Impact of Indigenous Mycorrhizal Fungi on Lead Speciation during Phytoremediation of Contaminated Landfill Soils in Bonoua, Côte d'Ivoire

Abstract

This study evaluates the changes in lead (Pb) speciation induced by phytoremediation in contaminated soils from the M'Ploussoué Park landfill in Bonoua, Côte d'Ivoire. *Acacia mangium* was cultivated, with or without inoculation by indigenous mycorrhizal fungi (IMF), on landfill soils artificially contaminated with three lead concentrations: 0 mg/kg (Soil $_{Pb0}$), 250 mg/kg (Soil $_{Pb250}$), and 500 mg/kg (Soil $_{Pb500}$).After five months of cultivation, the distribution of Pb among soil fractions before and after phytoremediation was assessed by sequential extraction according to the BCR scheme. Before the trial, lead was distributed among five fractions: Pb-$_{Hydro}$, Pb-$_{Exch}$, Pb-$_{Oxy}$, Pb-$_{OM}$, and Pb-$_{Resi}$ and was predominantly bound to iron and manganese oxides (45-52%).After the trial, extraction rates were consistently higher in the presence of IMF regardless of soil type: 82% versus 76% under Soil $_{Pb0}$, 95% versus 92.8% under Soil $_{Pb250}$, and 96% versus 94.3% under Soil $_{Pb500}$, relative to the initial total Pb content. These results indicate that IMF enhance lead extraction. In addition, the easily mobilizable fractions (Pb-$_{Hydro}$, Pb-$_{Exch}$) showed the highest extraction rates (up to 95%) under Soil $_{Pb0}$ and Soil $_{Pb250}$, whereas under Soil $_{Pb500}$ the highest extraction rates (up to 97%) were observed in the fractions bound to oxides and organic matter (Pb-$_{Oxy}$, Pb-$_{OM}$).

Key words: Mycorrhizal fungi, sequential extractions, polluted soil, Bonoua.

1. Introduction

The industrial and domestic waste we produce is generally sent to landfills without any prior treatment. These wastes, during their decomposition, produce liquid effluents that can introduce trace metal (TM) such as lead into the soil[1]. Lead remains widely used in numerous industrial processes, including battery manufacturing, automotive fuels, paints, waste-processing and recycling facilities, accumulators, soldering, crimping, radiation shielding, and hunting and shooting ammunition[2, 3].Such uses have contributed to a marked increase in lead concentrations in landfill soils, with consequences extending beyond soil contamination to the pollution of adjacent water resources. Moreover, once accumulated in soil, this persistent metal

can be transferred to surrounding ecosystems and pose a risk to human health: lead is classified among the potentially toxic metals, associated with anemia, hypertension, and lead poisoning, among other condition[4-6].Given these risks, the rehabilitation of landfill sites is a pressing need, and within a sustainable-development framework, phytoremediation offers an environmentally friendly alternative that harnesses the capacities of plants and microorganisms to remove pollutants[7]. Understanding the chemical forms taken by metals in soil is essential, since the incorporation of a metal into the soil and its subsequent transfer to plants involve changes in its speciation[8]. The speciation of metals in the solid phases of soil is therefore a key parameter to consider during phytoremediation.

It is against this background that the present study aims to evaluate the changes in lead speciation induced by phytoremediation in the solid phases of contaminated soils from the M'Ploussoué Park landfill in Bonoua, with particular attention to lead fractionation throughout the remediation process.

2. Materials and Methods

2.1.Sampling and preparation of culture substrates

Soil samples were collected from a depth of 0-30 cm at 35 points across the former M'Ploussoué Park landfill in Bonoua, Côte d'Ivoire (5°16′41″ N, 3°36′03″ W). Samples were sieved at 2 mm and homogenized to obtain a composite sample. To study lead fractionation, soils were artificially contaminated to achieve a gradient of lead concentrations (0, 250, and 500 mg/kg) following the procedure described by Redon [9]. The landfill soil samples were artificially contaminated with lead at different concentrations according to the following procedure:

Lead acetate powder [$Pb(CH_3COOH)_2$] was dissolved in distilled water and applied according to the following relationship:

$$\boldsymbol{Weight}_{\boldsymbol{of\ Pb\ acetate\ used}} = \frac{C\left(\frac{mg}{kg}\right) \times \boldsymbol{Weight\ of\ the\ soil}\ (kg) \times \boldsymbol{Molar\ mass\ of\ lead}}{\boldsymbol{Molar\ mass\ of\ lead}}$$

where C represents the target lead concentration[10]. For example, to contaminate 2 kg of soil at 250 mg/kg, 0.8 g of lead acetate was dissolved in 1 L of distilled water; this solution was mixed with 10% of the soil to be contaminated (0.2 kg), and the resulting mixture was blended with the remainder of the soil by thorough homogenization in plastic bags. The contaminated soil was

then aged for three weeks at room temperature under shelter. After this ageing period, total Pb content was measured to confirm successful artificial contamination prior to the experiment. Part of the contaminated soil was air-dried for physicochemical characterization, and the remainder was used to set up the experimental trial.

2.2. Plant Material

Seeds of *Acacia mangium*, obtained from the National Center for Agronomic Research (CNRA) of Côte d'Ivoire, were scarified in 95% concentrated sulfuric acid (H_2SO_4) prior to pre-germination, following the method ofBongoua-Devisme, Koffi [7].Seeds were placed on a sterile 0.8% agar medium in Petri dishes, sterilized at 110 °C, then wrapped in aluminum foil and incubated in the dark at 37 °C for 72 hours.

2.3. Biological Material

The biological material consisted of an inoculum of arbuscular mycorrhizal fungi (AMF) produced in the laboratory from indigenous spores that had colonized the polluted soils of the M'Ploussoué Park landfill (Figure 1).

The inoculum was multiplied in planting boxes (1 m × 45 cm × 50 cm) containing a sand/soil/vermiculite substrate (2:1:1, v/v/v)[11], using maize as a host plant for two months. Mycorrhizal colonization was confirmed at 45 days, after which the plants were subjected to two weeks of stress to promote spore multiplication. After two months, the aerial parts were removed and discarded, and the root-associated soil was harvested. The resulting inoculum was stored in sealed bags under controlled humidity (45%) and shade, consistent with AMF preservation requirements.

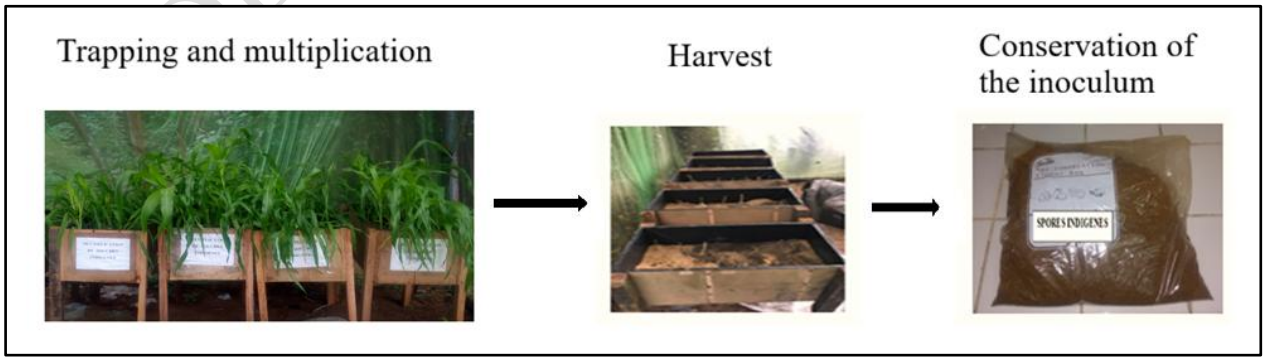


Figure 1: Inoculum Production

2.4. Experimental Setup

The experiment was conducted under a shelter at the National Floristic Center (CNF) of Félix Houphouët-Boigny University, Abidjan, where average greenhouse temperature ranged from 28 to 36 °C and relative humidity from 37% to 66% over the course of the day.

Pre-germinated *Acacia mangium* seedlings in manypetri dishes were transplanted into polyethylene nursery bags (15 × 40 × 150 cm) containing 2 kg of soil at each of the three lead concentrations (0, 250, and 500ppm). Each bag was abundantly watered with sterile distilled water to facilitate the transplanting of *Acacia mangium* seedlings. Using tweezers, three small holes were made in the bags containing the previously watered soils.

Inoculation was performed using indigenous mycorrhizal fungi (IMF) isolated from the landfill soils: 20 g of inoculum were added per bag and mixed into the substrate before transplanting the pre-germinated seedlings. The experiment thus comprised six treatments, each replicated nine times: Soil $_{Pb0}$ inoculated (Soil $_{Pb0}$-I) and non-inoculated (Soil $_{Pb0}$-NI); Soil $_{Pb250}$ inoculated (Soil $_{Pb250}$-I) and non-inoculated (Soil $_{Pb250}$-NI); and Soil $_{Pb500}$ inoculated (Soil $_{Pb500}$-I) and non-inoculated (Soil $_{Pb500}$-NI).

After 5 months of cultivation, the plants were harvested and the soil clods around the roots were collected, air-dried to perform the sequential extraction of the different forms of lead in the soil fractions during phytoremediation.

2.5. Sequential Extraction Protocol

Following five months of cultivation, sequential extraction was performed on the artificially contaminated soils, both before and after phytoremediation, to determine the distribution of lead across soil fractions according to the Community Bureau of Reference (BCR) method. The extraction steps are summarized in Table I. Lead concentrations in the resulting digests were measured by inductively coupled plasma mass spectrometry (ICP-MS) at the Central Environmental Laboratory of the Ivorian Anti-Pollution Center (CIAPOL).

Table I: Protocol for sequential extractions performed on soil samples for 100 mg of dry soil

Steps	Fractions	Reagents	Volume ofthereagent	Conditions
1	Hydrosoluble (hydro)	Distilled water	40 ml	16 hours of rotational agitation, 300 minutes, centrifugation at 3000 rpm
2	Exchangeable (Ech)	Acetic acid (CH_3COOH) at 0.11 M	40ml	16 hours of rotational agitation, 300 minutes, centrifugation at 3000 rpm
3	Oxidizable (Oxy)	Hydroxylamine hydrochloride (NH2OHHCl) at 0.5 M	40ml	16 hours of rotational agitation, 300 minutes, centrifugation at 3000 rpm
4	Related to Organic Matter (OM)	2 times 10ml of hydrogen peroxide (H_2O_2) at 8.8 M + Ammonium acetate (CH_3COONH_4) at 0,5 M	10ml, 10ml, 40ml	1 h digestionat room temperature, then 1 hour at 85°C,followed by 16 hours under constant agitation at room temperature
5	Residual (Resi)	1 mL of aqua regia (HNO_3: HCl; 1:3, v/v) 3 mL of concentrated 48% pure HF + 10 mL boric acid solution 50 ml	50 ml	120°C for 3 hours (according to total mineralization)

2.6. Statistical Data Analyzes

The obtained data were processed in Excel software and subjected to an analysis of variance (ANOVA) allowing for a comparison of multiple means. A significant difference between the treatments was determined using SAS 9.0 software and the Student Newman-Keuls test at $P < 0.05$.

3. Results

3.1. Chemical Characteristics of soils before phytoremediation

Analysis of the soils following artificial lead contamination revealed slightly acidic conditions, with pH values ranging from 6.38 to 6.44 across all soil types (Soil $_{Pb0}$, Soil $_{Pb250}$, Soil $_{Pb500}$) (Table II). Nitrogen (730-740 mg/kg), phosphorus (96-98 mg/kg), and organic matter (30,400-30,700 mg/kg) contents were similarly low across treatments, and mean CEC ranged from 9 to 10 cmol/kg.

Table II: Chemical Characteristics of Soil Samples Contaminated with Lead (Pb)

Parameters	Soil $_{Pb0}$	Soil $_{Pb250}$	Soil $_{Pb500}$
pH_{water}	6,43	6,38	6,44
N (mg/kg)	730	735	740
P_{assi} (mg/kg)	98	96,75	97,25
OM(mg/kg)	30600	30700	30400
CEC (cmol/kg)	9,18	10,09	9,85

$Soil_{Pb0}$: Non-contaminated soil; Sol_{Pb250}:Contaminated soil with 250lead; $Soil_{Pb500}$: Contaminated soil with 500; pH_{water}: hydrogen potential in water; N: Nitrogen; Passi: assimilable phosphorus; OM: organic matter; CEC: cation exchange capacity.

Sequential extraction showed that lead was distributed among five fractions (Table III; Figure 2):

- the water-soluble fraction (Pb-$_{Hydro}$, 4–9% of total Pb),
- the exchangeable fraction (Pb-$_{Exch}$, 7–29%of total Pb),
- the fraction bound to iron and manganese oxides (Pb-$_{Oxy}$, 45–52%of total Pb),
- the fraction bound to organic matter (Pb-$_{OM}$, 15–18%of total Pb),
- the residual fraction (Pb-$_{Resi}$, 2–24%of total Pb).

With increasing soil Pb content, most fractions except the water-soluble and residual fractions showed an increase in relative Pb content.

The exchangeable fraction rose from 7.46% under Soil $_{Pb0}$ to 28.83% under Soil $_{Pb250}$ and 19.73% under Soil $_{Pb500}$. The oxide-bound fraction increased from 45.24% ($_{Soil\ Pb0}$) to 46.78% (Soil $_{Pb250}$) and 51.94% (Soil $_{Pb500}$), while the organic-matter-bound fraction rose from 14.60% to 17.85% and 18.27%, respectively. In contrast, the residual fraction was higher under Soil $_{Pb0}$ (23.5%) than under Soil $_{Pb250}$ (2.49%) or Soil $_{Pb500}$ (2.07%), and the water-soluble fraction declined from 9.12% (Soil $_{Pb0}$) to 4.04% (Soil $_{Pb250}$) and 8% (Soil $_{Pb500}$) (Figure 2).

Overall, lead distribution followed the order (Table III):

- Soil $_{Pb0}$: Pb-$_{Oxy}$ ≥ Pb-$_{Resi}$ ≥ Pb-$_{OM}$≥ Pb-$_{Hydro}$ ≥ Pb-$_{Exch}$
- Soil $_{Pb250}$: Pb-$_{Oxy}$ ≥ Pb-$_{Exch}$ ≥ Pb-$_{OM}$≥ Pb-$_{Hydro}$ ≥ Pb-$_{Resi}$
- Soil $_{Pb500}$: Pb-$_{Oxy}$ ≥ Pb-$_{Exch}$ ≥ Pb-$_{OM}$≥ Pb-$_{Hydro}$ ≥ Pb-$_{Resi}$

These results indicate that, prior to phytoremediation, lead was predominantly associated with the oxidizable fraction.

Table III: Pb content (mg/kg dry soil) in the different fractions of the soils studied before and after contamination with lead acetate ([Pb (CH3COOH)2]) and aging for three weeks.

Fractions	Soil $_{Pb0}$	Soil $_{Pb250}$	Soil $_{Pb500}$
Total Pb	15,96	243,46	494,6
Pb-$_{Hydro}$ (Hydrosoluble)	1,5 (9.12%)	9,8 (4.04%)	39,5 (8%)
Pb-$_{Exch}$ (Exchangeable)	1,2 (7.46%)	70,2 (28.83%)	97,6 (19.73%)
Pb-$_{oxy}$ (oxydable)	7,2 (45.24%)	113,9 (46.78%)	256,9 (51.94%)
Pb-$_{OM}$(OrganicMatter)	2,3 (14.6%)	43,5 (17.85%)	90,4 (18.27%)
Pb-$_{Resi}$ (Residual)	3,8 (23.5%)	6,1 (2.49%)	10,3 (2.07%)

Soil$_{Pb0}$: Non-contaminated soil; Soil$_{Pb250}$:Soil contaminated with 250 lead; Soil$_{Pb500}$: Soil contaminated with 500 lead.

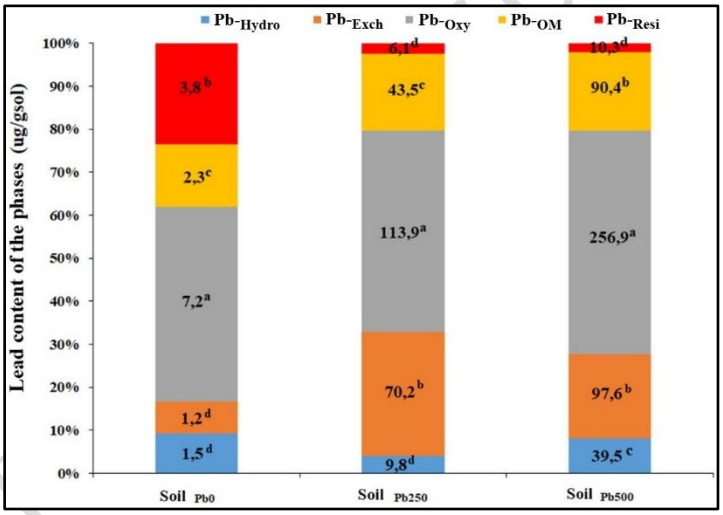


Figure 2: Fractionation of lead (Pb) before phytoremediation.

Soil$_{Pb0}$: Non-contaminated soil; Soil$_{Pb250}$:soil contaminated with 250 lead; Soil$_{Pb500}$:soil contaminated with 500 lead. Pb-$_{Hydro}$:water-soluble form; Pb-$_{Exch}$:exchangeable form; Pb-$_{Oxy}$:iron and manganese oxides bound form; Pb-$_{OM}$:organic matterbound form(OM); Pb-$_{Resi}$:residual form. The histograms followed by the same letter (a, b, c, d) are not significantly different according to the Newman-Keuls Student's test at<Pr 0,05.

3.2. Chemical characteristics of soils after artificial lead contamination and after phytoremediation

A slight increase in pH was observed after five months of phytoremediation in both inoculated and non-inoculated treatments, regardless of initial Pb content (Table IV): pH rose from 6.43 to

6.61 under Soil $_{Pb0}$, from 6.38 to 6.53 under Soil $_{Pb250}$, and from 6.44 to 6.68 under Soil $_{Pb500}$. This increase was more pronounced in the presence of IMF. Furthermore, nitrogen (N) and phosphorus (P) levels increased more markedly in the presence of the indigenous mycorrhizal fungi (IMF) than in the non-inoculated treatments (Table IV), demonstrating a beneficial effect of IMF on the availability of nitrogen and plant-assimilable phosphorus.

Organic matter (OM) content and cation exchange capacity (CEC) likewise increased slightly after five months of phytoremediation, irrespective of inoculation status or soil Pb content: OM rose from 30,400-30,700 mg/kg to 30,900-32,800 mg/kg, and CEC from 9.18-10.09 cmol/kg to 10.14-11.3 cmol/kg. This increase, however, was more pronounced in IMF-inoculated soils (Table IV).

Table IV: Evolution of soil chemical characteristics after five months of phytoremediation of lead-acetate-contaminated soils.

Soil types	Treatments	pH_{water}	N (mg/kg)	P_{assi} (mg/kg)	OM (mg/kg)	CEC (cmol/kg)
Soil $_{Pb0}$	AV phyto	6,43	730	98	30600	9,18
	AP phyto NI	6,5	820	124,25	31600	10,14
	AP phyto I	6,61	870	132,5	32800	11,01
Soil $_{Pb250}$	AV phyto	6,38	780	85,75	30700	10,09
	AP phyto NI	6,48	880	105	31000	11,2
	AP phyto I	6,53	880	117,5	31500	11,3
Soil $_{Pb500}$	AV phyto	6,44	840	93,25	30400	9,85
	AP phyto NI	6,57	910	105	30900	11,02
	AP phyto I	6,68	920	122,5	31800	11,16

pH: hydrogen potential; N: nitrogen; Passi: assimilable phosphorus; OM: organic matter; CEC: cation exchange capacity; Pb: lead; I: Inoculated; NI: non-inoculated; AV phyto: Before phytoremediation; AP phyto: After phytoremediation.

The total Pb content of the soils decreased markedly after phytoremediation (Table V). Under Soil $_{Pb0}$, Pb content fell from 15.96 mg/kg to 3.83 mg/kg without IMF and 2.89 mg/kg with IMF.

Under Soil $_{Pb250}$, Pb content declined from 243.46 mg/kg to 27.56 mg/kg without IMF and 12.92 mg/kg with IMF. Under Soil $_{Pb500}$, Pb content dropped from 494.55 mg/kg to 52.25 mg/kg without IMF and 18.84 mg/kg with IMF.

Extraction rates in the absence of IMF were 76%, 88.7%, and 89.4% under Soil $_{Pb0}$, Soil $_{Pb250}$, and Soil $_{Pb500}$, respectively, relative to the initial total Pb content (Figure 3). In the presence of IMF, extraction rates rose to 82%, 94.7%, and 96.2%, respectively. These differences were statistically significant ($P < 0.05$, SNK test), confirming that inoculation with IMF significantly enhances lead extraction from these soils.

Table V: Evolution of lead content (mg/kg dry soil) in lead-acetate-contaminated soils after five months of phytoremediation.

Treatments	**Soil types**		
	Soil $_{Pb0}$	**Soil $_{Pb250}$**	**Soil $_{Pb500}$**
AV phyto	15,96[a]	243,46 [a]	494,6 [a]
AP phyto NI	3,83 [b]	27,56 [b]	52,25 [b]
AP phyto I	2,89 [b]	12,92 [c]	18,84 [c]

I: Inoculated; NI: non-inoculated; AV phyto: Before phytoremediation; AP phyto: After phytoremediation.

In the same column, the numbers followed by the same letter (a, b) are not significantly different according to the Newman-Keuls Student's test at Pr< 0.05.

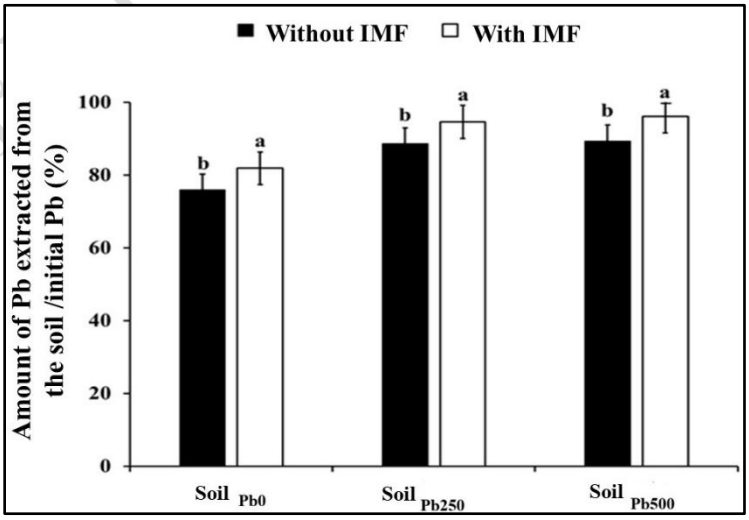


Figure 3: Proportion of Pb extracted from the soil (%) after phytoremediation, relative to the

initial Pb content.

Soil $_{Pb0}$:Non-contaminated soil; Soil $_{Pb250}$:Soil contaminated with lead 250 mg/kg; Soil $_{Pb500}$:Soil contaminated with lead 500 mg/kg; AMF: Indigenous arbuscular mycorrhizal fungi. The histograms followed by the same letter (a, b) are not significantly different according to the Newman-Keuls Student's test at Pr< 0.05.

3.3. Evolution of Lead Speciation Following Phytoremediation

Overall, the comparison of the lead content of the fractions before phytoremediation, in the absence and presence of IMFafter phytoremediation, shows that lead (mg of Pb per kg of dry soil) is retained in the following order:

- Under Soil $_{Pb0}$
  - Before phytoremediation: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{Resi} \geq Pb\text{-}_{OM} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Exch}$
  - After phytoremediation, without MIC: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{Resi} \geq Pb\text{-}_{OM} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Exch}$
  - After phytoremediation, with MIC: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{Resi} \geq Pb\text{-}_{MO} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Exch}$

Under the Soil $_{Pb0}$, lead is more retained in the oxydable fraction [Av phyto (7.22), Ap phyto without IMF (2.3), and Ap phyto with IMF (1.69)], followed by the residual fraction [Av phyto (3.75), Ap phyto without IMF (1.16), and Ap phyto with IMF (0.87)] and bound to organic matter [Av phyto (2.33), Ap phyto without IMF (0.32), and Ap phyto with IMF (0.26)]. On the other hand, low levels were observed in the exchangeable fractions [Av phyto (1.19), Ap phyto without IMF (0.01), and Ap phyto with IMF (0.02)] and water-soluble fractions [Av phyto (1.46), Ap phyto without IMF (0.04), and Ap phyto with IMF (0.04)]. We observe a similar trend in the distribution of lead across all fractions (Table VI).

- Under Soil $_{Pb250}$
  - Before phytoremediation: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{Exch} \geq Pb\text{-}_{OM} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Resi}$
  - After phytoremediation, without MIC: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{OM} \geq Pb\text{-}_{Resi} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Exch}$
  - After phytoremediation, with MIC: $Pb\text{-}_{Resi} \geq Pb\text{-}_{Oxy} \geq Pb\text{-}_{OM} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Exch}$

Under the Soil $_{Pb250}$, a variation in speciation is noted before and after phytoremediation.

- Before phytoremediation, lead is more retained in the oxydable fraction (113.9), followed by the exchangeable fraction (70.2), and then bound to organic matter (43.46). The lowest concentrations were observed in the hydrosoluble (9.84) and residual (6.06) fractions.
- After phytoremediation and in the absence of MIC, our results indicate high levels in the oxydable fraction (8.48), followed by the fraction bound to organic matter (6.4) and then

the residual fraction (5.86). The lowest concentrations were observed in the water-soluble fraction (3.44) and the exchangeable fraction (3.38).

- After phytoremediation and in the presence of MIC, high levels are observed in the residual fraction (4.97), followed by the oxydable fraction (4.71) and then the fraction bound to organic matter (3.04). The water-soluble (0.13) and exchangeable (0.07) fractions show the lowest concentrations (Table VI).

➢ Under the $Soil_{Pb500}$

- Before phytoremediation: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{Exch} \geq Pb\text{-}_{OM} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Resi}$
- After phytoremediation, without MIC: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{Exch} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{Resi} \geq Pb\text{-}_{OM}$
- After phytoremediation, with MIC: $Pb\text{-}_{Oxy} \geq Pb\text{-}_{Exch} \geq Pb\text{-}_{Resi} \geq Pb\text{-}_{Hydro} \geq Pb\text{-}_{OM}$

Under the soil Pb500, lead is more retained in theoxydablefraction [Av phyto (256.85), Ap phyto without IMF (14.07), and Ap phyto with IMF (7.93)], followed by the exchangeable fraction [Av phyto (97.55), Ap phyto without IMF (12), and Ap phyto with IMF (5.05)], then by the fraction bound to organic matter for the soil Av phyto (90.37), the hydrosoluble fraction for the soil and Ap phyto without IMF (7.71), and the residual fraction for the soil Ap phyto with IMF (3.03). However, the lowest concentrations were observed for the Av phyto soil in the residual fraction (10.25) and the hydrosoluble fraction (39.53), for the Ap phyto soil without IMF in the organic matter-bound fraction (6.09) and the residual fraction (6.38), and for the Ap phyto soil with IMF in the organic matter-bound fraction (0.83) and the hydrosoluble fraction (1.99) (Table VI).

Table VI: Evolution of lead speciation (mg/kg dry soil) in lead-acetate-contaminated soils after phytoremediation.

Soil types	**Treatments**	**Fractions (mg/kg)**				
		$Pb\text{-}_{Hydro}$	**$Pb\text{-}_{Exch}$**	**$Pb\text{-}_{Oxy}$**	**$Pb\text{-}_{Mo}$**	**$Pb\text{-}_{Resi}$**
Soil $_{Pb0}$	AV phyto	1,46	1,19	7,22	2,33	3,75
	AP phyto NI	0,04	0,01	2,3	0,32	1,16
	AP phyto I	0,04	0,02	1,69	0,26	0,87
Soil $_{Pb250}$	AV phyto	9,84	70,2	113,9	43,46	6,07
	AP phyto NI	3,44	3,38	8,48	6,4	5,86

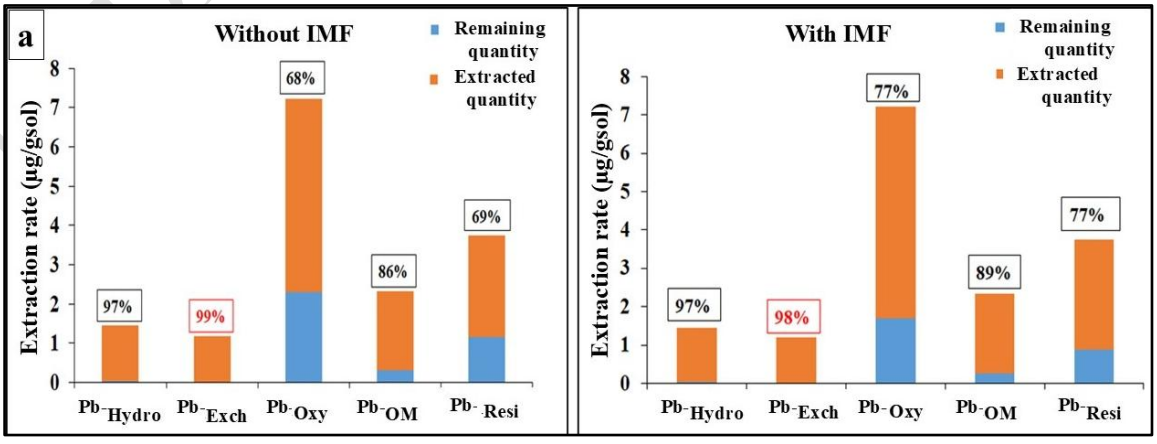
	AP phyto I	0,13	0,07	4,71	3,04	4,97
	AV phyto	39,53	97,55	256,85	90,37	10,25
Soil $_{Pb500}$	AP phyto NI	7,71	12	14,07	6,09	6,38
	AP phyto I	1,99	5,05	7,93	0,83	3,03

Pb-$_{Hydro}$: soluble form; Pb-$_{Exch}$: exchangeable form; Pb-$_{Ox}$: iron and manganese oxides bound form; Pb-$_{MO}$: form bound to organic matter (OM); Pb-$_{Resi}$: residual form. I: Inoculated; NI: non-inoculated; AV phyto: Before phytoremediation; AP phyto: After phytoremediation.

The analysis of the evolution of lead speciation after phytoremediation shows a considerable decrease in the lead content of the different fractions (water-soluble, exchangeable, oxydable, organic matter-bound, and residual) in the studied soils (Soil $_{Pb0}$, Soil $_{Pb250}$ et Soil $_{Pb500}$). Overall, the remaining lead content is higher in the oxydable fraction (associated with iron and manganese oxides) and lower in the presence of the isolated fungal consortium compared to that observed in the absence of the MFC (Table VI).

3.4. Extraction rate (%) of lead content after phytoremediation

After phytoremediation, our results indicate under Soil $_{Pb0}$ an extraction rate of 99% in the absence of IMF and 98% in the presence of IMF in the exchangeable fraction (figure 4.a). Under soil $_{Pb250}$, a 99% extraction rate is also noted in the absence of IMF and in the presence of IMF in the exchangeable fraction (figure 4.b). Under Soil $_{Pb500}$, the extraction rate is 98% in the absence of IMF and 99% in the presence of IMF in the fraction bound to organic matter (Figure 4.c). The lowest extraction rates (less than or equal to 50%) were observed in the residual fraction under soil $_{Pb250}$ in the absence of IMF (53%) and in the presence of IMF (18%) (Figure 4.b), under soil$_{Pb500}$, in the absence of IMF (38%) (Figure 4.c).



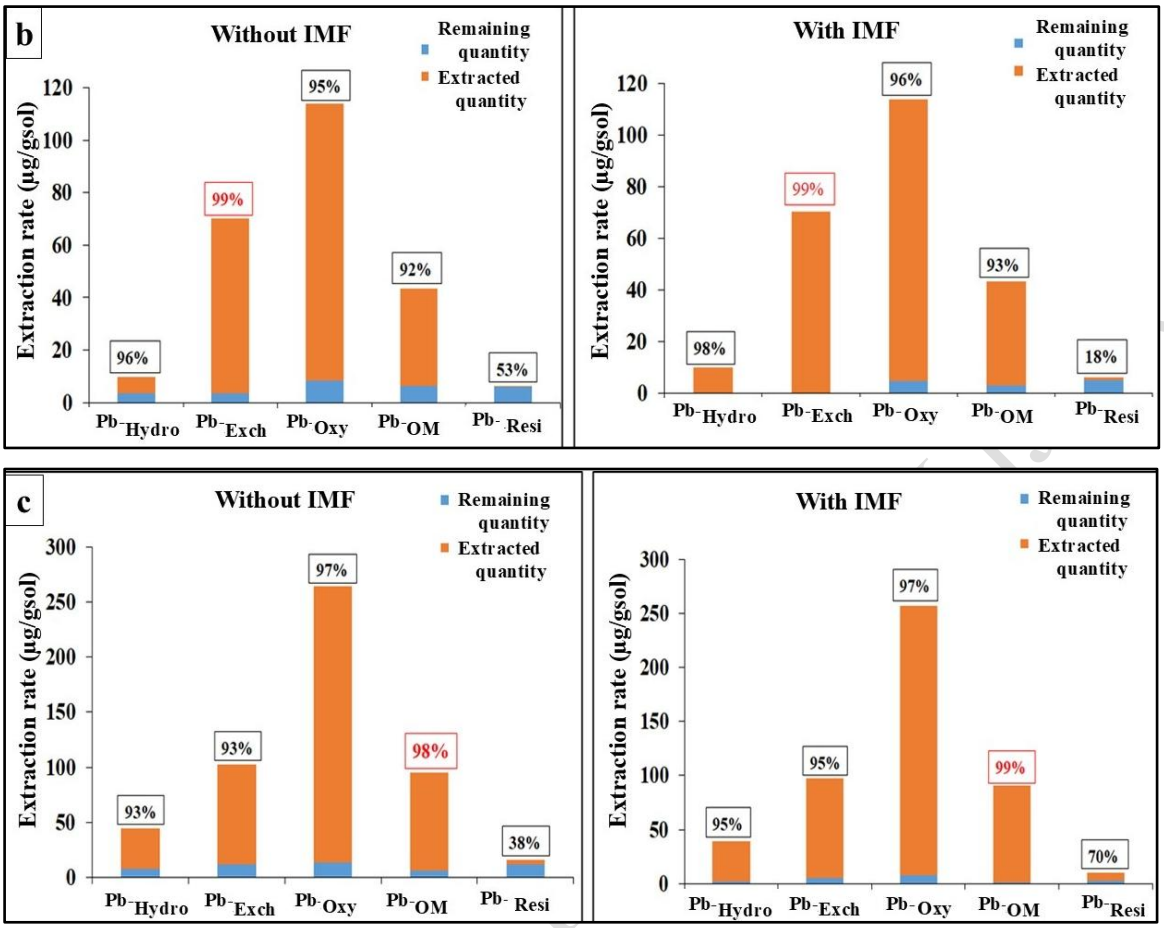


Figure 4: Extraction rate of lead content (µg/gsol) under the different studied soils ($Soil_{Pb0}$ (a), Soil $_{Pb250}$ (b), Soil $_{Pb500}$ (c)) in the absence (Without IMF) and presence (With IMF) of fungal isolates after five months of phytoremediation of the different fractions.

Pb-$_{Hydro}$:water-soluble form; Pb-$_{Exch}$:exchangeable form; Pb-$_{Oxy}$:iron and manganese oxides bound form; Pb-$_{OM}$:organic matter bound form(OM); Pb-$_{Resi}$:residual form; IMF:indigenous mycorrhizal fungi.

4. DISCUSSION

4.1. Soil Characteristics after Phytoremediation

Soil analysis after phytoremediation revealed an increase in pH from 6.4 to 6.7, attributable to the effect of indigenous mycorrhizal fungi (IMF) and reflecting their influence on reducing soil acidity. Comparable results were reported by Ndonda, Mahungu [12], who observed a pH increase from 4.6 to 6.3 following AMF inoculation. Such an increase would tend to promote the immobilization of trace metal elements through the formation of insoluble compounds and the expansion of exchange sites available for their retention[13]. This is consistent with the decrease in lead content observed in the easily mobilizable fractions (Pb-$_{Hydr}$o, Pb-$_{Exch}$) and the

corresponding increase in the residual fraction in IMF-inoculated soils, particularly under Soil Pb_{250}, suggesting that a portion of the lead not taken up by the plant becomes stabilized in a more recalcitrant, less available form.

A pH between 6.6 and 7.2 is generally considered favorable for microbial activity and, by extension, for nutrient bioavailability [14, 15]. And our results indicate an increase in the nitrogen rate after experimentation with a perceptible effect of the MIC on the non-polluted soil ($Soil_{Pb0I}$) and the soil polluted with 500 lead ($Soil_{Pb500I}$) compared to the soil polluted with 250 lead ($Soil_{Pb250}$). The effect of PGPR on the soils ($Soil_{Pb0I}$, $Soil_{Pb500I}$) could be related to the action of PGPR that contribute to the mobilization of nitrogen[12].Indeed, arbuscular mycorrhizal fungi play an important role in the nitrogen cycle by facilitating the acquisition and transfer of this element to plants, particularly through the extraradical mycelial network[16-18]. Lazcano, Barrios-Masias [19]also showed that the symbiosis of AMF with plant roots contributed to the availability of nitrogen in the soil. It appears that when the AMF inoculum is added, the nitrogen concentration in the soil is modified[12].

Moreover, it is observed that the levels of assimilable phosphorus (Passi) under the inoculated treatments ($Soil_{Pb0I}$, $Soil_{Pb250I}$, $Soil_{Pb500I}$) are higher compared to the non-inoculated treatments ($Soil_{Pb0NI}$, $Soil_{Pb250NI}$, $Soil_{Pb500NI}$). Our results show a favorable effect of PGPR on the availability of assimilable phosphorus for plants. This positive impact of AMF on the availability of P in the soil would be attributed to their ability to solubilize complexed phosphorus in the soil. Grant, Mortimer [20]reported that association with arbuscular mycorrhizae improves phosphorus uptake in several crop species, and Aboubacar, Ousmane [21]achieved a 51% increase in cowpea grain yield following co-inoculation with mycorrhizal fungi, attributed to enhanced soil phosphorus availability. Ndonda, Mahungu [12], similarly reported a 7.5% increase in soil phosphorus content following AMF inoculation, and concluded that local AMF isolates could be multiplied and formulated as inoculants to mobilize soil phosphorus for plant uptake. Salvioli, Novero [22]had earlier recognized the potential of AMF as biofertilizers for vegetable crops.

Organic matter (OM) content increased overall following the experiment, most markedly in the non-polluted soil ($Soil_{Pb0}$), likely reflecting the accumulation of dead leaf and root residues during and after cultivation.Similar enrichment in organic matter following phytoremediation was reported by Belouchrani, Abdi [23],who attributed it to root residue biomass. The increase in OM may also be linked to the production of glomalin, a glycoprotein secreted abundantly by AMF

and recognized as a stable component of the soil organic matter pool[24].

Regarding cation exchange capacity (CEC), higher values were observed under soils with greater lead concentration, irrespective of inoculation status. These findings are consistent with those of Huynh [10], who similarly reported significantly higher CEC values in Pb-treated soils. The relative increase in CEC observed under IMF inoculation may be related to the incorporation of fungal biomass into the soil, which would increase the density of negative charges and, consequently, the cation exchange capacity of the soil [25, 26].

4.2. Effect of inoculation with indigenous mycorrhizal fungi on the fractionation of lead in the solid phases of lead after 5 months of cultivation

Sequential extraction enables estimation of pollutant localization and the potential mobility of heavy metals in soil [27]. The present study examined the distribution of lead among solid fractions of artificially polluted soils from the M'Ploussoué landfill following phytoremediation.

Our results show a consistently higher extraction rate of lead in the presence of IMF, indicating that inoculation promotes lead extraction, most likely through fungal-mediated mobilization or remobilization of lead in the rhizosphere. These findings are consistent with those of Gadd [28], who suggested that the metabolic activity of the fungal consortium would promote the complexation and local mobilization of lead. Moreover, various researchers have reported that the native microbiota often proves to be a better ally for phytoremediation than the introduced one [29-31].

Comparison of extraction rates among soils showed that, under Soil $_{Pb0}$ and Soil $_{Pb250}$, the highest extraction rates occurred in the exchangeable fraction (Pb-$_{Exch}$), indicating limited lead availability in this fraction under the experimental conditions, consistent with the generally low absolute Pb content recorded there. These results align with those ofBodjona, Tchegueni [32], who likewise observed a low proportion of lead in the exchangeable and acid-soluble fractions and concluded that the mobile, exchangeable portion of lead is closely linked to carbonate fractions and therefore less bioavailable overall[33]. Under Soil $_{Pb500}$, by contrast, the highest extraction rates occurred in the fractions bound to organic matter and to iron and manganese oxides. These findings are consistent with those ofRaskin and Ensley [34]and [35], who identified organic matter as an important sink for lead accumulation in polluted soils. Qasim [35], further reported a strong association of lead and copper with the iron- and manganese-oxide-

bound fraction, indicating a high affinity for this fraction and, consequently, a potentially greater environmental risk should these metals become mobile.

Across treatments, the amount of lead extracted or remaining was consistently highest in the fraction bound to iron and manganese oxides, supporting a strong affinity of lead for these oxides and suggesting reduced plant uptake in their presence. This is consistent with the findings ofDiallo [36], who similarly reported a high affinity of lead for iron oxides, and with those ofHodomihou [37], who described lead as predominantly present in stable forms bound to organic matter and to oxides and hydroxides. Bodjona, Tchegueni [32]likewise found a strong tendency for lead to associate with the oxide-bound and residual fractions,while Baize [38]reported a non-uniform distribution of lead in soil profiles, with strong association to iron and manganese hydroxides. Several authors have similarly noted that lead is primarily bound to clays, oxides, hydroxides, and organic matter, becoming mobile mainly when soluble organic complexes form or when the soil's adsorption capacity for lead is exceeded[34, 39].

More broadly, the availability of a given heavy metal is influenced by edaphic properties, particularly soil pH[40-42]. which appears to be the most decisive factor governing metal mobility. A decrease in pH tends to enhance the mobility of trace metals, notably through the dissolution of metal salts or the breakdown of retention phases, whereas an increase in pH promotes immobilization via the formation of insoluble compounds or an increase in cation exchange capacity[13].

In the present study, the slightly acidic to neutral pH observed (6.4-6.6) is likely unfavorable to lead bioavailability, consistent with the low proportions recorded in the easily mobilizable exchangeable and water-soluble fractions. These results are consistent with those ofDiallo [36], who similarly reported low lead concentrations at pH values between 4.9 and 6.4, and with those ofAhmadipour, Bahramifar [43]who reported low proportions (9%) of lead in the soluble and exchangeable fractions. Overall, these findings support the view that lead becomes relatively immobile in soils with pH values above 6.5 5 [33].

4.3. Possible mechanism of lead mobilization by indigenous mycorrhizal fungi (IMF)

The mobilization of lead by IMF may be attributed to the extensive extraradical mycelial network of arbuscular mycorrhizal fungi, which substantially increases the volume of soil explored and the contact surface between mycelium and soil particles, thereby facilitating access to lead trapped within soil micropores[44, 45]. Mycorrhizal hyphae are also known to exude low-

molecular-weight organic acids (oxalate, citrate, malate) and siderophores capable of complexing and solubilizing metals bound to mineral phases, particularly iron and manganese oxy-hydroxides[28]. In our study, this localized solubilization likely accounts for the more pronounced decrease in Pb content within the oxidizable fraction observed in IMF-inoculated soils.

In addition, glomalin a glycoprotein secreted by AMF is recognized for its capacity to sequester trace metal elements at the hyphal surface and within the rhizosphere, thereby limiting their free diffusion while facilitating their localized uptake by fungal structures and, indirectly, by the host plant[24]. This hyphal sequestration mechanism, combined with the increase in root absorption surface associated with mycorrhizal symbiosis [46], may explain both the overall increase in Pb extraction from the soil and the preferential accumulation of lead previously reported in the root tissues of *Acacia mangium* under AMF inoculation. Together, these findings underscore the value of incorporating an indigenous fungal consortium, already adapted to the metal-stress conditions of the site, into assisted phytoremediation strategies.

5. Conclusion

The study of the impact of the isolated indigenous fungal consortium (IFC) on the fractionation of lead during the phytoremediation of artificially lead-contaminated landfill soils showed a positive effect of the presence of IFC on the evolution of the characteristics (pH, N, C, OM, CEC, Passi) of the studied soils and consequently on the availability of lead.

A notable effect of the indigenous fungal isolate was also observed on the transfer and accumulation of lead in the soil-plant system, as well as on its extraction and distribution in the different soil fractions. These results suggest that indigenous mycorrhizal fungi play an important role in the dynamics and mobilization of lead during the phytoremediation process. Indigenous arbuscular mycorrhizal fungi could therefore be considered a promising biological approach to improve the rehabilitation and biorestoration processes of lead-contaminated sites, particularly landfill sites.

Acknowledgements

We sincerely thank the Education and Research Ministry of the Ivory Coast, as part of the Debt Reduction Development Contracts (C2Ds) managed by IRD, for the financial support rendered to carry out the projection ReSiPol research. We also thank Bonoua City Hall for permission to carry out the studies at the dumpsites.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Référencesbibliographiques

1. Bongoua-Devisme, A., et al., *Hazardous Impacts of Open Dumpsite of Municipal Solid Wastes on Soil: Case of M'Ploussoue Park Dump at Bonoua in Ivory Coast.* International Journal of Sciences, 2018. **7**(05): p. 32–38.
2. Alloway, B.J., *Heavy metals in soils: trace metals and metalloids in soils and their bioavailability*. 2012: Springer Science & Business Media.
3. Tchounwou, P.B., et al., *Heavy metal toxicity and the environment.* Molecular, clinical and environmental toxicology: volume 3: environmental toxicology, 2012: p. 133–164.
4. Flora, G., D. Gupta, and A. Tiwari, *Toxicity of lead: a review with recent updates.* Interdisciplinary toxicology, 2012. **5**(2): p. 47–58.
5. Wuana, R.A. and F.E. Okieimen, *Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation.* International Scholarly Research Notices, 2011. **2011**(1): p. 402647.
6. Ali, H., E. Khan, and M.A. Sajad, *Phytoremediation of heavy metals—Concepts and applications.* Chemosphere, 2013. **91**(7): p. 869–881.
7. Bongoua-Devisme, A., et al., *Response of Acacia mangium to inoculation with symbiotic microorganisms in the remediation of metal-contaminated soil at Bonoua, Ivory Coast.*Journal of Chemical, Biological and Physical Sciences, 2019. **9**(4): p. 544–560.
8. Kirpichtchikova, T., *Phytoremédiation par Jardins Filtrants d'un sol pollué par des métaux lourds: Approche de la phytoremédiation dans des casiers végétalisés par des plantes de milieux humides et étude des mécanismes de remobilisation/immobilisation du zinc et du cuivre*. 2009, Université Joseph-Fourier-Grenoble I.
9. Redon, P.-O., *Rôle de champignons mycorhiziens à arbuscules dans le transfert du cadmium (Cd) du sol à la luzerne (Medicago truncatula).* Science des sols. Université Henri Poincaré–Nancy, 2009.
10. Huynh, T., *Impact des métaux lourds sur les interactions plante/ver de terre/microflore tellurique (Thèse de Doctorat).* Université Paris-Est, France. 170p, 2009.
11. Chave, M., et al., *Fiche technique (2): Multiplier des champignons mycorhiziens sur son*

exploitation. 2017.

12. Ndonda, A., et al., *Effet des champignons mycorhiziens Arbusculaires sur le phosphore des sols tropicaux et implication dans la biosynthèse du caroténoïde du manioc.* 2019.
13. Perrono, P., *Les micro-polluants métalliques et les sols amendés par des boues résiduaires urbaines.*Mémoire DUESS, Université de Picardie, Amiens, 1999.
14. Rousk, J., P.C. Brookes, and E. Bååth, *The microbial PLFA composition as affected by pH in an arable soil.* Soil Biology and Biochemistry, 2010. **42**(3): p. 516–520.
15. Fierer, N. and R.B. Jackson, *The diversity and biogeography of soil bacterial communities.* Proceedings of the National Academy of Sciences, 2006. **103**(3): p. 626–631.
16. Smith, S.E. and D. Read, *Mycorrhizal symbiosis*. 2008: Elsevier.
17. Hodge, A., C.D. Campbell, and A.H. Fitter, *An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material.* Nature, 2001. **413**(6853): p. 297–299.
18. Veresoglou, S.D., B. Chen, and M.C. Rillig, *Arbuscular mycorrhiza and soil nitrogen cycling.* Soil Biology and Biochemistry, 2012. **46**: p. 53–62.
19. Lazcano, C., F.H. Barrios-Masias, and L.E. Jackson, *Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes.* Soil Biology and Biochemistry, 2014. **74**: p. 184–192.
20. Grant, S., et al., *Facilitated transport of dioxins in soil following unintentional release of pesticide-surfactant formulations.*Environmental science & technology, 2011. **45**(2): p. 406–411.
21. Aboubacar, K., et al., *Effet de la co-inoculation du rhizobium et de mycorhizes sur les performances agronomiques du niébé [Vigna unguiculata (L.) Walp.] au Niger.* Journal of applied biosciences, 2013. **72**(1): p. 5846.
22. Salvioli, A., et al., *The impact of mycorrhizal symbiosis on tomato fruit quality.*2008.
23. Belouchrani, A., et al., *Etude expérimentale de la phytoremédiation dans la restauration d'un sol pollué par les métaux lourds (cas du zinc)*. 2014.
24. Gonzalez-Chavez, M., et al., *The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements.* Environmental pollution, 2004. **130**(3): p. 317–323.

25. Hernández-Ortega, H.A., et al., *Arbuscular mycorrhizal fungi on growth, nutrient status, and total antioxidant activity of Melilotus albus during phytoremediation of a diesel-contaminated substrate.* Journal of Environmental Management, 2012. **95**: p. S319–S324.
26. Yang, Y., et al., *The effects of arbuscular mycorrhizal fungi on glomalin-related soil protein distribution, aggregate stability and their relationships with soil properties at different soil depths in lead-zinc contaminated area.*PloS one, 2017. **12**(8): p. e0182264.
27. El Ass, K., A. Laachach, and M. Azzi, *Étude de la biodisponibilité des métaux lourds dans des sols agricoles irrigués par des eaux polluées.*Environnement, Ingénierie & Développement, 2003.
28. Gadd, G.M., *Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation.* Mycological research, 2007. **111**(1): p. 3–49.
29. Baum, C., et al., *Heavy-metal mobilization and uptake by mycorrhizal and nonmycorrhizal willows (Salix× dasyclados).* Journal of Plant Nutrition and Soil Science, 2006. **169**(4): p. 516–522.
30. Kidd, P., et al., *Trace element behaviour at the root–soil interface: implications in phytoremediation.* Environmental and Experimental Botany, 2009. **67**(1): p. 243–259.
31. Sessitsch, A., et al., *The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils.*Soil Biology and Biochemistry, 2013. **60**: p. 182–194.
32. Bodjona, B.M., et al., *Extraction chimique des métaux lourds des argiles de la décharge finale d'Agoè-Nyivé au Togo.*Environnement, Ingénierie & Développement, 2018.
33. Rahman, M.M., S.M. Azirun, and A.N. Boyce, *Enhanced accumulation of copper and lead in amaranth (Amaranthus paniculatus), Indian mustard (Brassica juncea) and sunflower (Helianthus annuus).* PloS one, 2013. **8**(5): p. e62941.
34. Raskin, I. and B.D. Ensley, *Phytoremediation of toxic metals*. 2000.
35. Qasim, B.H., *Détermination, spéciation et biodisponibilité des éléments traces métalliques dans les sols contaminés et technosols*. 2015, Université d'Orléans.
36. Diallo, B.A., *Fractions chimiques du plomb dans le sol et phyto-extraction du plomb par le saule (Salix miyabeana) cultivé dans un sol calcaire riche en éléments traces métalliques traité avec des amendements acides.* 2019.

37. Hodomihou, R., *Comment concilier l'intensification de l'agriculture périurbaine à l'aide des intrants organiques et la préservation durable des agrosystèmes tropicaux?* 2016.
38. Baize, D., *Teneurs totales en éléments traces métalliques dans les sols (France): Références et stratégies d'interprétation. Programme ASPITET*. 1997.
39. Morlot, M., *"AGHTM Aspects analytiques du plomb dans environnement ".* lavoisier TEC&amp, 1996.
40. Kabata-Pendias, A., H. Pendias, and H. Pendias, *Trace elements in soils and plants*. Vol. 4. 2011: CRC press London.
41. Cui, J., et al., *Influence of soil pH and organic carbon content on the bioaccessibility of lead and copper in four spiked soils.* Environmental Pollution, 2024. **360**: p. 124686.
42. Bravo, S., et al., *Influence of the soil pH in the uptake and bioaccumulation of heavy metals (Fe, Zn, Cu, Pb and Mn) and other elements (Ca, K, Al, Sr and Ba) in vine leaves, Castilla-La Mancha (Spain).* Journal of Geochemical Exploration, 2017. **174**: p. 79–83.
43. Ahmadipour, F., N. Bahramifar, and S. Mahmood Ghasempouri, *Fractionation and mobility of cadmium and lead in soils of Amol area in Iran, using the modified BCR sequential extraction method.* Chemical Speciation & Bioavailability, 2014. **26**(1): p. 31–36.
44. Khan, A.G., *Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation.* Journal of trace Elements in Medicine and Biology, 2005. **18**(4): p. 355–364.
45. Miransari, M., *Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals.* Biotechnology advances, 2011. **29**(6): p. 645–653.
46. Joner, E. and C. Leyval, *Uptake of 109Cd by roots and hyphae of a Glomus mosseae/Trifolium subterraneum mycorrhiza from soil amended with high and low concentrations of cadmium.* The New Phytologist, 1997. **135**(2): p. 353–360.