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Assessing the bioaccumulation and translocation potential of vetiver grass for dioxins phytoremediation in Bien Hoa airbase, Viet Nam

Nguyen Thi Thanh Thao[1, 3], Nguyen Quoc Dinh[2, 5], Martine Leermakers[3], Yue Gao[3], Dinh Van Huy[1], Nguyen Thi Loi[1], Ngo Thi Thuy Huong[4, 5, *]

[1]*Vietnam Institute of Geosciences and Mineral Resources, Vietnam, 67 Chien Thang Street, Van Quan, Ha Dong, Ha Noi, Viet Nam*

[2]*External Engagement Office, Phenikaa University, Nguyen Trac Street, Yen Nghia, Ha Dong, Ha Noi, Viet Nam*

[3]*Analytical, Environmental, and Geo-Chemistry, Vrije Universiteit Brussels, Plaine 2, 1050 Ixelles, Brussels, Belgium*

[4]*Faculty of Biotechnology, Chemistry and Environmental Engineering, Phenikaa University, Nguyen Trac Street, Yen Nghia, Ha Dong, Ha Noi, Viet Nam*

[5]*Environmental Chemistry and Ecotoxicology Lab, Phenikaa University, Nguyen Trac Street, Yen Nghia, Ha Dong, Ha Noi, Viet Nam*

[*]Emails: *huong.ngothithuy@phenikaa-uni.edu.vn*

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Abstract. Dioxins (PCDD/Fs) are harmful organic pollutants that accumulate and transfer through the food chain, endangering humans and the environment. Dioxin-contaminated hotspots in Vietnam are the result of herbicide use during the US-Vietnam War. Low-cost and environmentally friendly phytoremediation has increased in recent decades. Vetiver grass (Chrysopogon zizanioides) can remediate organic and inorganic pollutants. Hence, an experiment at Bien Hoa airbase, Vietnam was used to investigate the uptake and transport of dioxins in Vetiver grass from dioxin-contaminated soil. Three Vetiver grass plots (FT1, FT2, FT3) and three control plots (FC1, FC2, FC3) with initial dioxin concentrations as follows: FC2>FC3>FC1>FT2>FT3>FT1 were set up. Vetiver grass grew well in dioxin-contaminated soils, producing lots of biomass. Dioxin levels in Vetiver roots were found to have a significant correlation ($r2 = 0.67$; $p < 0.01$) with growth rate. Vetiver roots accumulate high levels of dioxin, particularly during the initial year when the BAF is greater than 1. The dioxin translocation from the roots to the shoots was significantly lower than the BAF and less than one (TF < 1), with first-year samples being greater than the others. Dioxin concentrations in soils do not provide a reliable indicator of its bioavailability, but rather a variety of other parameters, including soil physicochemical properties and microorganisms. Our study aims to contribute significant insights into the capability of Vetiver grass to accumulate and translocate dioxins, hence to the arsenal of dioxin remediation. Furthermore, we envisage translating our findings into practical applications in other areas, considering optimised planting techniques, growth conditions, and long-term sustainability.

Keywords: Dioxin concentration, Vetiver grass, bioconcentration factor, translocation factor

Classification numbers: 3.2.3, 3.4.3, 3.7.2.

1. INTRODUCTION

Environmental toxins such as polychlorinated dibenzo-p-dioxins (PCDDs) and their relatives polychlorinated dibenzofurans (PCDFs) are well-known. There are 210 chemically distinct PCDD/Fs, referred to as "congeners", including 75 possible PCDDs and 135 possible PCDFs. The 210 chemicals are collectively referred to as "dioxins" [1,2] and only 17 of the 210 that are theoretically feasible are of concern from a toxicological standpoint. The most well-known and harmful dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD, TCDD), a molecule with four chlorine atoms. Risks to human health have been determined using these congeners [3]. Concerns have been raised about dioxin contamination in Southern Vietnam where chemical defoliants have been historically used [4]. Agent Orange herbicide containing equal amounts of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was the main herbicide used. Agent White (2,4-D; picloram), Agent Blue (cacodylic acid), Agent Purple (2,4-D; 2,4,5-T), Agent Green (2,4,5-T), and Agent Pink (2,4,5-T) were among the other herbicides used in Vietnam [5,6]. The three dioxin hotspots in Vietnam are Bien Hoa, Da Nang and Phu Cat airbases, which served as bulk storage facilities for Agent Orange during the Vietnam War [7]. Among these airbases, the Bien Hoa airbase had the highest concentration of dioxins in its soils, with a TCDD concentration of up to 365,000 ng $TEQ.kg^{-1}$ dw in the topsoil [5]. Furthermore, there was an accumulation of 3,300 ng $TEQ.kg^{-1}$ dw at a depth of 2.6 m at Bien Hoa airbase [8].

In paddy soils, the half-life of PCDD/Fs was estimated to be 10 to 20 years [9]. Their accumulation in the soil causes contamination of food products such as vegetables and animal food products, forming the major human exposure route for PCCD/Fs [10], with high-fat animal products serving as the main source of dioxin contamination [11 - 13]. The purpose of bioremediation technologies, which include bioaugmentation, biostimulation, and phytoremediation are designed to eliminate contaminants from soil by utilizing plant species that possess a high biomass and natural microbial communities. They are regarded as optimal and ecologically friendly methods [14]. Phytoremediation requires plants with rapid growth, high biomass, an extensive root system and the ability to grow in harsh environmental conditions. They form an ideal habitat for microorganisms and fungi in the rhizosphere. They can form a favourable environment for higher absorption and breakdown of toxic compounds in the soil [14, 15]. Vetiver grass (*Chrysopogon zizanioides*), strain Monto, is one of the few plants that meet all the requirements for phytoremediation [16]. Therefore, in our project, it was used for the remediation of dioxin-contaminated soil in the Bien Hoa Airbase, specifically at the Pacer Ivy area which was used as a herbicide storage and filling location during the Vietnam War. The objective of this research is to determine whether vetiver grass can bioaccumulate dioxin from contaminated soils and whether it can translocate dioxin from roots to the shoots to aid in the remediation of such soils.

2. MATERIALS AND METHODS

2.1. Sampling collection

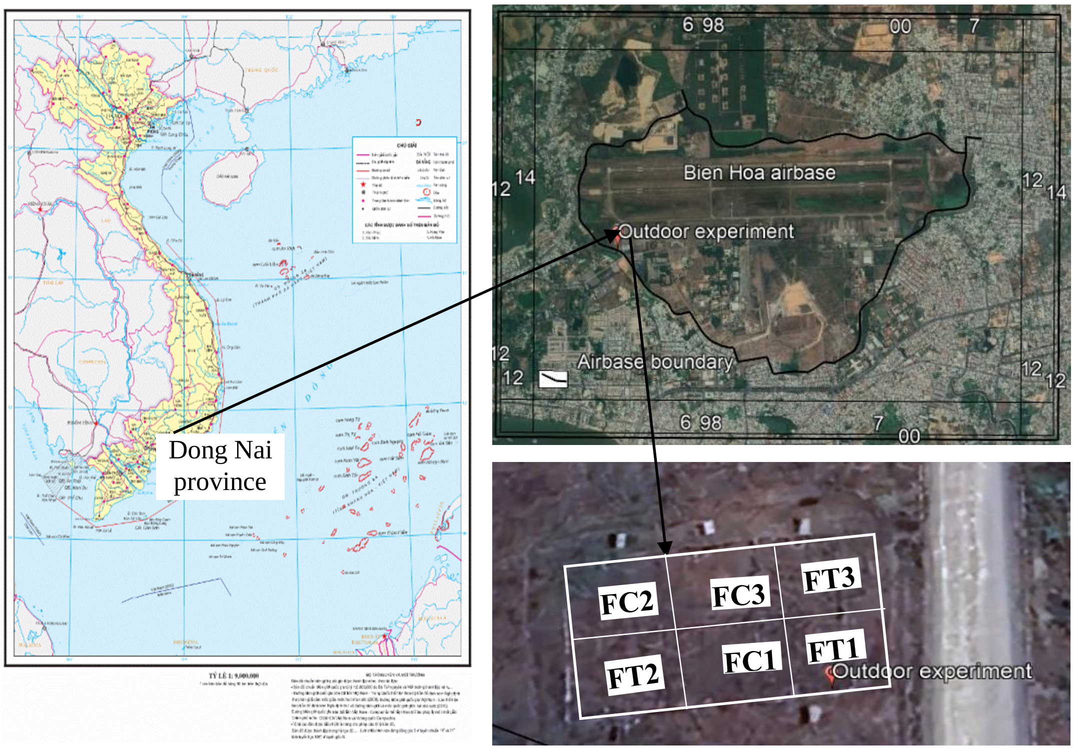

Figure 1. The outdoor experiment and the study area at Bien Hoa Airbase (From Google Map 2023).

The outdoor experiment had a total area of 600 m² and was situated at the southwest corner of the runway in the Pacer Ivy area at the Bien Hoa airbase (106°50'22.13"E-106°50'21.66"E (Figure 1). The experimental area was divided into six plots, each measuring 100 m² (10 m×10 m). Three plots (FT1, FT2 and FT3) were planted with Vetiver grass, while the other three (FC1, FC2 and FC3) were not. The species of Vetiver grass employed in this research was *C. zizanioides* var Monto Australia. This grass was taken from the experiment of the previous project. In the previous project, this grass was purchased from Phuong Mai Agricultural Processing and Trading Company Limited, Duc Trong district, Lam Dong province. In November 2018, Vetiver grass was planted in an outdoor experiment, and initial samples, including roots and shoots, were systematically collected at the same time. Selected vetiver slips were cut into 25 cm long shoots and 5 cm long roots before being transplanted. The grass was planted in rows about 50 cm apart. Each clump had 3 - 5 tillers and was about 25 - 30 cm apart from the next. An O-twist drill with a T-handle extension was used to collect the soil samples at a depth of 0 - 50 cm. In each plot, thirty soil samples were collected and mixed into a composite sample from which three subsamples were taken for dioxin analysis. The sample collection procedure was performed following the standards of UNEP and UNEP/POPs/COP.5/INF/27 [17]. Like soil sampling techniques, three shoot samples and three root samples were obtained from each plot during each designated sampling time and each sample was made up of 30 component sub-samples. The root samples were taken to a depth of 50 cm using a garden shovel and scissors, while the shoots were cut into 5-7 cm sections. In this study, 6 soil samples were collected in October 2018, and 42 plant samples were taken during the experiment, from October 2018 to March 2022.

Before conducting any sampling, all tools were carefully washed with double distilled water, then hexane and acetone, to prevent any potential cross-contamination between samples and from other sources. Then, all the samples, including soil, shoot and root samples, were transported to the Analytical Laboratory for Environment, Dioxin and Toxins at the Northern Center for Environmental Monitoring, Vietnam Environment Administration (NCEM).

2.2. Analysis method and quality control

Sample analysis: Samples (soil, shoot and root samples) were analyzed using the standard analytical EPA method 1613B at the Analytical Laboratory for Environment, Dioxins and Toxins at the Northern Center for Environmental Monitoring, Vietnam Environment Administration (NCEM). The analytical procedure known as EPA Method 1613B, which was established in 1994 [18], employed gas chromatography and a high-resolution mass spectrometer (HRGC/HRMS) to determine the 17 dioxin congeners of PCDD/Fs as recommended by the World Health Organization (WHO, 2005). After drying at room temperature, the soil samples were crushed and sieved to less than 1 mm, and the root and shoot samples were also air-dried and ground three times. The soil and plant samples were individually placed within a thimble filter and thereafter subjected to extraction using the Soxhlet method. The extractions were conducted using a mixture of n-hexane, dichloromethane, and ethanol for a duration exceeding 24 hours. The obtained plant-based samples (roots, shoots and stems) were evaporated to determine the weight-based measurement of the lipid content. The extracted samples underwent purification using multi-layer silica columns coupled with dual-layer activated carbon thereafter. Finally, the concentrated eluate of the sample was evaporated using a nitrogen stream. The WHO-TEF 2005 was used to calculate TEQ.

Quality control analysis: The blank tests were performed with each sample batch. The average recovery value for the 13C-PCDD/F surrogate standard ranged from 50 to 99 %, 52 to 89 %, and 55 to 96 % for soil, root, and shoot samples, respectively. The method detection limit for soil samples ranged from 0.1 to 1.0 ng/kg, but for root and shoot samples they ranged from 0.02 to 0.1 ng/kg.

2.3. Data processing and analysis

Bioaccumulation factor (BAF) and translocation factor (TF): BAF and TF of dioxin in plant samples were calculated following (1) and (2) and described by [19].

$$BAF = \frac{\sum Dioxins_{Root}}{\sum Dioxins_{soil}} \quad (1)$$

where $\sum Dioxins_{root\ or\ shoot}$ is the concentration of dioxins (ng TEQ.kg^{-1} dw) in roots of plants; $\sum Dioxins_{soil}$ is the concentration of dioxins (ng TEQ.kg^{-1} dw) in soil.

$$TF = \frac{\sum Dioxins_{shoot}}{\sum Dioxins_{root}} \quad (2)$$

where $\sum Dioxins_{shoot}$ is the concentration of dioxins (ng TEQ.kg^{-1} dw) in shoots of plants; $\sum Dioxins_{root}$ is the concentration of dioxins (ng TEQ.kg^{-1} dw) in roots of plants.

With R Studio software, principal components analysis (PCA) was performed to elucidate any differences in the dioxin composition of soil and plant samples and to establish the link between dioxin concentrations in the soils and plant samples.

3. RESULTS AND DISCUSSION

3.1. The concentration of dioxin in the shoots and roots of Vetiver grass

Dioxin levels in soils collected right before planting Vetiver ranged from 805 (ng TEQ.kg^{-1} dw) to 3065 (ng TEQ.kg^{-1} dw) with an average level of 1726 ± 961 (ng TEQ.kg^{-1} dw) which is higher than permissible levels in soils recommended by Vietnam (300 ng TEQ.kg^{-1} dw for agricultural soils; 1200 ng TEQ.kg^{-1} dw for commercial soil) [20], Finland (500 ng TEQ.kg^{-1} dw) and the Netherlands (1000 ng TEQ.kg^{-1} dw) [21]. The most hazardous dioxin congener, 2,3,7,8-TCDD [22], accounted for 98 % of the TEQ (Fig 2.). Typical background levels of dioxins generally range from 1–20 ng TEQ.kg^{-1} dw, with TCDD accounting for up to 20 % of the TEQ [23]. This confirms that the herbicides are the source of the dioxins at Bien Hoa Airbase. The concentrations of dioxins in the different plots were in the following order FC2>FC3>FC1>FT2>FT3>FT1. The highest dioxin concentrations are thus found in the northwest part of Pacer Ivy, most likely around the herbicide storage location. This contrasts with arsenic concentrations, which are the highest in the eastern half of the study area [24]. Thus, Vetiver grass was cultivated in areas with higher trace element concentrations and lower dioxin concentrations.

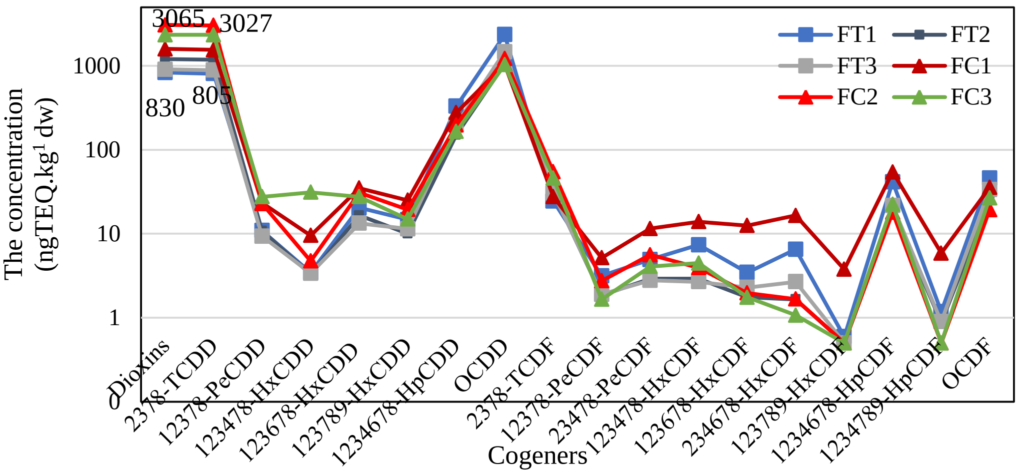

Figure 2. Distribution of dioxins and congeners (ng TEQ.kg^{-1} dw) in soils at the outdoor experiment, Pacer Ivy area, Bien Hoa Airbase.

3.2. The concentration of dioxin in the shoots and roots of Vetiver grass

The concentration of dioxins in the shoots and roots of plants during the experiment is shown in Figure 3. The root samples at the FT3 plot which were collected in October 2019 are not presented due to sample contamination.

Dioxin absorption in the roots of Vetiver grass increased from October 2018 to October 2019, then decreased between October 2019 and May 2020 (visible for FT1 and FT3) and remained constant from May 2020 to March 2022. The highest absorption occurred in FT1 and

FT3 during the first year. Dioxin concentrations in Vetiver's root increased from 130 to 944 ng TEQ.kg^{-1} dw in FT1 and from 117 to 875 ng TEQ.kg^{-1} dw in FT3. On the other hand, in FT2, a peak was observed after 6 months, with an increase from 100 to 522 ng TEQ.kg^{-1} dw in April 2019. Different physicochemical conditions of the plots (FT1 and FT3 on the east side and FT2 on the west side) should be investigated further. The growth pattern of Vetiver grass in FT2 is significantly different from FT1 and FT3 in the first year, but not in the subsequent years (Figure 3). Initial growth for FT2 is greater than that of FT1 and FT3.

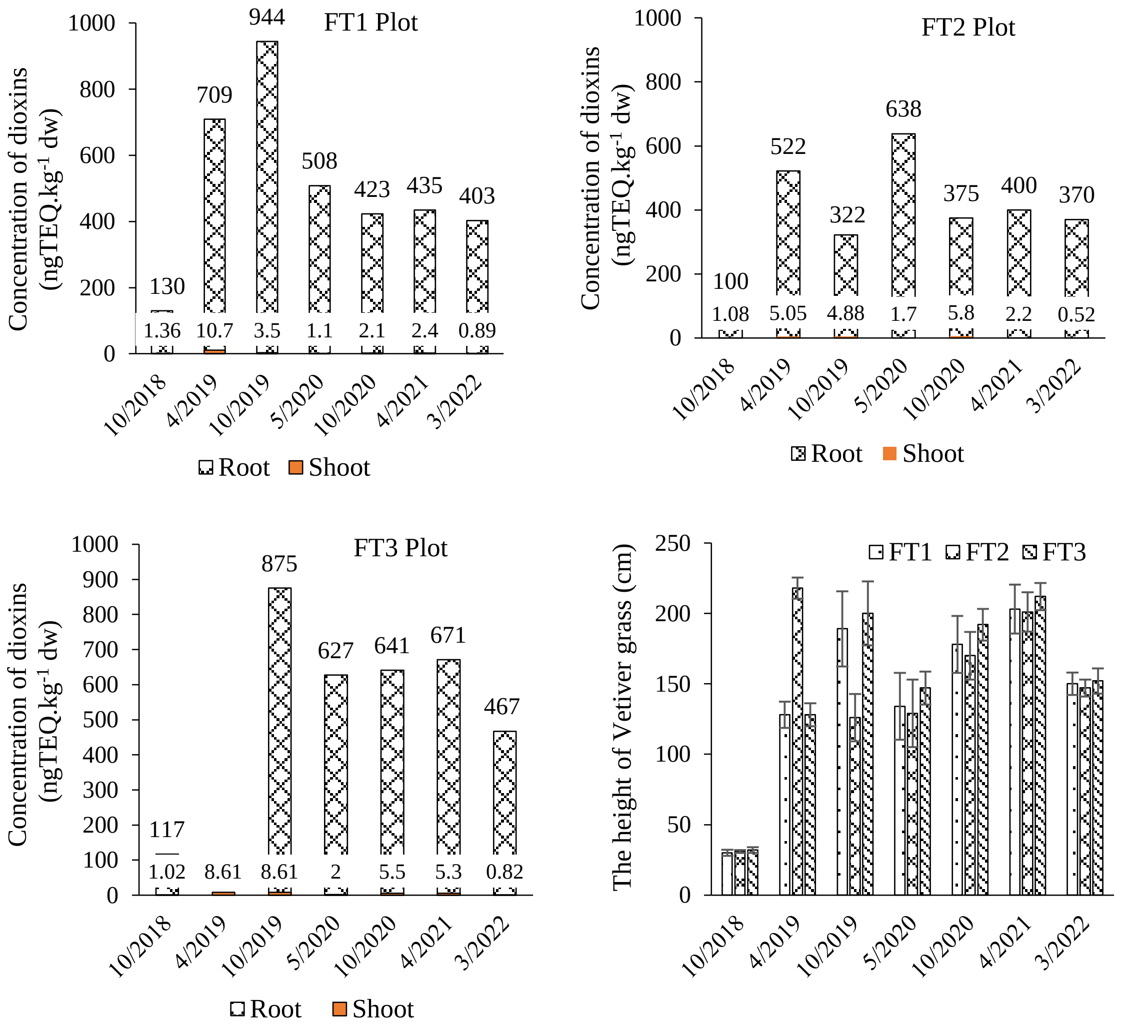

Figure 3. Dioxin concentrations (ng TEQ.kg^{-1} dw) in the roots and shoots of FT1, FT2 and FT3, as well as the height of Vetiver grass in the outdoor experiment (FT3 root data missing at the time of sampling in April 2019).

After 12 months, the concentration of dioxins in the root of Vetiver grass decreased in the FT1 and FT3 plots. Following an initial rapid decline from October 2019 to May 2020 (from 944 to 508 ng TEQ.kg^{-1} dw in FT1 and from 875 to 627 ng TEQ.kg^{-1} dw in FT3), the subsequent 22 months of the experiment, dioxin concentrations remained relatively stable. FT1 and FT3 exhibited dioxin accumulation in the roots of these plots varied between 508 - 403 ng TEQ.kg^{-1} dw in FT1 and 627 - 467 ng TEQ.kg^{-1} dw in FT3. As can be seen in the FT2 plot, most of the time during the experimental period, the dioxin concentrations in root fluctuated from 322-400 ng TEQ.kg^{-1} dw, with the highest amount recorded in May 2020 at 638 ng TEQ.kg^{-1} dw.

The accumulation of dioxins in the roots is correlated with the growth pattern of Vetiver grass. The growth in Vetiver grass can be divided into two main periods. The first period was extended one year, from October 2018 to October 2019. The grass reached its maximum height during this time, with a mean height of more than 2 m (Figure 3). Based on an eight-month field investigation [25], the Vetiver grass roots have been shown to have a maximum length of 2 m and a width of 25 cm. As a result of the regeneration process, Vetiver grass regrew during the second period. This period was estimated to be from May 2020 to April 2021. Likewise, there was a positive correlation ($r^2 = 0.67$; $p < 0.01$) between the growth rate of Vetiver grass and the concentration of dioxins in its root.

Conversely, the dioxin concentrations in the shoots are extremely low, ranging from 0.82 to 10.7 ng TEQ.kg^{-1} dw (Figure 3). This indicates that this plant is capable of phytodegrading pollutants in the shoots and phytostabilizing them in the roots. Perhaps the metabolism of plant roots is closely linked to the synthesis of vetiver essential oil and could be a factor in the accumulation of dioxins here [26]. Vetiver grass is a plant with 5.12 % to 7.42 % essential oils in roots [27]. After 6 months, the volume of oil in the roots of Vetiver grass was found to be twice as much as it had been grown for 2 months and decreased after 6 months [26]. Furthermore, the oil content in the root tip was found to be lower in comparison to the oil content in the crown roots of the explant [28]. Following the initial year, the decline in dioxin levels, observed in both the shoots and roots, could potentially be attributed to either a diminished capacity for the plant to absorb and adapt to the hazardous environmental conditions, or an active breakdown mechanism occurring within the roots.

3.3. Bioaccumulation and translocation

The dry-weight ratio of the dioxin content in the root to that in the soil of the experiments was used to compute the bioaccumulation factor (BAF) (Table 1). In April 2019, after 6 months of growth, the roots of plants started to bioaccumulate dioxins from the soil (BAF > 1) with the highest BAF value of 1.09 found in FT1. Then, in both FT1 and FT3 plots, BAF values remained greater than one for another 6 months (October 2019). After a year, the BAF values between the plots varied in distinct ways, with FT1 and FT2 having values less than 1 (0.6 - 0.8), while the FT3 plot was nearly unchanged and was higher (0.84 - 1.17).

The increased BAF values observed in the root samples demonstrate that Vetiver grass has mostly performed its phytostabilization mechanism to remediate dioxin contaminants due to certain properties of this grass. Besides, In addition, the amount of lipids in plant roots was positively correlated with the quantity of organic contaminants [29]. Root accumulation of PCDD/Fs from soil solutions could be anticipated by extractable lipid content in plant roots, and lipophilic adsorption dominated root uptake of PCDD/Fs from nutrient solution [30]. This observation suggests that a chemical transformation has taken place from the soil to the roots of Vetiver grass.

The translocation factor (TF) is calculated as the ratio of the accumulation of dioxins in plant shoots to root accumulation. The result of TF values (Table 1) showed that the translocation factor values were much lower than 1 (TF < 1), and TF values for the first and second sampling times were higher than the TF values of other times. The results also showed that translocation values (TF) were much lower than BAF values. On the contrary, a significant positive correlation ($r^2 = 0.55$, $p < 0.05$) was established between the concentration of dioxins in shoots and roots of Vetiver grass. Furthermore, it is worth noting that the initial sampling time (April 2019) revealed a dioxin concentration in grass shoots of Vetiver grass that was 4.67–2.44 times greater than that of newly planted plants (October 2018). This finding provides evidence for the occurrence of translocation, as illustrated in Figure 3. In an earlier investigation, researchers observed the absorption of polycyclic aromatic hydrocarbons (PAHs) from a liquid solution through the roots of wheat plants, followed by their subsequent transportation to the shoots [31]. A transpiration stream may facilitate the translocation of dioxins from roots to shoots [32]. Then, the process of detoxification in plants takes place and is influenced by various degradation-related enzymes [33, 34]. Furthermore, previous research has documented low translocation factors for non-essential trace elements [16]. As a result, Vetiver grass can be considered a rich in nutrient forage option for livestock.

Table 1. Dioxins' the bioaccumulation factor (BAF) and translocation of dioxins (TF) of Vetiver grass at the treatment experiments throughout experimental time.

Plots	Factors	Experimental time					
		4/2019	10/2019	5/2020	10/2020	4/2021	3/2022
FT1	BCF	1.09	1.26	0.64	0.75	0.63	0.69
	TF	0.015	0.004	0.002	0.005	0.006	0.002
FT2	BCF	0.51	0.43	0.80	0.58	0.58	0.62
	TF	0.010	0.015	0.003	0.015	0.006	0.001
FT3	BCF	-	1.16	0.86	1.11	1.17	0.84
	TF	-	0.010	0.003	0.009	0.008	0.002

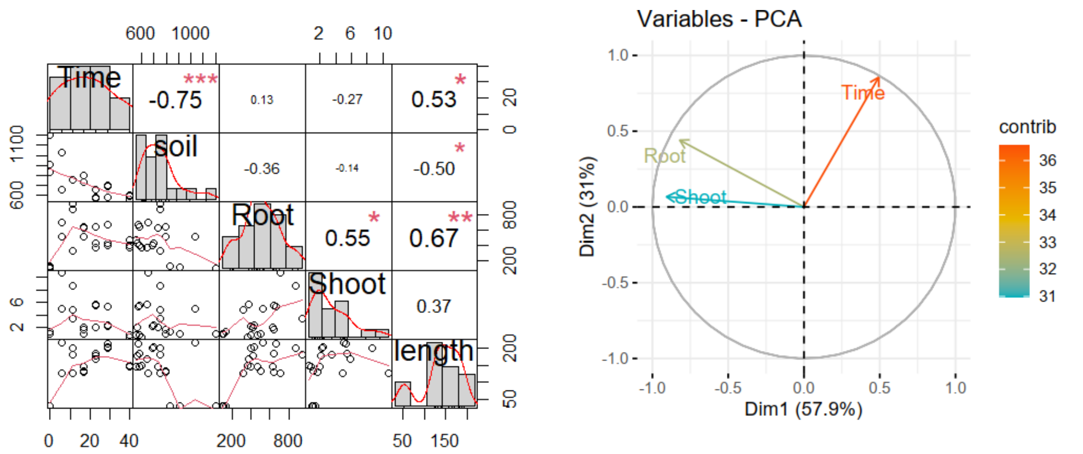

Figure 4. Principal component analysis of dioxins in soils, roots, and shoots; Matrix correlation between the dioxin concentration in plant samples and the height of Vetiver grass over the sampling intervals.

Correlation and principal component analysis (PCA) revealed that dioxin concentrations in the roots are highly correlated with the height of Vetiver grass and that the concentrations in the shoots are also correlated to the concentrations in the roots, even though the translocation factor is low. The highest dioxin concentrations in the treatment plots were found in FT2, which had the lowest BAF. Therefore, the total dioxin concentrations do not provide a reliable indicator of the bioavailability of the substance. However, numerous other factors, like the physicochemical properties of the soil and the population of soil-dwelling microbes, influence plant uptake.

4. CONCLUSIONS

The growth dynamics of Monto Vetiver grass were the main determinant of the absorption of dioxins from the soil. The bioaccumulation capacity of Vetiver grass was found to be substantially higher during its initial year of growth, followed by a subsequent decline. Overall, Vetiver demonstrated an immediate ability to accumulate dioxins from the soil and store them in its extensive root system, but limited translocation of these dioxins into the aboveground portion of the plant was seen.

In addition to absorption by plants, other processes that affect dioxin concentration in soils (decrease over time – data not shown), include microbial breakdown, photodegradation, weathering, and erosion.

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CRediT authorship contribution statement. All authors contribute in different ways and/or extend to this study. Nguyen Thi Thanh Thao: Investigation, Methodology, writing original draft, Review & Editing; Nguyen Quoc Dinh: Conceptualization, Validation; Yue Gao: Resource, Validation; Martine Leermakers: Resource, Validation, Review & Editing; Dinh Van Huy and Nguyen Thi Loi: Field sampling, Investigation; Ngo Thi Thuy Huong: Conceptualization, Investigation, Methodology, Resource, Validation, Writing, Review & Editing, Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest. The authors assert the absence of any identifiable conflicting financial interests or personal relationships that might have been perceived as exerting influence on the findings presented in this paper.

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