Comparative assessment of the state of growth and bioaccumulation of manganese in Poaceae growing on the Mbembele manganese mine in Gabon

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Abstract. The study conducted at the Mbembele manganese mine in Ndjolé, central Gabon, aimed to evaluate the manganese (Mn) levels in soil from the tailings dam and assess the phytoremediation potential of selected Poaceae species. Four species, namely *Phacelurus gabonensis*, *Cenchrus setosus*, *Panicum* sp, and an undetermined gramineae species, were chosen for their rapid growth, high biomass production, and adaptation to the mine's soil conditions. Soil analysis revealed elevated Mn concentrations, surpassing the average composition of the upper continental crust (UCC), indicating significant Mn contamination. The Poaceae species exhibited substantial biomass growth after six months, suggesting their adaptation to high Mn levels in the soil. Mn accumulation was generally higher in the roots than in the leaves, except for *Phacelurus gabonensis*, which showed higher Mn concentrations in the leaves. Interestingly, Mn concentrations in plant tissues were higher at three months than at six months, implying a limited biological uptake capacity over time for these species. While the bioconcentration factor (BCF) was less than 1 for all plants, only *Phacelurus gabonensis* demonstrated a translocation factor (TF) greater than 1, indicating its potential for moving Mn from roots to shoots. The findings suggest that, among the studied Poaceae species, *Phacelurus gabonensis* could be a promising candidate for phytoremediation of Mn-contaminated soils in Gabon. Renewing this species every three months may enhance its effectiveness in rehabilitating mining soils impacted by Mn contamination. The study provides valuable insights into the potential use of indigenous plant species for sustainable environmental remediation efforts in the region.

Key words: Phytoremediation, Poaceae, *Phacelurus gabonensis*, manganese, translocation, bioconcentration

1. Introduction

The bioavailability of trace metals (TMEs) in soils has increased sharply in recent years with the expansion of industrial, agricultural, mining and petroleum activities. This is a serious problem that requires a great deal of global attention if soil contamination and the risks to human health and the environment are to be considerably limited [1]. In Gabon, the intensive development of mines exploiting Mn oxides contributes significantly to the destruction of arable layers and above all to the mobility of Mn in residual soils and groundwater. Since the 1960s, large deposits of Mn have been located in the Bangombé and Okouma plateaux in Moanda in the Franceville sub-basin in Gabon [2,3]. Other manganese-bearing plateaux have been discovered in several Gabonese localities, namely: Franceville, Okondja and Ndjolé, all of which are currently exploited by various companies, the main ones being Compagnie Minière de l'Ogooué (COMILOG) and Nouvelle Gabon Mining (NGM). This phenomenon favours soil contamination by Mn and endangers human health through the bioaccumulation process due to the soil-plant transfer of TMEs in the trophic chain [4]. Locally, high concentrations of Mn have been detected in the soil and leaves of cassava (*Manihot esculenta Crantz*) grown in vegetable gardens near the Moanda mine [5]. However, manganese bioaccumulation in humans is likely to lead to cryptogamic disorders and other health problems due to regular consumption of these contaminated foods [6]. Under these conditions, soil remediation seems necessary to protect human health.

There are several conventional physical, thermal, chemical and biological methods for rehabilitating contaminated soils, including moving contaminated materials to another location [7]. These conventional methods are generally very costly and destructive of edaphic parameters and the environment [8]. Phytoremediation is the only method that is both less costly and non-destructive of environmental ecosystems [9,10]. It uses plants to decontaminate environmental compartments (soil, water and air) [11]. Phytoremediation is a technology based on the transfer of pollutants from the soil to plant tissue without damaging soil structure and fertility [12].

The uptake of metal by plants depends on their concentration in the soil, the nature of the soil and

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the physiology of the plant [13,14]. One of the advantages of phytoremediation is that it provides an environment conducive to the proliferation of microbes in the rhizosphere, capable of degrading synthetic chemicals [15]. This is why, in West Africa, vegetated buffer zones are being set up to reduce soil and water pollution by metals [16, 17].

Plants commonly considered for phytoremediation are characterized by their ability to exhibit rapid growth, produce high biomass, and demonstrate tolerance to challenging environmental conditions, including fluctuations in temperature, soil acidity, and resistance to elevated concentrations of both organic and inorganic micropollutants [18,19].Several studies have shown that certain grasses are able to accumulate and tolerate moderate to high concentrations of heavy metals in the vegetative parts of the plants [20,21]. In addition, grasses are considered good remediation agents due to their dense rooting and wide species diversity [22]. In the study area, grasses of the Poaceae family grow naturally on the Mn-rich soils of the tailings dam. It is possible that these species are tolerant of high TME concentrations and have a good Mn uptake capacity, which could make them hyperaccumulative plants if they can accumulate more than 10,000 ppm Mn (1%) in their leaves [23]. The aim of this study was therefore to investigate the biomass production of four Poaceae species grown on the soil of the mine tailings dam, as well as their ability to extract Mn from the soil and accumulate it in their aerial and root parts. Mn concentrations in soils and plants were analyzed inductively coupled plasma atomic emission spectroscopy (ICP-AES) to determine performance indices such as the bioconcentration factor (BCF) and translocation factor (TF). These indices make it possible to compare the phytoaccumulation of Mn in the species studied and to determine the most effective Poaceae for the rehabilitation of Mn-contaminated soils in the vicinity of the Mbembele/Ndjolé mine.

2. Materials and methods

2.1. Sampling and characterisation of the soils studied

Soil samples were taken from the surface (0-20 cm and 20-40 cm) of the tailings dam at the Mbembele mine in Gabon. Due to the technogenic nature of the soil sampled in the mine's tailings dam, two sampling zones were selected. These were zone A, regularly irrigated by leachate from the mine washing plant (wet) and zone B (dry) (Fig.1).

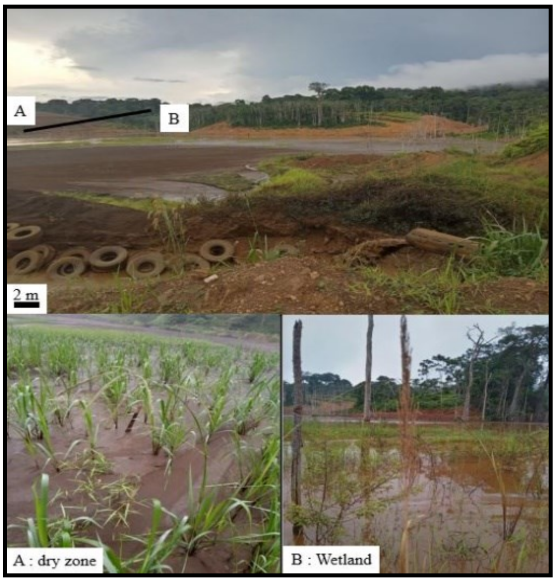


Fig. 1. Mbembele mine tailings dam soil sampling area: Dry zone (A) and wetland (B)

Each sample point was taken at two levels: from surface to depth (0 - 20 cm and 20 - 40 cm). The various samples were oven-dried at 105°C for 48 hours, then pulverized to obtain powders (< 75 µm). The following analyses were carried out using the following methods and standards: pH (NF ISO 10390) and manganese (Mn) content were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) Mn quantification limit 10mg/kg dry matter.

To dissolve the Mn, 250 mg of powder from each soil sample was acid digested with a mixture of 39.5% hydrofluoric acid, 37% hydrochloric acid and 50% nitric acid in Teflon beakers heated to 250°C on a hot plate. The cooled contents were transferred to 1000 ml flasks and diluted with distilled water to the limit for ICP-AES analysis.

The major element content of the soil samples was obtained by melting 1g of powdered soil sample in lithium tetraborate containing 15% lanthanum oxide. Each molten glass sample was poured into platinum cups. At the end of preparation, each bead was analyzed by X-ray fluorescence.

2.2 Plant species studied and experimental protocol

A total of four (4) Poaceaes specimens were used (Fig.1): three (3) specimens taken directly from the soil of the tailings dam, namely: *Panicum* sp, *Cenchrus setosus*, an undefined Poaceae noted nd, and *Phacelurus gabonensis*, which was supplied by the Raponda Walker Arboretum and first described in 2016 by Olivier Lachenaud (Fig.2).

Fig. 2. First description of *Phacelurus gabonensis* provided by the National Herbarium of Gabon.

A descriptive study of the four specimens in Gabon's National Herbarium enabled us to identify each plant
In situ experimentation was carried out under natural site conditions, with no external input to the soil of the tailings dam at the Mbembele manganese mine. A total of twenty (20) plants, distributed as follows: seven (7) plants of *Panicum sp* seven (7) plants of *Cenchrus setosus* and six (6) plants of *Poaceae* nd were marked from young shoots for complete monitoring over six months.

Ten (10) *Phacelurus gabonensis* plants, trimmed to 40 cm (leaf) and 8 cm (root), were planted directly on the soil of the mine tailings dam.

The four plant specimens (Fig.3): *Panicum sp*, *Cenchrus setosus*, *Poaceae* nd and *Phacelurus gabonensis* were grown on the soil of the mine tailings dam and monitored from August 2021 to March 2022. Irrigation was carried out in accordance with the natural climatic conditions of this region of Gabon, characterized by a nine (9) month rainy season and a dry season from March to May. Average rainfall in the region (central Gabon) varies from 1400 mm to 2000 mm [24].

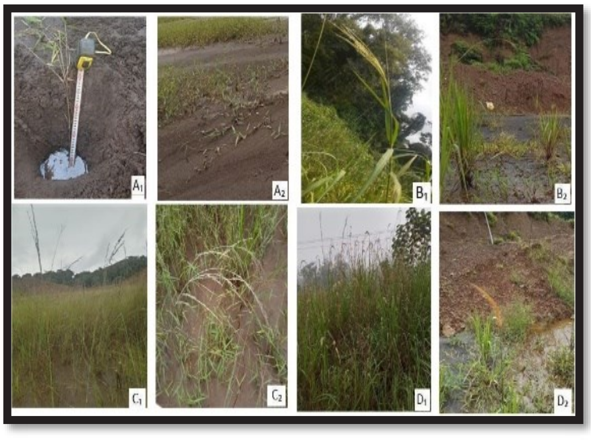


Fig. 3. A: images of some Poaceae specimens found at the Mbembele manganese mine site. A: Undefined Poaceae (1 close-up, 2 distant views); B: *Phacelurus gabonensis* (1 close-up, 2 distant views); C: *Panicum sp* (1 distant view, 2 distant views); D: *Cenchrus setosus* (1 distant view, 2 distant views).

2.3 Plant harvesting and Mn analysis

After three (3) and six (6) months of growth, each harvested plant specimen was washed with tap water and rinsed with distilled water. The plants were then divided into two parts (aerial part, root part) and dried in an oven at 60°C for 72h. The dry biomass of each part was weighed and the different dried parts were ground to powders using a mill.

For Mn extraction, 250 mg of powder from each plant sample was acid digested with a mixture of 39.5% hydrofluoric acid, 37% hydrochloric acid, 50% perchloric acid and 50% nitric acid in Teflon beakers heated to 250°C on a hot plate. The cooled contents were transferred to 100 ml flasks and diluted with distilled water to the limit for ICP-AES analysis (limit of quantification 10 mg/kg dry matter).

Samples with a high manganese content are adjusted by multiplying each volume analysed by the dilution factor (Df) :

Df= Volume of solution / Test sample (1)

2.4. Data analysis

The ability of Poaceae to absorb and accumulate Mn and transfer it to aerial parts is assessed in this study by two parameters: the bioconcentration factor (BCF) and the translocation factor (TF). When these two indices are >1, they indicate that the plant is potentially capable of being used for phytoextraction [25]. They are determined using the following formulas:

$$BCF = \frac{Concentration\ of\ contaminant\ in\ roots}{Concentration\ of\ contaminant\ in\ soil} \quad (2)$$

$$TF = \frac{Concentration\ of\ contaminant\ in\ leaves}{Concentration\ of\ contaminant\ in\ roots} \quad (3)$$

2.5. Statistical analysis

Tukey's multiple comparison test was used to assess the significant difference in Mn uptake in aerial and root parts of specimens at T3 and T6 after observing the Shapirow-Wilk normality test ($p>0.05$) at a 95% confidence interval.

3. Results and discussion

3.1. Physico-chemical properties of soil samples

Due to the technogenic nature of the soil in the Mbembele mine tailings dam, the pH of the two selected areas of the tailings dam is acidic (Tab.1). Repeated and increased irrigation with washing water containing heavy metals has considerably affected the soil quality parameters of the wetland, resulting in a pH change towards neutrality. Manganese is highly concentrated in both sample areas of the tailings dam. This observation is consistent with previous works [20,21]: the movement of heavy metals in soils irrigated with metal-laden water is very slow (Tab.1).

Table 1. Characteristic of the tailings dam soil environment and pH variation: zone A (wet), zone B (dry). Mn content in 250mg soil test samples is mean of three replicates. Standard deviation (σ ≤ 2.58).

	Mn (ppm)	Soil environment
Zone A	329600	Discontinuous ground cover ranging from 20% to 80%. The thickness of the cover is thin, more or less 10cm, in the course of formation. The soil is young and poorly differentiated, resulting from residue treatment of Mn.
Zone B	333600	
Soil sample taken 2 km north of the mine tailings dam	0,07	Continuous burnished soil profile covering more than 80%. Thin cover of less than 40cm, strongly influenced by physico-chemical environment processes(high rainfall in the area).

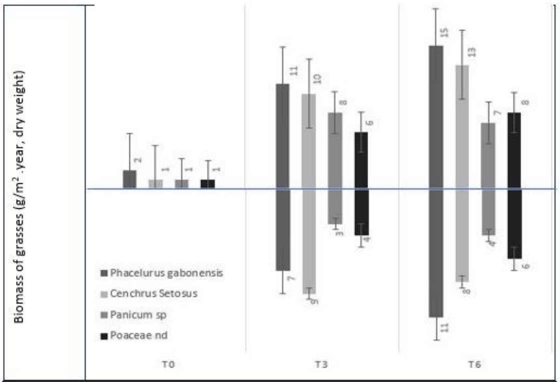
Mine tailings are known to release metals in response to surface weathering and leaching [26]. The percentage of major elements obtained from XRF analysis of the various points sampled on the tailings dam at the Mbembele manganese mine is presented in Table 2. The main chemical constituents of the samples (in decreasing percentage) are manganese dioxide (MnO2), iron (III) oxide (Fe2 O3), aluminium oxide (Al2O3), potassium oxide (K2O), phosphorus oxide (V) (P2O5), titanium dioxide (TiO2), silica (SiO2), calcium oxide (CaO), magnesium oxide (MgO), sodium oxide (Na2O). Concentrations of MnO2, Fe2O3 and Al3O2 are higher, with oxide contents in excess of 10%. When analyzing specimens, only the absorption capacity of manganese is analyzed

Table 2. Mean major element content (n=5) as a function of depth for each soil sample.

Ech	Al_2O_3	CaO	Fe_2O_3	K_2O	MgO	MnO_2	Na_2O	P_2O_5	SiO_2	TiO_2	P-Fire	Total
Zone A	10,19 (%)	0,06(%)	18,39(%)	0,39(%)	0,03(%)	32,67(%)	0,00(%)	0,65(%)	0,15(%)	0,45(%)	19,84(%)	83,41(%)
Zone B	4,65 (%)	0,26(%)	21,10(%)	1,66(%)	0,12(%)	31,99(%)	0,06(%)	0,25(%)	0,12(%)	0,17(%)	20,45(%)	81,69(%)

3.2- Effect of manganese on biomass production

The total biomass of each part of the four specimens (roots and aerial parts) obtained at T3 and T6 is shown in Figure 4. A notable disparity in biomass was observed between *Phacelurus gabonensis*, *Panicum* sp, and *Poaceae* nd. This contrast was evident for all parts of the plant (roots and aerial parts).However, there was no difference in the biomass between *Phacelurus gabonensis* and *Cenchrus setosus*. On the soil of the tailings dam, plants grown and identified at all observation points showed variable growth in height. At timepoint T3, the average heights were recorded as follows: 139 cm for *Phacelurus gabonensis*, 139 cm for *Cenchrus setosus*, 67 cm for *Panicum* sp, and 41 cm for *Poaceae* nd. By timepoint T6, the heights increased to 166 cm for *Phacelurus gabonensis*, 145 cm for *Cenchrus setosus*, 83 cm for *Panicum* sp, and 51 cm for *Poaceae* nd.The *Cenchrus* setosus-Phacelurus gabonensis group grows on different substrates on the tailings dam (dry and wet). The *Panicum* sp - *Poaceae* nd group grows best at the edge of the artificial lake formed by the water from the washing plant. In 2004, the work of Lidon [27] defined the symptoms of toxicity that could be attributed to Mn uptake, namely: brown spots on leaves; chlorosis or necrosis; deformation of young leaves, burning of leaf tips. At T3 and T6, none of these symptoms were observed on any of the four plants. This result is consistent with previous work [28,29]. Some grasses can grow on mining sites without showing symptoms of toxicity.



ANOVA		
	Shoots	
Phacelurus - Cenchrus setosus	ns	ns
Phacelurus - Penicum	ns	*
Phacelurus - Poaceae nd	*	*
Levene Statistics	P>0.05	P>0.05
	Roots	
Phacelurus - Cenchrus setosus	ns	ns
Phacelurus - Penicum	*	*
Phacelurus - Poaceae nd	*	*
Statistique de Levene	P>0.05	P>0.05
time effect	a	b

Fig. 4. Biomass of plants collected from the tailings dam. The standard deviation of the means of the different parts of each plant calculated is between 1<σ< 3.98. The total biomass of Poaceae was analysed by ANOVA. A Tukey test was used to assess significant differences between dry weights at different times with a 95% confidence interval. Significant differences are indicated by (*) p < 0.0 5 ; (**) p < 0.01 ; (***) p < 0.001 and ns : not significant.

The root architecture of *Panicum* sp and *Poaceae* nd is characterized by fine (*Poaceae* nd) and fibrous (*Panicum)* roots, which are shallow and extend horizontally over the soil. Root length varies from 37 cm at three months to 40 cm at six months. On the other hand, *Cenchrus setosus* and *Phacelurus gabonensis* have the advantage of a taproot and small lateral feeder roots. Root growth is often an indicator of the plant's ability to adapt to drought [30]. The *Cenchrus setosus* and *Phacelurus gabonensis* group shows an ability to grow on the various wet and dry substrates of the tailings dam at the Mbembele manganese mine.

3.3 Mn concentration in Poaceae

The manganese concentrations obtained in the roots and aerial parts of the four specimens are presented in Table 3. Statistical analysis reveals a highly significant difference (P<0.001) between *Phacelurus gabonensis, Poaceae* nd, *Panicum* sp. This difference is moderately significant (P<0.05) between *Phacelurus and Cenchrus setosus*. Manganese concentration in aerial parts of *Cenchrus setosus* is 10100 ppm (T3); 71^{2}00 ppm (T6), in *Panicum* sp 6900 ppm (T3); 4100 ppm (T6) in *Poaceae* nd 21100 ppm (T3); 17900 ppm (T6). There is a decrease in the accumulation of Mn concentration in the aerial parts of all four specimens at T6 and all concentrations in the aerial parts are lower than those found in the roots.

Certain authors [31] have demonstrated that, in practical terms, leaves tend to accumulate more manganese than stems and roots. In the context of this study, it was observed that only *Phacelurus gabonensis* exhibited elevated concentrations in the aerial parts, with lower concentrations in the roots.

Plants such as *Cenchrus setosus, Poaceae* nd and *Panicum* sp are highly tolerant of heavy metals [28,32].

Generally speaking, the average quantities of Mn accumulated in the leaves are > 10,000 ppm in *Phacelurus gabonensis* (22,200 ppm), and *Poaceae* nd (21,100 ppm) whether at 3 or 6 months of growth (Table 3). These results indicate that these specimens store high levels of Mn in their aerial parts and can be considered Mn hyperaccumulating plants.

Table 3. Values are ppm means, statistically significant at P≤ 0.05. The mean Mn accumulate of each specimen at different times in the experiment is analyzed by ANOVA. Levene's normality test is performed (p>0.05) to verify the homogeneity of accumulation, followed by Tukey's multiple comparison test for the observation of a significant difference in manganese accumulation in each part of the plants over a 95% confidence interval. Significant differences are indicated by (*) p < 0.05 and (***) p < 0.001.

		Aerial part of the plant				Root part of the plant			
		T3		T6		T3		T6	
	number of analyses		Average (ppm)		Average (ppm)		Average (ppm)		Average (ppm)
Phacelurus gabonensis	6	[1100 - 48000]	**22200**	[900 - 33300]	**11000**	[10100 - 26500]	**20100**	[9800 - 20300]	**10100**
Cenchrus Setosus	7	[2200 - 19100]	**10100**	[1000 - 17400]	**7100**	[8900 - 40400]	**20300**	[6600 - 26400]	**12200**
Panicum	5	[2500 - 8800]	**6900**	[800 - 6800]	**4100**	[3400 - 9800]	**7100**	[1200 - 10200]	**7700**
Poaceae nd	5	[1600 - 43600]	**21100**	[1100 - 38700]	**17900**	[13300 - 60800]	**28200**	[7800 - 32100]	**21700**
Anova									
Phacelurus - Cenchrus			*		*		*		*
Phacelurus - Panicum			***		***		***		***
Phacelurus - Poaceae nd			***		***		***		***
Time effect			a		a		a		a
Levene statistics			P>0,05		P>0,05		P>0,05		P>0,05

N.B.: The average is calculated as follows:
(Df) = Volume of solution / Test sample (1)
(Df= 200; 400; 1000) for samples with a high content: analyses were carried out in duplicate
Average = (sum of concentrations according to dilution factor) /number of total factors

$$M: \sum_{Fd=200}^{1000} \left(\frac{Concentration \frac{Df(200)}{Nb} + Concentration \frac{Df(400)}{Nb} + Concentration \frac{Df(1000)}{Nb}}{Number\ of\ total\ factors} \right) \quad (4)$$

Nb = Number of analyses
Df= Dilution factor

3.4. Translocation factor and Bioconcentration factor

Plant response to Mn in soil depends on plant specimens, concentration and bioavailability of Mn in relation to soil physico-chemical properties. To comprehensively evaluate the capacity of the four plant specimens to accumulate manganese (Mn) from the soil of the mine tailings dam in their tissues (through uptake and translocation), the study examined the bioconcentration factor (BCF) and translocation factor (TF), as presented in Table 4. A BCF value of ≤ 1 suggests that the plant can absorb the metal but does not accumulate it in its tissues. On the other hand, tissue accumulation is indicated when the BCF is > 1. The four specimens taken at three and six months from the soil of the tailings dam at the Mbembele mine contain a BCF <1. The percentage uptake of the mineral and the BCF depend on the physico-chemical conditions of the medium and the initial concentration of the metal. In general, the higher the initial concentration, the lower the BCF [33]. The translocation factor values vary among the different specimens, with specific values such as 0,5 for *Cenchrus setosus*, 0,7 for *Poaceae* nd, approximately 1 for *Panicum* sp, and 1,11 for *Phacelurus gabonensis* at three months. At six months, the translocation factor values are 0.5 for *Panicum* sp, 0,6 for *Cenchrus setosus*, 0,7 for Panicum sp, 0,8 for Poaceae nd, and 1,09 for *Phacelurus gabonensis*. Plants with high heavy metal transfer have a good capacity to repair pollution in mining areas [34]. According to the data, *Phacelurus gabonensis* transfers Mn from roots to the aerial parts at a rate greater than 1 (FT>1) after 3 and 6 months of growth. This indicates a phytoaccumulative character of *Phacelurus gabonensis* and suggests that this plant will be potentially effective for phytoremediation of Mn in soils. However, for the other three specimens, a translocation factor < 1, with a bioconcentration factor < 1 indicate that these specimens are not effective for phytoremediation methods.

Table 4. Manganese translocation factor from roots to leaves (FT=average concentration in leaves/average concentration in roots)

	Mn			
	T3		T6	
	FBC	TF	FBC	TF
Phacelurus gabonensis	0,1	**1,11**	0,1	**1,09**
Cenchrus setosus	0,1	**0,5**	0,1	**0,6**
Panicum sp	0,01	**~1**	0,01	**0,5**
Poaceae nd	0,1	**0,7**	0,1	**0,8**

4 Conclusion

The growth of four Poaceaes specimens, *Phacelurus gabonensis*, *Cenchrus setosus*, *Panicum* sp and *Poaceae* nd and their ability to absorb and accumulate Mn were evaluated under natural conditions on the soil of the tailings dam at the Mbembele mine in Gabon. The results show that the plants adapted well, particularly *Phacelurus gabonensis* and *Cenchrus setosus*, whose biomass increased significantly over the 6-month experimental period. Furthermore, on the basis of the high Mn concentrations in the aerial parts, these specimens can be classified in the following order: *Phacelurus gabonensis* > *Paceae nd* > *Cenchrus setosus* > *Panicum* sp. However, only the first two specimens regularly concentrate more than 10,000 ppm Mn in their aerial parts, which would indicate that they can be classified as potential candidates for phytoremediation of surrounding Mn-rich soils. However, the low values of BCF < 1 for all plants show that their ability to absorb Mn from mining soils is limited. Based on values TF >1 for *Phacelurus gabonensis*, this study shows that this specimen can be considered a potential candidate for Mn phytoremediation. However, its efficacy would need to be demonstrated on a controlled area of contaminated soil and over a very short experimental period.

Autors' contributions

Rudy BEKOUNG ASSOUMOU: Methodology, Field study, Formal analysis, Writing - original version, Software and revision. Norbert ONDO ZUE ABAGA: Field study, Drafting - revision. Mohamed TAYEBI: Conceptualisation, Validation, Supervision, Writing - revision and editing.

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