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THE EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION ON Pb REMOVAL OF FERN (*Pteris vittata* L.) FROM POLLUTED SOIL

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ABSTRACT

The aim of this study to evaluate the effects of mycoroot bio-product to the Pb accumulation capability of ferns. This pot experiment was carried out in a greenhouse with 4 treatments arranged in a randomized complete block. Soil of the Dong Mai village, Chi Dao commune, Van Lam district, Hung Yen province, Vietnam was analyzed and its Pb contamination was 37 times higher than the permissible standard (QCVN 03: 2008 / BTNMT). After 40 days of experimentation, mycoroot treated ferns had increased biomass and Pb content of plant parts. The increase of plant biomass depended on the dose of the inoculant applied to the soil. The Pb content accumulated up to 834.63 mg/kg in the roots and 121.19 mg/kg in the stalk-leaves when the ferns were treated with 40g mycoroot bio-product/plant. Ferns had lower a Pb content in each fresh biomass unit and a higher biomass weight when mycoroot bio-product was applied at a rate of 80 g/plant compared to 40 g/plant. Thus, the total amount of Pb removed from the soil was higher after the ferns were treated with 80 g/plant mycoroot bio-product (7.27 mg Pb/pot). The total amount of Pb accumulated in the roots was always higher than in the stalk- leaves. The flexibility of Pb could be increased when soil was mixed with mycoroot bio-product before ferns were transplanted.

Keywords: Fern, mycoroot mycorrhizal fungi bio-product, Pb pollution.

Ảnh hưởng của nấm cộng sinh rễ đến khả năng hấp thu chì của cây dương xỉ từ đất bị ô nhiễm

TÓM TẮT

Mục đích của nghiên cứu nhằm đánh giá ảnh hưởng của chế phẩm nấm rễ Mycoroot đến khả năng tích lũy chì của cây dương xỉ. Thí nghiệm chậu vại trong nhà lưới được tiến hành với 4 công thức và sắp xếp theo khối ngẫu nhiên hoàn chỉnh. Kết quả phân tích đất thí nghiệm tại thôn Đông Mai, xã Chỉ Đạo, huyện Văn Lâm, tỉnh Hưng Yên, Việt Nam cho thấy, đất đã bị ô nhiễm chì vượt hơn 37 lần so với tiêu chuẩn cho phép (QCVN 03:2008/BTNMT). Sau 40 ngày thí nghiệm, chế phẩm nấm rễ đã làm tăng sinh khối cây dương xỉ cũng như kích thích sự hấp thụ Pb trong các bộ phận của cây. Sinh khối của cây tỷ lệ thuận với liều lượng chế phẩm bón vào đất. Sinh khối tươi lớn nhất đạt được là 55,98 g thân lá và 40,66 g rễ (công thức 4). Hàm lượng chì tích lũy lớn nhất ở công thức 3 (tích lũy 834,63 mg/kg rễ và 121,19 mg/kg thân lá), nhưng do có ưu thế về sinh khối nên tổng lượng chì được lấy đi khỏi đất ở công thức 4 là lớn nhất (7,27 mg Pb/chậu). Tổng lượng chì tích lũy trong rễ luôn cao hơn trong thân lá. Việc bổ sung chế phẩm Mycoroot vào đất trồng cây dương xỉ có khả năng làm gia tăng tính linh động của chì.

Từ khóa: Cây dương xỉ, chế phẩm nấm rễ Mycoroot, ô nhiễm chì.

1. INTRODUCTION

Currently, environmental pollution, including heavy metals in the soil, is destroying the planet and threatens human health. The source of emissions of heavy metals are varied, such as metal recycling villages, waste from factories and industrial parks, mining exploitation, improper use of fertilizers, and plant protection chemicals. Heavy metal pollution from metal recycling villages is a major problem in many countries around the world, including Vietnam, due to the dangerous impacts on ecosystems and people.

In recent years, many articles have reported about metal pollution in the soil. When research was conducted on heavy metal content in soils in Tan Long commune, Dong Hy district, Thai Nguyen province, Luong Thi Thuy Van (2012) showed that the soil samples contained levels of heavy metals exceeding permitted standards of the QCVN 03:2008/BTNMT many times. Among the samples, one contained very high levels of arsenic (As) and cadmium (Cd). Arsenic content was 949.15 mg/kg, 79 times over the limit, and the concentration of Cd was 195.20 mg/kg, 97.6 times over the limit. Ho Thi Lam Tra (2009), who analyzed the contents of copper (Cu), lead (Pb), zinc (Zn), and Cd (in both total and digestible forms) in 11 soil samples collected from the Dai Dong commune, showed that farmland was contaminated with heavy metals. Ten soil samples were polluted with Cu and 10 soil samples were polluted with Pb, and the 11 samples analyzed had concentration levels that exceeded permissible standards. The contaminated soil samples exceeded the allowable limit from 1.1 to 5.6 times (for Cu) and from 1.1 to 24.3 times (for Pb).

In her research, Cao Viet Ha (2012) showed that 10 of the total 50 soil samples from Van Lam district were contaminated with Pb. Two samples taken near Dong Mai and Nghia Lo hamlet of Chi Dao commune had very high Pb content that exceeded the limit 10-13 times compared with QCVN 03:2008/BTNMT. Lead poisoning in rural environments in Dong Mai hamlet is very high. According to the analysis of humans contaminated with Pb, Pb content in urine ranged from 0.25 to 0.56 mg/l and in blood was 135 mg/l, exceeding 1.5 times the permitted level (Ministry of Natural Resources and Environment, 2012).

There are many different methods used to treat heavy metals in the soil. However, recent methods using plants to treat heavy metals in soils is appealing because it is seen as an environmentally friendly approach and reduces costs significantly when compared to physical and chemical methods.

Watercress (*Thlaspi caerulescens*) grown for 391 days removed more than 8 mg Cd/kg from soil and 200 mg Zn/kg from soil, corresponding to reductions of 43% for Cd and 7% for Zn in contaminated soil (Luong Thi Thuy Van, 2012). According to a study by Dang Dinh Kim and researchers of the Institute of Environmental Technology (Ministry of Science and Technology, Vietnam) in 2008, Vetiver grass (*Vetiveria zizanioides*) grown on soil contaminated with 1400.5 ppm – 2530.10 ppm Pb was well developed after 90 days. The ability of Vetiver grass to extract Pb from soil ranged from 87% - 92.56% after 90 days of the experiment. In the study results of Bui Thi Kim Anh (2011), two fern species, *Pteris vittata* L. and *Pityrogramma calomelanos,* could absorb and accumulate As in their trunks up to 5876.5 ± 99.6 and 2426.3 ± 104.5 mg/kg, respectively.

The research of some scientists have demonstrated that mycorrhizal arbuscular fungi (AMF) will not only increase the growth capacity of the plant's development, but also can increase the absorption of minerals (such as P, Cu, Zn...) in the soil, and reduce the level of "shock" of the plant when it is grown in soils with a high salinity, soils that are too moist, high soil temperatures, and many other causes. In 2004, Tran Van Mao studied the efficiency of nutrient uptake of P by *Glomus* fungi when symbiotic in maize and showed that P content in maize root cells increased 35% for the *Glomus mosseae* fungus and 98% for the *Glomus fasciculatum* fungus. Regarding the ability to protect the host against pathogens of AMF, Schonbeck and Dehne (1989) studied 11 common crops, beans, barley, wheat, carrots, corn, onion, tobacco, tomato, cucumber, lettuce, and pepper, and found that AMF reduced common root diseases by 40% on the host plants. In addition, in 1989, Vancura and Kunc also found, along with the ability to increase the biomass and increase the stalk/root, that the AMF infection increased the activity of the nitrogenase enzyme and increased the level of phosphorus assimilation of their bean crops.

The plants used to treat soils contaminated with heavy metals must be capable of accumulating heavy metals, producing large amounts of biomass, and tolerating soil polluted highly with heavy metals, but since the general biomasses of these crops are low, adding mycoroot to the polluted soil is essential. The natural ability of plants to remove pollutants can be integrated and improved by symbiotic mycorrhizal fungi. Symbiotic mycorrhizal fungi are also considered a key to plant survival in contaminated soils by increasing metal resistance in plants and also improving the absorption of essential nutrients. The objective of this study was to evaluate the symbiotic relationship between native ferns and AMF fungi in the treatment of Pb contaminated soil in Dong Mai lead recycling rural village, Van Lam district, Hung Yen province in experimental pot conditions.

2. MATERIALS AND METHODOLOGIES

2.1. Materials

Five soil samples were collected in the upland, cultivation floor with a depth from 0 - 30 cm from Dong Mai village, Chi Dao commune, Van Lam district, Hung Yen province.

Ferns (*Pteris vittata* L.) originated from the Dong Mai village, Chi Dao commune, Van Lam district, Hung Yen province.

AMF mycorrhizal bio-product: Bio-product Mycoroot (Green Times Co., ICDC Building, I2 Lot, D2 Street, Hi-Tech Park, District 9, HCMC)

2.2. Location and time of the study

The pot experiment was conducted in a net house - Department of Microbiology - Faculty of Environment - Vietnam National University of Agriculture from January to April, 2014.

2.3. Research methodology

Soil sampling was conducted as shown in TCVN 4046: 1985 and TCVN 5297: 1995.

The pot experiment was designed as a randomized complete block (RCB) with 4 treatments and 3 replications, each pot was one replication. A mixture was made of the soil samples and sand at a ratio of 3:1 and then sterilized at 121°C for 2h. Before being filled with 3 kg of the mixture, the pots were sterilized with alcohol 70º, and 4 ferns with 20 cm stalk-leaves of length were planted in each plastic pot.

- Treatment 1: mixture
- Treatment 2: mixture + 20g mycoroot bio-product/plant
- Treatment 3: mixture + 40g mycoroot bio-product/plant
- Treatment 4: mixture + 80g mycoroot bio-product/plant

The number of mycorrhizal inoculants was determined by weighing 1g bio-product and then dissolving it in water, spores were collected by using an average sieve-ray beam to all spores in the Petri dish, and the number of spores was counted via stereoscopic microscope.

Sample preparation

- Soil samples were dried, ground and sieved through 2mm, and stored in polyethylene bags at room conditions.
- Ferns were harvested after 40 days, soil attached to roots was cleaned off by water flow. Stalk-leaves and roots were detached and weighed fresh. Samples were dried at 70ºC until they reached a constant weight before determining the dry weight, then samples were ground and stored in polyethylene bags at room conditions.

Soil analytical methods

+ pH (KCl) was determined by pH meter (HQ11D, USA).

+ Mechanical composition was determined by the pipette method (Robinson straw) (Nguyen Huu Thanh *et al.*, 2006).

+ The cation exchange capacity (CEC) was determined by the ammonium acetate method.

+ Soil organic matter (OM) was determined by the Walkley – Black method (Nguyen Huu Thanh *et al.,* 2006).

+ Total Pb content: Samples were homogenized by aqua regia solution (a mix of HCl and HNO_3 acid solution at a ratio of 3:1) and measured by atomic absorption spectroscopy (AAS 240FS, USA).

+ Available Pb: the samples were extracted by HCl 0.1M solution then measured by atomic absorption spectroscopy.

- Fern sample analysis

Weighed 0.5 g of dried fern sample, baked it at 550°C for 4 hours and cooled it in ambient conditions, then added 5 ml of HCl 6N and boiled it for 15 minutes to completely dissolve the residue. After being cooled in ambient conditions, 50 ml distilled water was added and Pb content then measured by a portable atomic absorption spectroscopy (AAS 240FS, USA).

Amount of Pb that the ferns absorbed from the soil: Based on the Pb content of each fern and number of ferns in each pot.

Data were analyzed by ANOVA using IRRISTAT 5.0. The least significant difference ($LSD_{0.05}$) was used to determine significant differences among treatments at $P \leq 0.05$.

QCVN 03:2008/BTNMT (Vietnam) was used for the standard limitation of heavy metals in the soil.

3. RESULTS AND DISCUSSION

3.1. Soil properties of polluted soil

The determination of some physical and chemical properties of the experimental soil was necessary for the general determination of Pb accumulation potential.

Soil samples taken in the Dong Mai village contained 12.7% clay, up to 45.1% silt, and about 42.2% sand (Table 1). According to the USDA's classification of soil textures, the sample soil was Dystric Fluvisols. This soil was also the same as the Red River's alluvial soil according to Vietnam soil classification because its CEC: 10-15 cmolc kg^{-1} and OM% was an organic average group (Cao Viet Ha, 2012). This type of soil can hold contaminants on the average level (Cao Viet Ha, 2012).

pH_{KCl} of the soil was as low as 4.3, indicating acidic soil (Nguyen Huu Thanh *et al.*, 2006). Improper battery recycling and overuse of chemical fertilizers could be the main causes of low pH. Low soil pH promotes flexibility of the cations in the soil and is a high risk factor of heavy metal contamination (Cao Viet Ha, 2012).

The total Pb concentration of the soil soared up 2622.14 mg kg^{-1}, over 37 times the standard limit. The available Pb was 5.4 times higher than the standard of QCVN 03:2008/BTNMT. Cao Viet Ha (2012) reported that Pb content of soil sampled at Dong Mai of Chi Dao commune exceeded 10-13 times the limit compared with QCVN 03: 2008/BTNMT. These results indicated that the soil of Dong Mai village (Chi Dao commune) was seriously contaminated with Pb. However, this soil has been used for farming, such as rice cultivation, and there was a high risk of Pb accumulation in agricultural products. Public health could be vulnerable if using Pb contaminated products.

Table 1. Physical and chemical properties of the soil

No.	Criteria	Value
1	Sand (%)	42.2
2	Silt (%)	45.1
3	Clay (%)	12.7
4	pH_{KCl}	4.3
5	OM (%)	3.03
6	CEC (cmolc kg^{-1})	13.2
7	Total Pb (mg kg^{-1}dry soil)	2,622.14
8	Available Pb (mg kg^{-1}dry soil)	378.2

3.2. Effects of the mycoroot product on biomass and Pb absorption of ferns

Ferns that were treated with an increased mycoroot bio-product amount produced fresh biomass 1.1 to 1.6 times greater (Table 2)

Table 2. Fresh and dry biomass of fern plant after 40 days add mycoroot

Treatments	Fresh biomass of				Dry biomass of			
	Leaves (g/pot)	Roots (g/pot)	Total	Control comparison (%)	Stalk-leaves (g/pot)	Roots (g/pot)	Total	Control comparison (%)
Mixture	37.66	24.43	62.09	-	6.18	5.28	11.46	-
Mix. + 20g bio-product	40.06	27.88	67.94	9.42	6.20	6.06	12.26	6.98
Mix. + 40g bio-product	49.41	31.06	80.47	29.60	8.24	6.92	15.16	32.29
Mix. + 80g bio-product	55.98	40.66	96.64	55.64	9.11	8.06	17.17	49.83
CV %	4.50	3.80	4.70		2.50	3.80	4.20	
$LSD_{0.05}$	3.88	2.20	6.57		0.35	0.47	0.73	

compared with the control. This result was consistant with Bui Thi Kim Anh's (2011) report showing that when mycorrhizal fungi bio-products were used to treat ferns for absorbing As in an area after coal mining.

Fresh weight of leaves and roots of AMF treatments were significant higher than the control ($p < 0.05$) (Table 2). Moreover, the dry biomass of fern plants decreased when less mycorrhizal fungi bio-product was added. Of which, stalk-leaves and root dry weight of treatments 3 and 4 were significantly higher ($p < 0.05$) than that of treatment 1 (Table 2).

Plants increased their biomass due to symbiotic relationships with AMF. Root symbiotic fungi (AMF) increased the area of contact between roots and soil thereby increasing the absorption surface of the roots. Roots interact with small particles of soil, absorbing the nutrients and water from where hair roots did not rise. Additionally, the decomposition process, in which AMF promoted the transformation of organic compounds in the soil from indigestible organic matter into digestible inorganic substances, and increased the solubility of iron and phosphorus. This allowed plants to absorb nutrients more easily and increased plant biomass. In addition, AMF could secrete antimicrobial substances to inhibit infection of disease microorganisms and secrete other useful substances such as amino acids, vitamins, enzymes, and indole acetic acid (IAA). Thus AMF could stimulate beneficial microorganisms in the root zone and increase the growth and development of plants. These results are entirely consistently with the results of many previous reports (Schonbeck and Dehne, 1989; Vancura and Kunc, 1989; Tran Van Mao, 2004).

Mycoroot product contributed to increased plant biomass and incited more removal of heavy metals from the soil.

Pb concentrations of stalk-leaves and roots were analyzed before and after being treated with mycoroot products were 32.55 and 115.79 mg Pb/kg of dry biomass, respectively. After 40 days of treatment with mycoroot bio-product and transplanting, the cumulative Pb content of fern stalk-leaves ranged from 85.05 mg Pb/kg to 123.93 mg Pb/kg dry biomass and increased 2.61 to 3.81 times (Figure 1). On the other hand, the increase of Pb accumulation in fern roots were 5.20 and 6.56, 7.21 and 6.58 times in each treatment, respectively (Figure 2).

Pb accumulation in the ferns' stalk-leaves and roots positively correlated to the dose of bio-product added to the soil. The highest Pb accumulation in the fern stalk-leaves of treatment 4 and roots of treatment 3 reached 123.93 and 834.63 mg Pb/kg dry biomass, respectively (Figures 1 and 2). However, Pb accumulation in the roots was 6 times higher than that in the fern stalk-leaves 40 days after being treated with bio-product. The same results were reported (Luong Thi Thuy Van, 2011; Phan Quoc Hung *et al.*, 2012).

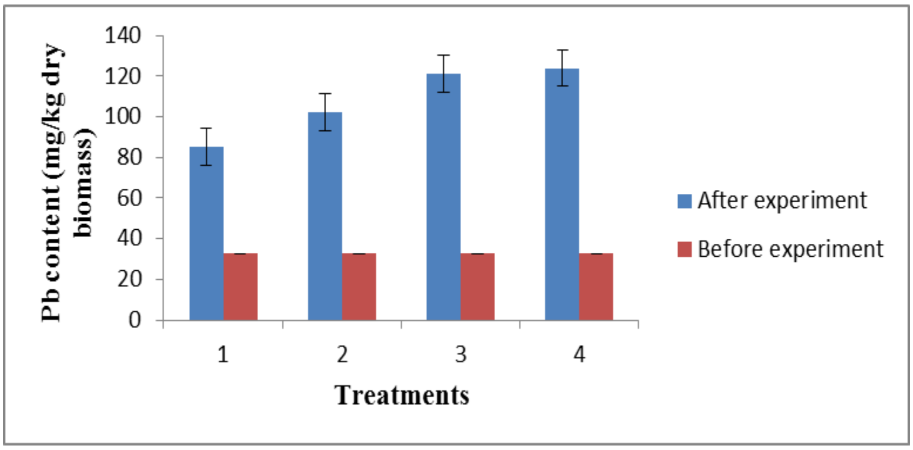

Figure 1. Pb accumulation of fern stalk-leaves before and after 40 days treated mycoroot bio-product

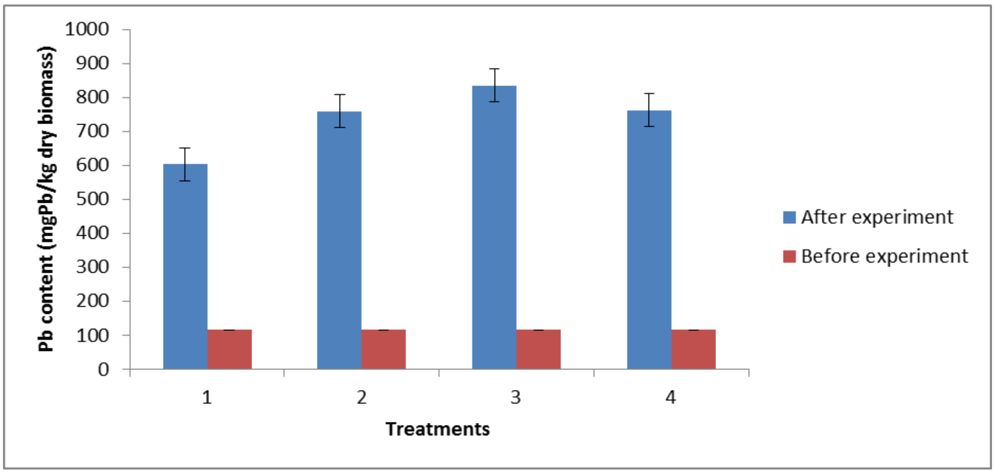

Figure 2. Pb accumulation of fern roots before and after 40 days treated mycoroot bio-product

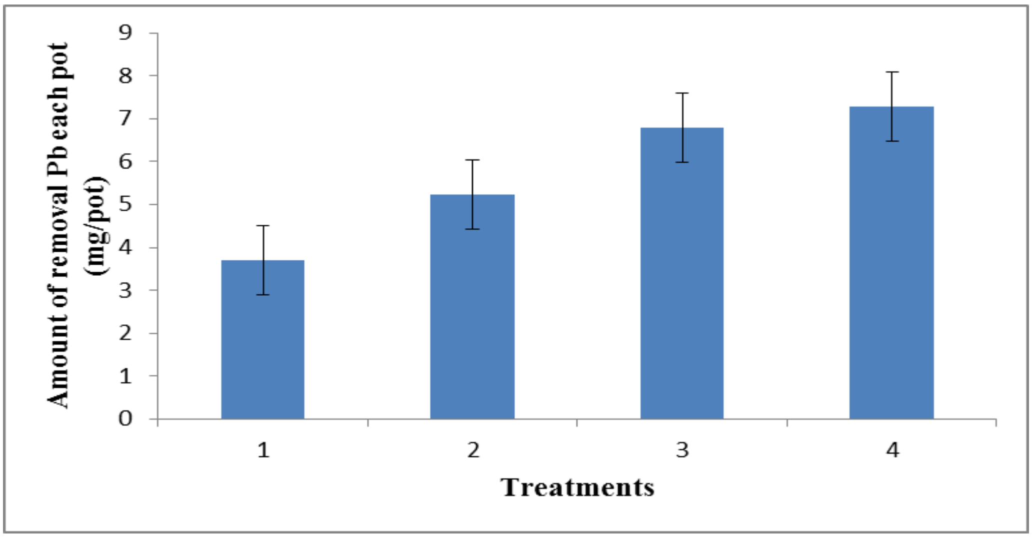

Figure 3. The amount of Pb removed from the soil by ferns

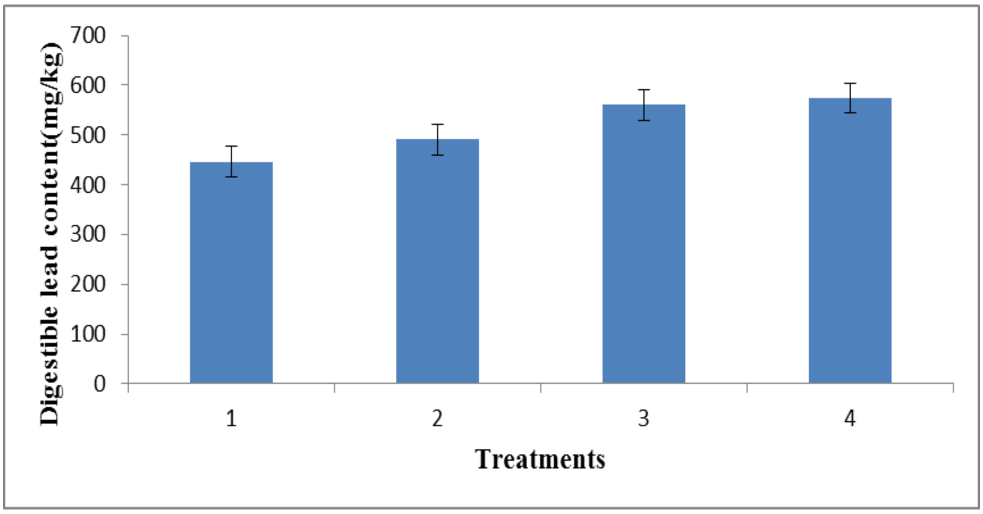

Figure 4. Digestible Pb content of the soil 40 days after transplantation of ferns

Pb accumulation of roots and stalk-leaves of supplemented mycoroot-product treatments were higher than the control. This showed a positive influence of root symbiotic fungi (AMF) to the accumulation of Pb. Similar results were mentioned by Bui Thi Kim Anh (2011). The results recommended that the dose of mycoroot inoculants should be 40 - 80g bio-products/plant for the Pb removal by ferns.

3.3. The Pb removal of ferns from polluted soil

The amount of Pb removed from the soil due to accumulation in the mycoroot-product treated ferns was readily higher than in the control (Figure 3). By adding mycoroot-product at 40-80 g/plant, the amount of Pb removed from the soil was nearly double compared to control.

The digestible Pb content of the rhizosphere soil samples increased to 128.2 mg Pb/kg dry soil after being treated with mycoroot-product. Samples from treatments 3 and 4 were nearly 1.26 and 1.29 times greater than control, respectively. Highly digestible Pb content favored Pb absorption by plants and accumulation into biomass. AMF could secrete organic acids, enzymes, etc. and these substances disintegrated matter in the soil and increased the flexibility of Pb. AMF indirectly supported the ferns by removing Pb from the polluted soil (Schonbeck, 1989; Vancura, 1989).

4. CONCLUSION

The total Pb content of all soil samples collected was 2622.14 mg/kg dry soil, 37 times higher than standard for agricultural land (QCVN 03: 2008 / BTNMT).

After 40 days of experimentation, application of 80g/plant of mycoroot bio-product resulted in increased biomass of ferns, as well as stimulated the Pb uptake of ferns upto 7.27 mg Pb/pot.

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