E3S Web of Conferences **412**, 01089 (2023) https://doi.org/10.1051/e3sconf/202341201089
ICIES'11 2023

Remediation of soil contaminated with Mn, Cu and Zn around the Mbembele mine in Gabon: Phytoremediation approach (*Vetiveria zizanioides* and *Phacelurus gabonensis)*

Rudy.BEKOUNG ASSOUMOU[1], *Norbert.ONDO ZUE ABAGA*[2], *Mohamed.TAYEBI*[1]

[1]Geosciences Laboratory, Department of Geology, Faculty of Science**,** Ibn Tofail University, Kenitra, Morocco.
[2]Laboratoire des Sols et Altérations de Surface, Unité de Recherche en Sciences de la Terre et de l'Environnement (URESTE), Université des Sciences et Techniques de Masuku (USTM), Franceville, Gabon.

Corresponding author: Email: rudy.bekoungassoumou@uit.ac.ma

Abstract. The ability of Vetiveria zizanioides and *Phacelurus gabonensis* to accumulate copper (Cu), zinc (Zn) and manganese (Mn) in the aerial parts and to mobilise them in the roots is applied. This investigation is based on the in situ observation of these plants, their biomass and the evaluation of the translocation capacity of each metal from roots to aerial parts.

A proliferation of both plants in culture was observed for three months, then harvested and analysed. Significant metal contents, on 250 mg of samples taken, are observed in the aerial parts of *Phacelurus gabonensis* (2028 ppm of Cu, 9 ppm of Zn and 7456 ppm of Mn) whereas (3854 ppm Cu, 268 ppm Zn 43005 ppm of Mn) in the roots. *Vetiveria zizanioides* records in the aerial parts 83 ppm Cu, 31 ppm Zn and 8761 ppm, Mn while in the roots there are 83 pm Cu, 1974 ppm Zn and 11930 ppm Mn. The ability to transfer these metals from the roots to the aerial parts is greater in *Vetiveria zizanioides* than in *Phacelurus gabonensis* despite the high metal concentrations in the aerial parts in *Phacelurus gabonensis.*The high concentrations of metals purified by *Phacelurus gabonensis and Vetiveria zizanioides* place them among the top ranks of phytoremediation.

Key words: Copper, Manganese, *Phacelurus gabonensis*, phytoremediation, soil, translocation factor, *Vetiveria zizanioides* , Zinc,

1 - Introduction

In recent decades, the contamination of terrestrial ecosystems by heavy metals has accelerated [1].The presence of metals such as Manganese (Mn), Copper (Cu) and Zinc (Zn) in the environment as well as their persistence, mobility and bioaccumulation are of great concern [2]. This accumulation (pollution) leads to serious risks for public health via the food chain and the degradation of biodiversity [3].

Gabon is the world's second largest producer of high-grade manganese (Mn) [4], after South Africa and before Brazil [5]. This mineral is gradually becoming one of the riches of the Gabonese economy after oil and timber exploitation [6,7,]. Manganese has antagonistic roles for plants where it acts as a micronutrient at low concentrations, while it becomes toxic when its concentration rises in the soil [8,9]. This is particularly the case in Hawaii where its phytotoxicity has been reported on oxisols at pH <6 [10].

In Gabon, similar situations have been reported by Marchal and Fouré (1983) [11] and more recently by Eba (2007) [12]. All these potential risks point to the need for remediation of contaminated soils, in order to protect human health and the environment. In addition to Mn, Zn and Cu are also being analysed for their implications in the photosynthetic response and their integration in cellular metabolism [13].

The rehabilitation of mining soils, including in Gabon, requires several physical, chemical and biological methods to decontaminate soils polluted by heavy metals [14,15,16]. Many of these methods are expensive, difficult to implement and, above all, destructive to ecosystems. Phytoremediation remains one of the ecological and cost-effective techniques using plants that tolerate abiotic stress and remove elemental pollutants from the soil [17,18]. Metal uptake and accumulation depends on the metal concentration, the nature of the soil but also the physiology of the plant [19].

The Mbembele mining area in Gabon has soils heavily contaminated with Cu, Zn and Mn. To rehabilitate these soils, two species were chosen in thi study:*Phacelurus gabonensis* and *Vetiveria zizanioides*. The objective of this study is to evaluate the capacity of these two plants to absorb and accumulate Cu, Zn and Mn in their root and aerial parts. While *Vetiveria* is known for its resistance to environmental stress and for its use in phytoremediation of polluted soils [20,21,22] *Phacelurus gabonensis* is newly included to assess its effectiveness for phytoremediation given its ability to grow on soils contaminated by heavy metals (Cu, Zn and Mn).

2 - Materials and Methods

2.1 - Soil sampling and characterisation

Fourteen young plants of *Phacelurus gabonensis* supplied by the Raponda Walker Arboretum in Gabon on 10 September 2021 and fourteen young plants of *Vetiveria zizanioides* supplied by the Laboratoire de Géosciences Faculté des Sciences Kenitra Maroc on 9 September 2021, are being monitored in situ. Each plant is pruned before its cultivation on 30 cm for the aerial part (leaves) and 10cm for the underground part (roots). Two zones are carefully selected on the dyke to receive the crops. These are the wet Zone A or water reception area of the dam (N0°03'73.6188" E10°46'28.17012") and a dry Zone B (N0°04776012 E10°4625734). Seven plants of *Phacelurus gabonensis* and seven plants of *Vetiveria zizanioides* were grown in Zone A and Zone B respectively, with a distance of 1m between the plants in succession. The soil pH and heavy metal content (Cu, Zn and Mn) of the soils from both zones are given in Table 1.

The collected soils are oven-dried at 105°C for 48 hours and then pulverised (< 75 µm). Due to the technogenic nature of the soil, the pH of the two planting areas gradually evolves towards neutrality. The water from the mine'swashing plant causes a decrease in pH when watered in zone A (wet). The Mn, Cu and Zn contents are of the same order of magnitude in both zones.

Table 1: Chemical parameters of the soil samples from the two study areas A and B. Values are means (n=3), statistically significant at P ≤0.05 levels

	Ph	Mn (ppm)	Cu (ppm)	Zn (ppm)
Zone A (wet)	5,79	329 600	250	219
Zone B (dry)	6,23	333 600	228	204
Witness	7,6	700	28	88

2.2 - Monitoring plant growth and harvesting

The growth of the plants (Fig.1) is observed over an interval of zero to six months. During the first month, the plants are monitored daily to assess the adaptation conditions of the plants. After three months (T3) and six months (T6) of growth, the stem length, leaf length and root length of the plants are measured. The time zero months (T0) corresponds to the state of the young plants before their growth in zones A and B.

After six months of cultivation under local conditions, the whole plants in each zone are uprooted, carefully washed with running tap water to remove soil and dust, and then rinsed with distilled water. Each plant is separated into aerial and root parts. The samples were dried in an oven at 60°C for 72 hours. The dry biomass is weighed and ground into a fine powder.

Figure 1: Images of plants used for the phytoremediation trial at the Mbembele manganese mine site. A:*Vetiveria zizanioides* (1 university reserve, 2 site view); B: *Phacelurus gabonensis* (1 site view, 2 national herbarium identification sheet)

2.3 - Soil and plant digestion

Soil and plant samples and blanks of the products used are prepared for elemental analysis using inductively coupled plasma-atomic emission spectrometry (ICP-AES)
For the solution of the analytical elements, each soil sample is accurately weighed to 250 mg in the digestion vessels. The preparation is carried out using a three acid digestion of the samples. 5 ml of 39.5% hydrofluoric acid and 5 ml of 37% hydrochloric acid are used as the first digestion. The digestion system is carried out on a hot plate at 250°C for 40 minutes. The second digestion is carried out with 5 ml of nitric acid and 5 ml of hydrochloric acid heated again for 40 minutes. After complete evaporation of the diacid mixture, a third attack is made with 50% hydrochloric acid heated for a few minutes. A blank solution comprising only the acids from each attack is also digested and diluted. The cooled contents are transferred to the 100ml flasks and dilutedwithdistilled water to the mark for ICP-AES analysis.

The analytical data is adjusted by triplicating each volume analysed by the dilution factor (Fd):

Fd= Volume of solution / Test sample (1)
(Fd= 200; 400; 1000) for high grade samples.

The digestion of roots and leaves is carried out in a three-step method to completely digest the plant material. The samples are digested in 5 ml of 39.5% hydrofluoric acid, 5 ml of 37% hydrochloric acid and 3 ml of perchloric acid to destroy the plant material in the first attack on a hot plate at 250°C for 1 hour. The second attack by a mixture of 10ml of 50% nitric acid and 50% hydrochloric acid heated for 40min. The third attack 50% hydrochloric acid heated for 10 minutes. The cooled contents were transferred to the 100ml flasks and diluted with distilled water to the mark for ICP analysis.
The analytical data is adjusted by tripling each volume analysed by the dilution factor (Fd):

Fd= Volume of solution / Test sample (Fd=200 ;400) (1)

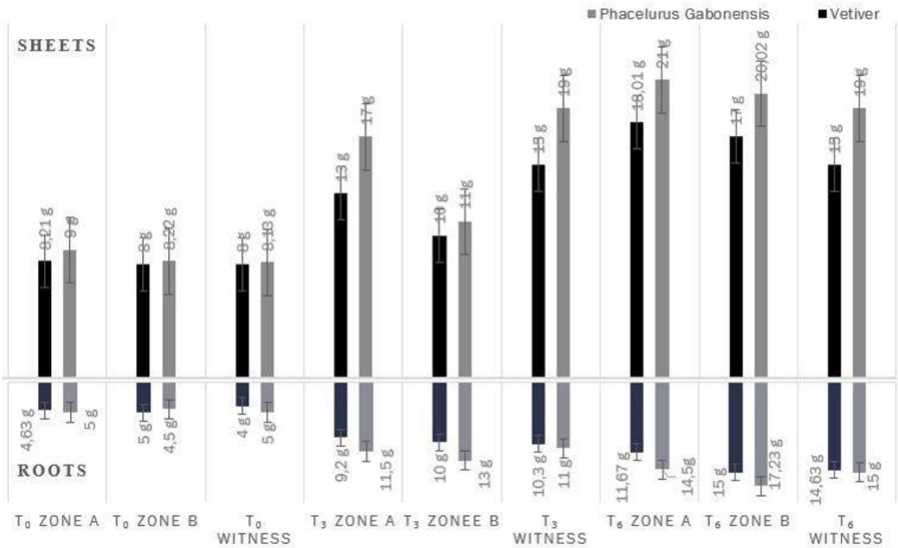
3 -Results

3.1 – Biomass Vetiveria zizanioides *and Phacelurus gabonensis.*

At T3 and T6 months of growth, a total of forty-six plants were studied, i.e. 26 Vetiver plants and 20 Phacelurus *gabonensis* plants were visually monitored for growth. At three months, 14 *Vetiver* plants and 12 Phacelurus gabonensis plants *were* taken from each zone (T3), followed by 10 *Vetiver* plants and 10 *Phacelurus gabonensis* plants taken at six months (T6).

At each sampling point, plant growth varied without any symptoms of toxicity. At T3, the plants reached average heights of 139cm for *Phacelurus gabonensis* and 108cm for Vetiveria *zizanioides*, and at T6 they reached heights of 166cm for *Phacelurus gabonensis* and 145cm for *Vetiveria zizanioides.*
These observations show that *Vetiveria zizanioides* and *Phacelurus gabonensis* adapt well *to* both types of substrate and show variability in the total biomass of each plant.

Figure 2 shows the total biomass of each part of the two plants (root and aerial) obtained at T3 and T6. At T3, no significant difference in total biomass was noted, whatever the substrate (zone A, B and control). However, at 6 months, the biomass of Vetiveriazizanioides *and Phacelurus gabonensis* grown in zone B (dry) was significantly higher than that in the control zone and zone A (wet). This variation affected the biomass of the root and aerial parts. However, the ratio of root biomass to above-ground biomass for each plant was similar, with no difference between zones A, B and the control.



A N OVA Leaf and Root			
vetiver ZA - phacelurus ZA	ns	ns	ns
vetiver ZB - phacelurus ZB	ns	ns	ns
Vetiver ZB - phacelurus ZA	ns	*	*
Time	a	b	b

Figure 2: Total biomass of both plants at zero, three and six months. Values are means (n=3), statistically significant at $p<0.05$. The total biomass of each plant per zone wasanalysed by ANOVA. A Tukey test was used to assess significant differences between plants at the 95% confidence interval.

3.2 - Concentration of Cu, Zn and Mn in biomass

Table 2 summarises the accumulation performance of Cu, Zn and Mn in the two plants as a function of time. The evaluation of the evolution of the uptake rate of each mineral according to the observation of their concentrations in the dry biomass during the experiment is defined at different times. In the aerial parts of *Vetiveria zizanioides* (Table 2), the initial Cu concentration increased from <10 ppm to 10.01 ppm at three months and reached 28.1 ppm at six months in samples of 250 mg. In *Phacelurus gabonensis,* concentrations in the aerial parts are higher. The initial Cu concentration rose from <10ppm to 1848ppm at month three and 830ppm at month six. The observation of concentrations of the same content in the roots is slightly lower than the values of the concentrations in the aerial parts for the same duration. This observation was made in both experimental zones by taking 250 mg of each *Vetiveria zizanioides* biomass. However, for 250 mg of biomass taken for each analysis from *Phacelurus gabonensis*, the highest concentrations were recorded in the roots.

For zinc (Table 2), the highest concentrations in both zones A and B were detected in the roots at different times: the maximum value detected was 2112.6 ppm at three months and 1165ppm at six months in *Phacelurus gabonensis* in zone B, while in *Vetiveriazizanioides it was* 1974 ppm at three months in zone B and 621 ppm in zone A at six months.

Manganese concentrations in the biomass of *Vetiveria zizanioides* and *Phacelurus gabonensis* differed according to genus and sampling zone. Mn concentrations varied from 0.01 ppm to 8761 ppm at three months and 318 ppm at six months for *Vetiveria zizanioides* in zone A. In *Phacelurus gabonensis* in the same zone A, the variation is 0.01 to 16008 ppm at three months and 1401 ppm at six months. Manganese values in above-ground parts in zone A were higher at three months than values in roots in both genera. The same observation was made at six months.

Mn levels in zone B are highly representative in the roots. The maximum concentrations detected were 43005ppm in *Phacelurus gabonensis* and 10920ppm in *Vetiveria zizanioides*. These levels are much higher than those recorded in the aerial parts.

In terms of uptake (Fig. 2), there was no significant difference in the accumulation of Mn, Cu and Zn in the aerial parts, irrespective of the duration of growth and the substrate. However, Fig. 3 shows that the elements Mn, Cu and Zn are more concentrated in the leaves at T3 and decrease at T6.

Table 2:Variability of Cu, Zn, Mn concentration in shoots and roots of two species: *Phacelurus gabonensis* and *Vetiveria zizanoides*, at different times (3 months and 6 months).

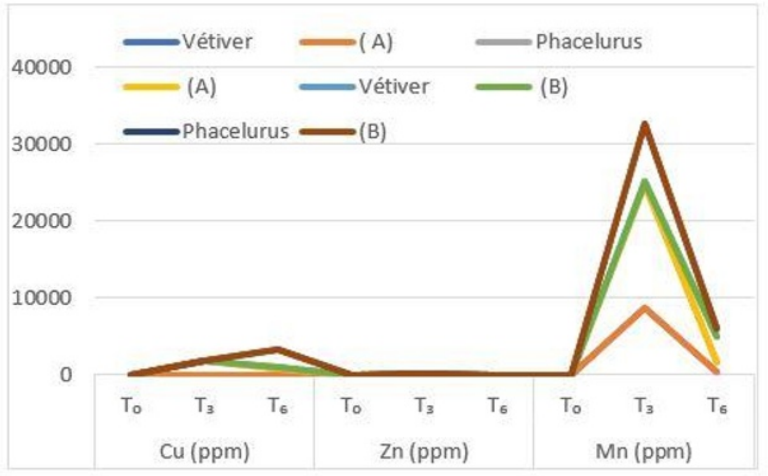
Sheets

Zone sampled	No. of tests per point sampled	Cu (ppm)			Zn (ppm)			Mn (ppm)		
		T_0	T_3	T_6	T_0	T_3	T_6	T_0	T_3	T_6
Vetiver (A)	5	<10	10,0	28,1	13	31	12	0,01	**8761**	318
Phacelurus (A)	7	<10	**1848**	830	20	79	17	0,01	16008	**1401**
Vetiver (B)	5	<10	51,5	83	0,1	19	26	0,01	451	**3328**
Phacelurus (B)	6	<10	20,7	2258	0,1	21	11	0,01	**7456**	976

Roots

Zone sampled	No. of tests per point sampled	Cu (ppm)			Zn (ppm)			Mn (ppm)		
		T_0	T_3	T_6	T_0	T_3	T_6	T_0	T_3	T_6
Vetiver (A)	5	28	8,9	27,1	89	112,1	124	0,01	4033	6823
Phacelurus (A)	7	4,3	2048	2536	21	268	621	0,01	14270	1 370
Vetiver (B)	5	32	24,1	48	21	1974	411	0,01	**11930**	10920
Phacelurus (B)	6	25	18,1	3854	88	2112,6	1165	0,01	**43005**	30100

For zinc, (Table 2), the highest concentrations in both zones A and B, are detected in the roots at different times. The maximum value detectedis2112.6ppm at threemonths and



Anova	
Vetiver - Phacelurus	ns
Time effect	a

Figure 3. concentration of Cu,Zn and Mn in the aerial parts of *Vetiveriazizanioides*and*Phacelurus gabonensis*during six monthsunder absorption conditions.ns: not significant

3.3 - Translocation of Cu, Zn and Mn in aerial parts

The calculation of the Translocation Factor (TF) shows that the variation in the rate of Cu, Zn and Mn transferredfromroots to leaves varies according to the plant, the sampling zone and the element Cu, Zn and Mn (Table 3). Cu in both zones isperfectlytranslocatedfromroots to leaves (FT>1) at different times. For *Phacelurus gabonensis*, the FT varies from 0.9 to lessthan 0.5 in zone A and from 1.1 to 0.6 in zone B. The FT of copperishigher in zone B for *Vetiveriazizanioides*and *Phacelurus gabonensis* with a better translocation capacity in *Vetiveriazizanioides*. For zinc, however, translocation isrelativelylow for both plants in both zones.

Table 3. Evaluation of FT of Cu, Zn, Mn in *Vetiveria zizanioades* and *Phacelurus gabonensis* at three and six months in zones A and B.

	Translocation Factor											
	Vetiveria zizanioides						*Phacelurus gabonensis*					
	Zone A			Zone B			Zone A			Zone B		
	Cu	Zn	Mn	Cu	Zn	Mn	Cu	Zn	Mn	Cu	Zn	Mn
3 months	1.1	<0.5	2.2	2.1	<0.5	<0.5	0.9	<0.5	1.1	1.1	<0.5	<0.5
6 months	1	<0.5	<0.5	1.7	<0.5	<0.5	<0.5	<0.5	1	0.6	<0.5	<0.5

4-Discussion

The high manganese concentrations associated with copper and zinc concentrations in the tailings dam at Mbembele in Gabon do not influence the adaptation or growth of *Vetiveria zizanioides* and *Phacelulurs gabonensis* in the two areas selected. There were no visible symptoms of toxicity, brown spots on the leaves, chlorosis, deformation of young leaves or leaf tip burns attributed to plant stress [23] despite the high levels of Mn (333,600ppm), copper (250ppm) and Zn (219ppm) present in the various 250mg samples of substrate from the Mbembele mine tailings dam analysed. These results are consistent with the work of [24, 25, 26, 22], which confirm the tolerance of *Vetiveria zizanioides* and other grasses to these metals.
At 6 months, the significant difference in the biomass of *Vetiveria zizanioides* and *Phacelurus gabonensis* grown on zone B (dry) is higher than that on the control zone and zone A (wet) and affects the biomasses of the root and above-ground parts. This result is contrary to those of [27] and [20], who observed a decrease in biomass in *Vetiveria zizanioides as a* function of treated metal concentrations. The ratio of root biomass to above-ground biomass was similar, with no difference between zones A, B and the control. There was therefore no visual environmental stress on *Vetiveria zizanioides* and *Phacelurus gabonensis under* the experimental conditions. In fact, plants grown in deficiency conditions increase their sorption of root surfaces: the leaf/root ratio [28, 29].
Metal concentrations in plants growing in the same soil vary according to species and soil conditions [30, 31]. The difference in the accumulation of the elements Cu, Zn and Mn observed in the two species decreases with increasing exposure time. Salas-Moreno's work in 2020 [32] is consistent with this observation, demonstrating that *Vetiveria zizanioides* and *phacelurus gabonensis* can be exposed to high levels of Mn, Cu and Zn. Their tolerance mechanism is not affected by Cu, Zn and Mn levels in this zone.
The Zn levels observed in the aerial parts during the study are largely low compared to the level in the roots. This result appears to be similar to those reported by Sbartai (2011) [33], who noted a high concentration of zinc in the roots of certain plants compared with the aerial parts.
Mn contents were 8761ppm at three months and 318ppm at six months for *Vetiveria zizanioides* in zone A. In *Phacelurus gabonensis,* in the same zone A, Mn contents were 16008ppm at three months and 1401ppm at six months. Manganese values in the aerial parts of zone A were higher at three months than values in the roots of both genera. The same observation was made at six months. The average Mn content in the 250 mg samples of aerial parts analysed in the two plants seemed to fall just short of the critical values (Mn and Zn at 100 mg kg^{-1}) [34, 35] above which toxic effects are possible; whereas for Cu (20-100 mg kg^{-1}) they were much lower.
The rate of translocation of Mn from roots to leaves is higher in zone A than in zone B. This may be associated with the difference in pH, which is relatively neutral in zone B and more acidic in zone A, and which may influence the solubility of Mn in the soil [36].
The work of Kolek (1992) [28] and *Chui (2006)* [29] shows that root surface sorption increases under deficiency conditions. This increase in root surface area would probably have led to these high concentrations in the drier zone B (water deficiency) than in zone A (wet). The highest concentrations of copper and zinc recorded on the dyke came from zone B. In the case of copper, both plants translocated copper from the roots to the leaves. The aerial parts of *Phacelurus gabonensis* concentrated more copper than those of *Vetiveria zizanioides*. However, *Vetiveria zizanioides* translocated copper best from the roots to the aerial parts. In accumulator plants, the metal content of the aerial parts is generally higher than that of the roots. This increases the capacity of plants to absorb, transport and store metals in their aerial parts [37].

https://doi.org/10.1051/e3sconf/202341201089

Zinc concentrations were particularly high in the roots. Both plants grown on the dyke translocated Zn weakly into aerial parts. This result seems compatible with those reported by Sivasankar et al (2012) [38], where they show that heavy metals are stored in the roots mainly in the vacuoles, limiting transport of the metal to the aerial parts. This result is also confirmed by Sbartai (2011) [33] who noted a high concentration of zinc in the roots of tomatoes in contrast to the aerial parts.

5 -Conclusion

The two plants *Vetiveria zizanoides*and *Phacelurus gabonensis*are used to evaluate the possibility of depolluting Mn, Cu and Zn from a site heavilypollutedwithmanganese, in particular from a manganese mine quarry in Gabon. The analysisisbased on the local conditions of the polluted site withoutanyexternal input. The twospecieschosen (*Vetiveria zizanoides*and *Phacelurus gabonensis)* adaptperfectly to high Mn levels and theirgrowthis not significantlyaffected by a multi-contamination in Mn, Cu and Zn. Theyshowed a strongcapacity to absorb Mn, Cu and Zn from the contaminatedsoil and accumulatethem in theiraerial and root tissues. The resultsobtained on the analysis of the twospeciesindicatethatthey can beused for phytoremediation of Mn, Cu and Zn contaminatedsoils. Bothspeciesshow a high accumulation of Mn and Zn.

The previously known phytoremediation capacity of *Vetiveria zizanioides* is well demonstrated in parallel with the newly tested *Phacelurus gabonensis* for the decontamination of the contaminated site of the Mbembele/Gabon mining dam. This plant also shows resistance to high concentrations of Mn whereasitwasconsidered a weed.

Acknowledgements

The authors are grateful to the Raponda Walker Arboretum in Gabon for material support. We also thank the Service d'Analyse de l'Office National des Hydrocarbures et des Mines de Rabat (ONHYM Rabat) for their analytical contributions.

conflicts of interest

none

References

1. E.K. Atibu, N. Devarajan, F. Thevenon, P.M. Mwanamoki , J.B. Tshibanda, P.T. Mpiana, J. Poté**.** Appl Geochem.**39**,26-32(2013).

2. M. Al-Wabel. JAppl Sci.**7**,242-247(2007).

3. K. Peng, C. Luo, L. Lou, X. Li, Z. Shen.Sci Total Environ. **392**, 22-29(2008).

4. Comilog**.**Rapp. Annu. 2017, 68(2018)

5. C. Boupassia. ThèseUniv. Bourgogne, 305(2004).

6. P. D. I. Ndala, and E. Moussone.Foreign direct investmentopportunities in Gab.**2**, no.14 (2011).

7. A. Aterianus-owanga, and M. Debain.Polit. africaine Cairn.info for Ed. Karthala. **144**, 157- 179(2016)

8. L.V. Kochian, O.A. Hoekenga, and M. A. Piñeros.AnnRev of Plant Biology.**55**,459-493(2004).

9. T.Dučić, and A. Polle.Brazil J of Plant Phy.**17**, 103-112 (2005)

10. N.V. Hue, S. Vega, J.A. Silva.SoilSci. Soc. Am. J. **65**,153- 160(2001)

11. J. Marchal, and E. Fouré.Fruits, **38**, 153-160(1983)

12. F. Eba, J.A. Ondo, M.S. Emane, M. Ollui-M'boulou, J. Omva-Zue.J. Soc. Ouest-Afr. Chim. **023**, 69-74(2007)-

13. A. V. Barker, and D. J. Pilbeam.Handbook of Plant Nutrition, nd Ed: CRC Press.(2015)

14. C. Garbisu, and I. Alkorta. The Eur J of Mineral Processing and Env Protection. **3**, No.1, 1303-0868, 58-66(2003)

15. M. Ghosh, and S.P. Singh. Appl Eco and Env Research.**3**, 1-18.(2005)

16. M.S. Rahman, M. Her, J.Y Kim, S.I Kang, K. Lee, M.J. Uddin, A. Chakrabartty, and S.C. Jung.Afr J of MicrobiologyResearch Vol. **6**, no 22, 4775-4781 (2012).

17. E. Azab, and A.K. HegazyPlants.**9**, no:9, 1057.(2020).

18. A. Doghbage, H. Boukerker, F.A. Belhouadjeb, B.Nedjimi. *Env Health Eng and Management :* 10, no:1, 17 – 22 (2023)

19. Y. Chen, Z. Shen, X. Li.Appl.Geochem. **19,**1553-1565 (2004).

20 .N. Ondo Zue Abaga, S. Dousset, C. Munier-Lamy, D. Billet**.** Int J of Phytoremediation **16**, no:1, 95-108(2014a)

21. N. Ondo ZueAbaga, S. Dousset, C. Munier-Lamy. J of Geosc and Env Protection, **9**, 73-88(2021).

22. N. Saidi, and A. Bouabdli. *Phyto trials using vetiver grass* (2020).

23. F.C. Lidon, M. Barreiro, J. Ramalho,. J. Plant Physiol. **161**,1235-1244 (2004)

24. H.M. Chen, C.R. Zheng, C. Tu, Z.G. Shen. Chem **41**,229-234(2000)

25. F. Itanna, and B. Coulman.Com in Soil Sc and Plant Analysis, **34,** no: 1-2, 111-124(2003)

26. M.M.A. Boojar and F. Goodarzi. Eco and envsafety **71** ,692-699(2008)

27. L.T.Danh, P. truong, R. Mammucari, N. Foster.Int.J. Phytoremediation **13**, 47-60. (2010).

28. J. Kolek, V. Kozinka, (Eds.) *Kluwer Acad Publishers, Dordrecht*, 80(1992),

29. K.K. Chiu, Z.H Ye, M.H. Wong.Bioresour.Technol. **9,**158-170. (2006).

30. A. Kabata-Pendias, and H. Pendias,*3rd Edition. CRC press, Boca Raton*, 403.(2001).

31. M. Laghlimi, B. Baghdad, H. El-Hadi, A. Bouabdli, A review. Open J of eco, **5**, 375-388(2015)

32. M. Salas-Moreno and J. Marrugo-Negrete. Int J of Phytoremediation, **22**, no:1, 87-97 (2020)

33. H. Sbartai, M.R. Djebar, R. Rouabhi, I. Sbartai, and H. Berrebbah Am Eurasian J of Toxicological Sc.**3**, no:1, 41-46.(2011)

34. A.J.M. Baker, P.L. Walker*Shaw AJ. Evolutionary Aspects. CRC Press, Boca Raton.* 155-177(1990).

35. A. Kabata-Pendias, and H. Pendias, *2nd Edition. CRC press, Boca Raton, FL,***365** (1992)

36. L.C. Batty, A.J.M.Baker, B.D. Wheeler, C.D. Curtis,Ann. Bot. **86**, 647-653.(2000).

37. R.D. Reeves, and A.J.M. Baker, Wiley, New York, pp. 193-229(2000).

38. R. Sivasankar, R. Kalaikandhan, and P. Vijayarengan. Int J of Research in EnvSc and tech **2**, no:1, 1-9(2012).