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Phytotolerance, Phytotoxicity and Phytoremediation of Cd and EDTA mixtures with Napier Grass

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Abstract

The main objective of this study was to investigate the cadmium resistance of Napier grass (Pennisetum purpureum) and the application of ethylene diamine tetraacetic acid (EDTA) on cadmium toxicity reduction. This study design includes batch experiments. These experiments were divided into three sequential parts which were: (1) Effect of cadmium concentration (20, 40, 60, 80, and 100 mg/L); (2) Effect of EDTA concentration (20, 40, 60, 80, and 100 mg/L), and (3) Effect of molar ratio of cadmium and EDTA (1:0.5, 1:1, and 1:2). The results from part 1 and part 2 were used in part 3. Napier grass were glowed the plant in distillated water (DI) and aeration was applied throughout the experiment. Percentage of phytotoxicity and relative growth rate (RGR) were used as the main indicators. The plant samples were collected on the 15th, 30th and 45th days of the experiment. At a cadmium concentration of 40 mg/L, the maximum cadmium accumulation in plants was observed. However, the Napier grass showed low phytotoxicity while the RGR was higher. This implied that cadmium could be used as a supplement for Napier grass. Moreover, applying EDTA could potentially increase the cadmium accumulation and lower the phytotoxicity. The highest cadmium accumulation in Napier grass was observed on the 45th day of the experiment at a mole ratio of cadmium to EDTA of 1:2. Thus, this research suggests that the ratio of cadmium and EDTA at 1:2 mole was appropriated for the application and further development. This is because cadmium and EDTA at 1:2 mole has the highest efficiency in terms of cadmium accumulation in the roots and shoots. In addition, lower cadmium toxicity was demonstrated in the other experiments.

Keywords: Napier grass; relative growth rate, cadmium, EDTA

1. Introduction

Cadmium (Cd) contamination in soil, water, and sediment soil is considered as a major environmental problem. This problem has been expanding due to the growth of industry sectors such as mining, metal coating (electroplating), metal casting, and tanning (Pendias and Pendias, 2001). In the case of Thailand, it has been found that there has been critical cadmium contamination of soil located in Mae Sot district, Tak province, due to the natural geography with heavy mining and agriculture activities (Department of Primary Industries and Mines (Thailand), 2006). The cadmium level in surface water was between 0.3 and 0.8 mg/L which far exceeded the standard of the Pollution Control Department, Thailand (National environment board, 1994). Therefore, several research groups have been interested in this area and attempted to solve this heavily cadmium-contaminated site using physical, chemical, and biological treatments via both in-situ and ex-situ processes (Krishna, 2008; Akkajit, 2015). Even though the performances of cadmium removal are promising, the treatment costs are very high (Ensley, 2000). Phytoremediation, which uses plants to extract and accumulate the heavy metal in plant tissue, might be a suitable treatment since the treatment cost is low with the performance of heavy metal removal is good (Aisien et al., 2012; Sampanpanish, 2015). The treatment could be applied for organic and inorganic pollutants in soil, sediment, and contaminated water (Peer el al., 2007, USEPA, 2000; Tananonchai and Sampanpanish, 2014). However, the limitation of the process is the long duration time of remediation (Aisien et al., 2012). Due to this problem, several research studies have sought to investigate the plants which can rapidly extract cadmium from contaminated soil. During 2012 and 2013, Napier grass (*Pennisetum purpureum* cv. Mott.) was intensively studied for its cadmium accumulation and its performance compared to that of sunflowers (*Helianthus annuus* L.) and sorghum (*Soghum biocolor* L.) (Ishii et al., 2015). The cadmium extraction and accumulation in Napier grass was significantly higher than those of other plants, and it also had a higher relative growth rate (RGR) (Abdel-Salam, 2012; Ishii, 2013; Sabeen, 2013). In order to further improve the cadmium extraction of plants, there are several techniques. Applying a chelating agent such as EDTA increases the heavy metal extraction and accumulation of the plant. Moreover, EDTA can reduce the phytotoxicity from heavy metals (Evangelou et al., 2007). Hence, in this study, EDTA was applied to enhance the cadmium removal from water by Napier grass (i.e. hydroponic). The objective was to investigate the effect of cadmium and EDTA on phytotoxicity in Napier grass and it's RGR along with the cadmium removal capacity of Napier grass.

2. Materials and Methods

Experiment preparation

1. A plastic bottle (1.5 L) was used as the pot for the hydroponic system. It was treated using 10% w/v nitric acid overnight and rinsed using distillated water.

2. The cadmium free Napier grass cuttings were cultured in growing media for one month. The cuttings were randomly sampled and analyzed for total cadmium based on the United States Environmental Protection Agency (USEPA) method 3052 (USEPA, 1996).

3. $Cd(NO_3)_2 \cdot 4H_20$ was used as the cadmium source and EDTA was prepared from $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$.

Experiment design

The experiments were divided into three sequential parts which were: (1) Effect of cadmium concentration (20, 40, 60, 80, and 100 mg/L); (2) Effect of EDTA concentration (20, 40, 60, 80, and 100 mg/L), and (3) Effect of molar ratio of cadmium and EDTA (1:0.5, 1:1, and 1:2). The results from part 1 and 2 were used in part 3. Cadmium and EDTA were added on the first day of the experiment. Three replications of Napier grass samples were applied in each treatment, and the plant samples were collected on the 15^{th}, 30^{th} and 45^{th} days during the experiment. RGR and percentage of phytotoxicity were applied to evaluate the toxicity of cadmium and EDTA in Napier grass based on direct observation following the instruction described in Brown et al. (1991) and Pangta (2009). The total cadmium concentration in Napier grass was evaluated by the atomic adsorption spectroscopy (AAS) technique based on the USEPA 3052 method (USEPA, 1996).

Sample analysis

The Napier grass were collected every 15 d during the 45 d experimental period. After collection, whole plant samples were rinsed using pipe water twice before a final rinse using distillated water. Samples were separated into two parts, namely roots and shoots. Each sample was dried at room temperature for 2-3 hour and weighed to obtain wet weight. Plant samples were then dried in a hot-air oven at 105 °C for 24-48 hours until reaching a constant weight and then were all measured in their dry weight. Next, the RGR was calculated using Equation 1 (Hoffmann and Poorter, 2002). Direct observation was used to evaluate the phytotoxicity in Napier grass as shown in Equation 2 (Brown et al,1991; Pangta, 2009). The score of each symptom is exhibited in Table 1.

$$RGR= ([\ln W_2-\ln W_1])/((t_2-t_1)) \quad (1)$$

where t_1 was the first date of the experiment; t_2 was the last date of the experiment; W_1 was the weight of Napier grass in t_1 (g); and W_2 was the weight of Napier grass in t_2 (g).

Phytotoxicity(%)

$$= \frac{\begin{bmatrix}(A_0 \times B_0)+(A_1 \times B_1)+ \\ (A_2 \times B_2)+(A_3 \times B_3)+ \\ (A_4 \times B_4)+(A_5 \times B_5)\end{bmatrix} \times 100}{(A_1 \times B_1)} \quad (2)$$

where A_0 was the number of normal leaves with no change in color; A_1 was the number of pale yellow leaves (<20% of leaf area); A_2 was the number of pale yellow leaves (<40% of leaf area); A_3 was the number of burned dark yellow leaves (leaf blight; <60% of leaf area); A_4 was the number of burned dark yellow leaves (leaf blight; <80% of leaf area); A_5 was the number of burned dark yellow leaves (leaf blight; >80% of leaf area); A_r was the total number of leaves, and B_0, B_1, B_2, B_3, B_4, B_5, and B_r were the scores of toxicity which were equal to 0, 1, 2, 3, 4, 5, and 5, respectively (Brown et al.,1991; Pangta, 2009).

Afterward, the subsample was oven-dried at 105°C for 2-3 d to determine the dry matter yield. One subsample was treated following the USEPA method 3052 (USEPA, 1996) and analyzed for the total Cd concentration using atomic absorption spectroscopy (AAS).

Statistical analysis

The results of cadmium accumulation in Napier grass were analyzed on the basis of variance (ANOVA) using Duncan's new multiple range test (DMRT). The SPSS program was applied to perform this statistical analysis analysis.

Table 1. Guidelines for evaluating the phytotoxicity of Napier grass based on direct observation

Score of toxicity	Appearance and symptoms
0	Normal, green leaf.
1	Slight toxicity, small area of pale yellow on leaf.
2	Slight moderate toxicity, large area of pale yellow on leaf.
3	Moderate toxicity, small area of blight leaf was observed.
4	High toxicity, large area of blight leaf was observed.
5	Critical toxicity, whole area of blight leaf was observed.

3. Results and Discussion

Effect of cadmium on RGR and phytotoxicity of Napier grass

The results showed that the RGR of Napier grass was high in the first 15 days of experiment. At low cadmium concentrations (20 and 40 mg cadmium/L) and in the control sample, the RGR of Napier grasses showed continual growth during the experimental period; however, the RGR of the last 15 days of the experiments became slightly lower than that for the first 15 days as shown in Fig. 1a. Comparing the RGR of the 45th day under low cadmium concentrations, there was no significant effect of cadmium concentration on RGR (p-value>0.05). In particular, the plants did not exhibit toxins, and also thrived and had high efficiency in terms of cadmium accumulation. On the other hand, the RGR of Napier grass at high cadmium concentrations (60, 80, and 100 mg cadmium/L) was dramatically reduced during the last 15 days of the experiment. Shah et al. (2008) reported that India rosewood (*Dalbergia sissoo*) was able to resist the cadmium sulfate solution at 0-40 mg cadmium/L. However, at higher cadmium concentrations, its growth rate was reduced. Similar results also reported by Namdjoyan et al. (2011) who stated that the growth rate of safflower (*Carthamus tinctorius*) was decreased under the cadmium chloride concentration of between 25 and 100 μM.

Based on direct observation, the phytotoxicity of Napier grass was correlated with the cadmium concentration and duration of the experiment. The higher the cadmium concentration and the longer the duration time of the experiment, the higher the phytotoxicity of Napier grass exhibited. At low cadmium concentrations (0, 20, and 40 mg cadmium/L), the phytotoxicity was between 0 and 3.330 as shown in Fig. 1b. Pale yellow leaves were observed. This was due to the degradation of chlorophyll. Farouk et al. (2011) and Hediji et al. (2010) found that in the presence of cadmium, plants produced lower chlorophyll levels leading to yellow leaf, leaf blight, and leaf spot symptoms. According to these results, a cadmium concentration of 40 mg cadmium/L was selected for further experimentation.

Effect of EDTA on RGR and phytotoxicity of Napier grass

Additional EDTA was able to increase the RGR of the Napier grass, especially in high concentrations (60, 80, and 100 mg/L) during the 45 days of the experiment. For low concentrations (20 and 40 mg/L), the RGR increased only during the first 30 days of the experiment as shown in Fig. 1c. Based on direct observation, under EDTA concentrations <100 mg/L there was no phytotoxicity of EDTA on Napier grass (phytotoxicity = 0) as exhibited in Fig. 1d. Thus, Napier grass might be able to tolerate EDTA concentrations greater than 100 mg/L.

Hernmandez et al. (2007) stated that exceeding EDTA concentrations might lead to low growth rate, decrease the stomata open/close system, and reduce the respiration of the plant.

Effect of cadmium and EDTA on RGR and phytotoxicity of Napier grass

At every different mole ration of cadmium to EDTA using 40 mg cadmium/L, the maximum RGR was observed at the 15th day of the experiment, and then decreased afterwards as shown in Fig. 1e. On the 45th day the phytotoxicity was between 6.67 and 11.11, and there was no significant effect of cadmium and EDTA on phytotoxicity (p-value >0.05). Lu et al. (2004) studied the effect of EDTA and nitrilotriacetic acid on cadmium phytoremediation using water hyacinth (*Eichhornia crassips*) and found that as the experimental duration increased, the RGR of the plant was reduced.

According to direct observation and phytotoxicity calculation, the percentage of phytotoxicity in Napier grass increased across the experimental duration. The lowest phytotoxicity was found when using a cadmium to EDTA mole ratio of 1:2 (Fig. 1f). The experimental sets, which had cadmium concentrations of 0, 20, and 40 mg cadmium/L, had low percentages of phytotoxicity between 0 and 3.330 as shown in Fig. 1b. Wu et al. (2004) used Chinese mustard (*Brassica juncea*) with EDTA to remediate the heavy metal. The results showed that EDTA potentially reduced the phytotoxicity of heavy metal to Chinese mustard and also enhanced the cadmium accumulation.

Cadmium extraction and accumulation of Napier grass without EDTA

The majority of cadmium was accumulated in the roots, followed by shoots during the first 30 days of the experiment. At the 45th day under cadmium concentrations of 80 and 100 mg cadmium/L, they showed the highest cadmium accumulation with 1,374 and 1,369 mg/kg, respectively (Fig. 2a). For cadmium accumulation in the shoot part, the values were 1,499 and 1,487 mg/kg in the presence of cadmium concentrations at 80 and 100 mg cadmium/L, respectively. These results were similar to the reports of Kongmuang and Sampanpanish (2010) who stated that an increase in cadmium concentration led to an increase in cadmium accumulation in plants but reduced the plants' growth rate as presented in Fig. 2b.

Effect of EDTA on cadmium extraction and accumulation of Napier grass

The results from AAS showed that the most of cadmium was accumulated in the roots, followed by shoots, respectively, within the first 30 days of the experiment (Fig 2c and 2d). Comparing between cases with and without EDTA, the cadmium accumulation in the root parts in the presence of EDTA was far higher than in the absence of EDTA.

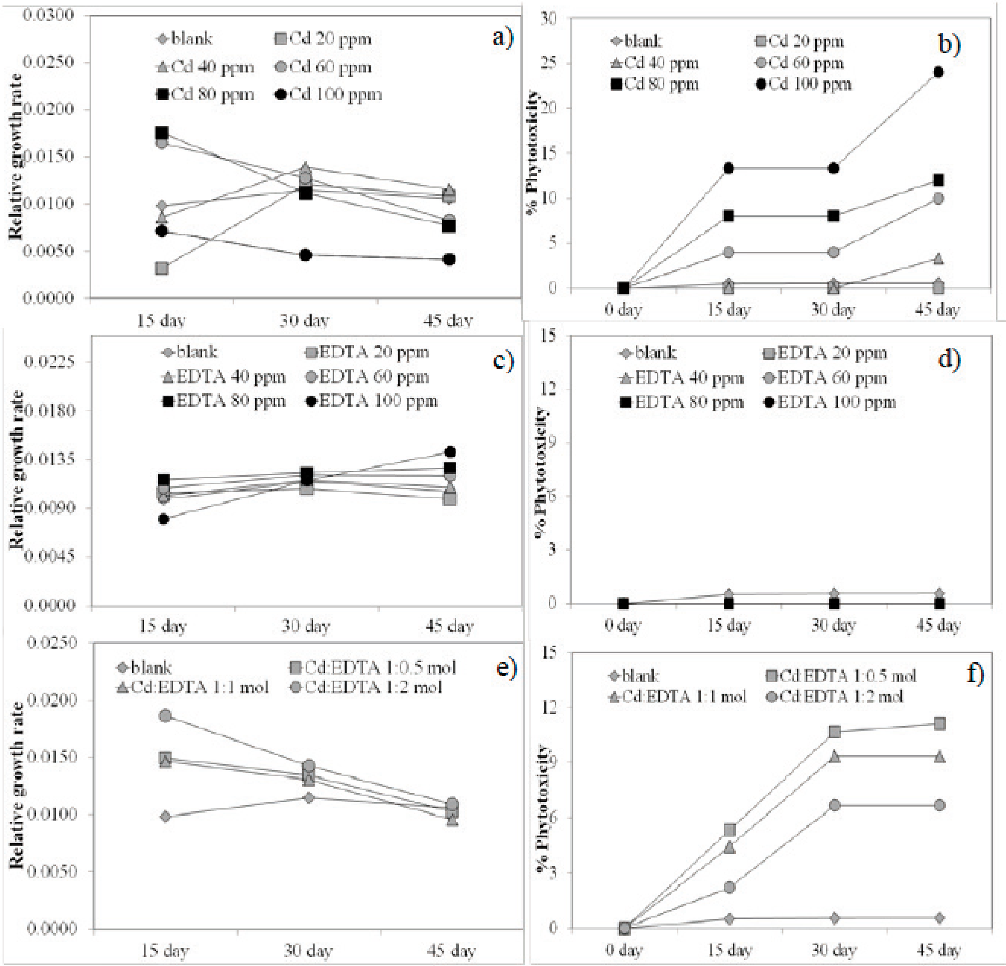

Figure 1. a) Relative growth rate of Cd concentration on Napier Grass, b) Phytotoxicity of Cd concentration on Napier Grass, c) Relative growth rate of EDTA concentration on Napier Grass, d) Phytotoxicity of EDTA concentration on Napier Grass, e) Relative growth rate of Cd-EDTA mixture on Napier Grass, and f) Phytotoxicity of Cd-EDTA mixture on Napier Grass

Under the cadmium to EDTA molar ratios of 1:2 and 1:1, the cadmium accumulation values in the roots were 1,042 and 948 mg/kg, respectively. These cadmium accumulation values were significantly higher than those obtained when using a cadmium to EDTA molar ratio of 1:0.5 and in the absence of EDTA (p-value <0.05).

In the shoot parts under a cadmium to EDTA molar ratio at 1:2, the highest cadmium accumulation value was obtained at 1,273 mg/kg which was significantly different from the other treatments (p-value<0.05). Hermandez et al. (2006) reported that EDTA had an ability to enhance lead, zinc, and

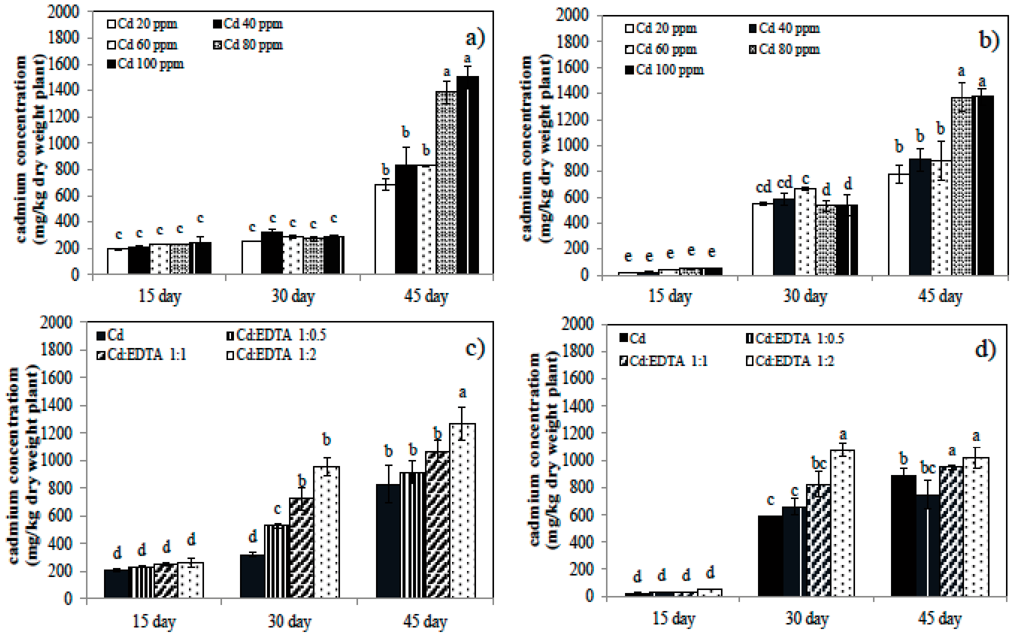

Figure 2. Cadmium extraction and accumulation in Napier grass a) roots, and b) shoots. Effects of EDTA on cadmium extraction and accumulation in Napier grass c) roots, and d) shoots.

cadmium extraction from aqueous solutions using artichoke (*Cynara cardunculus*). Muhammad et al. (2009) compared the performance of several chelating agents on cadmium extraction using narrow-leaved cattail (*Typha angustifolia*) and found that EDTA was the best chelating agent on cadmium extraction.

4. Conclusion

In this study, five concentrations of cadmium and EDTA (0, 20, 40, 60, 80, and 100 mg/L) were used to evaluate the phytotoxicity resistance of Napier grass as well as cadmium accumulation and RGR. The results showed that a cadmium concentration of 40 mg cadmium/L was the most suitable condition since the Napier grass had its highest RGR with low phytotoxicity and highest cadmium accumulation. In the case of EDTA, there was no significant negative effect of EDTA on RGR and phytotoxicity. For the effect of molar ratio of cadmium to EDTA, it was found that EDTA could enhance cadmium accumulation as well as reduce the phytotoxicity from cadmium. Especially, the Cd and EDTA at the ratio 1:2 was the highest efficiency to induce the cadmium accumulation in Napier grass and reduce the phytotoxicity from cadmium.

According to this study, the application of using Napier grass with EDTA could be applied in real cadmium - contaminated sites. Moreover, the phytotoxicity results could be used as the pre-indicator for growing Napier grass in the cadmium-contaminated site.

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