Trends in Applied Sciences Research

ISSN 1819-3579

Academic
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OPEN ACCESS Trends in Applied Sciences Research

ISSN 1819-3579
DOI: 10.3923/tasr.2020.281.292

Research Article
Phytoextraction Capacity of *Panicum maximum* Grown on Synthetic Heavy Metals Contaminated Soil

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Abstract

Background and Objective: Heavy metals contaminated soils pose serious environmental and health threats and there is a need to develop suitable cost-effective soil remediation techniques. This research aimed to determine the effective capacity of *Panicum maximum* to accumulate heavy metals (Pb, Cd, Ni, Zn, Cu) in a controlled environment. **Materials and Methods:** Phytoremediation of metals contaminated soils offers a low-cost method for soil remediation. *Panicum maximum* has been observed in a greenhouse pot experiment on synthetic soil. Seedlings of *P. maximum* were sown in plastic pots containing uncontaminated or contaminated soils. Experiments were conducted to compare the growth of *P. maximum* and its ability to uptake heavy metals. The bioaccumulation and transfer factors, as the location of the heavy metals in the tissues and cells of the plant, have been determined. Physico-chemical parameters were also analyzed. **Results:** *Panicum maximum* showed stress for Zn and Cu and accumulated more Ni and Pb than Cd. However, Cd and Pb are mainly retained in the roots while Ni is exported to the above parts. The Pb remains essentially fixed to the cell walls in the organs of the roots and leaves, in comparison to Ni which was accumulated preferentially in the cells whatever organ considered. **Conclusion:** *Panicum maximum* accumulated more Ni and Pb than Cd. The Pb is mainly retained in the roots while Ni is exported to the above parts. Moreover, Observations and microanalytic spectra indicated that Pb remains in the cell walls and Ni was accumulated in the intracellular compartment. The phytoextraction potential of *P. maximum* according to the level of soil contamination can be a phytotechnology for polluted soils remediating.

Key words: *Panicum maximum*, heavy metals, phytoremediation, synthetic soil, bioaccumulation factor, transfer factor, greenhouse

Citation: Coulibaly, H., A. Messou, J.M.P. Ouattara and L. Coulibaly, 2020. Phytoextraction capacity of *Panicum maximum* grown on synthetic heavy metals contaminated soil. Trends Applied Sci. Res., 15: 281-292.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soils have become increasingly polluted by heavy metals with increasing urbanization and industrialization and this threatens ecosystems, surface and ground waters, food safety and human health[1-3]. Pollution of the soils by heavy metals poses serious problems because of their toxicity[4]. Their bioaccumulation of and the contamination of the food chain is a major health risk[5]. The most common heavy metals founded in polluted soils are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and Nickel (Ni)[6]. In humans, these heavy metals can affect many biological processes and lead to neurological conditions such as lead poisoning, anemia and decreased fertility[7]. Some elements such as Pb, Cd and Hg are mutagenic and carcinogenic[8]. As a result, several physical and chemical remediation technologies for heavy metals contaminated soils have been developed. However, these technologies are generally expensive, greatly disrupt the biological activity of soils and alter their physical and chemical characteristics[9-10]. There is a need to develop suitable cost-effective biological soil remediation techniques to remove contaminants without affecting soil fertility. Phytoremediation could provide a sustainable technique for metal remediation[11]. Indeed, phytoremediation is a less expensive technique, more extensive and ecological[12]. This technology has received more attention[13] and has shown better results in several countries[14-17]. However, plant species previously experienced are not always present in Cote d'Ivoire. Consequently, their implementation may be confronted with problems of adaptation to local soils, hence the need to explore endogenous species with potential for accumulation. Therefore, the phytoaccumulation potential of some endogenous species, including *Panicum maximum*, grown on the Akouedo landfill were assessed[18]. The *P. maximum* has a high potential for the accumulation of heavy metals, including Cd, Pb, Cu, Ni and Zn[18-19]. However, given the complexity of this environment, which has the characteristics of polycontaminated natural environments. Thus, the present study proposed to determine the actual capacity of *P. maximum* to accumulate heavy metals (Pb, Cd, Ni, Zn, Cu) in a controlled environment. Specifically, this study involves in evaluating the effect of the heavy metals concentration on plant growth, determining the potential for extracting heavy metals by *P. maximum* and understanding the accumulation mechanisms.

MATERIALS AND METHODS

Study area: Research project was conducted from October 1st, 2019 to February 28th, 2020. That period was subdivided into two phases, of which one month for the establishment of infrastructure, soil preparation and the creation of nurseries and four months for conducting experiment.

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, 56 PVC pots (length = 27 cm, width = 20 cm, height = 45 cm) containing synthetic soil contaminated or not, at a height of 40 cm (Fig. 1), were arranged.

Synthetic soil: Synthetic soil was prepared based on the granulometric composition (clay: 2%; silt: 10% and sand: 78%) of uncontaminated soil obtained in the northern zone of Abidjan district[18]. To do this, white lagoon sand previously washed (acid treatment with 0.2 M HCl for soil heavy metals neutralization and rinsing with distilled water), dried and sieved to 2 mm, was mixed with kaolinite[20].

Plant selection: *Panicum maximum* was selected according to its availability, its rapid growth and its potential tolerance of heavy metals (Cu, Zn, Pb, Cd, Ni)[18]. In addition, *P. maximum* produces significant shoot and root biomass and has been described as a phytoaccumulator[18, 21-23].

Experimental design: The experiment was performed with plants grown in pots filled with uncontaminated (control, Te) and contaminated soil. Contaminated soil was treated with zinc (Zn), copper (Cu), nickel (Ni), lead (Pb), cadmium (Cd). Two groups were constituted of which one contaminated with one heavy metal and the other contaminated with all the heavy metals studied (polycontaminated, PC). Each treatment was replicated eight times. Heavy metals concentrations in soil at the start of experience were 2 ppm for Cd, 50 ppm for Ni, 100 ppm for Cu and Pb and 300 ppm for Zn. Moreover, seedlings of *P. maximum* were used to establish nurseries on the experimental site. Plants with the same morphological development were selected and cultured in each pot.

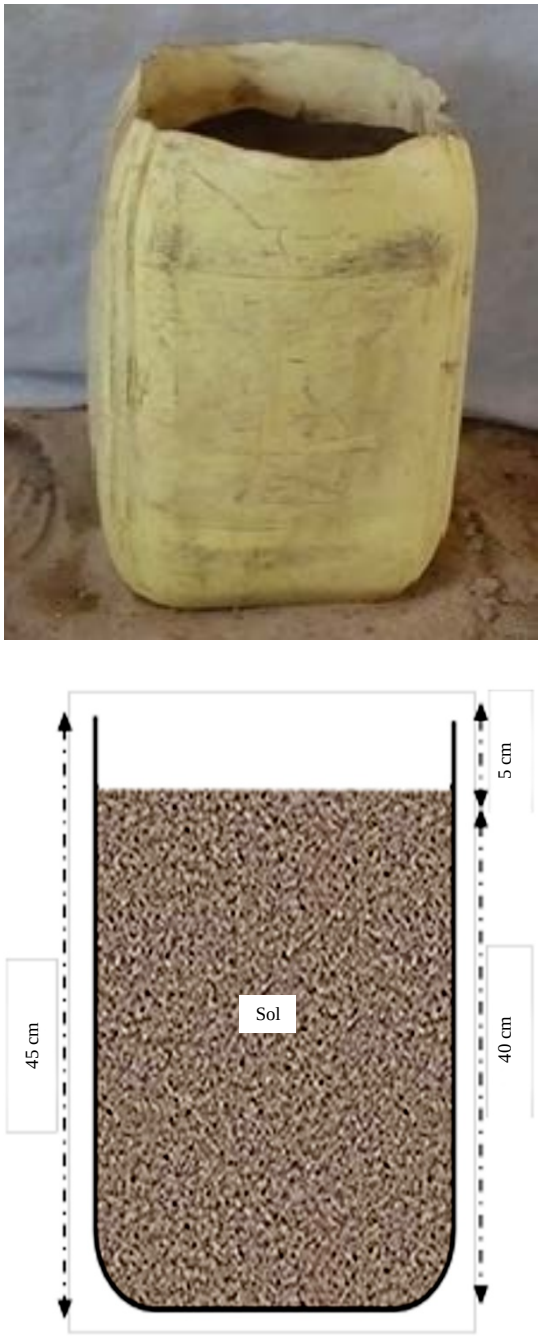

Fig. 1: Experimental pots

Plant growth and plant biomass produced: Growth monitoring was carried out by weekly measurement of the height of the studied plant stems using a tape measure in millimeters. Plants of two (2) replicates per heavy metals were harvested monthly and the plant biomasses produced were determined by weighing on a 10^{-3} precision Sartorius EB150FEG-I scale.

Physico-chemical properties and heavy metals concentration in soil: Soil pH was measured with a soil to deionized water ratio of 1:2.5 (w/v) by a pH meter. Concerning the redox potential, it was determined with a ORP-meter Humeau tester on a composite soil sample taken in the root zone and put in solution (ratio 1:5)[24]. Relative to CEC and heavy metals concentrations, they were analyzed on composite samples taken monthly from the horizons (0-10, 10-20, 20-30 and 30-40 cm) of soils. It was determined according to the standard[25]. That method consists in displacing all the adsorbed cations on the exchange sites and then saturating them with the ammonium ions (NH_4^+).

Heavy metals analysis: Soil heavy metals concentrations were carried out according to the standard[26]. The soil sample (0.5 g) was digested with a mixture of HCl and HNO_3 (7.5 mL of HCl and 2.5 mL of HNO_3). The content was filtered at 0.45 µm and diluted up to 50 mL with distilled water. Heavy metals concentrations were determined by Plasma-Coupled Induction Atomic Emission Spectrometry (ICP-AES).

Assessment of the accumulation potential of heavy metals by *P. maximum*

Sampling and pretreatment of plant material: In each pot, the harvested plants were separated into shoot and root parts. Each plant sample was washed with distilled water and high purity water to remove dust and soil. After air-drying, each plant sample was dried at 80°C to a constant weight. The dried samples were crushed using a stainless-steel plant tissue grinder (LD-Y500A).

Samples analysis: The mineralization of the samples was made according to the standard[27]. Subsample (20 g) of crushed plant material was oven-dried at 500°C for 2 hrs and 0.5 g of that burned sample was digested with 10 mL of aqua regia (7.5 mL of HCl and 2.5 mL of HNO_3). Then, the sample was put in an oven at 180°C for 30 min for ending digested process. The filtrate obtained after cooling was used for heavy metals analysis by Plasma Coupled Induction Atomic Emission Spectrometry (ICP-AES).

Phytoextraction efficiency: Two factors were calculated to evaluate plant phytoextraction efficiency. The Bioaccumulation Factor (BF) was calculated to estimate the metal uptake in the plant. It presents an index of the ability of a plant to accumulate a particular metal relative to the concentration in the medium[28]:

$$BF = \frac{\left[\begin{array}{l}\text{Metal concentration in the roots} + \\ \text{Metal concentration in the shoots}\end{array}\right]}{[\text{Metal concentration in the soil}]}$$

The Transfer Factor (TF) defined as the ratio between the metal concentration in plant shoots and its concentration in roots[29-30]. It determined the relative movement of heavy metals from roots to shoots:

$$TF = \frac{\text{Metal concentration in the shoots}}{\text{Metal concentration in the roots}}$$

- TF>1: Accumulation of ETM in shoot biomass
- TF<1: Accumulation of ETM in the root biomass

Heavy metals localization in tissues and cells of *P. maximum*: This study determined the distribution of heavy metals in plant roots and leaves, precisely at the tissue and the cell level. It was performed using a scanning electron microscope equipped with an X-ray detector connected to an EDS microanalyzer platform (SEM-EDX). For the analyses, the plant materials (leaves and roots) were collected at the end of the experiment, from the Ni and Pb contaminated pots where maximum values of transfer factor and bioaccumulation factor were obtained. Those samples were fixed for 24 hrs in 2.5% glutaraldehyde (pH 7.2). Then, they were rinsed two or more times with distilled water. A 2 mm cross-section of the samples (leaf or root) was followed by dehydration in successive baths of 30 min of ethanol (from 70-100%). Then, these samples were put in a solution composed of ethanol and acetone at 50, 70 and 90% acetone (30 min per bath), then in a solution of 100% acetone for 1 hr. The samples were subsequently dried in the open air and fixed on pads placed on a plate carried in the metallizer to spray them with gold. The plate was finally mounted on the stage of scanning electron microscope equipped with an X-ray detector connected to an EDS microanalyzer platform to perform heavy metals observations in the tissue and the cell.

Statistical analysis: Statistical analysis of the data was performed with R software version 3.3.2. The normality of the data distribution and the homogeneity of the variances were verified respectively with the Shapiro test. To examine differences between heavy metals concentrations and physico-chemical parameter values in different soil layers and also trace metals concentration in plant materials, 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 $p<0.05$.

RESULTS

Plant growth and biomass: During the treatment, plants grown on soils contaminated with nickel (Ni), lead (Pb), cadmium (Cd) and those grown on the control soil (Te) were resistant to experimental conditions and presented more or less regular growth (Fig. 2). This growth was more regular from 1-64 days and less regular from day 64 to the end of the experiment (Fig. 3). Comparing the average lengths of the stems, they were significantly higher on the control soil than on the contaminated soils ($p<0.05$). The order of the average lengths of stem at the end of the experiment is as follows: Te (120 cm)>Pb (103.5 cm)>Cd (94.5 cm)>Ni (68.5 cm). In addition, the plant growth on soils contaminated by copper (Cu), zinc (Zn) and by all the studied trace metals (PC), was limited and the plant died after 71 days for Cu and 36 days for PC and Zn. Mean length of stems at plant death were 36.8 cm (Cu), 25 cm (Zn) and 22.5 cm (PC).

Fig. 2: Overview of plants grown at the end of the experiment

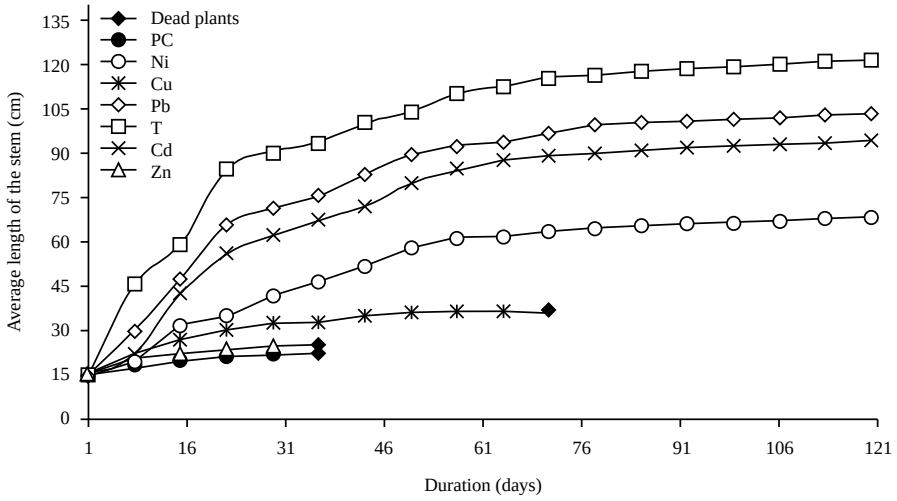

Fig. 3: Growth profile of *P. maximum* stems during the experiment
Te: Control soil, PC: Polycontaminated soil, Ni: Soil contaminated with nickel, Cd: Soil contaminated with cadmium, Pb: Contaminated lead soil

Table 1: Fresh shoot and root biomass produced by *P. maximum*

		Time of experimentation (months)			
Plant material	Pot of culture	1	2	3	4
Shoot biomass (g plant^{-1})	Soil-Te	40.5	50.8	56.2	61.3
	Soil-Pb	38.0	40.9	45.9	49.6
	Soil-Cd	28.4	36.6	40.9	44.2
	Soil-Ni	22.9	28.5	32.2	35.1
	Soil-Zn	15.1	nd	nd	nd
	Soil-Cu	15.9	20.8	nd	nd
	Soil-PC	13.8	nd	nd	nd
Root biomass (g plant^{-1})	Soil-Te	22.5	26.9	30.2	32.6
	Soil-Pb	17.1	25.7	28.8	31.1
	Soil-Cd	15.9	17.5	19.6	21.2
	Soil-Ni	10.7	12.6	14.5	15.9
	Soil-Zn	6.2	nd	nd	nd
	Soil-Cu	6.2	10.5	nd	nd
	Soil-PC	4.6	nd	nd	nd

Te: Control, Ni: Nickel, Cd: Cadmium, Pb: Lead, nd: Not determined

Monthly shoot and root biomasses harvested during the experiment are presented in Table 1. It is noted that these biomasses have increased during the experimental and the shoot part has remained higher than that root in all the pots. From the first to the fourth month of the experiment, shoot biomasses increased from 40.5-61.3 g on the control soil, from 28-44 and 2 g, from 38-49.6 g and from 22.9-35 g on soils contaminated respectively by Cd, Pb and Ni. As for root biomass, the values ranged from 22.5-32.6 g (control), from 15.9-21.2 g (Cd), from 17.1-31.1 g (Pb) and from 10.7-15.9 g (Ni). Overall, the evolution of the biomasses produced by *P. maximum* in the control and contaminated pots with Pb did not differ significantly ($p>0.05$). On the other hand, the biomass produced on the pots contaminated with Ni and Cd was significantly different from that obtained from control. Regarding to the shoot biomass of the plant in the pots contaminated by Ni, Pb and Cd they were significantly different ($p<0.05$). Concerning root biomasses produced by *P. maximum*, if no difference were observed between the control pot and the pot contaminated with Pb ($p>0.05$), it is found that the root biomasses of the control pot differs significantly from that of Cd and Ni contaminated pots ($p<0.05$). Also, root biomasses in the pot contaminated by Ni, Pb and Cd were significantly different ($p<0.05$).

Physico-chemical parameters and heavy metals concentrations in soils during the experiment: Table 2 shows soil physico-chemical parameters and heavy metals concentrations during the experiment. Soil pH ranged from 5.8-5.0, from 5.7-4.8, from 5.5-4.5 and from 5.2-4.3, respectively

Table 2: Variation of physic-chemical parameters and heavy metals concentrations on soils

		Time of experimentation (months)			
Parameters	Pot of culture	1	2	3	4
pH	Soil-Te	5.8	5.5	5.3	5.0
	Soil-Pb	5.7	5.1	5.1	4.8
	Soil-Cd	5.5	5.1	4.6	4.5
	Soil-Ni	5.2	5.0	4.3	4.3
	Soil-Zn	4.0	nd	nd	nd
	Soil-Cu	4.5	5.0	nd	nd
	Soil-PC	4.1	nd	nd	nd
Eh (mV)	Soil-Te	162.5	172.0	175.0	186.0
	Soil-Pb	173.0	179.2	180.0	204.0
	Soil-Cd	171.0	175.0	185.0	194.0
	Soil-Ni	185.0	181.5	185.0	227.0
	Soil-Zn	227.0	nd	nd	nd
	Soil-Cu	198.0	248.0	nd	nd
	Soil-PC	258.0	nd	nd	nd
CEC (meq/100 g)	Soil-Te	9.6	9.9	9.4	7.3
	Soil-Pb	9.0	8.9	8.4	6.8
	Soil-Cd	8.3	8.4	7.9	6.5
	Soil-Ni	8.3	8.1	7.4	6.1
	Soil-Zn	7.7	nd	nd	nd
	Soil-Cu	7.8	7.5	nd	nd
	Soil-PC	7.3	nd	nd	nd
Heavy metals concentrations (ppm)	Soil-Pb	95.26	92.6	81.13	75.92
	Soil-Cd	1.93	1.91	1.80	1.77
	Soil-Ni	47.74	44.12	42.29	39.91
	Soil-Zn	273.35	nd	nd	nd
	Soil-Cu	90.15	86.89	nd	nd
Soil-PC	Pb	91.76	nd	nd	nd
	Cd	1.92	nd	nd	nd
	Ni	46.41	nd	nd	nd
	Zn	273.35	nd	nd	nd
	Cu	90.15	nd	nd	nd

Soil-Te: Control soil, Sol-PB: Soil contaminated with Pb, Sol-Cd: Soil contaminated with Cd, Sol-Ni: Soil contaminated with Ni, Sol-Zn: Soil contaminated with Zn, Sol-Cu: Soil contaminated with Cu, Soil-PC: Soil contaminated with Ni, Pb, Cd, Zn and Cu, nd: Not determined

on the control soil and soils contaminated with Pb, Cd and Ni. On Zn-contaminated soil and polycontaminated soil (PC), the pH values were 4.0 and 4.1 respectively. Considering the control soil and soils contaminated with Pb, Cd and Ni, it is noted that the pH values obtained do not differ significantly ($p>0.05$). However, the pH of the control soil remains higher than Pb, Cd and Ni contaminated soils. During the experiment, Eh values recorded ranged from 185.0-227.0 mV (Ni), from 173.0-204.0 mV (Pb), from 171.0-194.0 mV (Cd) and from 162.5-186.0 mV (control). On Zn-contaminated soil and polycontaminated soil, Eh values obtained were 227.0 and 258.0 mV, respectively. Overall, Eh was lower in the control soil. However, Eh values of the control soil and those of the Ni, Pb and Cd contaminated soils were not significantly different ($p>0.05$). Moreover, CEC values ranged from 9.6-7.3, from 9-6.8, from 8.3-6.5 and from 8.3-6.1 meq/100 g, respectively for the control, Pb, Cd and Ni soils. Soil contaminated by Zn and polycontaminated soils had respective CEC values of 7.7 and 7.3 meq/100 g. In addition, although the control soil CEC was higher than that of the soils contaminated by Ni, Pb and Cd, there was no significant difference between these CEC values ($p>0.05$). Heavy metals concentrations in monocontaminated soils decreased from 95.26-75.92, from 1.93-1.77 and from 47.74-39.91 ppm, respectively for Pb, Cd and Ni, from month 1 to month 4. In the polycontaminated soil, heavy metals concentrations were 91.76 ppm Pb, 1.92 ppm Cd, 46.41 ppm Ni, 273.35 ppm Zn and 90.15 ppm Cu. Regarding to Cu-contaminated soil, the physico-chemical parameters ranged from 4.5-5 (pH), from 198-248 (Eh) and from 7.5- 7.8 (CEC) and Cu concentration ranged from 86.89-90.15 ppm.

Heavy metals accumulation potential of *P. maximum*: Heavy metals accumulation in the shoot and root biomasses of *P. maximum* (Table 3) indicated that the higher concentrations were recorded in the root. In the pots where the plants survived, the concentrations obtained in the shoot biomass of *P. maximum* ranged from 2.385-12.502 ppm Ni (shoot) and from 0.809-2.604 ppm Ni (root), from 0.394-8.262 ppm Pb (shoot) and 3.885-22.916 ppm Pb (root),

Table 3: Heavy metals concentrations in plant material

Culture soil	Heavy metals (ppm)	Plant material	Month 1	Month 2	Month 3	Month 4
Monocontaminated soils	Pb	Shoot	0.394	1.072	3.924	8.262
		Root	3.885	8.025	14.487	22.916
	Cd	Shoot	0.003	0.008	0.013	0.052
		Root	0.021	0.097	0.124	0.185
	Ni	Shoot	2.385	10.480	11.751	12.502
		Root	0.809	1.470	1.804	2.604
	Cu	Shoot	8.845	12.981	nd	nd
		Root	24.482	28.184	nd	nd
	Zn	Shoot	31.331	nd	nd	nd
		Root	89.150	nd	nd	nd
Polycontaminated soil	Pb	Shoot	0.089	nd	nd	nd
		Root	5.392	nd	nd	nd
	Cd	Shoot	0.007	nd	nd	nd
		Root	0.037	nd	nd	nd
	Ni	Shoot	0.730	nd	nd	nd
		Root	0.327	nd	nd	nd
	Cu	Shoot	6.272	nd	nd	nd
		Root	15.044	nd	nd	nd
	Zn	Shoot	27.261	nd	nd	nd
		Root	78.600	nd	nd	nd

nd: Not determined

Table 4: Bioaccumulation Factor (BF) and Transfer Factor (TF) of heavy metals

		Monocontaminated soil (month)				Polycontaminated soil (month)			
Factors	Heavy metals	1	2	3	4	1	2	3	4
BF	Ni	0.067	0.191	0.267	0.378	0.023	nd	nd	nd
	Pb	0.045	0.209	0.279	0.411	0.060	nd	nd	nd
	Cd	0.012	0.055	0.076	0.133	0.023	nd	nd	nd
	Cu	0.370[a]	0.474	nd	nd	0.236	nd	nd	nd
	Zn	0.368	nd	nd	nd	0.387	nd	nd	nd
TF	Ni	2.946	5.168	5.266	4.800	2.232	nd	nd	nd
	Pb	0.101	0.253	0.297	0.360	0.016	nd	nd	nd
	Cd	0.150	0.082	0.105	0.270	0.190	nd	nd	nd
	Cu	0.361	0.461	nd	nd	0.417	nd	nd	nd
	Zn	0.27	nd	nd	nd	0.347	nd	nd	nd

nd: Not determined

from 0.003-0.052 ppm Cd (shoot) and 0.021-0.185 ppm Cd (root). Regarding to plants grown on Zn and Cu contaminated soils, the concentrations recorded in the month 1, were 31.331 ppm Zn and 8,845 ppm Cu in the shoot biomass and 89.150 ppm Zn and 24,482 ppm Cu in the root biomass. Heavy metals concentrations in the shoot and root biomass on the polycontaminated soil were obtained only in the month 1 as follow: 0.730 ppm Ni, 6.272 ppm Cu, 27.261 ppm Zn, 0.089 pm Pb and 0.007 ppm Cd in the shoot and 0.327 ppm Ni, 15.044 ppm Cu, 78.600 ppm Zn, 5.392 pm Pb and 0.037 ppm Cd in the root.

Table 4 shows the Bioaccumulation Factor (BF) and Transfer Factor (TF) of heavy metals for *P. maximum*. On the monocontaminated soils, BF was higher for Pb (0.045-0.411), Cd (0.012-0.133) and Ni (0.067-0.378). These FB values were not significantly different (Anova test: $p>0.05$). Concerning TF values, they ranged from 0, 101-0.360 (Pb), from 0.150- 0.270 (Cd) and from 2.946-5.266 (Ni). The transfer factor for Ni was the highest and was greater than 1. Moreover, TF for Ni were significantly different of TF for Pb and Cd (ANOVA test: $p<0.05$).

Tissue and cellular localization of heavy metals: According to the bioaccumulation and the transfer factor calculated at the end of the experiment, the different results showed the high level of lead (Pb) and nickel (Ni) in the tissues and cells of *Panicum maximum*. Indeed, Nickel is widely detected in the central cylinder for the root (Fig. 4a). After magnification, Ni was found in the intracellular compartment, whatever the root or the leaf (Fig. 4b and d). While, for the leaf Ni was much in conductive bundles (Fig. 4c).

Unlike Ni, lead (Pb) was mainly present in the endoderm and mesophyll for the root and the leaf, respectively (Fig. 5a and c). The magnification on the root and leaf tissue compartment showed that Pb remained in the cell wall (Fig. 5b and d).

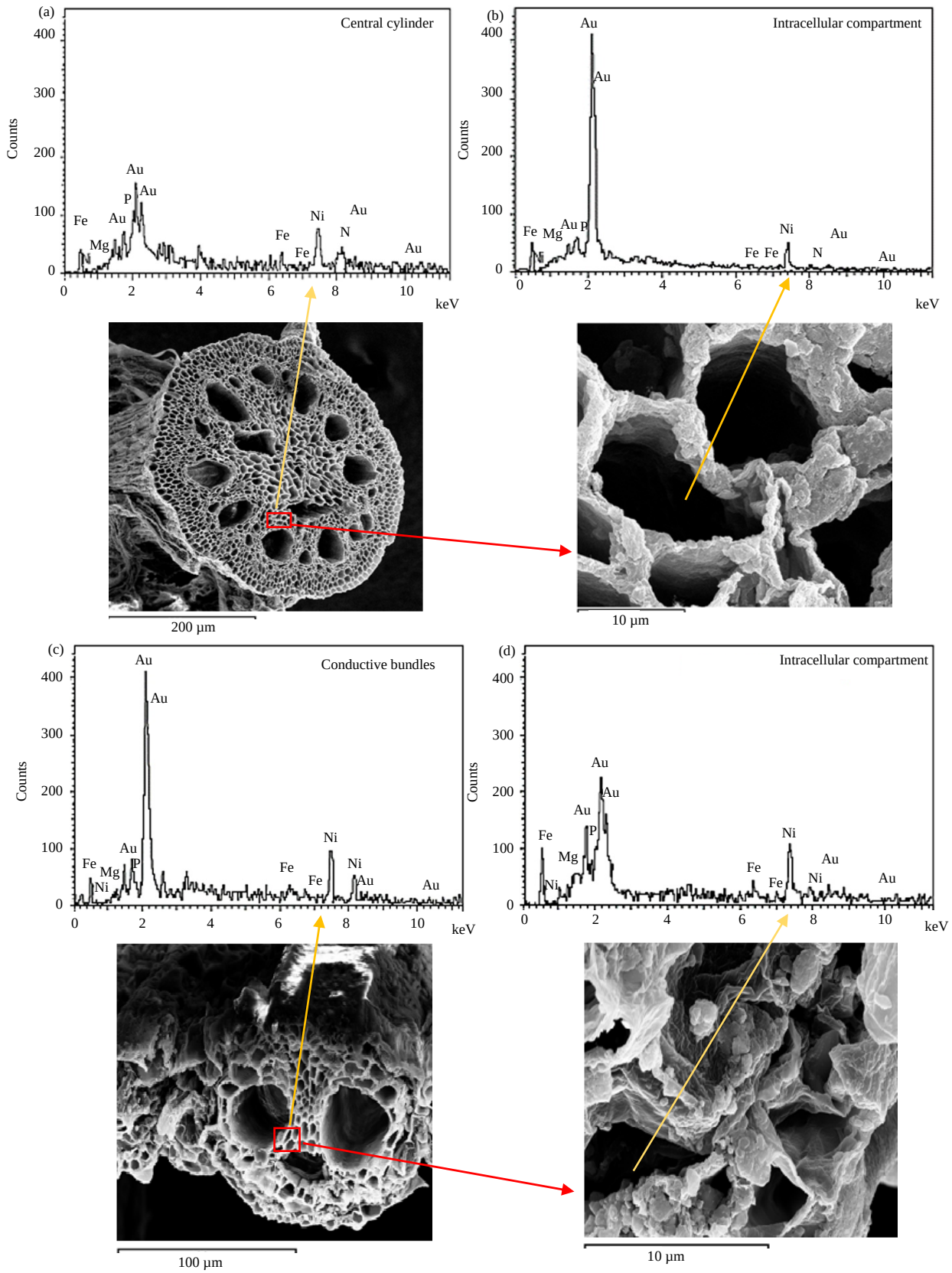

Fig. 4(a-d): SEM/EDS of root (a and b) and leaf (c and d) of *Panicum maximum* accumulated Ni

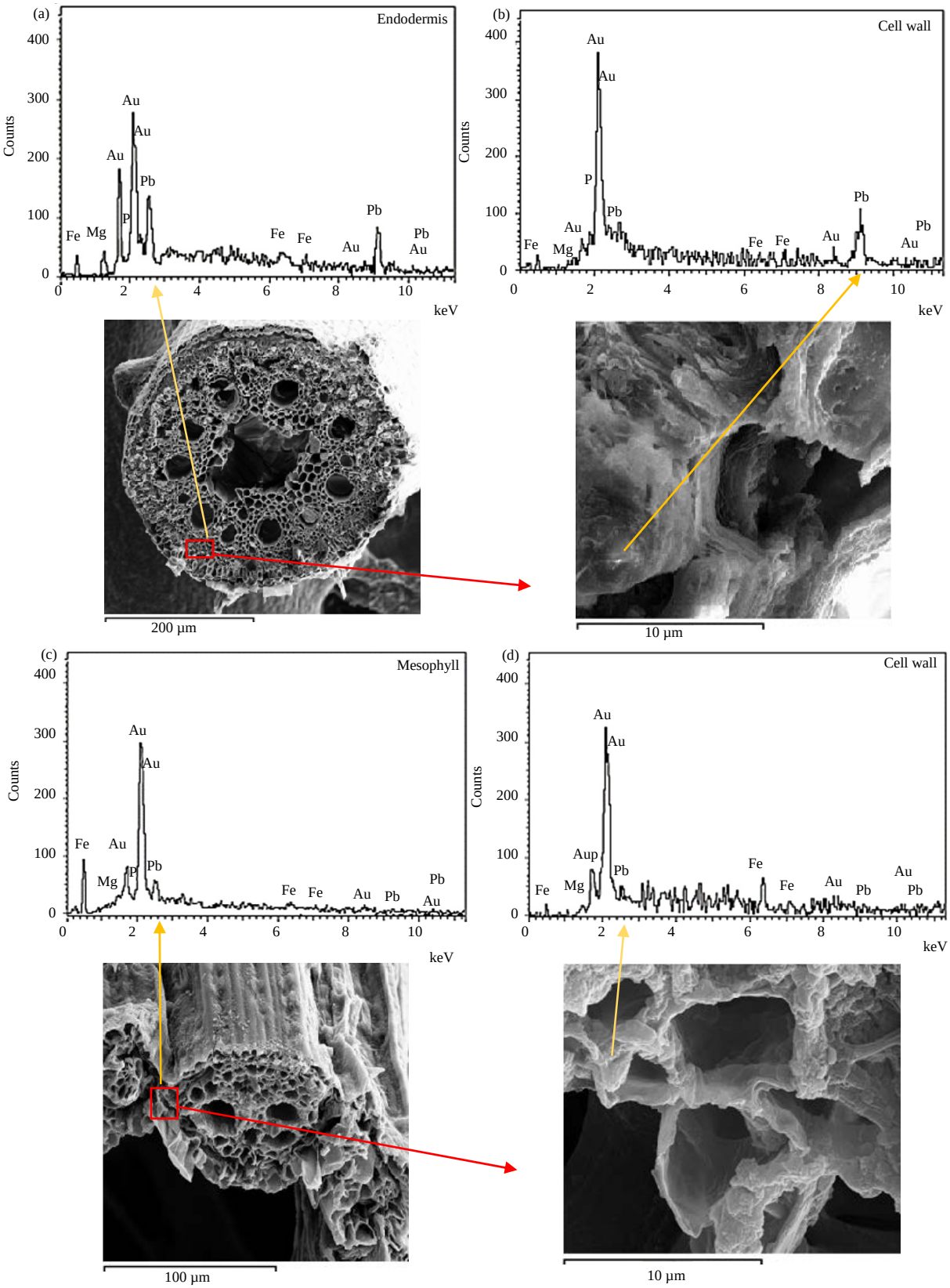

Fig. 5(a-d): SEM/EDS of root (a and b) and leaf (c and d) *Panicum maximum* accumulated Pb

DISCUSSION

The potential and mechanisms of heavy metals (Cd, Zn, Pb, Ni, Cu) accumulation by *Panicum maximum* on synthetic soil in a controlled environment was investigated. Regarding to soil physico-chemical parameters, their values recorded during the experiment decrease with time. Except redox potential, pH and CEC values were lower in heavy metals contaminated soil in comparing with control soil. Soil pH values were found to be acidic (pH<6.0), due to the presence of kaolinite in the culture soil which would induce a weak base sorption capacity. The decrease of CEC might be due to soil acidification increasing with time. Soil acidification promotes a decrease in cationic sorption[31] and cause high heavy metals mobility[32]. As for the range of CEC values observed, it would be related to soil clay consisting mainly of kaolinite (3-15 meq/100 g)[33]. Plant growth monitoring has revealed two trends in plant evolution according to the heavy metals in the soil. In fact, on uncontaminated soil (control) and soils monocontaminated with Ni, Pb and Cd, the plants showed relatively normal growth during the experiment, whereas on soils monocontaminated with Zn and Cu and on polycontaminated soil, the plants died during the test. The plant death observed on Zn and Cu monocontaminated soils and on the polycontaminated soil is explained by the concentration of Cu and Zn in the plant biomass (between 20 and 100 ppm for Cu and between 100 and 400 for Zn) which caused toxicity phenomena for the plant[8,34]. Moreover, even if Zn and Cu were micronutrients that are necessary for plant growth, they could be toxics in high concentrations[8,35-37]. Regarding to the plants which have developed during the four months of the experiment, the results obtained indicated that *P. maximum* regularly grown from 1-64 days, then stabilize phase was observed from day 64 to the end of the experiment. The first phase was probably due to the time required for *P. maximum* vegetative cycle to reach the optimum stage of exploitation which varies between 45 and 93 days[38]. Fresh plant biomass was also increased with the plant growth, due to the increasing of the stems number forming the plants tuft. The comparison of plant biomass produced shows that those of plants grown on non-contaminated soils (control) remain the highest. This difference was due to the presence of heavy metals, which have been reported to have a negative effect on soil functioning and soil biological parameters[39]. For heavy metals contaminated soils, plant biomass was higher on Pb and Cd monocontaminated soil. The concentrations of Pb and Cd obtained in the shoot biomass were lower than the normal concentration in plant biomass which ranges from 5-10 mg kg^{-1} DW for Pb and from 0.05-0.2 mg kg^{-1} DW for Cd[40]. Moreover, the bioaccumulation factors for all the heavy metals were lower than 1. Those values are justified by the experiment condition which was conducted in a controlled environment, i.e., without heavy metals exogenous sources. The analysis of the transfer factor shows that *P. maximum* accumulated Pb and Cd preferentially in the roots (FT<1). Others studies focused on *P. maximum* phytoaccumulation potential obtained similar results[18,22]. Indeed, the plant would behave like an exclusive plant[41] which limit the transfer of heavy metals to the above parts. Comparatively to Pb and Cd, Ni present the highest TF value above 1 (TF>1), which indicated that Ni was more concentrated in the upper part of the plant than in its root biomass was reported[18]. Considering the accumulation mechanisms, it was observed that Pb was accumulated more at the level of the cell walls, either at the roots or the leaves. This situation could be explained by the *P. maximum* reactions to the pollutant. In fact, to survive the pollution, two main strategies are adopted by many plants: the avoidance of metallic stress by fixing Pb on the urinary carboxyl groups of the cell walls and the rapid sequestration in the vacuoles, making it inactive[42-46]. In addition, more than 90% of Pb is found in insoluble form[47] and is strongly bound to cell envelopes. As for Ni, it has been found that it concentrated mainly in the intracellular compartments of the roots. Indeed, this element is a normal constituent of plant tissues that would be transported largely uncomplexed in raw sap[48].

CONCLUSION

The study showed the different potential accumulation of heavy metals by *Panicum maximum*. Many stress effects have been observed on Zn and Cu monocontamined soils as well as on polycontaminated soil (Cu, Zn, Cd, Pb and Ni). The results of the bioaccumulation and transfer factors showed that *P. maximum* accumulated more Ni and Pb than Cd. However, Cd and Pb are mainly retained in the roots while Ni is exported to the above parts. Observations and microanalytic spectra (SEM/EDS) indicated that Pb remains essentially fixed to the cell walls in the organs of the roots and leaves, in comparison to Ni which was accumulated preferentially in intracellular compartment whatever organ considered.

SIGNIFICANCE STATEMENT

This study which has been characterized in condition controls the phytoaccumulation potential of *Panicum maximun* and also the localization of trace metals in the tissues and cells of the plant. It constitutes a crucial stage in the development of phytotechnology for national researchers.

ACKNOWLEDGMENT

We sincerely thank the members of the research team in Biotechnology and Environmental Engineering of Nangui Abrogoua University (Abidjan, Côte d'Ivoire) for their help during the field sampling, their critical examinations and their useful suggestions, all of which have greatly improved this manuscript.

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