EFFECTIVENESS OF VETIVER GRASS IN PHYTOSTABILIZATION AND/OR PHYTOREMEDIATION OF DIOXIN-CONTAMINATED SOIL AT BIEN HOA AIRBASE, VIETNAM - AN OVERVIEW AND PRELIMINARY RESULT

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Abstract

Investigation of the phytoremediation technology for mitigation and/or bioremediation of soils contaminated with dioxin at low and moderate levels in the South of Vietnam is necessary. Vetiver grass (*Chrysopogon zizanioides* L.) with its unique morphological and physiological attributes is a potential candidate. The two main objectives of this project are to investigate:

- The capability of vetiver grass in phytostabilization of dioxin-contaminated sites, preventing its offsite contamination; and
- Its effectiveness in the bioremediation of the dioxin-contaminated soils.

Vetiver was planted on 25 November 2014 in two groups of 100 m^2 each with the initial dioxin levels in soil of about 1000 – 1800 ppt (part per trillion) TEQ. The first group (G1) received DECOM 1, a soil supplement promoting growth of indigenous microorganisms in the rhizosphere, and the second group (G2) as a control, without supplement. The growth rate of vetiver and the levels of toxic chemicals/dioxins in the soil, roots and shoots from the two groups will be compared as will soil samples from areas with and without vetiver grass (blank).

The initial results show that Monto vetiver can grow well in moderately dioxin-contaminated soils. One month after planting, there was no difference in growth (plant height) and tiller number per clump between two groups. The clear differences in growth were observed after one and a half months onward (6 weeks: 76 cm in G1 vs. 68 cm in G2; the and numbers of tilters per clump: 14 tilters in G1 vs. 10 tilters in G2). Especially, the difference in terms of the number of tillers between the two groups was significant from week 6 through week 16 ($p<0.05$). From 6 to 12 weeks, the number of tillers increased very fast, i.e., from about 14 to 26 tillers (G1) and from 10 to 20 tillers (G2). After that the growth of vetiver slowed down from week 12 to week 16 in terms of the number of tillers, particularly the height in G2 that nearly did not change. At week 16, the circumference of G1 and G2 were 25 and 24 cm, respectively, and did not differ from each other ($p>0.05$).

In general, the growth of vetiver in G1 is better than in G2 in terms of the number of tillers, but not in terms of circumference and the height at week 16. The result suggests that Monto vetiver is suitable for phytostabilization of moderately dioxin-contaminated sites. The addition of a soil supplement further enhances its potential as an effective phytostabilization agent.

This investigation is in progress and the final results are expected in the next 13 months with three sampling times in May 2015, October 2015 and in March 2016.

This project was approved and funded by the Ministry of Natural Resources and Environment of Vietnam (MoNRE).

Key words: Vetiver grass; Dioxin-contaminated soil; Phytostabilization; Phytoremediation.

INTRODUCTION

Dioxin contamination in Vietnam primarily originates from the war during the period 1961-1971, when herbicides were used extensively to defoliate forests, clear perimeters of military installations and destroy crops (Dwernychuk *et al.,* 2006). Over 80 million litres of herbicide were applied over approximately 10-12% of southern Vietnam (Stellman *et al.,* 2003). Dioxins contained in Agent Orange, which were sprayed in the South of Vietnam, was mainly TCDD (tetrachlorodibenzodioxin, aka dioxin), the most toxic one in this group, causing very serious environmental problems and human health risks. It has been estimated that there was about 170 kg TCDD sprayed over southern Vietnam together with Agent Orange (Westing, 1984; Gough, 1986). However, Stellman *et al.* (2003) have reported that there were about 366 kg of TCDD in total, which still does not take into account other sources of herbicides sprayed, i.e. by Republic of Vietnam forces, and by US Army and Navy forces by trucks, boats, hand sprayers and helicopters, etc., and also the dioxin contamination of Agent Pink. But he also stated that dioxin contamination of Agent Orange could be fourfold or more higher plus unaccounted-for Agent Pink (Stellman *et al.,* 2003). Studies have shown that Vietnam is one of the worst TCDD contaminated sites in the world with many hot spots, especially sites at army airbases, e.g., Da Nang, Phu Cat and Bien Hoa, etc, where large quantities of herbicides were stored or handled (Cecil, 1986). The dioxins accumulated at the hot spots that continue to be bio-available and disperse through different ways, pose a serious health problem, especially through the food chain (Dwernychuk, 2005). Recently, many efforts have been used to remediate and clean up the sites. Several remediation technologies have been applied in hot spots in Vietnam, such as Active Landfill technology, Ball Milling, Bio-remediation and Thermal Desorption Destruction technology (GEF/UNDP project, 2013). However, those technologies are expensive and only suitable in hot spots (small to medium scale). Hence, the investigation of the phytoremediation technology for stabilization, mitigation and/or remediation of soils contaminated with toxic chemicals/dioxins at low and moderate levels is necessary.

Vetiver grass (*Chrysopogon zizanioides L.*) with its unique morphological and physiological characteristics has been used extensively around the world for erosion and sediment control (Greenfield, 1995), mine rehabilitation, wastewater treatment and heavy metal remediation (Truong and Baker 1996; Truong *et al.,* 2004). Recently it was reported that vetiver could sequester and breakdown herbicides, particularly Atrazine (Marcacci *et al.,* 2006), and remove other persistent organic pollutants from aqueous and soil environments, such as 2,4,6-trinitrotoluene (Makris, 2007; Datta, 2010) and petroleum hydrocarbons (Infante *et al.*, 2012). However, so far there has been no research on the use of this grass for dioxin stabilization and remediation. This grass can be tolerant of extreme conditions, i.e., high concentrations of heavy metals, highly acidic soils (pH 2.7) and highly arsenic, highly saline, sodic, magnisic and alkaline, and the lack of major nutrients and organic materials, etc. (Truong and Baker 1996; Truong *et al.,* 2004). Additionally, vetiver can grow quickly to establish ground cover, has high biomass, a dense root system, and therefore might be a suitable candidate for phytostabilization (Raskin & Ensley, 2000) of toxic chemicals/dioxins contaminated soils. Vetiver also has a huge vertical root system, which produces an extremely chemically complex essential oil, containing more than 300 compounds, including bicyclic and tricyclic sesquiterpenoids - hydrocarbons, alcohols, ketones, aldehydes, and acids (Guzman and Oyen, 1999). Furthermore, owing to the special attributes of the vetiver root system, it provides a huge rhizosphere volume for bacterial and fungal growth and multiplication, thus enabling absorption and/or breakdown of contaminants, and perhaps also toxic chemicals/dioxins.

Therefore, the objective of this project is to investigate the capability of vetiver grass in phytostabilization and/or bioremediation of toxic chemicals/dioxins contaminated soils. It has been approved and funded by the Ministry of Natural Resources and Environment of Vietnam (MoNRE) over an 18-month period. The success of the project would see this low cost, but effective and sustainable technology practically implemented in a large-scale manner for remediation of moderately toxic chemicals/dioxins contaminated soils in southern Vietnam.

MATERIALS AND METHODS

Study site and Monto cultivar

Figure 1: A map showing the geographical location of the investigated site in a general view (upper image) and in a close-up view (lower image).

The experiment was conducted in Bien Hoa airbase, specifically at the Pacer Ivy area which was used as a herbicide storage and re-drumming location during Vietnam war. This is located at the South West corner of the Bien Hoa airbase, close to the runway (Figure 1). The site of 300 m^2 with a moderate dioxin-contaminated level (about 1000 – 2000 ppt

TEQ) was chosen to implement the experiment, after preliminary investigation of the dioxin level in this area.

Monto vetiver grass (*Chrysopogon zizanioides L.*) was purchased from P.M Co., LTD, Duc Trong district, Lam Dong province and transferred to Bien Hoa airbase with 5 tillers each in plastic cups filled with coir particle media. The Monto cultivar of vetiver grass was selected for this project so that the results can be directly compared with those obtained in Australia where it was used extensively in basic research on phytostabilization and phytoremediation (Truong and Baker 1996; Truong *et al.*, 2004).

Soil preparation and initial sample collection

The soil was hard and compact with lots of big limestone rocks. Therefore, before carrying out the experiment, an excavator was used to dig out and mix up the soil. After thoroughly removing the weeds and the big limestone rocks and gravel, the land was levelled before taking the initial sample and transplanting the vetiver grass slips.

The area was divided into three lots of 100 m^2 each and three soil samples were collected within each lot. The sampling procedure followed guidance from UNEP (2007b) and UNEP/POPS/COP.5/INF/27 (2011) with some modification. Briefly, in about 30 m^2, ten subsamples (60-cm deep core) were collected using an O-twist drill with a T-handle extension. They were then ground, mixed well, and evenly spread on a stainless steel tray. Then a 1 kg (approximately) soil sample was taken from 30 different portions with a stainless steel scoop. Collected soil samples were then cold stored at 4°C and transferred to the laboratory for further processing and analysis.

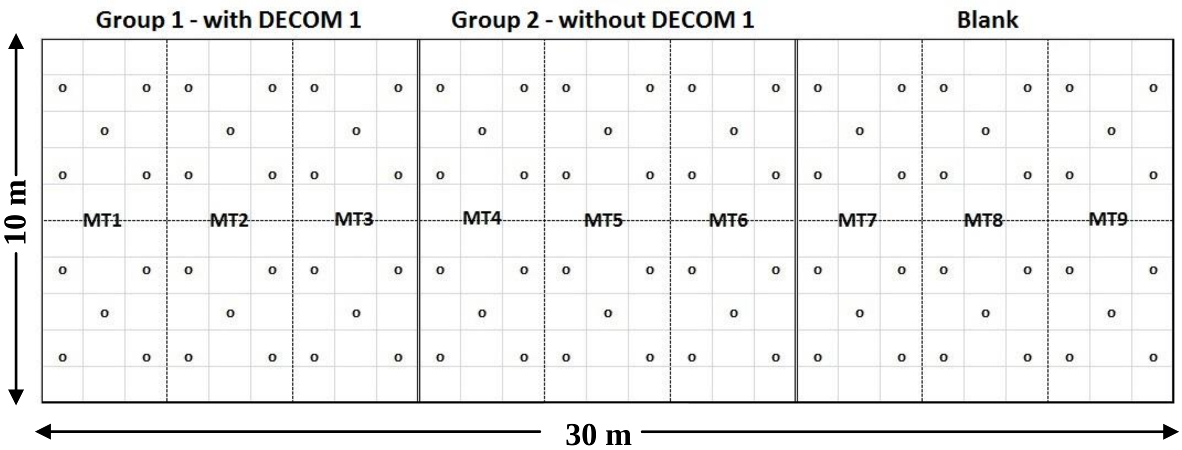

Figure 2: A schematic representation of the experimental design and sampling method.

Experimental design and growth monitoring

Monto vetiver grass (*Chrysopogon zizanioides L.*) was planted on November 2014 in two groups of 100 m^2 each with the initial dioxin concentration in soil of about 1000–1800 ppt TEQ. Vetiver slips selected for transplanting were pruned into 25 cm long shoots with 5 cm long roots. Bare root vetiver slips were transplanted in the rows, with 50 x 25 cm spacing between plants, during 24 - 26 November. Each slip contained 3-5 tillers. Before planting, all slips were dipped into a solution containing NAA in order to stimulate root development.

The first group (G1) had a soil supplement, DECOM 1, added to promote growth of indigenous microorganisms in the rhizosphere, and the second group (G2) served as the control, without a soil supplement. The growth rate of vetiver and the levels of toxic chemicals/dioxins in the soil, roots and shoots will be compared, as will soil samples from areas with and without vetiver grass (blank).

During cultivation, weeds and big stones were thoroughly removed. Vetiver grass was watered daily or twice daily to ensure that they could survive and thrive during the dry season. Plant height was measured and the number of tillers per clump are counted every two weeks until the end of experiment (after 15 months). Soil, root and shoot (new leave) samples are taken every five months. Root and shoot samples are collected at the same place as soil samples. They are rinsed thoroughly with bidistilled water, then with environmental grade hexane to remove soil residues. Then the samples are rinsed with environmental grade acetone to remove any residual materials and assist with hexane evaporation. Finally, the root and new leaf samples were air dried and stored at 4°C and transferred to the laboratory for further processing and toxic chemicals/dioxins analysis.

Dioxin analyses

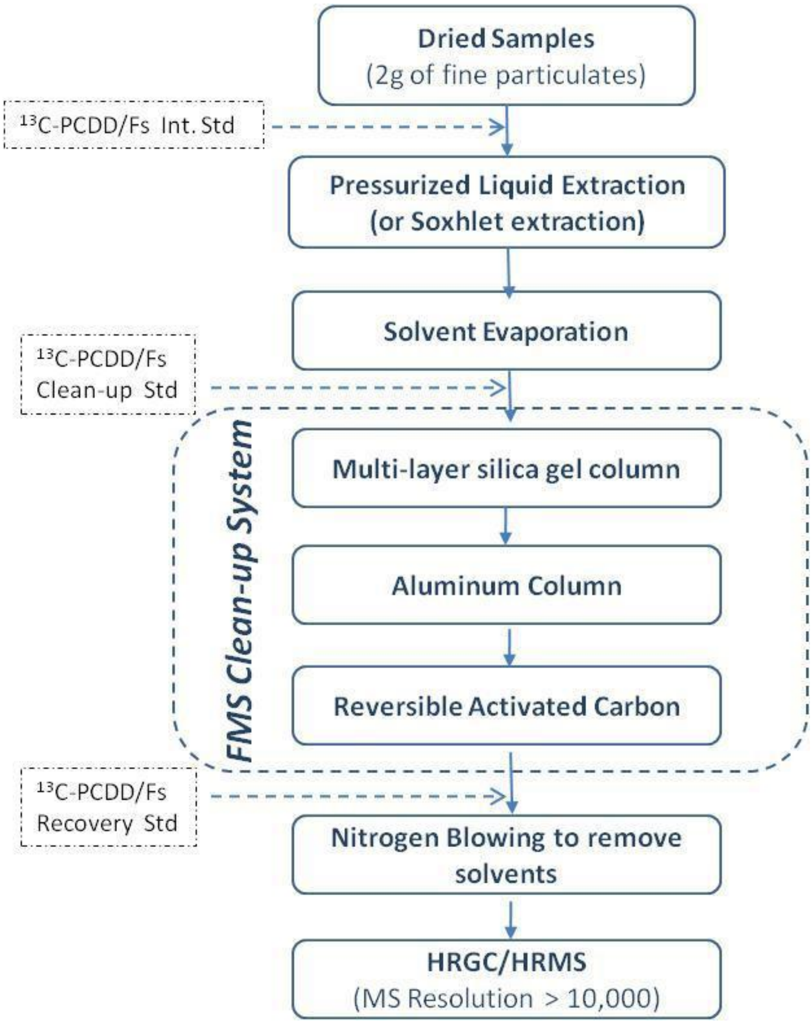

Figure 3. Analytical scheme for the determination of PCDD/Fs.

All the samples were processed and analyzed in the Dioxin Laboratory, Vietnam Environment Administration, Ministry of Natural Resources and Environment. Standard procedures for sample processing and analysis of toxic chemicals/dioxins analysis were applied (US EPA, 1994; UNEP, 2007a). All samples were analyzed for 17 PCDD/Fs recommended by the World Health Organization (WHO, 2005). Results were converted to

TEQ using TEF given by WHO and expressed on a dry weight basis. The US EPA Method 1613 for determination of PCDD/Fs by isotopic dilution HRGC/HRMS (high resolution mass spectrometer) was slightly modified to fit the available techniques advanced in recent years and was validated prior to regular usage (Figure 3).

Statistical analysis

The data were presented as means ± SD (standard deviation). A two-way analysis of variance was used to determine whether differences in growth rate between the two groups and sampling times were significant. When significant difference was found, the post-hoc test Student–Newman–Keuls test was applied (GraphPad Software, SanDiego, CA).

RESULTS AND DISCUSSION

Initial concentration of toxic chemicals/dioxins

In this study, nine samples were collected and analyzed at the Pacer Ivy area (Figure 1). TEQ concentrations (pg/g dry weight basis) are shown in Table 1. In general, dioxin contamination in this area is not evenly distributed. It is observed that eight out of nine samples (89%) exhibited concentrations above 1,000 ppt (the national guideline for remediation of soil in Agent Orange Hot Spots). The concentrations ranged from 686 to 4,425 ppt TEQ. However, lot 1 and lot 2 have lower levels of dioxins, less fluctuation (Table 1) and were chosen for vetiver planting (lot 1: group 1 and lot 2: group 2; Figure 2). The levels of dioxins from these three lots will also be examined every five months after planting the grass.

Table 1. Level of PCDD/Fs (ppt TEQ-WHO$_{2005}$) of the soil column (taken from 0-60 cm soil depth) from Pacer Ivy site, Bien Hoa airbase

Samples	Latitude	Longitude	2378-TCDD	TEQ$_{who}$	%TCDD
MT1	10°58'23.4"	106°48'21.7"	1,221.2	1,235.0	98.9
MT2	10°58'23.8"	106°48'21.6"	1,142.9	1,169.4	97.7
MT3	10°58'23.8"	106°48'21.5"	686.1	694.8	98.8
Average of lot 1 (G1)			**1,017 ± 167**	**1,033 ± 170**	**98.5 ± 0.4**
MT4	10°58'23.9"	106°48'21.5"	1,058.9	1,071.6	98.8
MT5	10°58'24.1"	106°48'21.5"	2,781.9	2,795.6	99.5
MT6	10°58'24.3"	106°48'21.4"	1,617.8	1,626.1	99.5
Average of lot 2 (G2)			**1,819 ± 507**	**1,831 ± 508**	**99.3 ± 0.2**
MT7	10°58'24.5"	106°48'21.4"	4,413.5	4,425.2	99.7
MT8	10°58'24.6"	106°48'21.3"	2,815.2	2,831.9	99.4
MT9	10°58'24.7"	106°48'21.4"	1,623.7	1,631.6	99.5
Average of lot 3 (Blank)			**2,950 ± 808**	**2,961 ± 809**	**99.6 ± 0.1**

Other contaminants such as As, 2,4-D and 2,4,5-T were also determined. As concentrations ranged from 16.2 to 39.5 mg/kg dry weight of collected soil (data not shown), which are significantly higher than the Vietnamese allowable limits (12 mg/kg dry wt.) and the US EPA limit (1.6 mg/kg, for industrial soil). This result raises concerns about As contamination for local environment and human health problem. It is known that one of the defoliants, Agent Blue, contains Dimethylarsinic acid (DMA – $(CH_3)_2As(O)OH$)) and its sodium salts as the active ingredients, could be a possible source of arsenic residues found here. However, no significant correlation between dioxins TEQ and As concentration in the soil were found. It is also known that As can also come from natural sources under certain geological conditions. Therefore, it may be necessary to analyze for Arsenic speciation (As(III), As(V), MMA and DMA) to understand further about the sources of Arsenic in local environment.

Analysis of 2,4-D and 2,4,5-T showed that their concentration are quite low, i.e., for 2,4-D: 0.012 – 0.067 mg/kg dry wt. and for 2,4,5-T: 0.017 – 0.274 mg/kg dry wt. These levels are much lower than the maximum level for residential land, i.e., 100 mg/kg, and are also lower than the level for Protection of Groundwater (2,4-D: 0.5 mg/kg and 2,4,5-T: 1.9 mg/kg) according to the New York Soil Cleanup Guidance (CP-51, 2010). These two herbicides are the main ingredients of Agent Orange widely used during the Vietnam war. Due to their short half-lives, i.e., 7-10 days with 2,4-D and 21-24 days with 2,4,5-T in soils, most of these two chemicals were degraded since the war until now. However, it was found that both 2,4-D and 2,4,5 T were highly correlated to the TEQ dioxins level with $r = 0.97$ and 0.99, respectively ($p<0.0001$). The correlation suggests a similar occurrence of Agent Orange and dioxins in the soil in the Pacer Ivy area.

Characteristics of the experimental soils

Table 2. Some physical and chemical characteristics of the experimental site soils at the Pacer Ivy site, Bien Hoa airbase

Samples	pH	Eh (mV)	EC (µS/cm)	TOC (%)	Soil Moisture (%)	Permeability coefficient of soil K ($x10^{-4}$) (cm/s)
MT1	5.93	-158.4	60	0.47	11.70	6.0
MT2	6.37	-124.5	55	0.39	18.54	12.3
MT3	6.22	-146.9	55	0.32	14.88	3.2
Ave. lot 1 (G1)	**6.2**	**-143.3**	**56.7**	**0.4**	**15.0**	**7.2**
MT4	6.68	-154.5	53	0.43	10.41	3.0
MT5	7.35	-150.5	77	0.33	14.66	25.0
MT6	6.65	-152.3	58	0.27	14.26	5.3
Ave. lot 2 (G2)	**6.9**	**-152.4**	**62.7**	**0.3**	**13.1**	**11.1**
MT7	7.39	-158.1	89	0.27	13.42	10.0
MT8	6.92	-156.3	63	0.33	12.19	25.0
MT9	8.3	-160.3	148	0.56	18.77	9.0
Ave. lot 3 (Blank)	**7.5**	**-158.2**	**100**	**0.4**	**14.8**	**14.7**

The soil texture is mainly coarse sands mixed with very fine sand, clay and limestone. The particle size distribution shows that the soils have very low clay contents (less than 5%).

Low electrical conductivity (EC) can be explained by the low percentage of total organic carbon (TOC) and low redox potential (Eh) due to low pH as well as the low clay contents of the samples. In general, with low pH, negative values of Eh, low EC, low K, and especially low TOC (Table 2), this type of soil shows its poor quality with high levels of reductants, low organic matter, low water holding capacity and the compact structure. This quality of soil is not suitable for growing many plants. However, with its special characteristics, vetiver has been found to be highly tolerant to extreme soil conditions, including heavy-metal contamination (Truong and Baker 1996). In this experiment, it has been growing well in this poor land after four months planting.

Growth performance

The survival rate of vetiver was about 96% in both groups. During the first four months of the experimental period, the heights of vetiver in both groups increased and they are all significantly higher than their initial height ($p<0.05$), especially from week 2 to week 8, their heights rose steadily. After eight weeks, the growth rate slowed down, and from week 12 to week 16 there was nearly no change in G1 ($p>0.05$).

The development of vetiver, in terms of the height and the number of tillers per clump, in G1 seems to be better than in G2 (Figure 4 & 5). Particularly, growth of the group that received a soil supplement – DECOM1 (G1) is faster than the second group (G2) after 6 and 12 weeks (Figure 4; $p<0.05$). At the other time points, the differences are not statistically significant (Figure 4; $p>0.05$).

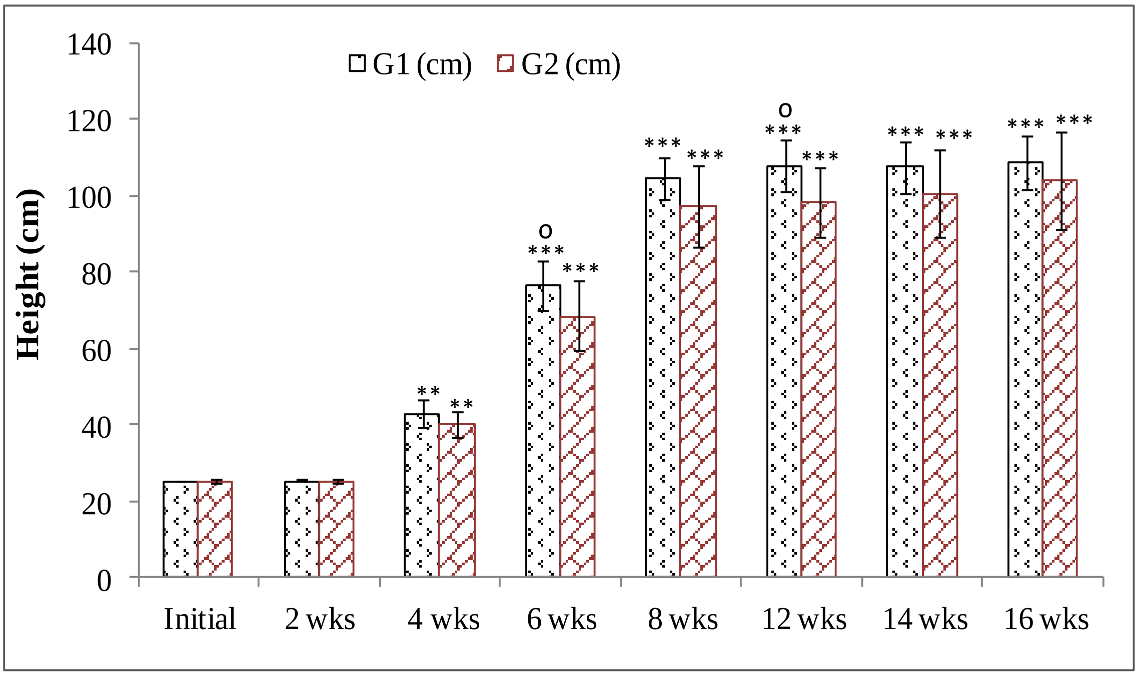

Figure 4. Height (cm) of vetiver grass (G1 and G2) planted in toxic chemicals/dioxins contaminated soil at different sampling time points. Significant differences for the height (cm) from the same group in comparison to the beginning (*) and between the two groups (o) are indicated (mean ± SD, n = 30, *p<0.05, **p<0.01, ***p<0.001; o: P<0.05; oo: P<0.01).

In terms of the number of tillers, both group 1 and 2 showed a similar increasing trend over time through to week 16 (Figure 5). From week 4, vetiver starts tillering with the number of tillers per clump higher than the initial (Figure 5; $p<0.05$). In both groups, the number of tillers from week 6 to week 16 was distinctively higher than their initial numbers ($p<0.001$).

Comparison of the number of tillers per clump between the two groups (G1 and G2) at each sampling time showed that from week 2 to week 4, no significant differences were observed ($p>0.05$). However, the differences between the two groups were clearly seen at other sampling time points: week 6, 8, 12, 14 and 16 (Figure 5; $p<0.05$).

The circumference of the vetiver grass clump was also measured at week 16. The data showed that there was no difference between G1 and G2 ($p>0.05$; data not shown). At week 16, some plants were flowering in both groups. This means that vetiver can establish itself well on this type of soil.

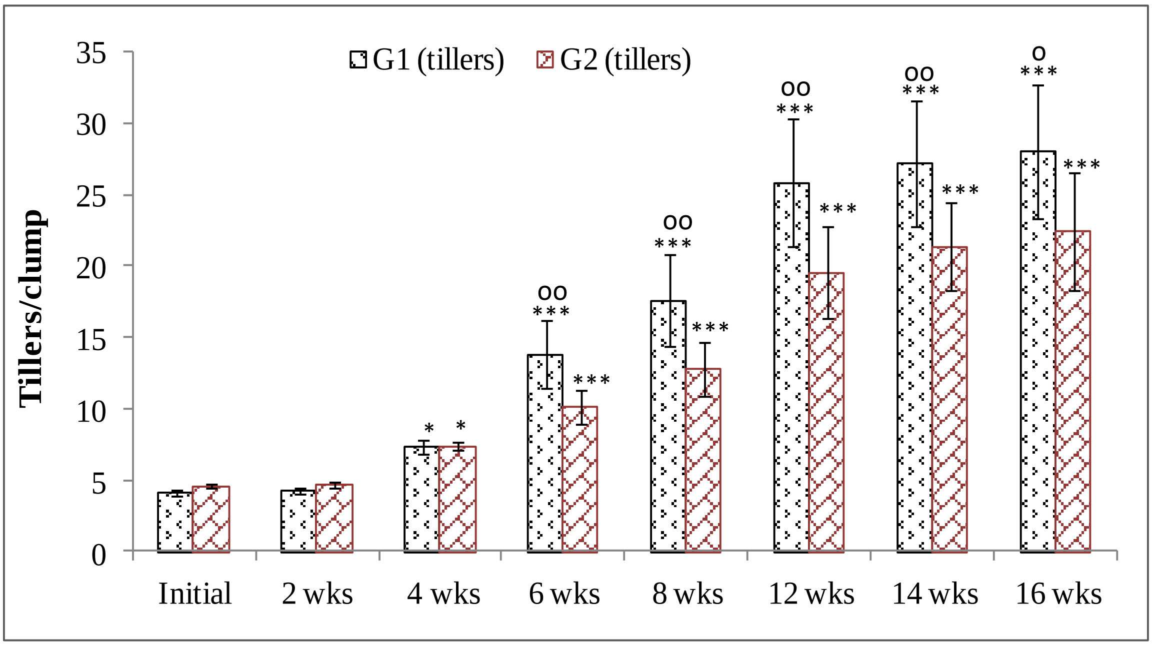

Figure 5. The size of the vetiver grass clump (number of tillers/clump) for G1 and G2 planted in toxic chemicals/dioxins contaminated soil at different sampling time points. Significant differences for the clump size (tillers/clump) from the same group in comparison to the beginning (*) and between the two groups (o) are indicated (mean ± SD, n = 30, *p<0.05, **p<0.01, ***p<0.001; o: P<0.05; oo: P<0.01).

In summary, vetiver grows quite well in the dioxin-contaminated soil at moderate levels with very little effects, i.e., it developed a little yellow colour on the leaves, especially during the dry and hot days. This is similar with the results of an experiment where vetiver was exposed to 80 mg/kg of 2,4,6 trinitrotoluene load in the soil (Das et al., 2010). Vetiver was also reported to grow well in an aqueous environment contaminated with Atrazin (Marcacci, 2006). It is also well known that vetiver can tolerate heavy metal contaminated soils (Raskin and Ensley, 2000; Shu *et al.*, 2002; Truong *et al.*, 2004). To the best of our knowledge, there have been no data on the growth of vetiver on dioxins contaminated soil so far.

CONCLUSIONS

Vetiver grass can grow well on poor quality and moderately toxic chemical/dioxin-contaminated soil. The plant growth in G1 is better than in G2 in terms of the number of tillers, but not in terms of the clump circumference and the plant height at week 16. It should be noted that flowering started in week 16 for some plants, which is a sign that vetiver is well established on this kind of contaminated soil. These results confirm that Monto vetiver is suitable for phytostabilization of moderately dioxin-contaminated sites. The addition of a soil supplement in G1 further enhances its potential as an effective phytostabilization agent.

This investigation is in progress and the final results are expected in March 2016. At that time, the question whether this grass is capable of remediating soils contaminated with toxic chemical/dioxins will be answered. If it is capable, large-scale implementation of this low cost, but effective and sustainable remediation method to rehabilitate moderately toxic chemicals/dioxins contaminated soils in Vietnam as well as elsewhere in the world, can be applied.

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