IDENTIFICATION OF TROPICAL NATIVE HIGH-ACCUMULATING PLANT SPECIES FOR PHYTOREMEDIATION [1]

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Abstract. Heavy metal contaminants from gold mine sites in southwest Nigeria are posing a high tendency of entry to the food chain. To avert the risk, phytoremediation of the sites is paramount. However, despite recent research advancements in phytoremediation and identification of over 400 hyperaccumulators plant species for Cd, As, Ni, Zn, and Mn, elements such as Pb, Cu, Cr, Hg are largely limited, moreover, less is known of tropical species. This study aims to identify native high-accumulating plants with a high level of heavy metals tolerance growing on three sites an abandoned gold mining site (Site 1); an active gold mining site (Site 2), and an undisturbed vegetation site (Control site) in southwestern Nigeria. Soil properties; pH, textural class, electrical conductivity, available P, percentage Total Nitrogen (TN), Organic Carbon (OC) and Organic Matter (OM), as well as total Pb, Cd, Fe, and Cu concentration, were analyzed at two sampling depths (0-20 and 20-40 cm). The accumulation and enrichment potential of Pb, Cd, Fe, and Cu in *Acanthus montanus, Chromolaena odorata*, *Crinum jagus*, *Melanthera scandens*, *Melochia corchorifolia, Palisota ambigua*, *Pteris togoensis*, *Musa sapientum*, and *Theobroma cacao*, were determined. High clay content, low nutrients, and elevated metal contamination characterized the mine sites soils, with significant differences indicated by PERMANOVA results and confirmed by nDMS analysis. All plants showed elevated metal content and high accumulating potential of Pb with high Fe. None of the plants meet the threshold criteria for hyperaccumulators, but they show high-accumulating potential for phytoremediation. *Crinum jagus* has the highest accumulation factor of 8.71, 37.47, 1.08, and 29.38 for Pb, Cd, Fe, and Cu, respectively. It is a novel recommendable plant species for phytoremediation and can be employed both in the tropical regions and in other climatic adaptable regions around the globe.

Additional Key Words: Hyperaccumulators, Phytoremediation, *Crinum jagus*, tropical regions, gold mining, Artisanal miners.

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Introduction

Although the benefits of mining are known, it induces several impacts on the environment (Ericsson, 1991; Kuter, 2013). Examples of such impacts include contamination and huge loss of viable agricultural lands to mining. Contrary to most developed countries, both the national and international stakeholders involved in mining in several developing countries usually show little or no concern towards appropriate remediation and sustainable management of the mine sites (Festin et al., 2019). However, the increasing demand for agriculture in highly populated countries, like Nigeria, calls for restoration of the agricultural sites that are lost to mining.

According to the Nigeria Mining Journal (2006), Nigeria mines over 34 solid minerals at more than 450 locations. In southwest Nigeria, gold mining dates back to 1942. However, due to the ongoing oil boom phase in the country, national interest in the mining has greatly declined. Recently, local artisanal miners (ASM) have invaded these mine areas (Makinde et al., 2013). Generally, mining and processing of the gold ores enrich the surrounding areas with its associated contaminants such as Pb, Cu, Cd, Fe, Hg, As, Zn, etc. (LaPerriere et al., 1985; Fashola et al., 2016; Wasiu et al., 2016; Petelka et al., 2019). After the gold mining, spoil heaps (mixture of overburden soil and rock) with its associated gold waste contaminants are after some abandoned period used for cultivation by the local farmers. This is because most of the mine sites are around farmlands (Eludoyin et al., 2017). This region is agriculture viable, typical for growing both cash and food crops, such as cocoa and plantain (Adesipo et al., 2020), and it's the main source of living of the local communities. The severe impacts characterizing these ASM activities have been highlighted (Oramah et al., 2015; Adeoye, 2016), but a major concern is connected with the transfer of the heavy metal contaminants (either directly or indirectly) into the trophic chain. Its entry into the food chain and direct ingestion has been recorded in the northern region of Nigeria (Lo et al., 2012; Plumlee et al., 2013; Bartrem et al., 2014), and efforts to avert similar occurrence are therefore necessary.

Previous studies within the study areas highlighted environmental impacts of the mining activities on the vegetation and soil (Salami et al., 2003; Adeoye, 2016), ecology (Oladipo et al., 2014), human health (Olujimi et al., 2015; Awomeso et al., 2017), and its floristic association (Adesipo et al., 2020). Results showed elevated heavy metals such as Pb, Cu, Cd, Fe, Hg, As, and Zn on the mine sites compared to control sites. Some of the contaminant concentrations are higher than the average limits for agricultural soils and are present in the soil and in the surrounding water bodies (Makinde, et al., 2013: Wasiu et al., 2016) as well as in both leaves and fruits of plant tissues growing on the sites (Salami et al., 2003). For Cd, Pb, and Fe, which two authors similarly analyzed in 2003 (Salami et al., 2003) and 2014 (Oladipo et al., 2014) on Itagunmodi, there has been an increase of 54 %, 1278 %, and 116 %, respectively. This reveals that continuous exploitation is further degrading the sites, increasing their acidity, and hence the solubility of the heavy metals. Therefore, immediate attention to alleviate the associated risks is paramount, in addition, enhancing food security and serving as a basis for other contaminated sites, both nationally and internationally.

For practical efforts towards amelioration, phytoremediation has been suggested as the preferable remediation technique. It was hypothesized that understanding the floristic association of the sites is necessary, the characterization and classification of the contaminants based on the chemical profile, age of contamination, and efficient high-accumulating native plants growing on the sites. This is important because despite the increasing knowledge in phytoremediation around the world (Mao et al., 2015), with over 400 experimentally confirmed hyperaccumulator species for elements such as Cd, As, Ni, Zn, and Mn, other elements such as Pb, Cu, Cr, Hg are largely limited (van der Ent et al., 2013). In addition, research on efficient hyperaccumulating species within the tropical region is largely limited. Moreover, the threshold criteria for defining hyperaccumulators needs critical review, some of which were highlighted by (van der Ent et al., 2013). In lieu of that, the aim of the present study is to identify native high-accumulating plant species with a high level of tolerance that are growing on the sites that can be employed for phytoremediation of multi-metal(loid) contaminated soils. This is hypothesized to help obtain appropriate native plant seed mixes for future remediation purposes.

Method

Experimental Areas and Design

The experimental areas are constituted of three main sites; an abandoned mining site (Site 1), an active mining site (Site 2), and the control site (or reference site), which is an undisturbed vegetation site (Table 1). Fig. 1 presents the scaled map of the study areas. The areas belong to the Ife-Ilesha schist belts; it is a known source of both alluvial and primary gold-field deposits, with a historical gold mining traceable back to 1942. It is situated in the lowland rainforest vegetation zone (Salami et al., 2003); mean annual rainfall of 1400 mm and temperature range of 23°C – 31°C. A previous study within the sites shows that the soil textural class is classified loamy (58 % sand, 10 % clay, and 32 % silt), with an average pH of 4.5 (Olabiyi et al., 2009).

Table 1. Description of the experimental areas

No	Site	Description	Situated	Location
1	Site 1	Abandoned mining site	Okutu-Omo	7° 30’ 30”N, 4° 38’15”E
2	Site 2	An active mining site	Itagunmodi	7°31’30”N, 4°39’03”E
3	Control	Undisturbed vegetation site	Igila	7°34’56”N, 4°39’50”E

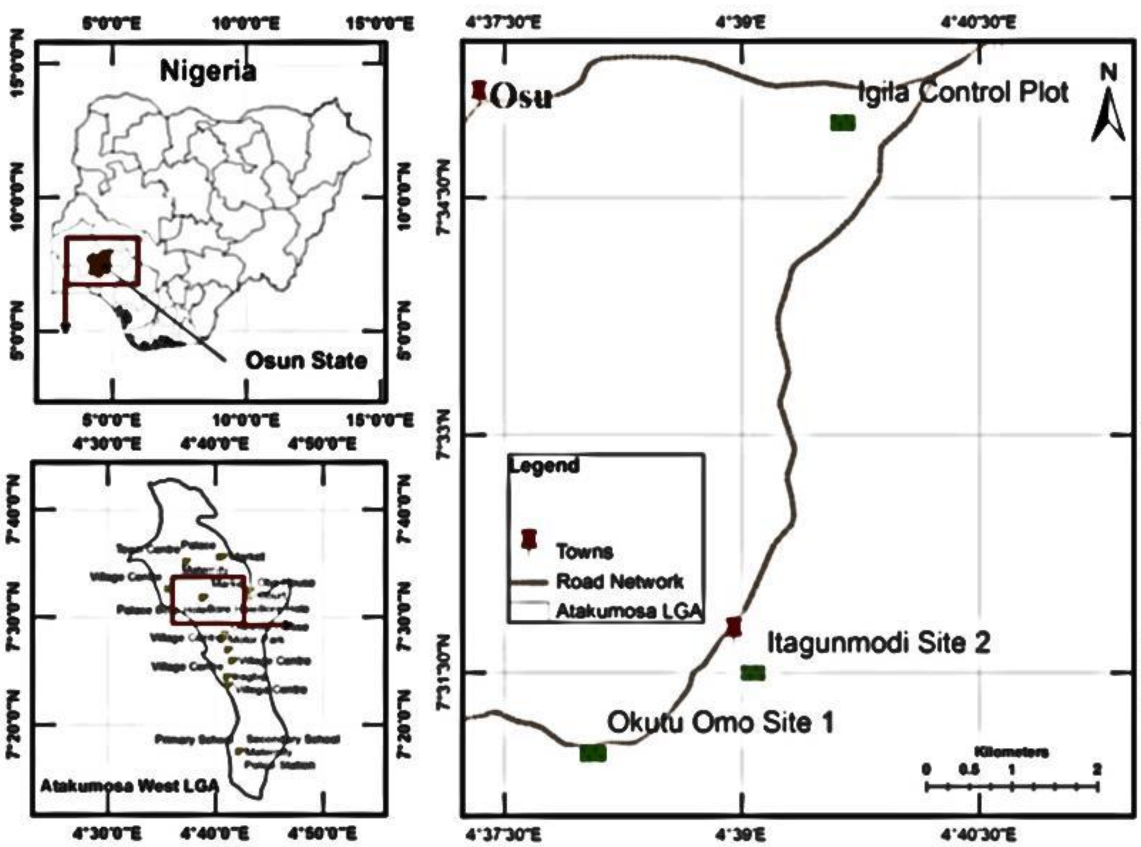

Figure 1. Scaled map of the study areas

Based on reconnaissance surveys, the three experimental sites were purposefully selected as representatives of the ongoing mining and farming activities within the region. Site 1 is occupied by degraded secondary lowland rainforest under regrowth with some relics of the original forest. Several uncovered pits (about 1.5 × 1 m) depict Site 2, with spoil heaps all around the site. However, the control site is an undisturbed vegetation site with a cocoa plantation; located about 7 kilometers from the mined sites, but within the same vegetation zone. On each of the three sites, experimental sample plots of 10 × 10 m were mapped out in duplicates; their GPS coordinates were recorded accordingly and the sites were barricaded from potential invaders.

Soil Sampling and Analysis

Soil samples were randomly collected at two sampling depths (0–20 and 20–40 cm) from each site to obtain a representative sample. The samples were mixed and homogenized, and transported to the laboratory in labeled polythene plastic bags. In the laboratory, soil samples were air-dried at ambient temperature to a constant weight, then thoroughly mixed and sieved for homogeneity in order to remove foreign materials such as stones and roots. To determine the soil particle size, the hydrometer method (Bouyoucos, 1962) was used for the soil particle size analysis after dispersion with Calgon™. Soil pH was determined in water and 0.01 M $CaCl_2$ extracts using glass electrode. Standard tests were employed to measure the electrical conductivity (EC) of extracts from the soil paste using conductivity meter, total nitrogen (TN), organic carbon (OC), and organic matter (Walkley and Black, 1934; Bremner and Keeney, 1966). Available P was extracted using the Bray I method (Bray and Kurtz, 1945). For heavy metal analysis, soil samples were first extracted using 0.1 M HCl and the total concentration of Pb, Cd, Fe, and Cu were determined using Atomic Adsorption Spectrometer (AAnalyst 400).

Plants Sampling and Analysis

Nine plant species were selected for assessment of their total metal accumulation (mg/kg) of Pb, Cd, Fe, and Cu (Table 2). However, seven species were considered for the identification of their phytoremediation and accumulating efficiency. The plants were chosen based on previous related studies (Salami et al., 2003), floristic association (Adesipo et al., 2020), ecological and economic significance, interest in phytoremediation, and adaptability to the environment.

The plant sampling was carefully done just after sunrise to ensure the activeness and freshness.

Data Analysis

To analyze the obtained data, a statistical package of Plymouth Routines in Multivariate Ecological Research (PRIMER-E) was employed. The significance of difference among the study sites was analyzed using PERMANOVA (Permutational multivariate analysis of variance) and pairwise comparison between sites (Anderson et al., 2008). The soil physico-chemical properties data were log-transformed (Log(X+1)) to improve the homogeneity of variances. A one-factorial Permanova design using Bray-Curtis distance measure and 9999 permutations with the site as a fixed factor for each site at 0.05 significant level was used to test for the significant differences of the composition of each site. In addition, a Non-Metric Multi-Dimensional scaling (nMDS) based on Bray-Curtis distance measure (Oksanen, 2015) was created to produce a 2D representation of the composition. The goodness of fit of the nDMS results was evaluated with a stress value.

Table 2: Selected plants species from the three considered mine sites

Plant	**Local name**	**Family**	**Habit**	**Sites**		
				Site 1	Site 2	Control
*Theobroma cacao**	Cocoa	Sterculiaceae	Tree	✔	✔	✔
*Musa sapientum**	Banana	Musaceae	Herb	✔	✔	✔
Chromolaena odorata	Siam weed	Asteraceae	Herb	✔	✔	✔
Melanthera scandens	Black anther	Asteraceae	Herb	✔	✔	✔
Melochia corchorifolia	Chocolate weed	Malvaceae	Herb	✔	✗	✗
Palisota ambigua	Flowering plant	Commelinaceae	Herb	✗	✔	✗
Pteris togoensis	Ferns	Pteridaceae	Fern	✗	✔	✗
Acanthus montanus	Mountain thistle	Acanthaceae	Herb	✗	✗	✔
Crinum jagus	Giant lilly	Amaryllidaceae	Herb	✗	✗	✔

*These are economic plants; only parts of the plants were investigated for heavy metal content.

Bioconcentration, Translocation, and Accumulation Factors

For the determination of the phytoremediation potentials, only the total metal concentrations in the soil samples from the rooting zone (0–20 cm) were employed for the calculations. Bioaccumulation factor (BCF) (Equation 1) was expressed as the ratio of the obtained total metal concentration in the roots to the soil (Yoon et al., 2006). It shows the plant's potential to take up, transport, and accumulate the metals in the shoots (McGrath & Zhao 2003; Wang et al., 2009). Translocation factor (TF) of each considered metal in each plant was estimated as the ratio of the metal concentration in the shoots to that in the roots (Zu et al., 2005), as shown in Equation 2. Plants with BCF and TF values greater than one (i.e., BCF and TF > 1) show the potential to be used for phytoextraction. Plants with BCF values greater than one and TF less than one (i.e., BCF>1 and TF < 1) show the potential to be used for phytostabilization (Yoon et al., 2006). Since the main interest is the accumulating efficiency of the plants, the accumulation factor (AF) (Equation 3) expressed as the ratio of metals in the plant shoots (i.e., harvestable part) to the concentration in the soil was also considered (Marrugo-Negrete et al., 2016). In this paper, high accumulators are defined as plants with AF and TF values greater than one (i.e., AF and TF > 1).

$$\textit{Bioaccumulation Factor BCF} = \frac{\textit{Total metal content in root}}{\textit{Total metal content in soil}} \quad \text{Equation 1}$$

$$\textit{Translocation Factor TF} = \frac{\textit{Total metal content in shoot}}{\textit{Total metal content in root}} \quad \text{Equation 2}$$

$$\textit{Accumulation Factor AF} = \frac{\textit{Total metal content in shoot}}{\textit{Total metal content in soil}} \quad \text{Equation 3}$$

Results

Soil Physicochemical Properties

The physical and chemical properties for the three sites are presented in Table 3. At each site, pH (both H_2O and $CaCl_2$) decreases with depth in the soil profile, ranging from slightly acidic (6.30) to acidic (4.30). The control site is more acidic followed by mine Site 1, while mine Site 2 showed the greatest pH values. Similarly, the percentage organic matter (OM) decreases down the soil profile, with the greatest values (2.21 %) on the control site and the least value (1.20 %) obtained on the mined sites. The electrical conductivity (EC) also decreases down the profile on the mined sites but vice versa on the control site. However, the topsoil of mine Site 2 showed an extraordinarily high EC of over 65 % higher than other sites. While the TN percent was greater on mine Site 1 (1.015 %) but TN was the same on both the control Site and mine Site 2 (0.945 %). On the other hand, available P was higher in the subsoil on both the control Site and mine Site 2, but vice versa on mine Site 1. The least available P (3.34 ppm) was obtained on the control site and the greatest (5.54 ppm) on Site 2. Percentage clay on the three considered sites increases down the soil profile (Fig. 2). Nevertheless, a greater percentage of clay was observed in the subsoil of the mine sites (i.e., Site 1 and Site 2) compare to the control Site. Site 2 has the greatest clay content on the topsoil while the control site has the greatest percentage silt.

Table 3. Mean values (n=3) for the physicochemical properties of the three sites.

Sites	**pH** (H_2O)	**pH** ($CaCl_2$)	**EC** (mSm^{-1})	**% TN**	**% OC**	**% OM**	**P** (ppm)
Site 1; 0-20 cm	5.50	5.03	23.70	1.015	1.25	2.15	5.03
Site 1; 20-40 cm	5.37	4.80	17.12	1.015	0.70	1.20	5.26
Site 2; 0-20 cm	6.30	5.83	71.50	0.945	1.05	1.81	5.54
Site 2; 20-40 cm	6.00	5.70	24.70	0.945	0.70	1.20	4.35
Control; 0-20 cm	4.60	4.60	18.46	0.945	1.29	2.21	3.34
Control; 20-40 cm	4.30	4.30	27.00	0.945	0.86	1.48	4.64

EC, TN, OC, OM and P represent electrical conductivity, total nitrogen, organic carbon, organic matter, and available P, respectively.

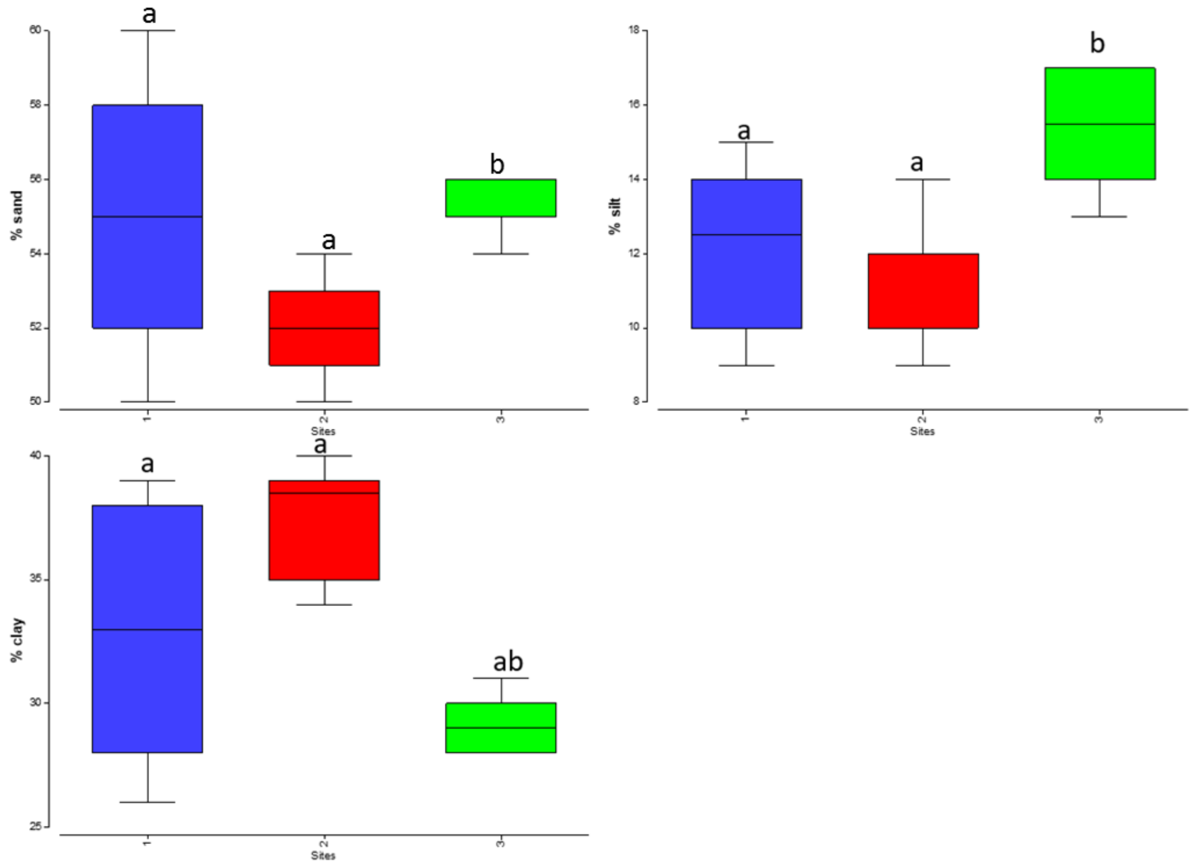

Figure 2. Box plot of the soil textural class (sand, silt, and clay) on Site 1 (Blue), Site 2 (Red), and the control site (green). Different letters indicate significant differences between the sites.

Concentration of Heavy Metals in Soil

The total content of Pb, Cd, Fe, and Cu (mg kg^{-1}) in the soils measured on the three investigated sites is presented in Table 4. Table 5 presents the heavy metal permissible limit by Finland Ministry of Environment (FME) (Ministry of Environment, 2007), National Institute for Public Health and the Environment, Netherlands (NIPHEN) (Crommentuijn et al., 1997), World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO), and the National Agency for Food and Drug Administration and Control (NAFDAC) of Nigeria (Lawal and Audu, 2011; Opaluwa, et al., 2012). All investigated metals (except Cu on the control site and in the subsoil of Site 1) exceed the natural concentration on uncontaminated soil by FME, but are all below the threshold values except Cd which shows elevated concentrations above the threshold values (from 23 – 153 %) on the mine sites. This is same in comparison to the Maximum Permissible Addition (MPA) of NIPHEN, except for Cu with MPA of 3.5 mg kg^{-1}.

Table 4. Mean values (n=3) for the total metal concentration (mg kg^{-1}) at the three sites.

Sites	**Pb** (mg kg^{-1})	**Cd** (mg kg^{-1})	**Fe** (mg kg^{-1})	**Cu** (mg kg^{-1})
Site 1; 0-20 cm	36.90	1.23	637.20	36.12
Site 1; 20-40 cm	29.64	1.30	684.60	13.40
Site 2; 0-20 cm	38.28	2.33	537.00	34.50
Site 2; 20-40 cm	12.96	2.53	663.00	29.10
Control; 0-20 cm	8.34	0.83	584.60	3.24
Control; 20-40 cm	16.86	0.80	483.60	3.72

Table 5. Permissible limits standards for heavy metals.

Standards	**Pb** (mg kg^{-1})	**Cd** (mg kg^{-1})	**Fe** (mg kg^{-1})	**Cu** (mg kg^{-1})
Finland Ministry of environment*	60 (5)	1 (0.03)	-	100 (22)
Maximum Permissible Addition; NIPHEN	55	0.76		3.5
FAO/WHO limits for food and vegetables	2	1	48	30
NAFDAC limit for fresh vegetables	2	-	-	20

* Threshold values (natural concentration)

The highest Pb concentration of 38.28 mg kg^{-1} was found on mine Site 2, while the lowest recorded concentration of 8.34 mg kg^{-1} was on the control site. On the mine sites, Pb concentration decreases down the soil profile, but vice versa on the control site; the Pb concentration in the subsoil was higher than the topsoil. The mean Cd concentration ranged from 0.80 mg kg^{-1} on the control site to 2.53 mg kg^{-1} on mine Site 2. Unlike Pb, Cd concentration increases down the soil profile on the mine sites, but not on the control site. Similar to the Pb reduction down the soil profile is the Cu concentration, which ranges from 3.24 mg kg^{-1} on the control site to 36.12 mg kg^{-1} on mine Site 1. The obtained concentration of Fe on the sites followed a different trend compared to other considered metals. Although the lowest concentration of Fe was observed in subsoil of the control site (483.60 mg kg^{-1}), the topsoil of mine Site 2 also has low Fe concentration compared to other sites. Box plots were used to present the relations between the heavy metal contents on the three sites (Fig. 3).

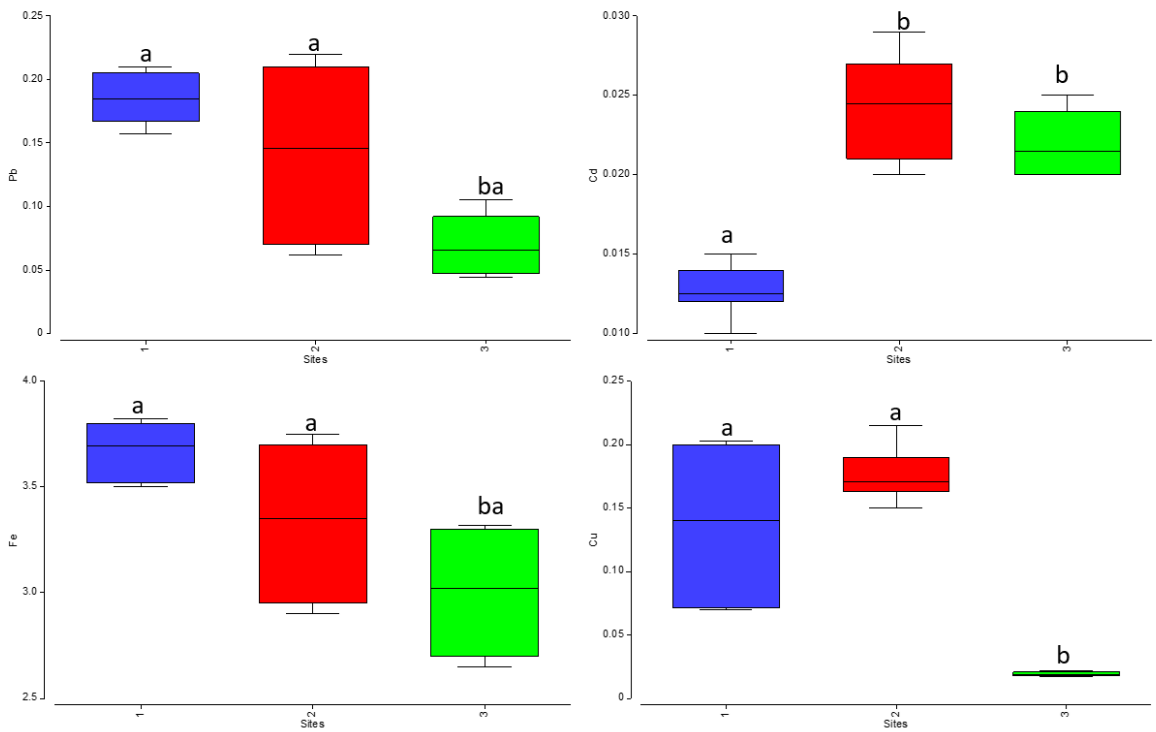

Figure 3. Box plots of the soil heavy metal content (Pb, Cd, Fe and Cu) on Site 1 (Blue), Site 2 (red) and the control site (green). Different letters indicate significant differences between the sites.

Analysis (soil physicochemical properties, heavy metals)

PERMANOVA results for TN showed significant differences in activity density among the three sites (Sites - $F_{2,15} = 35.549$, $P < 0.001$). Pairwise comparison revealed significant differences between Site 1 and Site 2, between Site 1 and the control site, but not between Site 2 and the control site. In terms of EC, significant differences were observed among the sites (Sites - $F_{2,15} = 7.002$, $P < 0.001$). Pairwise comparison revealed significant differences between Site 1 and Site 2, between Site 2 and the control site, but not between Site 1 and the control site. Similarly, pairwise comparison for the % OM indicates significant differences between Site 1 and the control site, between Site 2 and the control site, but not between Site 1 and 2. The nDMS analysis shows a distinct separation of the sites (Fig. 4) as supported by the stress value of 0.03. However, Site 2 and Site 1 share some similarities with both the blue and red triangles within the same sides. Likewise, Site 1 and Site 3 (the control site) show some similarities with the blue triangles and green squares on the same sides. Results of the nDMS analysis confirm the PERMANOVA results with a significant difference in some of the soil properties and similarities in others.

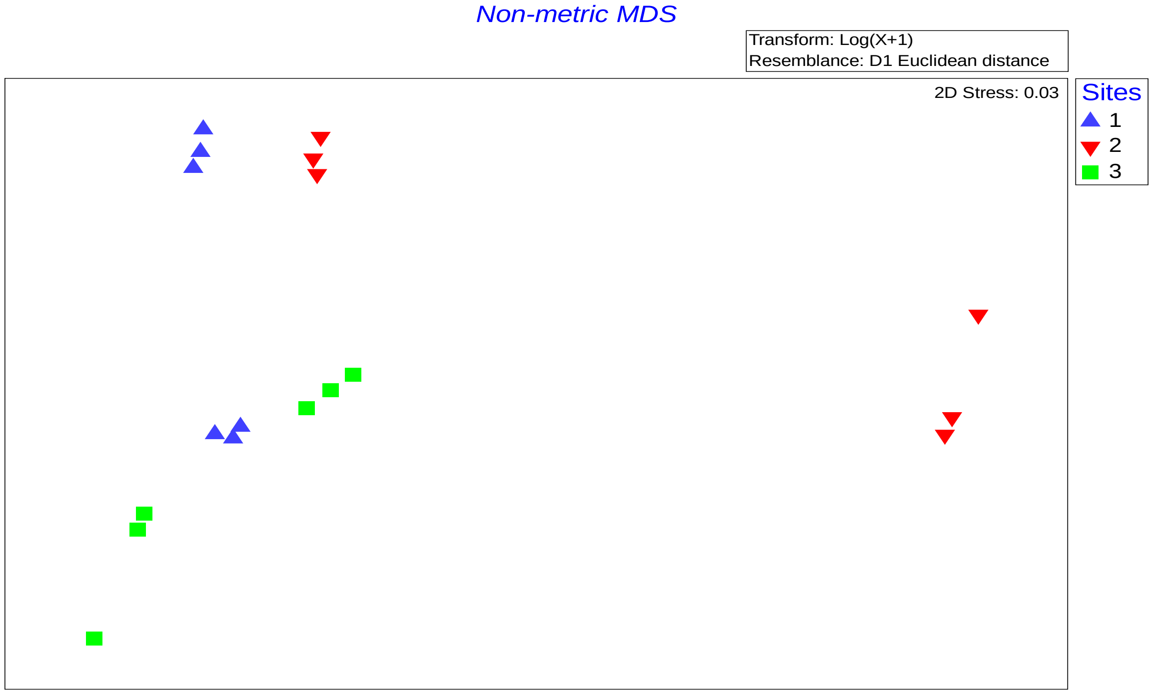

Figure. 4: Physicochemical composition of the 3 sites by nDMS analysis (with stress value of 0.03). It shows distinctive separation from the 3 sites, but Site 1 shares some similarities with the control site (the blue and green points) while Site 1 and Site 2 also shares some similarities (the blue and red points).

Plants Heavy Metal Content and Phytoremediation Potentials

As previously explained, the total heavy metal content from the obtained parts of *Theobroma cacao* and *Musa sapientum* are presented in Table 6. In *Theobroma cacao*, the mean concentration of Pb for both the beans and leaves range from 24.2 µg g^{-1} (on control site) to 50.8 µg g^{-1} (on Site 2); Cd, from 0.1 µg g^{-1} (on control site) to 5.1 µg g^{-1} (on Site 2). While for Fe, from 47.2 µg g^{-1} (on Site 2 beans) to 232.0 µg g^{-1} (on Site 2 beans), and Cu, from 6.6 µg g^{-1} (on control site) to 19.59 µg g^{-1} (on Site 1). In *Musa sapientum*, Pb was in concentration range of 12.1 µg g^{-1} (on control site) to 42.3 µg g^{-1} (on Site 1). Cadmium ranges from 0.1 µg g^{-1} (on Site 1) to 3.6 µg g^{-1} (on Site 2). Fe ranged from 27.9 µg g^{-1} (on control site) to 186.9 µg g^{-1} (on Site 2), and Cu is contained from 0.4 µg g^{-1} (on Site 2) to 7.9 µg g^{-1} (on Site 1). In comparison to the permissible limit by WHO/FAO and NAFDAC (Table 5), all the plants showed elevated Pb, Cd, and Fe in both *Theobroma cacao* and *Musa sapientum* on all the sites. However, concentration of Cu was low except in the leaves of *Theobroma cacao* on mine Site 1. Rather than presenting the total concentration of the other seven investigated plant species, their mean values (n = 3) were used to calculate its respective phytoremediation potentials; Bioaccumulation (BCF), Translocation (TF), and Accumulation (AF) factors. They were classified appropriately as phytoextraction (PE), phytostabilization (PS), and high-accumulators (HP) species. Results for each plant are presented in Table 7 accordingly. The Fe concentrations in several of the investigated plants were greater than that of the highest standard and were represented as (ND; not detected).

For Pb, all the plants showed hyperaccumulating potential for Pb (Table 7). The species *C. jagus* has the greatest BCF (5.44) and AF (8.71) while *A. monthanus* has the highest TF (3.74). Four plants showed high-accumulating potential for Cd; *C. odorata, C. jagus, M. scanetenes* and *P. ambigua*. *A. monthanus* showed potential for phytostabilization of Cu with BCF >1 but TF <1. The species *A. monthanus* has the highest BCF (2.01), and *C. jagus* the highest TF (34.56) and AF (37.47). Most of the plants showed high Fe content above the highest standard and were therefore not detected. However, *C. odorata, A. monthanus* and *C. jagus* showed high-accumulating potential for Cu. *A. monthanus* has the highest BCF (14.54), *C. odorata*; the highest TF (6.00) while *C. jagus* has the highest AF (29.38).

Table 5. Concentration of metals in *Theobroma cacao* and *Musa sapientum* in μg g^{-1}.

Species	**Sites**	**Pb**		**Cd**		**Fe**		**Cu**	
		Beans	Leaves	Beans	Leaves	Beans	Leaves	Beans	Leaves
T. Cacao	Site 1	29.1	45.7	0.7	0.3	48.7	90.3	12.3	195.9
	Site 2	31.9	50.8	5.1	2.4	232.0	47.2	8.0	9.1
	Control	-	24.2	-	0.1	-	113.4	-	6.6
M. Sapientum	Site 1	42.3	27.5	0.1	0.2	163.3	157.8	0.8	7.9
	Site 2	35.3	34.0	3.4	3.6	61.4	186.9	0.4	4.5
	Control	12.1	28.9	1.3	0.7	27.9	64.9	1.1	0.6

Table 6. Phytoremediation potential of the seven native plant species.

Species	**Pb**				**Cd**				**Fe**				**Cu**			
	BCF	TF	AF	Class	BCF	TF	AF	Class	BCF	TF	AF	Class	BCF	TF	AF	Class
C. odorata[1]	0.60	2.68	1.61	–	1.46	1.61	2.36	HP	0.37	2.54	0.94	–	0.07	3.00	0.21	–
C. odorata[2]	1.09	1.70	1.84	HP	0.94	2.09	1.97	–	ND	ND	ND	–	0.18	4.61	0.83	–
C. odorata[C]	4.29	1.22	5.22	HP	0.72	3.33	2.41	–	0.02	45.53	0.69	–	1.02	6.00	6.11	HP
A. Monthanus[C]	1.95	3.74	7.30	HP	2.01	0.53	1.08	PS	ND	ND	0.56	–	14.54	1.20	17.38	HP
C. Jagus[C]	5.44	1.60	8.71	HP	1.08	34.56	37.47	HP	ND	ND	1.08	–	12.19	2.41	29.38	HP
M. corchorifolia[1]	1.33	1.60	2.13	HP	0.24	4.67	1.14	–	ND	ND	0.37	–	0.20	0.19	0.04	–
M. Scanetenes[1]	1.71	1.46	2.49	HP	1.30	3.13	4.07	HP	ND	ND	0.90	–	0.11	0.88	0.10	–
P. ambigua[2]	1.67	1.01	1.68	HP	1.29	1.97	2.53	HP	ND	ND	ND	–	0.49	1.74	0.86	–
P. togoensis[2]	1.07	1.58	1.70	HP	0.94	2.68	2.53	–	ND	ND	0.84	–	0.22	0.43	0.09	–

BCF, TF and AF represent Bioaccumulation factor (root to soil), Translocation factor (shoot to root) and Accumulation factor (shoot to soil) respectively. HP and PS represents High-accumulator species and Phytostabilisation species, respectively, while 1, 2, and C represents species from Site 1, Site 2 and the Control site respectively, and ND represents Not Detected.

Discussion

Soil physicochemical properties

Soil textural class is a permanent physical property (Hartati & Sundarmadji, 2016), its distribution on the sites is not influenced by the crops, rather by the ASM activities. ASM involved activities such as excavation of the subsoil that is higher in clay content, piling them up as spoil heaps which are later used for farming in place of the original nutrient-rich topsoil. This influenced the textural class distribution of the sites. As a result, high percentage loss of silt and sand soil particles to erosion is unavoidable on mine sites, enhanced by heavy rainfall and the landscape characterizing the region. This is more evident on Site 2, which contains a high percentage of clay and less sand and silt compared to the other sites. Also, since high clay content supports lower plant growth, it might contribute to the noticeable lower vegetation on Site 2 (Adesipo et al., 2020). Moreover, the ability of clay minerals to regulate the mobility and bioavailability of heavy metals (Rieuwerts et al., 1998) might enhance retaining of metal ions on Site 2.

Despite the ASM activities, active mine sites show higher pH than the control sites, which is unusual compared to most related studies (Salami et al., 2003; Sheoran et al., 2010; Oladipo et al., 2014; Petelka et al., 2019). Nevertheless, a previous study by Olabiyi et al. (2009) within the region shows that the soil is characterized by a pH of 4.5. Moreover, low soil pH, low % OC, and low N contents limits cocoa production on old cocoa fields in the country (Ayanlaja, 1983). This could have influenced the pH of the control site, since Igila is an old cocoa farm with no record of anthropogenic activities except agriculture. However, obtained pH on the mine sites are comparable to previous studies (Oladipo et al., 2014), and suggest to limit plant growth, biological activity, nutrient availability, and toxicity of the heavy metal contaminants (Sheoran et al., 2010). Soil pH is one of the main factors influencing the mobility and availability of heavy metals to plants (Rieuwerts et al., 1998). Heavy metal solubility and leaching increases as the soil become more acidic (Liu et al., 2004; Yoon et al., 2006), it is therefore an important indicator of potential risk. Since none of the sites shows pH value greater than 6.5, the risk of its mobility and availability is high, with potential transfer to the food chain.

Soil OM positively influences nutrients supply to plant, soil physicochemical properties, as well as the binding and retention ability of metals (Rieuwerts et al., 1998; Sheoran, et al., 2010; Mensah et al., 2015). According to Landon, (1991), the obtained OM on all the sites are low, but are within the range of similar related studies (Areola et al., 1982; Abiya et al., 2019). The control site shows the greatest % OM (2.21 %), especially on the topsoil, which could be attributed to its high litter fall from cocoa production (Asiedu et al., 2013). Greater % OM on Site 1 compared to Site 2 can be attributed to the revegetation process the site is undergoing and its abandoned ASM activities, thereby increasing its litter accumulation, unlike Site 2 with ongoing intense ASM activities (Adesipo et al., 2020). Moreover, Site 1 is occupied by degraded secondary lowland rainforest, which is under regrowth with some relics of original forest capable of supporting rehabilitation of degraded soils.

Following N classification for tropical soils (Landon, 1991; Petelka et al., 2019), N values ≤ 0.2 %, ≤ 0.5 %, and ≥ 0.5 % are low, medium, and high, respectively. All sites show high N content typical of cocoa production (van Vliet and Giller 2017) and higher in comparison to other related studies (Eludoyin et al., 2017; Abiya et al., 2019). However, the highest % TN observed on mine Site 1 can be attributed to some N-fixing plant species growing on the site (Adesipo et al., 2020), as well as the ongoing agricultural activities which might require fertilizer application. The extremely high EC on mine Site 2 can be attributed to the intense ASM activities and its high clay content limiting the flow of salt on the site, as well as the low landscape condition of the site with a high tendency to accumulate more water (since salt moves with water). Soils dominated by clay minerals such as smectite have a high cation-exchange capacity (CEC) and EC, whereas kaolinite has a low CEC. Unfortunately, there is limited information on the clay minerals on this site. However, soils with high EC (sodic condition) limit soil structure, infiltration or drainage, as well as toxicity to crops. This might also have contributed to the low vegetation on the site compared to other sites (Adesipo et al., 2020). Previous studies in southwest Nigeria reported available P in most soil series is below the critical level of 8 mg kg^{-1} (Anetor et al., 2013). However, the very low available P observed on the sites can be attributed to elevated Fe (Okusami et al., 1997 and Soremi et al., 2017). In addition, due to limited mineralization that mining activities have on plant nutrients (Martinez and Motto 2000; Oladipo et al., 2014), the observed low nutrients on the mine sites can be attributed to the ASM activities and the elevated heavy metal concentration.

Soil Heavy Metal Concentration

The total content of Pb, Cd and Cu on Site 1 and 2 exceed the control site: Pb (from 43 – 78 %), Cd (from 36 – 68 %), and Cu (from 72 – 91 %) (Table 4). Similar high concentration differences were observed with Fe however, low Fe was as well obtained in the topsoil of mine Site 2. Since there are no existing standard threshold values in relation to heavy metal soil contamination in Nigeria, which might be in relation to its influencing factors such as the soil parent materials, there are limits to the extent of its assessment efficiency and comparison. However, standards according to the Finland Ministry of Environment (FME) (Ministry of Environment, 2007) and the Maximum Permissible Addition (MPA) of the National Institute for Public Health and the Environment, Netherlands (NIPHEN) (Crommentuijn et al., 1997) were employed accordingly. Concentration of Pb, Cd, and Cu on all the sites exceeds the natural concentration on uncontaminated soil (Table 3), except Cu on the control site as well as the subsoil of Site 1. Howbeit, except Cd on the mine sites, the concentrations of Pb, and Cu are below the threshold level (both FME and NIPHEN), and are within related studies on gold mine sites (Antwi-Agyei et al., 2009; Ekwue et al., 2012; Oladipo et al., 2014; Petelka et al., 2019), except Cd and Fe. The elevated Cd concentration suggest a prompt need for reclamation of these sites, most especially Site 2 with up to 153 % elevated Cd contamination. On Site 2 (Itagunmodi), comparing the result by Salami et al., (2003) and Oladipo et al., (2014), there is an increasing elevation of Pb (2.54, 35.00, and 38.28 mg kg^{-1}), Cd (0.13, 0.20 and 2.33 mg kg^{-1}), as well as Fe (137.27, 296.20 and 537 mg kg^{-1}), respectively. This consistent elevation is similar to previous related studies and can be attributed to the continuous exploitation by the ASM activities, which consequently induce variation in the soil chemical properties (Martinez and Motto 2000; Cooke and Johnson, 2002; Oladipo et al., 2014). Iron is not included in the threshold values set by both FME and NIPHEN. Neither is there a related threshold standard on its concentration in Nigeria. However, comparing results on Site 2 to previous studies, there is a percentage increase of 115 % and 81 % between 2003 – 2014 and 2014 – current study, respectively. Apart from the ASM activities, elevated Fe content on all the investigated sites can be attributed to the characteristics of the soil parent material within the southwest region of the country (Oladipo et al., 2014) and higher concentration has been observed in previous studies (Okoya et al., 2011). The high Pb concentration might be related to galena, which is a natural mineral form of PbS (lead sulfide) occurring in gold ores with high concentration of sulfide (Matocha et al., 2001). Cadmium occurs in gold bearing ore as an isometric trace element in sphalerite, which determines its level of concentration. Similar to that is Cu which occurs alongside gold ores, and distributed in sulfides, arsenites, chlorides, as well as carbonates (Fashola et al., 2016). Health risk of these metal toxicity has been widely discussed.

Though both Fe and Cu are trace elements, however, consequences of their toxicity need to be averted. Therefore, prompt remediation of the sites is recommended.

Analysis (Heavy Metals, Soil Physicochemical Properties)

The PERMANOVA and nDMS analysis of the soil physicochemical properties and total heavy metal showed the significant differences of the sites. The three sites are significantly different, with few similarities existing between Site 1 and Site 2, and Site 1 and the control site, but not between Site 2 and the control. Few of the soil properties with significant difference between Site 1 and Site 2 as obtained from the PERMANOVA result include % TN and EC, while between Site 1 and the control site include % OM, and % TN. Non-existing similarities between Site 2 and the control site indicate the severe impact of the ASM activities on Site 2, while the existing similarities between Site 1 and the control site confirms the ongoing self-rehabilitation of Site 1. Moreover, this can be explained from its floristic association highlighted by Adesipo et al., (2020). Leaving the mine sites after ASM activities gives the site the opportunity to restore and self-sustain its ecosystem.

Plants Heavy Metal Content and Phytoremediation Potentials

All the plants showed elevated Pb, Cd and Fe accumulation, but Cu was low except in *Theobroma cacao* leaves on mine Site 1. The elevated Fe can be attributed to the characteristics of the soil parent material in this region, while Pb and Cd can be attributed to the characterized gold ore mine on the sites. Therefore, prompt intervention is necessary to monitor, curb, and remediate the consequences of these illegal ASM activities, most importantly its entry into the food chain. Cases of Pb poisoning outbreak linked to ASM gold processing in the northwestern region of Nigeria, with over 400 deceased and thousands being affected have been reported (Lo et al., 2012; Plumlee et al., 2013; Bartrem et al., 2014). Similar occurrence needs to be averted. Apart from Pb, metals such as Cd which is highly mobile in soil-plant system, with exceptional long biological half-life >20 years can pose serious threat due to its high accumulation potentials in the food chain (Fashola et al., 2016). On the other hand, Cu and Fe are however essential nutrients for plants. In gold ores, Cu is widely distributed in arsenites, sulfides, and chlorides as well as carbonate, while Fe is one of the most abundant metals in the earth crust and all forms of life require it. However, a high concentration of either Cu or Fe is toxic; Fashola et al., (2016) have highlighted their toxic effects.

The high accumulation of the heavy metals in the investigated plants, despite the differences in the bioavailability of the metals (especially Pb, the least; and Cd, the most bioavailable), can be attributed first to the low pH of the soil and the perennial nature of the investigated plants (e.g.,a *Theobroma cacao, Musa sapientum* and *Crinum jagus*). Also, most of the considered plants suggest to have high tolerance for the contaminants and they have been existing on the sites for long time. The plants might be similar to examples of plants with mechanisms to accumulate the contaminants with less influence of the metal toxic effects (Wang et al., 2009; van der Ent et al., 2013; Petelka et al., 2019). Moreover, plants such as *Theobroma cacao* and *Musa sapientum* has deep roots, while *Musa sapientum* has a high-water content which might have also enhanced its high metal accumulation potential. Nevertheless, analysis of the plants secreted water in relation to bioavailability of heavy metals might be of interest. In addition to the plants' high accumulation potentials is their high biomass. This is also true especially for plants such as *Acanthus montanus*, as well as *Crinum jagus,* and *Melochia corchorifolia* which grow best in swamp forest and riverine vegetation (Adesipo et al., 2020).

Unfortunately, there are limited amount of research on the normal range of mineral nutrients in some of the investigated plants on uncontaminated soils. Since *Theobroma cacao* and *Musa sapientum* are food crops with high potential to enter the food chain, comparison with related studies is necessary (Lockard and Asomaning, 1964; Anhwange et al., 2009; Araujo et al., 2017; Assa et al., 2018). The level of Cu is within the normal nutrient range, while Pb, Cd and Fe are above. Araujo et al., (2017) highlighted that variations in Fe, Cu, and Cd as well as Mn, Zn, and Ba in cacao beans are in relation to the cropping system. Similar to that is the high range of Cd noticed by Arevalo-Gardini et al., (2017) in the species of cacao grown in Amazonas, Piura as well as the Tumbes regions in Peru. The Cd accumulation in *Theobroma cacao* is species related (for both the leaves and beans). Adequate screening of low metal accumulator of *Theobroma cacao* genotypes for safe production is therefore recommendable. Apart from the species, this variability factor might be true for other investigated metals as well. In addition, variation for within-species distribution of the heavy metals in the plants due to compartmentalization and translocation in the vascular system, which is poorly considered in most related studies (Kim et al., 2003).

Theobroma cacao and *Musa sapientum* are food crops and are not recommended for remediation purposes (Bansah and Addo, 2016). However, all the plant species showed high-accumulating potentials of Pb. *Chromolaena odorata, Crinum jagus, Melanthera Scanetenes,* and

Palisota ambigua showed high-accumulating potential of Cd, while *Acanthus Monthanus, Crinum jagus* and *Chromolaena odorata* showed high-accumulating potential for Cu. Most of these investigated plants are yet to be considered for phytoremediation by other authors, except *Chromolaena odorata* which has been efficiently confirmed to accumulate Pb (Tanhan et al., 2007; Wilberforce, 2015; Jampasri et al., 2016) and Cd (Fati, 2011), while *Melochia corchorifolia* was found efficient for As (Idris et al., 2016). None of the plants meets threshold criteria for hyperacumulators. However, a review of the threshold criteria is necessary, some of this has been suggested by van der Ent et al., (2013). The increasing demand for remediation in tropical regions, requires further investigation of plant phytotoxicity. Nevertheless, the accumulation potential of *Crinum jagus* is significantly high for all the metals, this is similar to the observations of (Salami et al., 2003). Interest in exploring its phytoremediation potentials is high, and it is a novel recommendable plant species for the tropics. Its remediation potentials can be employed with other remediation techniques such as bioremediation with agents such as bacterial, fungi, and algae.

Conclusions and Recommendations

Due to the ASM activities, tailing soils at the gold mine sites in southwestern Nigeria are characterized by elevated Pb, Cd, Fe, and Cu more than the control site, low nutrient, high heterogeneity and clay content. The elevated metal content of the mine site is also traceable to its acidic condition (below pH 6.5). However, Site 1 showed self-rehabilitation potential, and with no further ASM activities; the ASM induced adverse effects on the sites might be reduced. Cd on the mine sites exceeds recommendable threshold limits of both FME and NIPHEN. In comparison to previous studies, there is a consistent increase in the heavy metal contaminants, therefore prompt remediation of the sites is necessary. Effective approaches and policies to curb the ASM activities are necessary, this can be backed-up by empowering the youths, trace its supply chain, and discourage its illegal source of income. In addition, the economy needs to be stabilized with practicable conservation and management strategies that can deter the continuation of the ASM activities and enhance appropriate reclamation. More so, there is need to establish comprehensive threshold standards within this region. Moreover, due to limited access to the sites, the process the artisanal miners employ in processing the gold is not clear, this is however necessary, especially in case of cyanide. Nevertheless, determination of other related heavy metals such as As, Hg, Ni, Zn, and Cr, is important.

According to threshold criteria for defining hyperaccumulators, none of the plants shows hyperaccumulating potential. However, all the plants show high-accumulating potential for Pb, while *C. odorata, C. jagus, M. Scanetenes* and *P. ambigua* are high-accumulators for Cd, and only *C. odorata, A. monthanus* and *C. jagus* are high-accumulators for Cu. Most importantly is *C. jagus*, which showed high-accumulating potential for all the metals, with significant high accumulator factors of 8.71, 37.47, 1.08, and 29.38 for Pb, Cd, Fe, and Cu, respectively. It is a recommendable novel plant for all forms of remediation, and can be employed in climatic adaptable regions around the world. In addition, further studies on the plant with high heavy metal concentration is necessary to ascertain if it can attain hyperaccumulator threshold. Also, other similar viable plants for phytoremediation are to be explored in the tropics.

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