and 4.48 % Pb accumulation on the shoots, respectively. These values being greater than the threshold values, i. e. >1 mgg^{-1} (0.1%) of dry weight Pb suggest the potential of these plants to be lead metal-hyperaccumulator. This further suggests an effective ability of the plant to uptake and transport Pb from roots to shoots by accumulation.

This study also revealed that the highest concentration of Pb was found mainly on the roots with 43.0504 mgg^{-1} dry weight for *Tradescantia spathacea* Sw. and 215.6681 mgg^{-1} dry weight for *Chlorophytum orchidastrum*. Similar results of higher metal accumulation found in the roots have been reported in Vicia faba, Pisum sativum, and Phaseolus vulgaris [36], V. unguiculata [37], Nicotiana tabacum, [38], Zea mays [39], Lathyrus sativus [40], Avicennia marina [41] and Allium sativum [42]. As have been reported, the very limited translocation to the shoots despite the higher concentration found in the roots was mainly due to the susceptibility of Pb to be immobilized by molecule such as negatively charged pectins within the cell wall [43, 7], precipitation of insoluble lead salts in intercellular spaces [37] and the sequestration of Pb in the vacuoles of root cells [42].

CONCLUSION

Both *Tradescantia spathacea* Sw. and *Chlorophytum* orchidastrum plants were found to have the ability to hyperaccumulate Pb metal beyond the threshold level of 1 mgg^{-1} DW on their shoots without exhibiting significant deleterious or toxicity symptoms on the plants on Pb overload. These plants are potential plants for the phytoremediation of lead metal contaminated sites. It is recommended that other toxic metal studies be conducted to further establish the potential of these plants to hyperaccumulate in mix-metal condition.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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