COMPARISON OF SUNFLOWER MV-005 AND VETIVER GRASS IN SOIL DECONTAMINATION AND EVALUATION OF THE EFFECTIVE MICROORGANISMS AMENDMENT IN PHYTOREMEDIATION

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Received: 9 March 2020; Accepted: 2 November 2020

ABSTRACT

Phytoremediation is an alternative approach, that has attracted researchers and government concerns to against heavy metals pollution in soil. Phytoremediation is concerned as the next generation technique since itself is inexpensive fee, comfortable, and friendly to the environment. Sunflower and vetiver grass are known as the "hyperaccumulators" and effectively remove the trace of Lead (Pb) contamination. In this three-months study, the phytoremediation efficiency of sunflower MV-005 and Vetiver grass were evaluated in conditions of high/ mild/ low soil Pb (4000 ppm, 2000 ppm and 1000 ppm). Results confirmed the advantages of sunflower in soil Pb elimination as compared to Vetiver. Furthermore, amendment of effective microorganisms (EMs) facilitates sunflower MV-005 growth and the phytoremediation efficiency. This study, for the first evidence, provides the comparison between sunflower MV-005 and Vetiver grass in soil Pb elimination and opens new trend of research in sunflower based phytoremediation amended by EM.

Keywords: H. annuus, phytoremediation, *V. zizanioides*, effective microorganism, soil lead pollution.

1. INTRODUCTION

Conventional techniques to remediate heavy metals such as *in-situ* vitrification, soil incineration, excavation and landfill are expensive, requires the high skilled human resources and able to be applied in the limit space [1-2]. Furthermore, these practices have negative impacts to soil quality, infertility problem or unwanted residues depositing in soil [2]. Phytoremediation has been considered as an alternative approach, friendly to the environment, low cost and efficient to remove heavy metals [3]. Phytoremediation is the approach decontaminating the pollutants by immobilizing and depositing heavy metals to plants, preventing the expansion or leaching of heavy metals. Phytoremediation undergoes through several steps, including rhizofiltration, phytostabilization, phytovolatilization and phytoextraction [3]. Rhizofiltration is the phase that uptakes soluble heavy metals to the surfaces or inside of roots. Phytostabilization is the phase that plants stabilize the heavy metals and deposit them inside of plants. Phytovolatilization is the phase to extract several heavy metals (for instance, mercury, selen) and releasing them to the air from leaves. Phytoextraction is the phase to extract heavy metal from plants. Depending on type of plants, then, the methods may be varied [1, 4].

Nevertheless, phytoremediation itself has limiting tolerance to heavy metals and therefore to increase the decontamination yield, chelators such as ethylene diamine tetraacetic acid (EDTA) have been amended [5]. However, EDTA existence with high dose might affect and toxify the soil or might harm to other soil microorganisms and induce the chelators leaching to underground water [5-6]. Recently, the amendment of effective microorganisms (EMs) has been considered [3, 7]. Basic principle of this approach is to change the solubility of heavy metal via pH, redox reactions or adsorption and turns these into non-hazardous forms [8]. However, the environmental conditions are varied and depend on the soil types of contaminated areas, therefore partly limit the advantage of EMs amendment. Thus, more findings to surmount bioremediation limitation are necessary.

Sunflower (*Helianthus annuus*) meets all the requirements for "hyperaccumulator" in phytoremediation strategy such as high tolerance to heavy metals, short of growing season, efficiently stabilizing and deposit heavy metals and easy to harvest [9]. Furthermore, Sunflower is considered as one of the plants for bioenergy production and could be the natural scenery for the polluted area. Vetiver grass (*Vetiveria zizanioides*) is also employed to detoxify waste-water or soil phytoremediation [10-11]. With deep roots, fast growing, high resistance to extreme environmental conditions while stabilizing to the wide range of heavy metals types and other pollutants [11], Vetiver grass becomes the ideal choice for phytoremediation. Many evidences have been confirmed the virtues of Vetiver grass and Sunflower in anti- soil metal accumulation, however the prepotent of these plants to each other, remains elusive. The aims of this study are to compare the phytoremediation efficiency between sunflower MV-005 and Vetiver grass to contaminated soil (Lead-Pb served as the research model) and examine the aid of EMs in anti-metal accumulation. The study could be the reference in choosing plants for phytoremediation and emphasize the role of EM amendment during sunflower crops, in attempt to increase phytoremediation yield.

2. MATERIALS AND METHODS

2.1. Materials

Seeds of sunflower MV-005 (*Helianthus annuus)* were purchased from Sao Vang company and grown separately in pots. At the height of 10 cm, vigorous sunflowers were employed for experiment (three pulps/pot). Vetiver grass *(V. zizanioides*) was purchased from Nong An Phu Farm (Thuan An, Binh Duong). Three-pulps of vigorous Vetiver grass were grown in pots. Effective Microorganisms (EM) Fert-1 served as the amendment into soil, was purchased from Sao Vang company. To secure the Pb quantity during watering, all pots must be not pierced at the bottom (Figure 1).

2.2. Methods

2.2.1. Sample preparation

4 kg of soil in individual pots 23x25x25 cm (H x W x L) was added with the indicated Lead (II) Acetate - Pb $(CH_3COO)_2$ concentrations (1000 ppm, 2000 ppm, 4000 ppm) and mixed by homogenizer to generate homogenous Pb contaminated soil model for study. Pb contamination was evaluated prior to each experiment. To ensure each individual sample representative for each pot, soil sampling tube was employed to collect the soil from top to bottom of the pot. Soils, afterwards, were homogenized and 20 grams in each time point (1 month, 2 months and 3 months) were collected and qualified by The Environment and Energy Center (EEC, Tan Binh District, Ho Chi Minh city) as TCVN 6496:1999-ISO. In brief, soil Pb

level (mg/kg soil) was measured as technical protocols of flame and electrothermal atomic absorption spectrometric methods. Pb quantity in each soil samples were defined by standard curves. Soil samples without either sunflower or Vetiver treatment, were served as the control for study. The study was repeated two-times and independently (Figure 1).

2.2.2. EM FERT-1 preparation and amendment

Microorganisms and plants have been considered as the alternatively safe approach to the environment [3]. Mixture of microorganisms or effective microorganisms (EM) have the high resistance to heavy metal conditions and enables to degrade heavy metals and convert them to non-hazardous or soluble forms. These are symbiotic microorganisms and display the synergistic effect to heavy metals elimination. In this study, we used 20 grams of EM Fert-1 and dissolved in 1 lit of distilled water, followed by irrigating to each indicated pot. EM Fert-1 was employed in two phases: 1) at the day 5, this is the phase that sunflower is tender and EM Fert-1 would facilitate the survival rate of sunflower; 2) at the day 30, this is the phase that sunflower grows maturely/ flowering and EM Fert-1 would facilitate sunflower growth. The pots planted sunflower without EM Fert-1 serving as the control for study.

2.2.3. Phytoremediation efficiency

Determination of Pb quantity removed in the soil, was calculated by the following equation:

% Efficiency = $100 \times [Pb_0\text{-}Pb_t]/ Pb_0$

Pb_0: the concentration of the initial Pb (ppm)

Pb_t: the concentration of the time-point test (ppm)

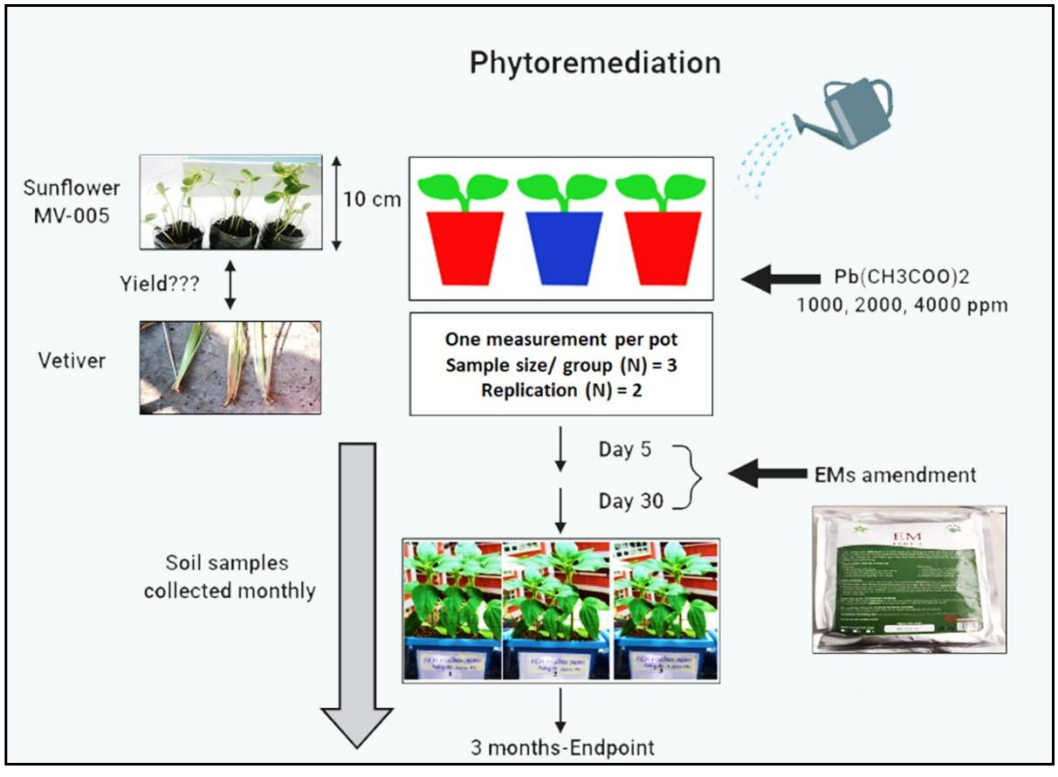

Figure 1. Experimental design

2.2.4. Statistical analysis

Data are expressed as mean ± SD (Standard Deviation). Experimental differences were examined using ANOVA and Student's *t*-tests, as appropriate by Graphpad prism 6.01. *P values* < 0.05 were considered to indicate statistical significance.

3. RESULTS AND DISCUSSION

3.1. Sunflower MV-005 and Vetiver grass possess Pb contaminated soil phytoremediation

Since both sunflower and Vetiver grass are able to eliminate soil Pb, therefore in this study, we aimed to compare these hyperaccumulators activities in soil polluted with indicated Pb concentration (4000 ppm). Due to the limit of time, we chose the sunflower MV-005 (short life time-3 months) for this study. Results showed that, the Pb level was significantly decreased in both plants in time dependent manner as compared to negative control (NC) (Figure 2). Sunflower displayed the notable remediation at second-month versus to first-month and reach to the deepest decrease at third-month ($P < 0.001$ and $P < 0.0001$, respectively). Regarding to Vetiver grass, first-month and second-months were not significantly different (*ns*) while soil Pb level was significantly reduced at third-month (Figure 2). In addition, there was a slight decrease at third-month in NC group ($P < 0.05$) while there was no change in first-month and second-month as compared to the initial. These data suggested that soil Pb (4000 ppm) was eliminated in sunflower and Vetiver models during 3 months study.

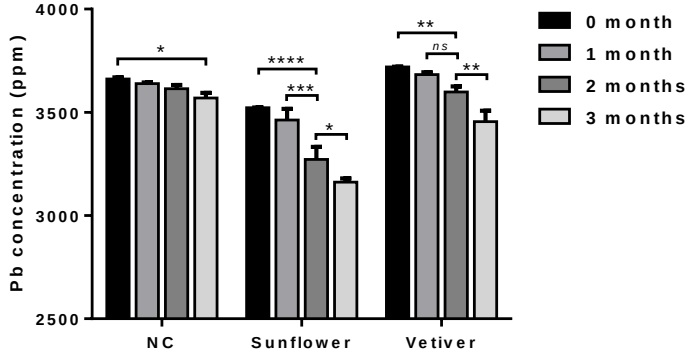

Figure 2. Sunflower MV-005 and Vetiver grass reduce soil Pb level within 3 months of study.

3.2. Sunflower MV-005 reflects higher yield of Pb elimination than Vetiver grass in 3-month study

Percentage of the soil Pb removal is considered as the criteria to compare the phytoremediation efficiency between sunflower MV-005 and Vetiver grass. Figure 3 showed that soil Pb (initial level 4000 ppm) in MV-005 treatment was slightly reduced at second-month as compared to either NC or Vetiver. Consequently, data showed that soil Pb level eliminated in MV-005, distinguished to Vetiver grass and NC ($P < 0.01$, $P < 0.0001$). These data indicated that in certain of time (3 months of treatment), sunflower MV-005 seems to efficiently remove heavy metals, better than Vetiver grass. Our study is consistent to the report by Boonyapookana *et al*. [12]. This study indicates the significant higher content of Pb accumulated in sunflower as compared to Vetiver grass and Tobacco in hydroponic condition. The study also indicated that overall potential Pb content deposited in sunflower was almost doubled as in Vetiver grass (171% and 88%, respectively). However, Vetiver grass has the

growth time relatively longer than sunflower MV-005 [11] while three-months is the harvest time of MV-005. Therefore, it is possible that Vetiver grass was not enough "mature" at phytoremediation, resulting in lower capacity of Pb elimination as compared to sunflower MV-005. It is necessary to conduct further studies adequately between comparable long-life plants. In addition, Pb might not be the optimum heavy metal for Vetiver grass [11]. Therefore, this characteristic also partially reflects the lower phytoremediation efficacy in Vetiver as compared to sunflower.

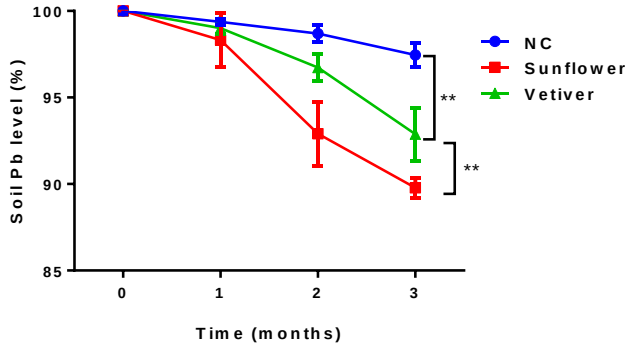

Figure 3. Sunflower MV-005 and Vetiver in soil Pb reduction

3.3. Low dose of soil Pb facilitates the phytoremediation efficiency both in sunflower MV-005 and vetiver

It is possible that, soil Pb at 4000 ppm is relatively high the phytoremediation efficiency of both sunflower MV-005 and Vetiver grass, leading to the percentage of soil Pb reduction was low (Figure 3). To test this hypothesis, MV-005 and Vetiver grass were exposed to lower soil Pb concentrations (1000, 2000 ppm) and soil Pb at 4000 ppm served as the control. Figure 4 (left) showed that lower soil Pb level facilitated the phytoremediation efficiency in MV-005 as compared to high dose of soil Pb (4000 ppm) (1000 ppm > 2000 ppm, $P < 0.01$; 2000 ppm > 4000 ppm, $P < 0.01$). Regarding to Vetiver grass, there was the same pattern to the lowest soil Pb concentration (1000 ppm) (Figure 4, right). However, there was not significantly difference between 4000 ppm and 2000 ppm soil Pb treatment. These data suggested that both sunflower MV-005 and Vetiver grass possess the tolerance of phytoremediation. Indeed, metal dose exceeding might result in irreversible effects to plant growth, root function, and heavy metal accumulation [13-14] and therefore lower soil Pb eliminating capacity of both plants. Therefore, before using these "hyperaccumulators", it is necessary to examine the initial condition of soil Pb level and estimate the crop number or time point for phytoremediation to deliver the highest effectiveness.

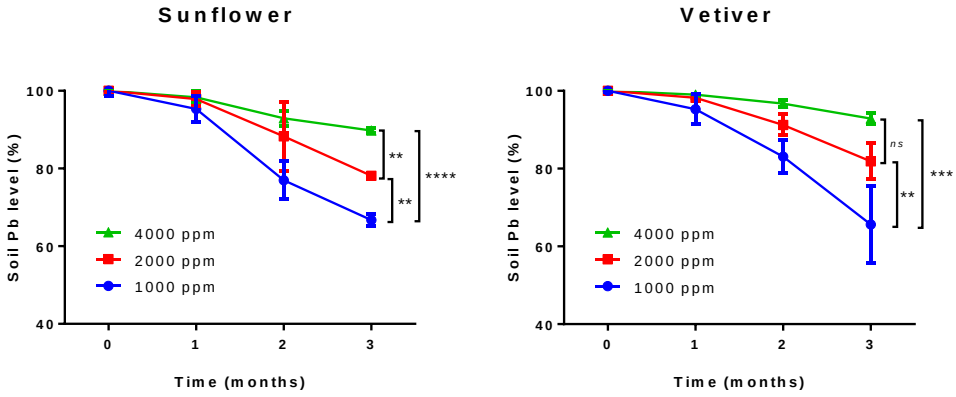

Figure 4. The lower dose of soil Pb facilitates the phytoremediation efficiency in sunflower MV-005 and vetiver grass.

3.4. Amendment of effective microorganisms (EM) increases the yield of phytoremediation by sunflower *MV-005*

Due to the efficiency of phytoremediation in sunflower MV-005 was higher than those in Vetiver grass (Figure 3), therefore MV-005 was chosen to further examine the effects of EM FERT-1 on the phytoremediation. Data showed that treatment with EM FERT-1 seems promote plant growth and no signs of development dysfunction as compared to NC (Figure 5, left). Furthermore, amendment with EM FERT-1 enhanced the efficiency of phytoremediation in MV-005 after three months ($P < 0.05$) while there is no difference at 1-2 months (Figure 5, right). These data suggested that EM FERT-1 amendment acts effectively to support the phytoremediation by sunflower MV-005, however this amendment takes longer time (3 months) to enhance soil Pb elimination.

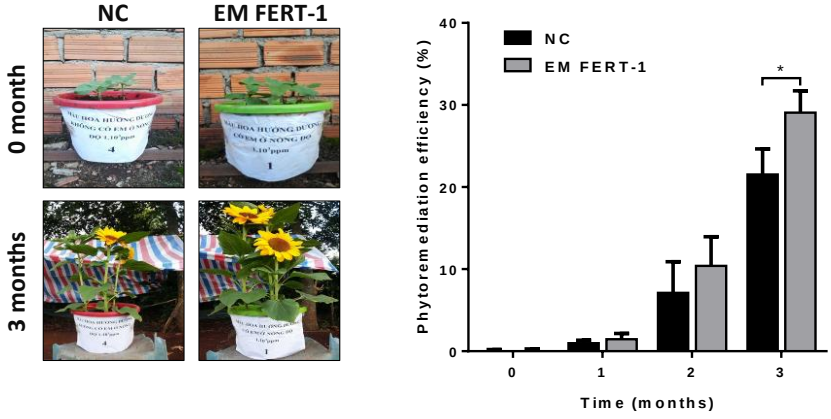

Figure 5. Amendment of EM FERT-1 enhances the phytoremediation by sunflower MV-005.

The amendment of EDTA might increase the phytoremediation yield and leaves/stem Pb deposition in sunflower, resulting in the higher resistance and better efficiency in Pb elimination. Therefore, adding chelators such as EDTA might be the good option for further experiment in sunflower based-soil Pb phytoremediation. The main problems as using chelators as EDTA, are that might poison soil quality or microorganism population and hardly degradable. These might induce the secondary pollution and leaching to the underground water [5, 6]. Therefore, more alternative findings in combination with sunflower-based phytoremediation are encouraging.

In this study, we have tested the effect of EM FERT-1 in combination with sunflower MV-005 and initially brings back the satisfying results after three months of study. This would inspire us to have more research to discover the optimal constituents of EM and conditions to apply EM in phytoremediation. Furthermore, some of genetic modified microorganisms (GEMs) have been employed to enhance the bioremediation and achieves some of initial successes [4]. However, it is seriously to reevaluate the ecology effects from GEM spreading out to nature, which might significantly affect to wildtype micro-organisms balance. Moreover, this study did not investigate the soil Pb depositing areas of sunflower MV-005, which might be occurred at roots, shoots or leaves. However, Lin et al. indicated that the heavy metals might be accumulated mainly at the root of sunflower (73% for copper) [15]. By contrast, other study showed that heavy metals (Zinc) accumulated in leaves and flower [16]. These different studies reaffirmed that the heavy metal depositing location might depend on the type of heavy metal and bio-transfer/ bioaccumulation of plants. The determination of Pb localization in sunflower would facilitate the phytoextraction phase or for further biofuel production [16].

4. CONCLUSION

The results of this study indicated that sunflower MV-005 (*H. annuus*) eliminates soil Pb pollution effectively as compared to Vetiver grass (*V. zizanioides*). Low soil Pb content might facilitate the phytoremediation. The phytoremediation efficiency can be enhanced by the addition of EM FERT-1. However, it is necessary to discover some of specific EMs and test whether these EMs fit sunflower-based phytoremediation in different soil conditions and tested with insoluble heavy metal forms. Furthermore, for the severe heavy metal-polluted areas, it is required to combine different strategies such as soil incineration, excavation or landfill together with phytoremediation based-sunflower, since the phytoremediation has itself tolerance to dose of heavy metals. Nevertheless, phytoremediation-based sunflower meets the requirements for long-term soil Pb elimination, economical solution, biofuel production.

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TÓM TẮT

SO SÁNH KHẢ NĂNG KHỬ KIM LOẠI CHÌ TRONG ĐẤT GIỮA CÂY HOA HƯỚNG DƯƠNG MV-005 VÀ CỎ VETIVER VÀ ĐÁNH GIÁ HIỆU QUẢ SỬ DỤNG PHÂN BÓN VI SINH-EM TRONG KHỬ KIM LOẠI NẶNG

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Việc sử dụng thực vật trong xử lý kim loại nặng gọi tắt là "phytoremediation", là cách tiếp cận đang thu hút nhiều nhà nghiên cứu và các chính phủ trong quản lý và xử lý ô nhiễm kim loại. Phytoremediation được xem là có tiềm năng lớn trong tương lai, do đặc tính chi phí rẻ, dễ thực hiện, thân thiện với môi trường. Hoa hướng dương (sunflower - *H. annuus*) và cỏ vetiver (*V. zizanioides*) được xem là thực vật có khả năng tích lũy cao kim loại nặng, "Hyperaccumulator", và được kiểm chứng ứng dụng hiệu quả trong loại bỏ kim loại nặng tại vùng ô nhiễm. Trong nghiên cứu này, chúng tôi tiến hành so sánh hiệu quả xử lý kim loại Chì trong đất, giữa hoa hướng dương chủng MV-005 và cỏ vetiver, trong các nồng độ Chì khác nhau (cao, vừa, thấp). Kết quả sau 3 tháng xử lý chỉ ra rằng, hoa hướng dương MV-005 cho kết quả tốt hơn trong xử lý kim loại chì trong đất. Bên cạnh đó, việc bổ sung phân bón vi sinh (Effective Microoranisms-Fert 1) trong các giai đoạn phát triển của MV-005, giúp thực vật tăng trưởng và tạo ra hiệu quả xử lí kim loại Chì trong đất cao hơn. Nghiên cứu này, lần đầu tiên cho thấy sự ưu điểm của hoa hướng dương MV-005 so với cỏ vetiver khi xử lí kim loại Chì trong đất, đồng thời mở ra hướng nghiên cứu mới trong việc tìm ra sự kết hợp phân bón vi sinh EMs để tăng tối ưu khả năng xử lý vùng đất bị ô nhiễm chì.

Từ khóa: H. annuus, phytoremediation, *V. zizanioides,* chế phẩm sinh học, ô nhiễm chì trong đất.