A TWO-YEAR FIELD STUDY OF PHYTO REMEDIATION USING
SOLANUM NIGRUM L. IN DONGNAI, VIETNAM

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ABSTRACT

A two-year in-situ phytoremediation trial was conducted in Dongnai province. The phytoremediation efficiency of Solanum nigrum L. was detected, by monitoring the change of soil Cadmium level in the 0 - 20 cm soil depth. The results indicate that soil Cd decreased significantly by planting S. nigrum. The Cd concentrations decreased averagely from 2.75 mg kg⁻¹ to 2.45 mg kg⁻¹ in the first year and 2.33 mg kg⁻¹ to 1.53 mg kg⁻¹ in the second year, separately. Decrease by a factor of 10.6% in first year and 12 % second year. After two years phytoremediation by S. nigrum, Cd concentrations of the seven experimental plots with S. nigrum growth decreased from 2.75 mg kg⁻¹ to 1.53 mg kg⁻¹, and decrease by a factor of 24.9%. Therefore, using S. nigrum for phytoremediation of Cd contaminated farmland soils seems very promising, and we can conclude that S. nigrum will get a better performance in the warmer area, as the temperature of the experimental area is relatively lower.

Key words: In-situ, phytoremediation, cadmium, Solanum nigrum L., efficiency.

1. INTRODUCTION

Among the heavy metals, Cd has a very high mobility in soil-plant systems, with propensity to damage both human health and the functioning of ecosystems [1]. Therefore, it is necessary to put remediation of Cd-contaminated soils into action.

Until now, many published papers suggested that phytoremediation could potentially be used to remediate heavy metal contaminated soils [2], as compared with physical and chemical techniques, phytoremediation has been advocated as a cost-effective and environmental friendly, green technology that utilizes the capacity of hyper-accumulator plants to extract heavy metals from soil [3, 4]. However, only a few attempts have been conducted to evaluate the phytoremediation efficiency of accumulators in field trials [5]. Thus field trials or commercial operations that demonstrate successful phytoremediation of metals have been limited [6]; Therefore, it is essential to test the phytoremediation possibility of defined hyper-accumulator
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plants on a field scale.

Solanum nigrum has been defined as a Cd accumulator on both a laboratory and field scale [7]. Some field experiments have shown that S. nigrum has a potential application for phytoextraction of Cd from contaminated soils, and the plant growth can be enhancing by some agriculture measures [8]. It is necessary to conduct a multi-year field experimental to determine the relationship between plant accumulation amount and the decreasing amount of soil Cd.

Since many years, together with the growth of agriculture in general, producing vegetables in Bien Hoa City, Dong Nai has provided dozens of tons of vegetables to the market, which has met the demand for quantity as well as quality of vegetables supplemented daily with meals. However, the massive application and lack of selectivity of technological advances such as fertilizers, growth stimulants, plant protection drugs, the existence of heavy metals in the soil has polluted not only the environment but also the cultivation of vegetables, which affects users’ health.

In this study, a two-year experimental study using S. nigrum was conducted in agricultural land planting vegetables in Dong Nai. The changes of the concentration of cadmium in the top soil layer of 20 cm was observed during the study, in the effort to evaluate the effectiveness of the phytoremediation under remediation towards a natural soil ecosystem beside the Cd pollution.

2. MATERIALS AND METHODS

2.1. The experimental site

In this study, we chose four typical large vegetable villages in Dong Nai (Tan Bien, Tan Binh, Trang Dai, Ho Nai), collecting 40 soil samples at different locations, and then analyzed the content of heavy metal Cd in soil AAS method. Results showed that 85% samples had Cd concentrations in excess of permitted levels (QCVN 03:2015/BTMNT, heavy metal Cd content of the livelihood land is ≤ 2 mg/kg of dry biomass).

Table 1. The properties of the upper cm of soil in the experimental field. Dada is shown as Mean ± SD (n = 40).

| Parameter                  | Units | Value       |
|----------------------------|-------|-------------|
| Gravel (> 2 mm)            | %     | 6.7 ± 0.4   |
| Coarse sand (0.2 - 2.0 mm) | %     | 22.5 ± 2.8  |
| Fine sand (0.02 - 0.2 mm)  | %     | 31.0 ± 1.6  |
| Silt (0.002 - 0.02)        | %     | 27.5 ± 2.6  |
| Clay (< 0.002 mm)          | %     | 2.4 ± 0.3   |
| pH                         | %     | 6.3 ± 0.7   |
| Total CEC (Cation Exchange Capacity) | c mol kg⁻¹ | 16.8 ± 1.5 |
| Organic matter             | %     | 1.3 ± 0.1   |

The study area is located in the tropical monsoon region with the annually average air
temperature of 25.7 - 26.7 °C. The average temperatures in dry and rainy season is 25.4 - 26.7 °C and 26 - 26.8 °C, separately. The annual average rainfall is 1,700 - 1,800 mm. Although climatic conditions and soil in four study areas were relatively same, Trang Dai vegetables village was chosen as study site because of convenient traffics as well as conditions serving experimental work. Some soil properties in this location with the area of 500 m² were shown in Table 1.

2.2. Experiment layout

In this study, the phytoremediation efficiency of S. nigrum were introduced by calculating the decrease of soil Cd in the soil after phytoremediation was conducted. For the study, seedlings of S. nigrum were cultured obey the following method: On 15th March, 2014, seeds were sown uniformly in the soil before propagation was carried out using a greenhouse-like chamber covered with polyethylene membrane and cotton quilt, which was maintained at the following conditions: natural sunlight, temperature 22/25 °C (day/night); relative humidity 45 – 71 %. Clean water is applied to achieve about 80 % of the soil water holding capacity (WHC). The whole operation cost about one month until six mature leaves developed.

On 15th March, 2014, eight independent experimental plots (as shown in Table 3, marked as P-1, P-2, P-3, P-4, P-5, P-6, P-7, and P-CK, respectively) were ploughed up to homogeneity by normal agronomic machinery before seedlings were transplanted. Each plot was 50 m² (10m×5m). Ten soil samples were collected from each plot on April 15th. Seedlings were transplanted to all the plots except P-CK on April 15th following this scheme: 0.3 × 0.3 m. Agricultural management and fertilizer application was maintained until 15th October, when the plants were harvested by cutting aboveground part. After harvesting, ten soil samples were collected in each plot immediately in the same method. The entire experiment was repeated in 2015, using the same plots. In addition, all the harvested plants was transported to the municipal landfill.

2.3. Sampling and Cd analysis

All the soil samplings conducted by using a stainless steel drill with a diameter of 24 mm, and about 100 g fresh soil sample can be collected once. Ten soil samples of 0 - 20 cm depth were collected in each plot on the day when S. nigrum transplantation. The sampling sites were marked by placing a plastic tube 40 cm long (with a diameter about 2 cm) into the drill hole. The subsequent sampling were conducted around the plastic tube within a distance less than 100 cm. Soil samples were collected by polyethylene bags in the field and transported to the laboratory; air-dried at room temperature to a constant weight; then they were homogenized by grounding in a micro mill and finally screened through a 1.5 mm mesh screen. The soil Cd analyses were conducted by a flame Atomic Absorption Spectrometer (AA-400, PerkinElmer, USA) after digested by strong acid.

2.4. Statistical analysis

All experimental results were statistical analyzed using the SPSS 16.0 package. All results are the means of 10 soil samples. Different among the plots and times were tested by analysis of variance (One way ANOVA). The statistical significance of the differences between groups was evaluated by HSD Tukey’s test at p < 0.05.
3. RESULTS AND DISCUSSION

3.1. Soil Cd extracted by *S. nigrum*

Table 2 indicates the amount of soil Cd extracted by *S. nigrum* in the phytoremediation process. The statistical analysis shows that, according to the Cd contamination level of this experiment, the soil Cd concentration (given in Table 3) had no effect on the growth of *S. nigrum*. The Cd concentrations of Cd in the above ground part of the hyper accumulator was significantly increased by the increasing of soil Cd concentration; the Pearson correlation coefficients were 0.9945 and 0.9564 in 2014 and 2015 respectively. At the same time, the amount of Cd extracted by *S. nigrum* was also significantly positively correlated to the soil Cd concentrations; the Pearson correlation coefficients were 0.865 in 2014 and 0.9152 in 2015. For example, in the first year, the lowest soil Cd concentration was 1.94 mg/kg in plot P-1, and the highest soil Cd concentration was 3.69 mg/kg in plot P-6 (from Table 3). In Table 2, the P-1 plot had the lowest Cd concentration (9.7 ± 0.8 mg/kg) in *S. nigrum* and the lowest Cd extraction amount (4612.4 mg/plot); while the P-6 plot had the highest Cd concentration (19.6 ± 0.7 mg/kg) and the highest Cd extraction amount (9615.8 mg/plot). This indicated that, according to the soil Cd concentrations of this experiment, the phytoremediation efficiency was mainly dictated by the soil Cd concentrations.

Table 2. Cd extracted by *S. nigrum* in the two year experiment. Data of the Cd concentrations in the above ground part of *S. nigrum* shown as Mean ± SD (n = 10).

| Plot | Cd concentrations in the above ground part of *S. nigrum* (mg/kg) | Year 2014 above ground dry biomass of *S. nigrum* (kg/plot) | Cd extracted amount (mg/plot) | Cd concentrations in the above ground part of *S. nigrum* (mg/kg) | Year 2015 above ground dry biomass of *S. nigrum* (g/plot) | Cd extracted amount (mg/plot) |
|------|-------------------------------------------------|-------------------------------------------------|-----------------|-------------------------------------------------|-------------------------------------------------|-----------------|
| P1   | 9.7±0.8                                        | 476.5                                          | 4612.4          | 9.6±0.2                                        | 495.3                                          | 4759.9          |
| P2   | 11.4±0.5                                       | 512.3                                          | 5840.2          | 10.9±0.6                                       | 521.0                                          | 5678.9          |
| P3   | 13.8±0.6                                       | 491.1                                          | 6777.2          | 13.2±0.5                                       | 486.4                                          | 6420.2          |
| P4   | 16.9±0.3                                       | 485.9                                          | 8211.7          | 15.8±0.6                                       | 501.6                                          | 7925.3          |
| P5   | 16.5±0.3                                       | 483.3                                          | 7974.5          | 15.9±0.1                                       | 479.2                                          | 7619.3          |
| P6   | 19.6±0.7                                       | 490.6                                          | 9615.8          | 19.3±0.4                                       | 493.6                                          | 9526.5          |
| P7   | 10.5±0.1                                       | 500.4                                          | 5254.2          | 10.8±0.6                                       | 506.8                                          | 5473.4          |

3.2. Change in Soil Cd concentrations

As shown in Table 3, in the first year, Cd concentrations in the upper 0-20 cm layer of soils decreased significantly in all the plots with *S. nigrum* growth. From P-1 to P-7, the Cd concentrations in all samples collected in October were significantly lower than in the samples collected in April. Cd concentrations decreased from an average of 2.75 mg kg⁻¹ to 2.45 mg kg⁻¹. The reduction in Cd concentration in individual plots ranged from 0.18 mg kg⁻¹ to 0.41 mg kg⁻¹, corresponding to a reduction of 8 - 12%, compared to an insignificant decrease of only 2.1% in
the control plot, thus indicating the effectiveness of the *S. nigrum* in Cd removal. The results of the second year are similar to those of the first year. The Cd concentrations decreased in surface soils in all plots with *S. nigrum* growth. The Cd concentrations in the soils of the seven remediation plots decreased from an average of 2.33 mg kg$^{-1}$ to an average of 1.53 mg kg$^{-1}$, and the reduction ranged from 10% to 14%. The statistical analysis confirmed that there is a significant positive correlation between the reduction in Cd concentration and the initial Cd concentration in the contaminated soils. This conclusion is similar to a former study in which the higher the concentration in the soil, the higher the concentration in the accumulator plants [9]. The rate of Cd decrease in the second year was slightly higher than in the previous year. This may be caused in two ways: Firstly, simply from lower starting values for the second year but a similar uptake rate; and secondly, through chemical and microbial effects induced by the decomposition of leaf and root litter, the plants might have changed the Cd mobility and availability by chemical changes in the rhizosphere [10]. Notwithstanding, the comparison between the plots with and without *S. nigrum* growth indicated the same statistical results as in the first year. After the two years remediation, the Cd concentrations decreased from 2.75 mg kg$^{-1}$ to 1.53 mg kg$^{-1}$, in average, corresponding to an overall average decrease in the Cd concentrations of the seven plots with *S. nigrum* growth of 24.9%. This remediation efficiency is very promising one, better than the results reported by Macci *et al.* [11] who found that real scale phytoremediation using *Populus nigra* (var.italica), *Paulownia tomentosa* or *Cytisus scoparius* can decrease the soil Cd by 25–30% in three years. Even though the remediation plants are totally different, *S. nigrum* show us another possibility of phytoremediation, at least. Compared to *Populus nigra* (var.italica), *Paulownia tomentosa* and *Cytisus scoparius*, and other reported phytoremediation trees or bushes, *S. nigrum*, as a wild weed, has the advantage of easy harvesting, and this may be very important for phytoremediation use in a practical scale.

| Plot | April 2014 | April 2015 | Reduction rate for two year period |
|------|------------|------------|-----------------------------------|
| P-1  | 1.94a      | 1.76b      | 18.0                              |
| P-2  | 2.39a      | 2.20b      | 16.3                              |
| P-3  | 2.68a      | 2.36b      | 30.2                              |
| P-4  | 3.23a      | 2.88b      | 32.5                              |
| P-5  | 3.27a      | 2.88b      | 27.2                              |
| P-6  | 3.69a      | 3.28b      | 24.7                              |
| P-7  | 2.05a      | 1.82b      | 25.4                              |
| Average of above (n=7) | 2.75a | 2.45b | 24.9 |

| Plot | April 2014 | October 2014 | Cd (mg kg$^{-1}$) |
|------|------------|--------------|-------------------|
| P-CK | 2.33a      | 2.28a        | 2.1C              |

Different lowercase letters indicate statistically different values (plot effect) within time according to HSD Tukey’s test (*p* < 0.05). Different uppercase letters indicate significantly different values (in the same column) between plots according to HSD Tukey’s test (*p* < 0.05).
4. CONCLUSIONS

After two years, the results of this in-situ phytoremediation study indicated that the Cd concentrations in the contaminated soil can be decreased significantly by planting S. nigrum in each year; and in this study, the phytoremediation efficiency was found to be mainly dictated by the soil Cd concentrations. After two years, the Cd concentration in the experimental plots had decreased by a factor of around 25%. Therefore, it is reasonable to predict that, S. nigrum has the potential to be useful for practical in-situ phytoremediation application.

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