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Influence of Lead and Cadmium Concentration on the Accumulation Capacity of *Panicum maximum*

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Abstract

The restoration of soils polluted by trace metals (Pb and Cd) by phytoremediation is an innovative and ecologically sustainable solution. The objective of the study was to develop a process of phytoaccumulation of trace metals (Pb and Cd) in soils with the species *Panicum maximum*. For this purpose, 30 buckets containing soil were used. These included six (06) buckets per dose of soil contamination by Pb and Cd (3 mg/kg and 9 mg/kg of Cd and 100 mg/kg and 300 mg/kg of Pb) and six (6) buckets containing uncontaminated soil (control). During a period of 90 days of experimentation, the concentrations of trace metals in the plant biomass and in the soils were measured. Also, the bioaccumulation (BF) and translocation (TF) factors, the mass of Pb and Cd taken up by the plant were determined. The results showed that the biomass produced was negatively influenced by increasing Pb and Cd concentration. The concentrations of Pb and Cd accumulated by *P. maximum* varied in the aboveground biomass from 6.48 ± 0.55 to 18.09 ± 0.71 mg/kg (Pb100); from 10.93 ± 0.38 to 23.04 ± 0.79 mg/kg (Pb300); from 0.91 ± 0.02 to 1.50 ± 0.03 mg/kg (Cd3); and from 3.05 ± 0.08 to 5.43 ± 0.09 mg/kg (Cd9) from day 30 to day 90. However, in the root biomass, trace metals (Pb and Cd) ranged from 8.09 ± 0.58 to 22.57 ± 0.86 mg/kg (Pb100); from 29.45 ± 0.49 to 62.35 ± 0.82 mg/kg (Pb300); from 0.66 ± 0.01 to 1.11 ± 0.07 mg/kg (Cd3); and from 2.22 ± 0.08 to 3, 97 ± 0.09 mg/kg (Cd9), from day 30 to day 90. Pb was concentrated in the root biomass and Cd in the aboveground biomass. Bioaccumulation factor values ranged from 0.26 ± 0.02 to 0.99 ± 0.04 (Pb100); from 0.21 ± 0.04 to 0.50 ± 0.06 (Pb300); from 0.83 ± 0.09 to 1.72 ± 0.18 (Cd3); and from 0.70 ± 0.08 to 1.54 ± 0.18 (Cd9). High concentrations of Pb and Cd show a negative effect on the accumulation potential of *P. maximum*.

Keywords

Phytoaccumulation, *Panicum maximum*, Lead, Cadmium

1. Introduction

Contaminated soils are an environmental and health concern. The various pollutants, including trace metals (lead, cadmium, copper, zinc, etc.) are non-biodegradable and therefore persistent in the environment. The accumulation of these inorganic pollutants in the environment is a threat to human and animal health [1]. Some of the trace metals, including Zn and Cu, are essential trace elements that become toxic at high concentrations in soil. In contrast, Pb and Cd are toxic even at trace levels [2] [3]. Bioaccumulation of these toxic metals and contamination of the food chain is a major health risk, as these metals exhibit carcinogenic and teratogenic effects in living things [4] [5]. Therefore, thermal, physical, chemical and biological methods have been developed. Among these processes, phytoremediation exploiting the properties of plants has many advantages. Phytoremediation is the use of plants to remove or degrade organic and inorganic contaminants from soil and water [6] [7] [8]. The various phytoremediation technologies include phytodegradation, phytoextraction, phytoremediation, phytovolatilization, and rhizofiltration. Phytoremediation technology has been successfully implemented in several countries [9] [10] [11]. However, the plant species used are not always present in Côte d'Ivoire. Consequently, their implementation may be confronted with growth problems on local soils, hence the need to exploit endogenous species with accumulation potential. Thus, the studies of [12] on the Akouedo landfill made it possible to evaluate the accumulation capacities of certain endogenous species, including *Panicum maximum*. This study showed that the species *P. maximum* has a high potential for accumulation of trace metals including Cd, Pb, Cu, Ni and Zn [12]. Moreover, [13] has proved *P. maximum* capacity for accumulating trace metals. In addition, the concentrations of trace metals in polluted soils vary and can sometimes be very high in some environments. Thus, the application of the process with *P. maximum* requires studying the adaptation levels and accumulation potentialities of the plant in highly polluted environments. The present study therefore proposes to investigate the influence of Pb and Cd concentration on the accumulation potential of *P. maximum*. Specifically, the aim is 1) to evaluate the effect of soil Pb and Cd concentration on the development of *P. maximum* and 2) to determine the accumulation capacity of Pb and Cd by *P. maximum* with increasing concentration.

2. Methodology

2.1. Experimental Procedure

The experimental was performed in a greenhouse (length = 13 m and width = 11

m) at the experimental site of the biotechnology and environmental engineering research unit of Nangui Abrogoua University, Cote d'Ivoire. It was equipped with a fan powered by a solar plate to regulate the temperature and the flow of air inside. Inside the greenhouse, buckets of 0.01 m^3 capacity (Figure 1) containing contaminated and uncontaminated soil at a height of 20 cm were placed. This height was considered to account for the average root length of the plant.

2.2. Experimental Soil Description

Soil culture was taken from an uncultivated plot at NANGUI ABROGOUA University. It was air-dried and sieved to 2 mm, then homogenized and placed in each bucket. The physico-chemical characteristics of this soil were determined (Table 1).

The soil used was artificially contaminated with Pb and Cd in order to obtain 3 mg/kg and 9 mg/kg for Cd, and 100 mg/kg and 300 mg/kg for Pb. The concentrations of Pb and Cd were chosen according to data from studies conducted in Côte d'Ivoire. According to these works, the concentrations vary from 2 to 9.87 mg/kg for Cd [12] [14] and from 90 to 300 mg/kg for Pb [12] [15]. In addition, the limit values for Cd (2 mg/kg) and Pb (100 mg/kg) concentrations in soils [16] were considered. Minimum concentrations of 3 mg Cd/kg and 100 mg Pb/kg and maximum concentrations of 9 mg Cd/kg and 300 mg Pb/kg, were used for contamination, in order to assess the resistance capacity and accumulation potential of the plant. This contamination was made from lead ($PbCO_3$) and cadmium ($CdSO_4$) salts. For this purpose, the soils were saturated with an amount of lead salt and cadmium salt determined by Equation (1) [16] [17]. The whole (soil-solution) was then homogenized and air-dried under the greenhouse for a week, and then arranged in buckets.

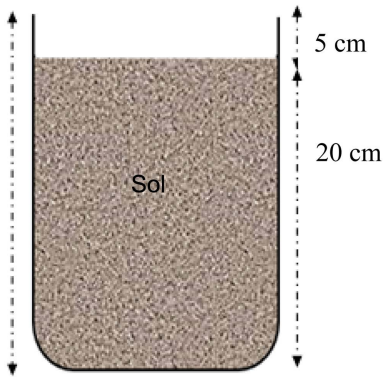

Figure 1. Bucket with culture soil.

Table 1. Physico-chemical characteristics of experimental soil.

Parameter	N %	P (mg/kg)	K (Cmol/kg)	CEC (Cmol/kg)	MO %	pH	Pb (mg/kg)	Cd (mg/kg)
Values	0.1	2.01	0.03	3.04	2.04	5.50	<0.005	<0.0005

$$m_{\text{metallic salt}} = \frac{C \times m_{\text{soil}} \times M_{\text{metallic salt}}}{M_{\text{heavy metals}}} \quad (1)$$

$m_{\text{metallic salt}}$ = mass of heavy metals (mg);
$M_{\text{metallic salt}}$ = Molar mass of heavy metals (g/mol);
$M_{\text{heavy metal}}$ = Molar mass of heavy metals (g/mol);
m_{soil} = Mass of soil in the bucket (kg);
C = Theoretical concentration of heavy metals (mg/kg).

2.3. Treatment Test

To conduct the treatment test, 30 buckets containing soil were used (Figure 2). These included six (6) buckets per dose of soil contamination with lead and cadmium (3 mg/kg and 9 mg/kg of Cadmium and 100 mg/kg and 300 mg/kg of Lead), *i.e.* 24 pots and six (6) buckets serving as control, containing uncontaminated soil. Uncontaminated soils (control) were used to understand the effect of contamination on biomass. Each bucket was planted with *P. maximum* and the experiment lasted ninety days. Every thirty days, two (2) plant replicates per dose of trace metals were collected, as well as two replicates from the uncontaminated soil (control).

2.4. Monitoring of Plant Development

It consisted in studying the evolution of the plant biomass produced during the period of the experiment. It was determined thirty day, by weighing shoot and root biomass.

2.5. Evaluation of the Accumulation Potential

2.5.1. Plant and Soil Sampling

Plant sampling was conducted each thirty days, from two culture pots per dose of trace metals. Plant samples were washed with tap water and rinsed with distilled water. Shoots and roots were separated and placed in kraft envelopes, then oven dried at 65˚C for 72 h. The samples were then ground using a RESPSCH S100 ball mill.

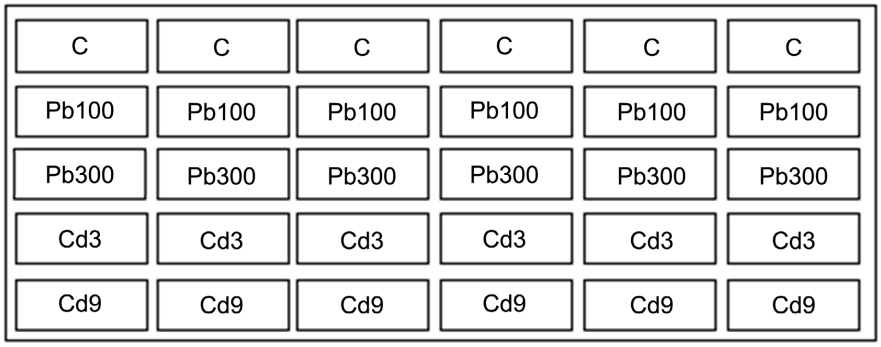

Figure 2. Schematic diagram of the treatment test (Pb100: soil contaminated at 100 mg/kg; Pb300: soil contaminated at 300 mg/kg; Cd3: soil contaminated at 3 mg/kg; Cd9: soil contaminated at 9 mg/kg: C: control (uncontaminated soil)).

For the soil, sampling consisted of core sampling using a 15 mm diameter polyvinyl chloride (PVC) sampler. The [0 - 20 cm] horizon was sampled. Four (4) incremental samples were mixed to obtain a composite sample. The collected samples were stored in hermetically sealed jars until analysis.

2.5.2. Trace Metals Analysis in the Plant Samples

The mineralization of the samples was done according to a method derived from the NFX 31-151 standard. It starts with the calcination step. Indeed, 20 g of plant shreds were put in a crucible and the whole was heated in an oven at 500˚C for 2 h. The solution for the determination of the trace metals (Pb and Cd) was carried out by acid etching of 0.5 g plant sample with 10 mL aqua regia (7.5 mL HCl and 2.5 mL HNO_3). Then, the whole was boiled in an oven at 180˚C for 30 min. After cooling, the solution was filtered into a 25 mL volumetric flask. The filtrate obtained was made up to the mark with distilled water. The determination of the TMEs (Pb and Cd) in the solution was performed by plasma-coupled induction atomic emission spectrometry (ICP-AES).

2.5.3. Pretreatment and Analysis of Soil Samples

The collected soil samples were air-dried, then crushed and sieved using a 2 mm diameter AFNOR type sieve. Then, intermediate sampling was performed on the sieved fraction using the quartering technique, in order to minimize the risk of error on the composition of the soils related to their heterogeneity, and to obtain the mass of residues needed for the analyses [18]. Subsequently, the protocol used for the determination of TMEs (Pb and Cd) was derived from NF ISO 11466 of June 1995. During this determination, 0.5 g of soil previously crushed and sieved to 63 microns was moistened with 1 mL of distilled water, 7.5 mL of a concentrated hydrochloric acid solution (Normapur HCl solution) and 2.5 mL of a concentrated nitric acid solution (Normapur HNO_3 solution). The whole is transferred to an oven at 180˚C for 30 min. The digestate was filtered to 0.45 µm and diluted in a 50 ml Erlenmeyer flask with distilled water. The digestate obtained was then analyzed by induction coupled plasma atomic emission spectrometry (ICP-AES).

2.5.4. Phytoextraction Efficiency

Two factors were calculated to evaluate plant phytoextraction efficiency. The bioaccumulation factor (BF) was calculated to determine the degree of metal accumulation in the plants grown (Equation (2)) [19]. However, the translocation factor (TF) defined as the ratio between the metal concentration in plant shoots and its concentration in roots (Equation (3)) [20]. It indicated the capability of plants to take up trace metals from their roots and to translocate them to their shoots. These factors were calulated as follows:

$$\mathrm{BF} = \frac{[\mathrm{Metal}]_{\mathrm{roots}} + [\mathrm{Metal}]_{\mathrm{shoots}}}{[\mathrm{Metal}]_{\mathrm{soil}}} \quad (2)$$

$$\mathrm{TF} = \frac{[\mathrm{Metal}]_{\mathrm{shoots}}}{[\mathrm{Metal}]_{\mathrm{roots}}} \quad (3)$$

$[Metal]_{roots}$ = Metal concentration in roots;
$[Metal]_{shoots}$ = Metal concentration in shoots;
$[Metal]_{soil}$ = Metal concentration in soil.
ØTF > 1: accumulation of trace metals in the shoot biomass of plants;
ØTF < 1: accumulation of trace metals in the root biomass of plants.

2.5.5. Statistical Analysis

Statistical analysis of the data was performed with R software version 3.3.2. The normality of the data distribution of the variances was verified with the Shapiro test. To evaluate differences between biomass produced by the plant, trace metals concentration in the plant biomass, bioaccumulation factor and translocation factor, data were analyzed using the parametric test (t-test, ANOVA test) and the non-parametric test (Mann Whitney). Statistical significance was defined at the level of $p < 0.05$.

3. Results

3.1. Plant Biomass

3.1.1. Shoot Biomass

The shoot fresh biomass produced by *P. maximum* increases with time on crop soils (Figure 3). Furthermore, they decrease with increasing Pb and Cd concentration. From day 30 to day 90, the mass of aerial part increased from 13.5 ± 0.06 to 22.2 ± 0.09 g, from 10.4 ± 0.07 to 17.6 g ± 0.12, from 7.3 ± 0.08 to 12.3 ± 0.13 g, respectively in control soil and soils contaminated with 100 mg Pb/kg (Pb100) and 300 mg Pb/kg (Pb300). In the Cd-contaminated soils, aboveground biomass

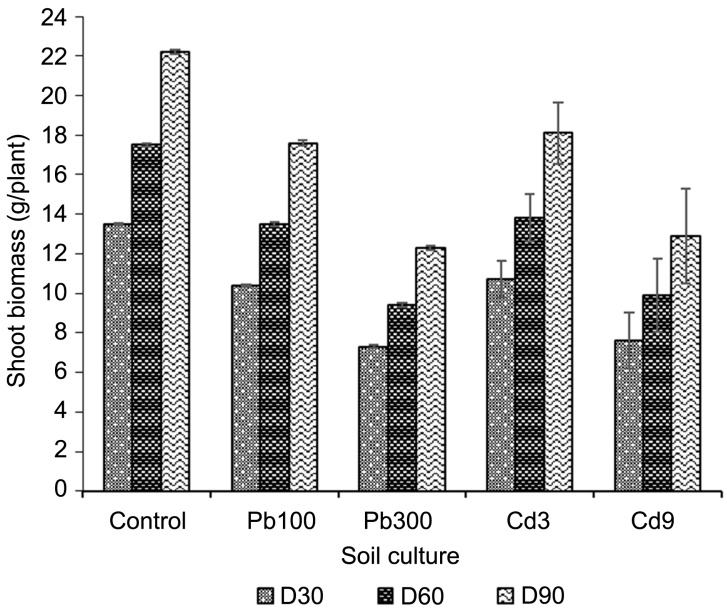

Figure 3. Average shoot biomass produced by *P. maximum* during the experiment (Pb100: soil contaminated at 100 mg Pb/kg; Pb300: soil contaminated at 300 mg Pb/kg; Cd3: soil contaminated at 3 mg Cd/kg; Cd9: soil contaminated at 9 mg Pb/kg; Control: uncontaminated soil).

changed from 13.5 to 22.16 g in the control soil, from 10.70 ± 0.91 to 18.1 ± 1.56 g in the soil contaminated with 3 mg Cd/kg (Cd3), and from 7.60 ± 1.41 to 12.90 ± 2.40 g in the soil contaminated with 9 mg Cd/kg (Cd9), from day 30 to day 90.

3.1.2. Root Biomass

Root biomass was higher in the control soil (Figure 4).

It is followed by the biomass from soils contaminated at 3 mg Cd/kg and 100 mg Pb/kg. Root biomass ranged from 11.40 ± 0.46 to 18.31 ± 0.60 g in the control, from 10.11 ± 0.35 to 16.10 ± 0.56 g in Pb100, and from 8.32 ± 0.70 to 13.31 ± 1.13 g in Pb300, from day 30 to day 90. In Cd-contaminated soils, it ranged from 8.22 ± 0.14 to 13.12 ± 0.23 g (Cd3) and from 7.91 ± 0.85 to 12.64 ± 1.36 g (Cd9).

3.2. Concentration of Pb and Cd in Culture Soil

Average concentrations of Pb Cd in cuture soil decreased during experimentation (Table 2). It ranged from 55.11 ± 0.14 (D30) to 40.82 ± 0.19 mg/kg (D90) for Pb100. and from 188.12 ± 0.13 (D30) to 168.63 ± 0.15 mg/kg (D90) for Pb300. Concerning Cd3 et Cd9, concentration ranged from 1.91 ± 0.08 (D30) to 1.50 ± 0.07 mg/kg (D90), and from 7.52 ± 0.09 (D30) to 6.10 ± 0.08 mg/kg (D90), respectively.

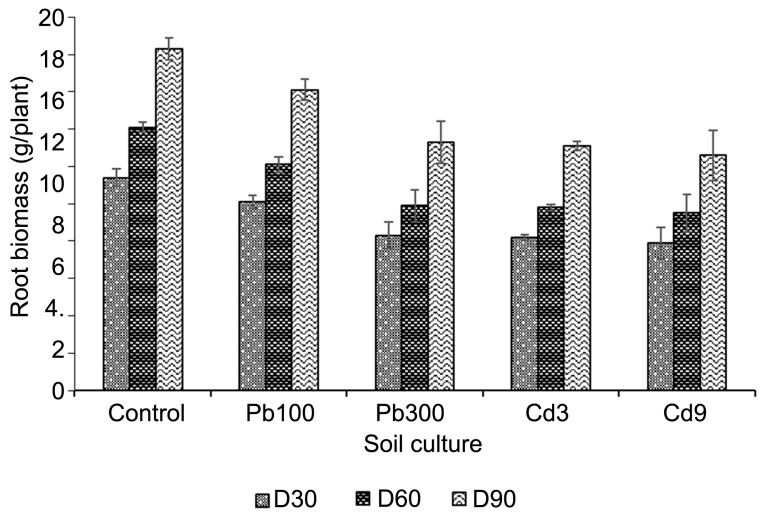

Figure 4. Average root biomass produced by *P. maximum* during the experiment (Pb100: soil contaminated at 100 mg Pb/kg; Pb300: soil contaminated at 300 mg Pb/kg; Cd3: soil contaminated at 3 mg Cd/kg; Cd9: soil contaminated at 9 mg Pb/kg; Control: uncontaminated soil).

Table 2. Average concentrations of Pb and Cd in culture soil during experimentation.

	D30 (mg/kg)	D60 (mg/kg)	D90 (mg/kg)
Pb100	55.11 ± 0.14	49.91 ± 0.18	40.82 ± 0.19
Pb300	188.12 ± 0.13	182.84 ± 0.16	168.63 ± 0.15
Cd3	1.91 ± 0.08	1.80 ± 0.09	1.50 ± 0.07
Cd9	7.52 ± 0.09	6.92 ± 0.08	6.10 ± 0.08

3.3. Concentration of Pb and Cd in Shoot and Root Biomass of *P. maximum*

3.3.1. Culture Soil Contaminated with Lead

Overall, regardless of the rate of Pb applied to the soil, there was higher accumulation of Pb in the root biomass compared to the shoot biomass (Figure 5).

The accumulated Pb concentrations increase with the duration of the experiment. In shoot biomass, they increase from 6.48 ± 0.55 to 18.09 ± 0.71 mg/kg (Pb100) and from 10.93 ± 0.38 to 23.04 ± 0.79 mg/kg (Pb300) from day 30 to day 90. For root biomass, accumulated Pb concentrations ranged from 8.09 ± 0.58 to 22.57 ± 0.86 mg/kg and from 29.45 ± 0.49 to 62.35 ± 0.82 mg/kg in the 100 and 300 mg Pb/kg contaminated soils, respectively.

3.3.2. Culture Soil Contaminated with Cadmium

Cd concentrations were higher in shoot biomass compared to root biomass (Figure 6). Accumulated Cd concentrations in biomass increased with the duration of the experiment. Cd concentrations ranged from 0.91 ± 0.02 to 1.50 ± 0.03

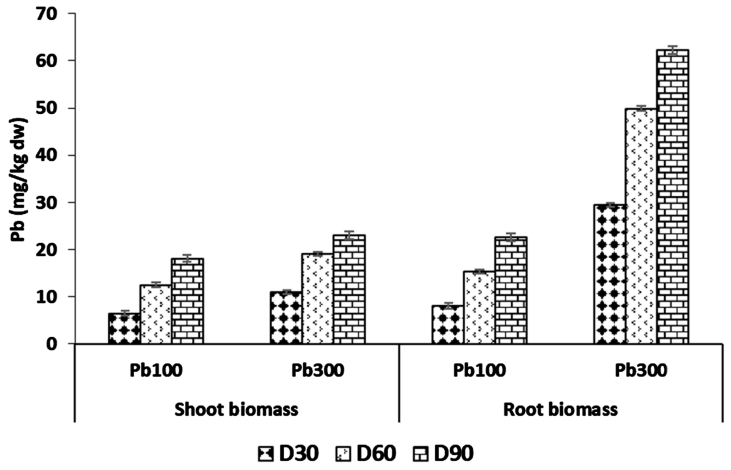

Figure 5. Average concentration of Pb in the shoot and root biomass of *P. maximum* (Pb100: contaminated soil at 100 mg Pb/kg; Pb300: contaminated soil at 300 mg Pb/kg).

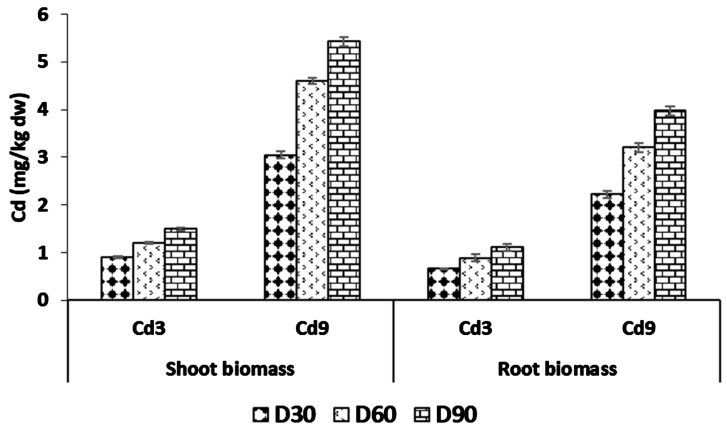

Figure 6. Average concentration of Cd in shoot and root biomass of *P. maximum* (Cd3: contaminated soil at 3 mg Cd/kg; Cd9: contaminated soil at 9 mg Pb/kg).

mg/kg (Cd3) and from 3.05 ± 0.08 to 5.43 ± 0.09 mg/kg (Cd9) in aboveground biomass of *P. maximum* from day 30 to day 90. Regarding Cd concentrations in root biomass, it ranged from 0.66 ± 0.01 to 1.11 ± 0.07 mg/kg (Cd3) and from 2.22 ± 0.08 to 3.97 ± 0.09 mg/kg (Cd9).

3.4. Bioaccumulation Factor (BF)

The BF values obtained during (Figure 7) vary from 0.27 ± 0.02 to 1.00 ± 0.04 (Pb100) and from 0.21 ± 0.04 to 0.50 ± 0.05 (Pb300). However, statistical analysis shows that there are no significant differences between the BF values (t-test: $p > 0.05$),), at days 30, 60 and 90.

The values of the bioaccumulation factor (BF) of *Panicum maximum* in the different Cd contaminated soils ranged from 0.83 ± 0.09 to 1.74 ± 0.18 (Cd3) and from 0.70 ± 0.08 to 1.54 ± 0.17 (Cd9). Bioaccumulation factors are higher with Cd3 compared to Cd9. However, this difference is not significant (t-test: $p > 0.05$).

3.5. Translocation Factor (FT)

The transfer factor values (Figure 8) were less than 1 for Pb and greater than 1 for Cd. They are between 0.80 ± 0.12 and 0.82 ± 0.13 (Pb100) and between 0.37

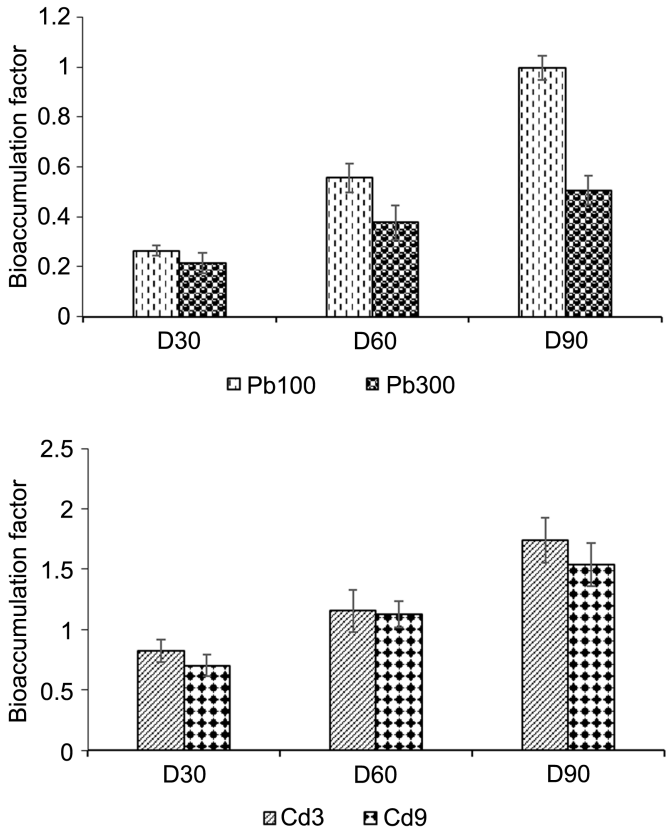

Figure 7. Bioaccumulation factor of Pb and Cd (Pb100: contaminated soil at 100 mg Pb/kg; Pb300: contaminated soil at 300 mg Pb/kg; Cd3: contaminated soil at 3 mg Cd/kg; Cd9: contaminated soil at 9 mg Pb/kg).

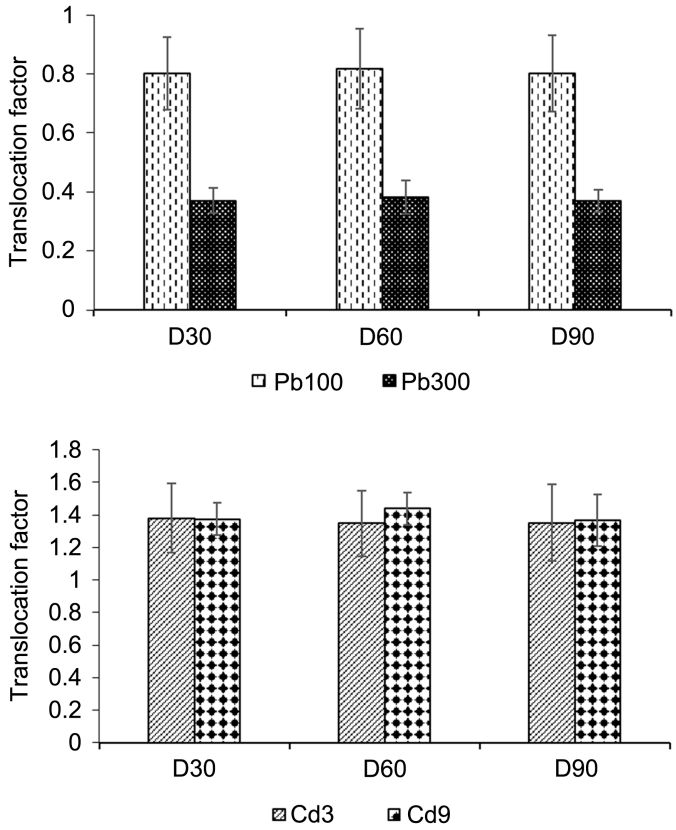

Figure 8. Translocation factor of Pb and Cd (Pb100: contaminated soil at 100 mg Pb/kg; Pb300: contaminated soil at 300 mg Pb/kg; Cd3: contaminated soil at 3 mg Cd/kg; Cd9: contaminated soil at 9 mg Pb/kg).

± 0.04 and 0.38 ± 0.05 (Pb300). The transfer factors obtained with Pb100 were higher than those obtained with Pb300. However, these factors were not significantly different (test t: $p > 0.05$).

Concerning the transfer factors recorded with Cd, they vary from 1.35 ± 0.20 to 1.38 ± 0.21 (Cd3) and from 1.37 ± 0.16 to 1.44 ± 0.10 (Cd9). The values of the transfer factor obtained with the soils at 3 and 9 mg Cd/kg, did not differ significantly (test t: $p > 0.05$).

4. Discussion

The present study examined the potentiality of Pb and Cd accumulation by *Panicum maximum* under controlled conditions. The results show a significant influence of the concentration of trace metals (Pb and Cd) on the development of *P. maximum* and its ability to accumulate Pb and Cd. The concentrations of trace metals used in this study were tolerable and the plants grew. Indeed, from one month to the next, an increase in fresh biomass was recorded. This observed increase could be explained according to [21] by a significant growth of *P. maximum* buds over time, which increases the number of stems forming plant clumps and consequently, increases plant biomass. Relative to the fresh biomass produced in the contaminated pots, the masses produced are higher in the

Pb100 and Cd3 contaminated soils, compared to, Pb300 and Cd9. These results would probably be related to the very high concentrations that could be toxic to the plant. Indeed according to [22], the excess or toxic content of Cd for plants would be between 5 and 30 mg/kg.

This phenomenon explains the bioaccumulation factors obtained. Indeed, the bioaccumulation factors are higher with Pb100 and Cd3. Therefore, the accumulation capacity of *P. maximum* could be limited beyond the concentrations 300 and 9 mg/kg. Furthermore, the shoot of *P. maximum* concentrates more Cd, compared to the root biomass which accumulates more Pb. Overall, the root biomass of *P. maximum*, concentrates the studied trace metals (Pb and Cd). These results are similar to those of [23], who indicated that the species *P. maximum* concentrated more Pb, in the root biomass. In addition, [24] showed that vetiver also accumulated Pb in root biomass. TF values were higher than 1. It indicated that P. maximum showed suitable potential for Cd-contaminated soil.

5. Conclusion

The present study allowed to evaluating the capacity of accumulation of trace metals (Pb and Cd) by *P. maximum*. After analysis of the different results of the experimental process, a reduction of the plant biomass was observed with the increase of Pb and Cd concentrations in the cultivation soil. Concentrations of Pb and Cd, accumulated in the shoot of *P. maximum* varied from 6.48 ± 0.55 to 18.09 ± 0.71 mg/kg (Pb100); from 10.93 ± 0.38 to 23.04 ± 0.79 mg/kg (Pb300); from 0.91 ± 0.02 to 1.50 ± 0.03 mg/kg (Cd3) and from 3.05 ± 0.08 to 5.43 ± 0.09 mg/kg (Cd9), from day 30 to day 90. On the other hand, in the root part, concentrations of Pb and Cd, changed from 8.09 ± 0.58 to 22.57 ± 0.86 mg/kg (Pb100); from 29.45 ± 0.49 to 62.35 ± 0.82 mg/kg (Pb300); from 0.66 ± 0.01 to 1.11 ± 0.07 mg/kg (Cd3) and from 2.22 ± 0.08 to 3.97 ± 0.09 mg/kg (Cd9) from day 30 to day 90. Overall, Pb is highly accumulated in the root of the plant compared to Cd, which is highly concentrated in the shoot of *P. maximum*. The plant bioaccumulation factor ranged from 0.26 ± 0.02 to 0.99 ± 0.04 (Pb100); from 0.21 ± 0.04 to 0.50 ± 0.06 (Pb300); from 0.83 ± 0.09 to 1.72 ± 0.18 (Cd3) and from 0.70 ± 0.08 to 1.54 ± 0.18 (Cd9). In contrast, the transfer factor values are less than 1 for Pb and greater than 1 for Cd. They ranged from 0.80 ± 0.12 to 0.82 ± 0.13 (Pb100) and from 0.37 ± 0.04 to 0.38 ± 0.05 (Pb300), and for Cd they fluctuated between 1.35 ± 0.20 and 1.38 ± 0.21 (Cd3) and between 1.37 ± 0.16 and 1.44 ± 0.10 (Cd9).

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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