J. Viet. Env. **2018**, 9(2):67-72
DOI: 10.13141/jve.vol9.no2.pp67-72

JOURNAL of VIETNAMESE ENVIRONMENT

Effect of fertilizer on lead (Pb) accumulation ability of *Polygonum hydropiper* L.

Ảnh hưởng của phân bón lên khả năng hấp thụ chì (Pb) của nghể răm Polygonum hydropiper L.

Research article

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Polygonum hydropiper L. was cultivated on alluvial soil (Pb = 2.6 mg/kg, dry weight) and Pb contaminated soil (Pb = 1,380 mg/kg dry weight) without and with amendment of 2 g organic fertilizer/kg soil and 2.5; 5.0; 10.0 g NPK fertilizer/1kg soil. After 45 days of cultivation, the growth in height and biomass of *P. hydropiper* in Pb contaminated soil without amendment of fertilizer was lower than that in alluvial soil, but the Pb content in the above-ground part of the *P. hydropiper* was higher. In the formula 4, on Pb contaminated soil (Pb = 1,380 mg/kg dry weight), when amending 2 g of microbiological organic fertilizer + 5 g NPK fertilizer per 1 kg of soil (with the total nutrients amended were: N = 0.25, P_2O_5 = 0.52, K_2O = 0.15, and organic matter = 0.21 g/kg soil), growth of *P. hydropiper* was optimal (its height and biomass were up to 244.0% and 284.9% in comparison to that of before experiment) and their Pb extraction potential was promoted to the highest level among the formulae used. The average level of Pb accumulated in the above-ground part of *P. hydropiper* cultivated at formula 4 was 1,098.3 mg/kg dry weight (DW) after 45 days of cultivation that was 1.6 time higher than the one of formula 2 without fertilizer amendment (687.8 mg/kg DW). The potential of Pb extracted and stored in the above-ground part of *P. hydropiper* cultivated at formula 4 after 45 days was 479.2 g/ha that was 2.85 time higher than the one of formula 2 without fertilizer amendment (168.02 g/ha).

Nghể răm Polygonum hydropiper L. được trồng theo 5 công thức trên đất phù sa không ô nhiễm chì (Pb = 2,6 mg/kg khô) và đất ô nhiễm chì (Pb = 1380 mg/kg khô) không bón phân và có bón phân với các liều lượng 2 g phân hữu cơ vi sinh/1 kg đất và 2,5; 5,0; 10,0 g phân NPK /1 kg đất. Sau 45 ngày thí nghiệm trồng cây, tăng trưởng về chiều cao và sinh khối của cây trên đất ô nhiễm Pb không bón phân thấp hơn trên đất phù sa, nhưng hàm lượng Pb trong phần trên mặt đất của cây cao hơn. Ở công thức (CT) 4, trên đất ô nhiễm chì (Pb = 1380 mg/kg khô) khi bón phân với liều lượng 2 g hữu cơ + 5 g NPK/1 kg đất (với tổng hàm lượng dinh dưỡng được bón là: N = 0,25, P_2O_5 *= 0,52,* K_2O *= 0,15, và chất hữu cơ = 0,21 g/kg đất) thì tăng trưởng của cây nghể răm đạt tối ưu (chiều cao và khối lượng đạt 244,0 % và 284, 9% so với trước thí nghiệm) và tiềm năng hút thu Pb của chúng cũng được thúc đẩy cao nhất trong số các công thức được sử dụng. Lượng Pb trung bình tích lũy trong phần trên mặt đất của nghể răm ở CT 4 đạt 1.098,3 mg/kg khô sau 45 ngày trồng, cao gấp 1,6 lần so với cây ở công thức 2 không bón phân (687,8 mg/kg khô). Khả năng loại bỏ Pb từ đất ô nhiễm của nghể răm khi được bón phân ở CT4 đạt 479,2 g/ha sau 45 ngày trồng, cao gấp 2,85 lần so với cây ở CT 2 không bón phân (168,02 g/ha).*

Keywords: fertilizer, growth, heavy metal pollution, lead accumulation, Polygonum

1. Introduction

Lead (Pb) is a heavy metal that is of much concern in environmental protection because of its widespread use and long-term harmful potential. In plant and animal life,

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http://dx.doi.org/10.13141/JVE
ISSN: 2193-6471

high Pb content suppresses most basic physiological processes (Michalak and Wierzbicka, 1998). In plants, Pb can induce cell membrane permeability, inhibit protein synthesis, inhibit some enzymes, affect respiration, photosynthesis, open stomata and transpiration (Fergusson, 1991). For human, when eating food containing Pb, it is not excreted out but accumulates in some important organs like the brain, bone marrow. The half-life of lead removal from the kidneys is 7 years, from the bone is 32 years, so the effects of lead are usually very long (Nies and Silver, 1995).

Many studies on heavy metal accumulation in different plant species were carried out in the world. Among them, there were some studies in Vietnam on the ability of heavy metal accumulation of vegetable species (Dang Thi An, Chu Thi Thu Ha, 2005); of vetiver grass (Vo Van Minh, 2007); of maize (Tang Thi Chinh, Bui Van Cuong, 2011), of reed (Tran Thi Pha, 2014). In recent years, many countries have been interested in the phytoremediation technology using plants to treat the environment pollution. Many plants are highly capable of absorbing and accumulating heavy metals that are known as hyperaccumulators (US EPA, 2000). There were over 400 species of heavy metal hyperaccumulators found in the world (Prasad and Freitas, 2003). However, most of the plant species that are able to accumulate heavy metals at high levels have limited small biomass. Therefore, to achieve high efficiency in heavy metal pollution treatment, it is necessary to enhance the biomass production of plants by creating favorable growth conditions.

Fertilizers, especially organic fertilizers, have the potential to improve soil properties, such as increased porosity, adsorption capacity, humus content in soil, contributing to nutrient balance in soil, enabling microorganism activities to help plants grow and develop well. Depending on the species, different fertilizer types and doses are required. (Nguyen Nhu Ha, 2006). Studies on the effects of fertilizers on heavy metal accumulation in plants also show the certain role of fertilizer (Bui Kim Anh et al., 2010; Chen Tong Bin et al., 2002).

Polygonum hydropiper L. was evaluated as having high Pb tolerance and accumulation with concentration of up to 4.650 $mg.kg^{-1}$, dry weight (DW) at the Pb pollution area in Hung Yen province (Chu Thi Thu Ha, 2014). Due to the high Pb tolerance and accumulation, *P. hydropiper* can be considered as a species having great potential for use in treating Pb contaminated soil. So far, there have been no report on evaluating the effect of fertilizer on Pb accumulation capacity of *P. hydropiper*. This study aims to find an effective fertilizer amendment formula that improves Pb removal from contaminated soil by *P. hydropiper*.

2. Materials and methods

2.1. Research location

Lead polluted soil samples and plant samples were collected from Dong Mai lead recycling village, Chi Dao commune, Van Lam district, Hung Yen province of Vietnam. Control soil samples were collected from the Institute of Ecology and Biological Resources, Ha Noi city. Planting experiment at pot scale with 1 kg soil / 1 pot, soil height in pot was about 9-10 cm was carried out at Institute of Ecology and Biological Resources, Ha Noi in the years 2016 and 2017.

2.2. Research subjects

Lead contaminated soil, *Polygonum hydropiper* L.

2.3. Methods

2.3.1. Plant identification

Using the morphometric method, the main document used in the process of identifying the scientific name was: Flora of Vietnam (Nguyen Thi Do, 2007).

2.3.2. Sampling

Sampling before planting experiment: Soil samples were taken at surface zone (0 - 20 cm) using stainless steel shovel (TCVN 7538-2: 2005). Plant specimens for planting were taken up with their whole root. Plant specimens for analysis were only taken their above-ground parts.

2.3.3. Experimental set up

Plants of approximately equal size were selected for the 5 experimental formulas. Prior to the experiment, 9 plants were selected, separated the above-ground parts to weigh fresh weight, measure height, determine moisture content and analyse Pb content. Each cultivated formula on soil amended different fertilizer dose was carried out in 9 replications using 9 trees.

Alluvial soil was taken from the Institute of Ecology and Biological Resources, Ha Noi (used in formula F1); Pb contaminated soil was collected from paddy fields in Dong Mai Pb recycling Village, Chi Dao commune, Van Lam district, Hung Yen province (used in experimental formulas from F2 to F5). The soil was removed from gravel, rubbish, then beaten with porcelain pestle, weighed and mixed with different doses of fertilizer for planting *P. hydropiper*.

Fertilizers used have composition as follows:

- Microbiological organic fertilizer: moisture content 30%; organic matter (OM) 15%; P_2O_5 mixture 1.5%; humic acid 2.5%; secondary nutrients: Ca 1%, Mg 0.5%, S 0.3%; useful microorganisms: *Bacillus* $1x10^6$ CFU / g, *Azotobacter* $1x10^6$ CFU / g, *Aspergillus* sp $1x10^6$ CFU / g.

- NPK fertilizer: N 5%, P_2O_5 10%, K_2O 3%, organic matter and trace elements > 12%.

The contents of N, P_2O_5, K_2O, and OM supplemented using microbiological organic fertilizer and NPK fertilizer in the soil of different planting formulas are shown in Table 1. The nutrient content per hectare of land was calculated on the basis of estimating each planted pot having surface square of 100 cm^2.

2.3.4. Experimental observation and sampling for analysis

- Plant care: Watering every morning and afternoon, catching worms and insect pests.
- Monitoring of plant growth in experimental formulas after 45 days of planting through their height and biomass. The height of plant was measured from top to the end point of stem close to the root, biomass of plant was counted for the above-ground part removing the yellow leaves.

2.3.5. Analytical methods

- The pH values of soil samples were determined by the hydrolysis selection method, using the Hanna pH meter.
- Moisture contents of soil samples and plant samples were determined following to Vietnam Standard (TCVN 4048: 2011).
- Analysis of N, P, K total following to Le Van Khoa et al. (2001).
- Pb Analysis:
+ Soil pre-treatment for analysis: Soil samples were dried at room temperature, removed from gravel, rubbish, then beaten with porcelain pestle to fine powder.
+ Plant sample pre-treatment for analysis: The above-ground part pf plants were washed with tap water, rinsed with distilled water and strained at room temperature then dried at 60^{o}C to get stable weight by oven (Memmert, Germany). Then the dried samples were cut and ground into fine powder using stainless steel scissors and porcelain mortar pestle.
+ Mineralization of soil and plant samples: Following US EPA 3052 (1996), using microwave: Speedwave 4, Berhof, Germany.
+ Determination of Pb content in the sample: Following to the method SMEWW 3125: 2012, using ICP-MS machine: ELAN 9000, Perkin Elmer, USA, with automatic sampler: SC-2 FAST - Elkin, USA.

2.3.6. Data processing methods

Experimental and analytical data are processed on Excel software.

Table 1. Contents of N, P_2O_5, K_2O, and organic matter supplemented in experiment soil

Formulas	Microbiological organic fertilizer supplemented (g/kg soil)	NPK fertilizer supplemented (g/kg soil)	N		P_2O_5		K_2O		OM	
			g/kg soil	kg/ha land	g/kg soil	kg/ha land	g/kg soil	kg/ha land	g/kg soil	kg/ha land
F 1	0	0	0	0	0	0	0	0	0	0
F 2	0	0	0	0	0	0	0	0	0	0
F 3	2	2.5	0.125	125	0.27	271	0.075	75	0.21	210
F 4	2	5	0.25	250	0.52	521	0.15	150	0.21	210
F 5	2	10	0.5	500	1,02	1021	0.3	300	0.21	210

3. Results and discussion

3.1. Characteristics of soil before experiment

Some parameters of soil analysis prior to the planting experiment are presented in Table 2. Before experiment, alluvial soil (control) had low Pb content of 2.6 mg / kg DW while Pb contaminated soil was collected from Dong Mai Pb recycling village had Pb content of 1,380 mg / kg DW exceeding the allowable value (70 mg / kg DW) nearly 20 times (QCVN 03-MT: 2015 / BTNMT).

The pH value in the alluvial soil was neutral 7.02, while the pH in the Pb contaminated soil was acidic 5.23 that was adjusted with lime to get pH values from 7.1 to 7.3. The contents of total nutrients including N, P2O5, K_2O and organic matter in alluvial soil before the experiment was 0.1; 1,1; 1.8 and 1.9%, respectively, whereas these values in Pb contaminated soil before the experiment were 0.16; 0,06; 0.95 and 1.92%. All of these values in two kinds of soil are within Vietnam standard values for soil (TCVN 7373: 2004; TCVN 7374: 2004; TCVN 7375: 2004; TCVN 7376: 2004; TCVN 7377: 2004).

Table 2. Characteristics of two soil kinds before experiment

Parameters	Alluvial soil	Pb contaminated soil	Vietnam standard	
			Value	Name
Pb (mg/kg DW)	2.6	1,380	70	QCVN 03-MT:2015/BTNMT
pH	7.0	5.3, adjustment: 7.1-7.3	3.57 - 7.84	TCVN 7377: 2004
N (%)	0.1	0.16	0.095 - 0.270 (mean 0.141)	TCVN 7373: 2004
P_2O_5 (%)	1.1	0.06	0.03 - 2.35 (mean 1.05)	TCVN 7374: 2004
K_2O (%)	1.8	0.95	0.03 - 2.35 (mean 1.05)	TCVN 7375: 2004
OM (%)	1.9	1.92	1.00 - 2.85 (mean 1.85)	TCVN 7376: 2004

3.2. Effect of fertilizer on height and above-ground biomass of *P. hydropiper*

Biomass is also an important index for evaluating the ability of plant removing heavy metals from soil. Research to increase the plant biomass will contribute to the heavy metal content taken by the plant after a certain period of time. After 45 days of experiment, the above-ground part of *P. hydropiper* was harvested to measure their height and determine their fresh weight, dry weight (Table 3). Before the planting experiment, the above-ground parts of 9 individuals of *P. hydropiper* were measured and weighed, with the average values of height, fresh weight and dry weight of above-ground biomass of a plant were 20.1 ± 1.08 cm, 10.6 ± 0.34 g and 1.2 ± 0.04 g, respectively. In the same condition of without fertilizer supplemented, growth of *P. hydropiper* after 45 days of planting in formula F2 containing Pb contaminated soil (1,380 mg / kg dry) was only 33.4 ± 0.92 cm; 17.1 ± 0.57 g and 1.9 ± 0.06 g in height, fresh weight and dry weight of above-ground biomass respectively compared to 45.3 ± 1.17 cm; 24.9 ± 0.46 g and 2.7 ± 0.05 g of those in formula F1 containing alluvial soil (with low Pb content of 2.6 mg / kg DW). In formula F3 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 2.5 g NPK fertilizer/ 1 kg soil), height, fresh weight and dry weight values of above-ground biomass of *P. hydropiper* after 45 days of planting were 45.9 ± 0.57 cm; 27.4 ± 0.84 g and 3.0 ± 0.09 g, equivalent to 228.1% and 257.3% higher than those before the experiment on height and weight, respectively.

Formula F4 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 5 g NPK fertilizer / 1 kg of soil, equivalent to N, P_2O_5, K_2O and OM contents added are: 0.25, 0.52, 0.15 and 0.21 g / kg of soil, respectively) showed the strongest positive effect of fertilizer on the height and weight of *P. hydropiper* among the all formulas used. Specifically, height, fresh weight and dry weight of above-ground biomass of *P. hydropiper* after 45 days of planting were 49.1 ± 0.53 cm; 30.3 ± 1.01 g; and 3.3 ± 0.12 g, equivalent to 244.0% and 284.9% higher than before the experiment on height and weight, respectively.

In the formula 5 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 10 g NPK fertilizer / 1 kg of soil), height, fresh weight and dry weight of above-ground biomass of *P. hydropiper* reached 47.8 ± 0.69 cm; 29.5 ± 0.80 g and 3.2 ± 0.09 g, equivalent to 237.7% and 276.8% higher than before the experiment on height and weight, respectively.

Table 3. Effect of fertilizer on height and above-ground biomass weight of *P. hydropiper*

Formulas	Height (cm)	Height increased comparison to those before experiment (%)	Fresh weight (g)	Dry weight (g)	Weight increased comparison to those before experiment (%)
Initial	20.1±1.08	-	10.6±0.34	1.2±0.04	-
F1, 45 days	45.3±1.17	225.2	24.9±0.46	2.7±0.05	233.8
F2, 45 days	33.4±0.92	166.2	17.1±0.57	1.9±0.06	160.2
F3, 45 days	45.9±0.57	228.1	27.4±0.84	3.0±0.09	257.3
F4, 45 days	**49.1±0.53**	**244.0**	**30.3±1.01**	**3.3±0.12**	**284.9**
F5, 45 days	47.8±0.69	237.7	29.5±0.80	3.2±0.09	276.8

As a result, when the fertilizer was added, the growth of *P. hydropiper* on the soil containing Pb 1,380 mg / kg DW (in formulas F3, F4, F5) increased significantly and was higher than that of alluvial soil without fertilizer supplemented (F1) and Pb contaminated soil without fertilizer supplemented (F2). This can be explained by the fact that microbiological organic fertilizer and NPK fertilizer contain the macronutrient, secondary nutrient and micronutrient elements essential for plant growth and development at a certain dose. In addition, it is also a suitable environment for microorganism development, especially microorganisms in root zone, which help dissolve organic matter into available nutrients easily absorbed by the plant. However, the growth of plant was not proportional to the amount of fertilizer. Similarly, other studies showed that each species needs different types of fertilizer (Nguyen Nhu Ha, 2006, Hoang Van Hong, 2008). In this study, the best amount of fertilizer applied to the *P. hydropiper* grown on soil contaminated with Pb 1,380 mg / kg was in formula F4.

3.3. Effect of fertilizer on Pb accumulation capacity of *P. hydropiper*

Before the experiment, Pb concentration in above-ground biomass of *P. hydropiper* was 99.83 mg / kg DW. After 45 days of planting experiment, Pb accumulation in above-ground biomass of *P. hydropiper* in the formulas F1, F2, F3, F4, and F5 was shown in Figure 1. Formula F1 was planting on alluvial soil with very low Pb content (2.6 mg/kg DW). The average Pb concentration in above-ground biomass of *P. hydropiper* after 45 days of planting experiment was insignificant lower than those before the experiment (88.3 compared to 99.83 mg / kg DW). In formula 2, on Pb contaminated soil (1,380 mg / kg DW), the average content of Pb accumulated in the above-ground biomass of *P. hydropiper* increased significantly to 684.7 mg / kg DW that was 6.86 times higher than those before the experiment (99.83 mg / kg DW).

In formula 3 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 2.5 g NPK fertilizer/ 1 kg soil), the average content of Pb accumulated in the above-ground biomass of *P. hydropiper* was 811.7 mg / kg DW, that was 8.13 times higher than those before the experiment (99.83 mg / kg DW) and 1.19 times higher than those in formula F2 without fertilizer (684.7 mg / kg DW).

In formula 4 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 5 g NPK fertilizer/ 1 kg soil, equivalent to N, P_2O_5, K_2O and OM contents added are: 0.25, 0.52, 0.15 and 0.21 g / kg of soil, respectively), the average content of Pb accumulated in the above-ground biomass of *P. hydropiper* was highest at 1,098.3 mg / kg DW, that was 11.0 times higher than those before the experiment (99.83 mg / kg DW) and 1.60 times higher than those in formula F2 without fertilizer (684.7 mg / kg DW). In formula 5 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 10 g NPK fertilizer/ 1 kg soil, the average content of Pb accumulated in the above-ground biomass of *P. hydropiper* was 790.7 mg / kg DW, that was 7.92 times higher than those before the experiment (99.83 mg / kg DW) and 1.15 times higher than those in formula F2 without fertilizer (684.7 mg / kg DW).

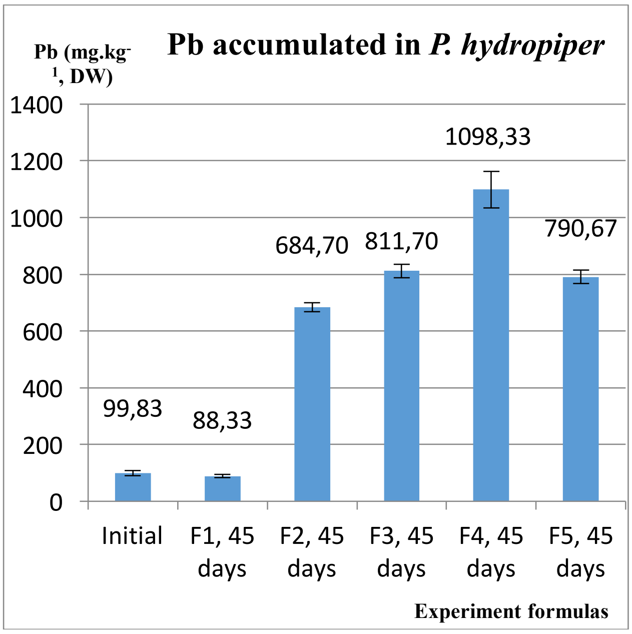

Figure 1. Concentrations of Pb accumulated in *P. hydropiper* after 45 days of planting

Thus, under the same weather conditions, at the pot scale, among the formulas used in this study, the formula F4 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 5 g NPK fertilizer/ 1 kg soil, equivalent to N, P_2O_5, K_2O and OM contents added are: 0.25, 0.52, 0.15 and 0.21 g / kg of soil, respectively) is optimal for Pb absorption. From this result it can be seen that the role of microbiological organic fertilizer and NPK fertilizer at a certain dosage not only promotes biomass weight but also increases the Pb accumulation in the above-ground parts. Research results on fern *Pteris vittata* showed that at P level amendment of 800 ppm (equal to 0.8 g P/kg soil) and N 100-200 ppm (equal to 0.1 – 0.2 g N/kg soil) the rate of growth and Pb accumulation of *Pteris vittata* was the best (Bui Kim Anh et al., 2010).

3.4. Assessing potential of using *P. hydropiper* in Pb pollution treatment

The results of the estimation of Pb uptake in the above-ground biomass of *P. hydropiper* were calculated based on biomass, accumulated Pb content, moisture content and plant density. The distance between 2 individuals of *P. hydropiper* is assumed to be 30 cm x 30 cm, so there are about 132,000 individuals of *P. hydropiper* planted in 1 ha of land. The amount of Pb extracted per 1 ha of soil in *P. hydropiper* before treatment and in the formulas F1, F2, F3, F4, F5 after 45 days of planting was: 15,29; 31.63; 168.02; 319.87; 479,18 and 335,22 g, respectively (Figure 2).

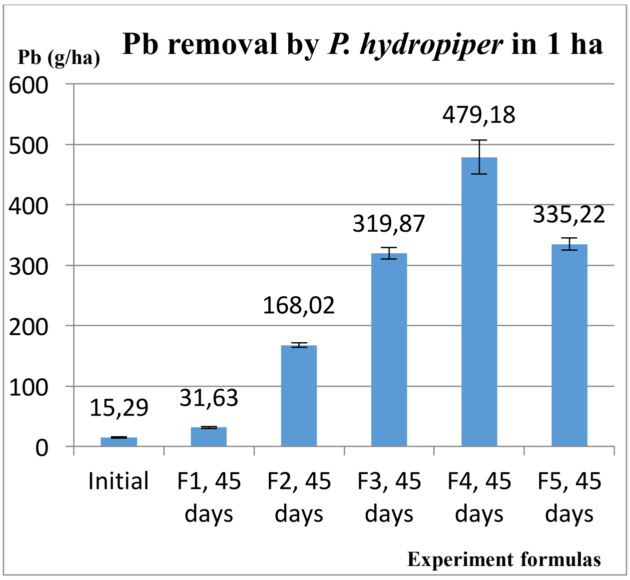

Figure 2. Potential for treatment of 1 ha of Pb contaminated soil after 45 days of planting *P. hydropiper*

The data in Figure 2 showed that, among the formulas studied, formula 4 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 5 g NPK fertilizer/ 1 kg soil, equivalent to N, P_2O_5, K_2O and OM contents added are: 0.25, 0.52, 0.15 and 0.21 g / kg of soil, respectively) are optimal for boosting Pb accumulation potential of *P. hydropiper* It could increase Pb content in the above-ground part of *P. hydropiper* per 1 ha of Pb contaminated soil (1,380 mg / kg DW) to 2.85 times higher than that of the formula 2 containing Pb contaminated soil (1,380 mg / kg DW) without fertilizer amendment (479.18 g / ha compared to 168.02 g / ha).

4. Conclusion

Microbiological organic fertilizer and NPK fertilizer had a positive effect on the growth of *P. hydropiper*, boosting biomass and increasing the amount of Pb absorbed and accumulated in the plant. Among the formulas used, formula 4 (soil with Pb content = 1,380 mg / kg DW, 2 g microbiological organic fertilizer + 5 g NPK fertilizer/ 1 kg soil, equivalent to N, P_2O_5, K_2O and OM contents added are: 0.25, 0.52, 0.15 and 0.21 g / kg of soil, respectively) was the optimum condition for the growth of *P. hydropiper* that promoted its height and biomass yield. At the same time, the amount of Pb accumulated in the above-ground biomass of this plant was increased. Calculated on 1 ha planting *P. hydropiper* with a distance of 30 cm x 30 cm after 45 days, the amount of Pb absorbed and accumulated in the above-ground part of *P. hydropiper* in formula 4 reached 479.18 g / ha that was 2.85 times higher than that in the formula 2 without fertilizer amendment (168.02 g / ha).

The results of the study will contribute scientific basis to determine the appropriate use of fertilizers to achieve optimum Pb absorption and accumulation in *P. hydropiper*. This makes it more feasible to apply phytoremediation technology - an environmentally friendly technology that is easy to handle for treatment of Pb contaminated soil.

Acknowledgment: This study belongs to the project code VAST07.03/16-17. The authors sincerely thank the funding from the Vietnam Academy of Science and Technology.

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