Journal of Experimental Biology and Agricultural Sciences, December - 2022; Volume – 10(6) page 1391 – 1400

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Phytoremediation study of mining soils: case of the Mibladen and Zaida mine (High Moulouya, Morocco)

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Received – June 22, 2022; Revision – October 16, 2022; Accepted – December 09, 2022
Available Online – December 31, 2022

DOI: http://dx.doi.org/10.18006/2022.10(6).1391.1400

KEYWORDS

Metallic trace elements

Phytoremediation

Cadmium

Plomb

Lolium multiflorum

ABSTRACT

The Upper Moulouya region, including the Mibladen (M) and Zaïda (Z) mines, is one of the highest lead (Pb) deposit areas in Morocco. These mines, abandoned without any measure of rehabilitation, constitute the main source of soil pollution by Metallic Trace Elements (MTEs) accumulation in the region. In this study, two greenhouse phytoremediation experiments (for the Mibladen and Zaida sites) were set up using Italian ryegrass (*Lolium multiflorum*) specie to assess its capacity and ability to remediate soils contaminated by zinc (Zn), cadmium (Cd), copper (Cu), and Pb. For both experiments, various factors including (i) three substrates (waste treatment [Wt]; clay uncovering [Cun]; and unpolluted control soil [Ucs]) and (ii) three treatments (no treatment, treatment with organic matter, and treatment with chemical fertilizers) were studied. The results before planting indicated that Wt substrates had poorer physicochemical properties than those of Cun, thus they are the most exposed to the degradation phenomena. This is confirmed by pollution index (PI) results that revealed the trend of PI (Z_{Wt})> PI (M_{wt})> PI (M_{Cun})> PI (Z_{Cun})> PI (Z_{Ucs})> PI (M_{Ucs}). The results of experiments indicated that ryegrass crops can grow on substrates contaminated with MTEs. Depending on the applied fertilizers, available metals, and the type of soil, the phytoremediation results showed that *L. multiflorum* can tolerate, hyperaccumulate, and translocate MTEs from polluted substrates. Our findings suggest that this plant can be a solution for remediating alkaline soils polluted by Cd, Pb, Zn, and Cu in Mediterranean conditions.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

1 Introduction

Soil is one of the most important sources for the continuity of life. However, this non-renewable natural resource, once degraded, could not be recovered (Rădoi 2021). Soils are subject to a range of threats such as erosion, loss of organic matter, compaction, acidification, salinization, and contamination (Khan and Al Shoumik 2022). Recent studies have shown that soil pollution is caused by many anthropogenic activities including mining, industrial activities, agriculture, smelting, fossil fuel combustion, and waste disposal (El Aafi 2016; Samsuri et al. 2019). These activities seriously threaten ecosystems and human health. Metallic Trace Elements (MTEs) from mining activity are one cause of soil pollution. Contrary to the organic elements, the MTEs are not biodegradable and accumulate for a long time in the soil. MTEs can also be transferred to human bodies causing serious health issues such as disabilities associated with malnutrition and Alzheimer's disease (Singh et al. 2023).

In Morocco, abandoned mine sites are a source of MTEs pollution (Laghlimi et al. 2022), particularly where no environmental management program has been put in place to reduce the extent of the impact. Located in Midelt city, the Mibladen and Zaïda mines, were the main source of lead mining during the 20th century. These mines were abandoned in 1985 without rehabilitation. Various studies have highlighted the potential risks posed by these mine tailings and the level of soil pollution by MTEs around abandoned sites. However, few research works have focused on the treatment of toxic and hazardous MTEs contamination from these mines using sustainable approaches based on natural processes such as phytoremediation. This last method directly uses green plants based on their ability to intercept, absorb, accumulate, sequester, stabilize, or transfer contaminants. Plants are particularly useful in the bioremediation process by preventing the spread of contaminants through climatic phenomena into nearby areas.

The main aim of this study was to highlight the capacity of Italian ray grass (*Lolium multiflorum*) to depollute the contaminated soils of the Mibladen and Zaïda mines with a focus on Cd, Pb, Zn, and Cu MTEs. The species chosen are grasses, which are generally used to be pioneers and adapted plants for covering polluted substrates.

2 Materials and Methods

2.1 Study areas

The sites chosen for this study are in the Upper Moulouya region, which is a lead mining district and includes the mines of Aouli, Mibladen, and Zaïda. This region contains the largest lead deposit in the country and covers an area of over 300 km^2 (figure 1).

The Zaida and Mibladen mine sites were selected for this study. The Zaïda mine (1490 m altitude; active exploitation was from 1972 to 1985) is located about 30 Km northwest of Midelt city. The temperature ranges from 6 to 36 °C and the mean annual precipitation is about 300 mm.

The mining area of Mibladen (1130 m altitude; exploitation was from 1936 to 1985) is situated 15 km Northeast of Midelt city and covers an area of 60 km^2. There is a foundry at this site, and the vegetation has been completely destroyed (El Aafi 2016). The climates of both sites are arid in nature.

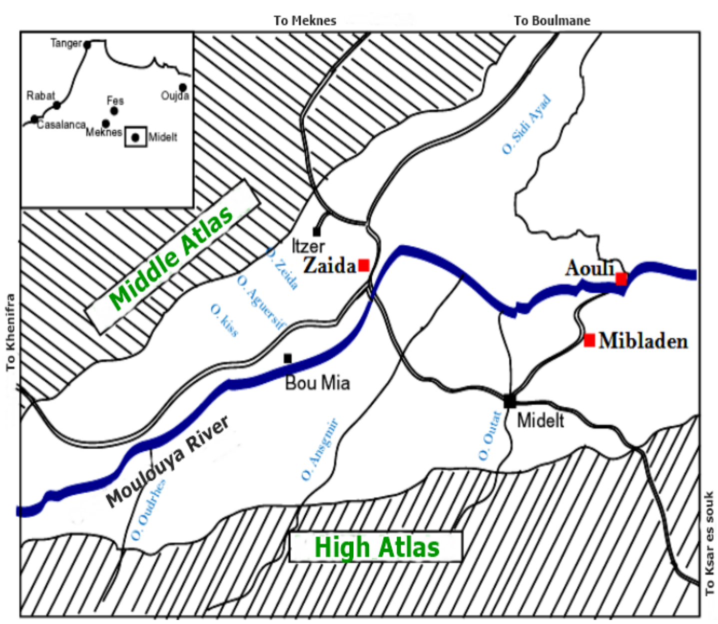

Figure 1 Geographic location of two studied sites Zaïda and Mibladen (El Aafi 2016)

2.2 Soil preparation and analysis

The metal contaminated substrates and unpolluted control soil (Ucs) samples were collected from a depth of 0-20 cm. Waste treatment (Wt) and clay uncovering (Cun) substrates were taken from the sites of Mibladen and Zaïda, while the Ucs was obtained from agricultural soil (uncultivated) of the National Institute of Agricultural Research (INRA) in Rabat, Morocco. Collected samples were taken to the soil laboratory and each one was air-dried, crushed, and sieved through a 2 mm sieve.

All samples were analyzed before planting the Italian ryegrass seeds to determine physicochemical proprieties and quantify MTEs. The physicochemical analysis of soils included the following: granulometry was measured by the Mériaux method (Mériaux 1954); organic matter (OM) was measured using the Walkley and Black method (Walkley and Black 1934); pH was measured using the potentiometric technique (in a soil/water ratios of $\frac{1}{2.5}$); electrical conductivity (EC) was determined with a conductivity meter in a saturated paste of soil $\frac{1}{5}$ (Montoroi 1997); total nitrogen (N) was obtained according to the Kjeldahl method (McGill and Figueiredo 1993); and the assimilable phosphorus (P) and the exchangeable potassium (K) were measured by Olsen et al. (1954) and Quemener (1979) procedures respectively. For MTEs quantification (Cd, Pb, Cu, and Zn), the Atomic Absorption Spectrometer (AAS Varian) method was used (Pinta 1976).

2.3 Greenhouse trials

For the greenhouse study, two experiments (one for the Zaïda site and one for the Mibladen site) were set up at INRA of Rabat. Various factors including (i) three substrates (Wt, Cun, and Ucs) and (ii) three treatments (no treatment, treatment with OM, and treatment with chemical fertilizers) were studied for each experiment. The number of fertilizers applied to the substrates used to sow Italian ryegrass seeds was calculated based on the physicochemical analysis parameters. In this study, four replicates were performed per pot. Fifteen grams per pot of OM along with peat was used for the Mibladen experiment and ovine manure for the Zaïda experiment. In the case of chemical fertilizers, we used 0.16 g/pot of ammonium phosphate and 0.50 g/pot of potassium sulfate for the substrates of Mibladen. For the Zaïda soils, we used 0.11g/pot of ammonium nitrate, 0.12 g/pot of potassium sulfate, and 0.08 g/pot of triple super phosphate. Five and seven seeds were grown in all substrates placed into plastic pots (15 cm × 20 cm, and 20 cm height) for Mibladen and Zaïda experiments respectively. The pots were irrigated three times per week with 200 ml of tap water from INRA. Harvesting time was six weeks for the Mibladen study site and seven weeks for the Zaïda study.

2.4 Plants and soil analysis

The below and the above-ground parts of the plant were cut, separated, and rinsed with distilled water. The fresh weight was measured and then left in the air to dry for four days before being dried in an oven at a temperature of 70 °C for 48 hours to evaluate their dry weight. Rhizospheric soil that adhered to the roots of *L. Multiflorum* was recovered and analyzed to determine the MTEs in substrates after planting. The bioavailability of the MTEs was measured using the AAS Varian method.

2.5 Phytoremediation indices calculation

In this study, two phytoremediation indices were calculated: i) the bioaccumulation factor (BAF) that evaluates the suitability of plants to accumulate MTEs from soil (Eq. 1), and ii) the translocation factor (TF) that shows the efficiency of a plant in transferring MTEs from the below-ground to the above-ground parts (Eq. 2). These indices were calculated using the following equations (Samsuri et al. 2019).

$$\mathrm{BAF}=\frac{[\mathrm{Metal}\ ]\mathrm{inshoot}}{[\mathrm{Metal}\ ]\mathrm{insoil}} \qquad (1)$$

$$\mathrm{TF}=\frac{[\mathrm{Metal}\ ]\mathrm{inshoot}}{[\mathrm{Metal}\ ]\mathrm{inroot}} \qquad (2)$$

2.6 Statistical analysis

All data obtained in this study were analyzed by applying the ANOVA test with a significant level of 5%. The software used in this study was Excel.

3 Results and Discussion

3.1 Physicochemical characteristics of the substrates

Results presented in table 1 showed the different substrate characteristics. Except for unpolluted control soil used for the Mibladen experiment (45.90 % clay), all the studied substrates were poor in clay (ranging from 7.2 to 22.7%) and the dominance of the sandy fraction was reported. For pH, the mining soils were weakly alkaline (pH ranges from 7.83 to 8.51). However, the suitable pH for the growth and development of plants in mining areas is near to neutral pH.

For other soil properties we found that the substrates were very poor in OM contents (ranging from 0.06 to 0.38 %); very low in total nitrogen (<0.1%); and potassium (2.81-54.22 $mg.kg^{-1}$) except Cun substrate of Mibladen site, which it was high in potassium (289.2 $mg.kg^{-1}$); and very low to low in phosphorous (0.39-11.5 $mg.kg^{-1}$ to 15.91$mg.kg^{-1}$). The main parameters (OM and NPK) are generally low for both sites. This could be because of mining activities. In addition, soils without vegetation which can reduce OM and NPK levels in the site soil can suffer the effects of intense

Table1 Basic physicochemical characteristics in the experiment substrates

Parameters	Wt		Cun		Ucs	
	M	Z	M	Z	M	Z
	Avg.± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
pH	8.51 ± 0.42	8.0 9 ± 0.81	7.89 ± 0.71	7.83 ± 0.63	7.37 ± 0.74	7.80 ± 0.70
EC (S/m)	0.033 ± 0.00	0.02 ± 0.00	1.94 ± 0.21	0.40 ± 0.03	0.07 ± 0.00	0.18 ± 0.02
Clay (%)	12.20 ± 0.73	7.20 ± 0.36	22.70 ± 2.27	18.60 ± 1.67	45.90 ± 5.97	10.60 ± 0.85
Silt (%)	15.70 ± 1.41	27.70 ± 2.49	27.30 ± 2.73	29.30 ± 2.64	31.10 ± 1.56	2.60 ± 0.18
Sand (%)	72.10 ± 4.33	65.10 ± 7.16	50.00 ± 2.50	52.10 ± 2.61	23.00 ± 2.07	86.80 ± 8.68
OM (%)	0.06 ± 0.00	0.08 ± 0.00	0.11 ± 0.01	0.38 ± 0.03	0.66 ± 0.04	1.02 ± 0.12
N (mg kg^{-1})	0.00 ± 0.00	56.62 ± 3.40	210.00 ± 14.70	134.92 ± 13.49	900.00 ± 63.00	78.30 ± 7.83
P (mg kg^{-1})	10.80 ± 0.76	0.39 ± 0.02	11.50 ± 1.04	15.91 ± 1.11	110.70 ± 5.54	41.53 ± 2.49
K (mg kg^{-1})	54.23 ± 5.42	2.81 ± 0.20	289.20 ± 17.35	9.24 ± 0.55	704.93 ± 35.25	5.62 ± 0.28

The values represent the mean of four replicates for each parameter. M: Mibladen; Z: Zaïda; Wt: waste treatment, Cun: clay uncovering, Ucs: unpolluted control soil; Avg.: Average; EC: electrical conductivity, OM: organic matter, N: nitrogen, P: available phosphorus and K: exchangeable potassium

erosion and can then be transported and dispersed and reach the water resources and the neighboring soils. Low nutrients in soils, particularly nitrogen and phosphorous, can be a limiting factor for plant growth.

In terms of soil health and physicochemical characteristics, the findings of this work indicated that the waste treatment substrates are poor (more alkaline, poor in clay content, very poor in OM and N, P, and K elements) than those of clay uncovering. These waste treatment soils are therefore the most exposed to degradation phenomena.

3.2 MTEs content before and after cultivation

The concentration of four MTEs, i.e., Cu, Zn, Cd, and Pb from the mining sites were determined to characterize the current MTEs of contaminated substrates and the effect of *L. multiflorum* on the phytoremediation of these MTEs (table 2). The results before planting showed a difference in MTEs concentrations between the tested substrates. Regardless of the site studied, the MTEs were higher in waste treatment compared to clay uncovering as confirmed by pollution index values (5.76 and 7.54 for Wt versus 1.27 and 1.18, for Cun for Mibladen and Zaïda respectively) (Table 3). This is probably attributed to the heterogeneous nature classically met for different mining substrates. A pollution index greater than one means that the soil is considered contaminated (Chon et al., 1998). In the case of this study, the substrates studied have a pollution index greater than one except for the unpolluted control soil, thus, they are all considered contaminated, particularly the waste treatment substrates. The level of pollution index is as $PI_{Z\text{-}Wt} > PI_{M\text{-}Wt} > PI_{M\text{-}Cun} > PI_{Z\text{-}Cun} > PI_{Z\text{-}Ucs} > PI_{M\text{-}Ucs}$. Similar findings were reported by EL Hachimi et al. (2013) who showed that except for the reference station all soil samples taken at the mining sites including those taken at 130 Km, have a PI higher than 1, which confirms the polymetallic contamination of the soils in the downstream area of the mine sites.

The pollution indices of Zaïdasite show that is more contaminated than the Mibladen site. This pollution is due to the very high levels of lead at Zaïda compared to Mibladen (2787.60 vs 1881 $mg.kg^{-1}$ for Wt and 350.04 vs 221.00 $mg.kg^{-1}$ for Cunat the Zaïda and Mibladen sites respectively). Further, the other MTEs (Cd, Cu, and Zn) are rather high in the Mibladen site compared to Zaïda. This finding was the same for both substrates.

From these results, it can be concluded that the plumbiferous deposit of Haut Moulouya of Morocco, which was abandoned without any pollution control or rehabilitation, constitutes an environmental and human hazard, especially when these areas are used for livestock.

Cultivation of Italian ryegrass on polluted substrates collected from both study sites resulted in a decrease in all MTEs concentrations. The greatest reductions in MTEs were achieved due to phytoremediation by the tested plant, Italian ryegrass, in the Mibladen site. This indicates that most metals had the best reductions without fertilizers for both substrates. The exceptions were Pb (for Wt), which had a better reduction when chemical fertilizers were added, and Zn (for Wt) and Cd (for Cun), which had a better reduction when OM was added. Whereas the results obtained for the Zaïda study site showed that apart from Zn of waste treatment (reduction with OM added), the best reductions in MTEs were obtained by the treatment with chemical fertilizers. However, the analysis of variance indicated that the different studied substrates

Table 2 MTEs contents ($mg.kg^{-1}$) in rhizospheric soils, before and after cultivation

MTEs ($mg.kg^{-1}$)	Bowen 1979 standards ($mg.kg^{-1}$)	Soil type	Before experiments		After experiments					
			Initial situation		No treatment		With OM treatment		With chemical Fertilizers treatment	
			M	Z	M	Z	M	Z	M	Z
			Avg.± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Cadmium	0.35	Wt	8.70±0.90	4.00±0.60	6.00±0.40	3.53±0.20	6.95±0.80	2.49±0.10	8.30±0.70	2.53±0.20
		Cun	6.90±0.60	2.00±0.60	6.80±0.50	1.74±0.10	6.00±0.70	1.45±0.40	6.05±0.80	1.02±0.30
		Ucs	5.40±0.70	4.60±0.30	2.50±0.20	2.99±0.70	3.70±0.70	3.03±0.60	4.00±0.50	3.03±0.20
Copper	30.00	Wt	33.00±4.30	30.30±3.60	18.00±1.30	30.23±3.80	24.50±3.40	29.92±2.30	29.50±3.40	20.27±2.30
		Cun	21.00±3.30	20.00±4.00	7.00±0.50	17.40±1.00	19.00±2.80	17.45±0.90	19.50±2.10	12.21±1.60
		Ucs	11.00±1.80	12.20±2.20	5.50±0.40	9.96±1.20	10.50±1.90	10.10±0.50	4.20±0.60	10.09±1.80
Lead	35.00	Wt	1881.00±21.00	2787.60±49.00	1632.00±114.20	2559.05±201.10	1640.00±89.00	2513.45±146.30	928.00±66.70	2442.78±132.00
		Cun	221.00±7.00	350.00±17.00	208.50±14.60	313.20±18.80	214.50±17.70	329.53±19.10	217.20±14.70	274.67±19.20
		Ucs	51.00±2.80	101.40±9.00	47.30±3.30	99.55±7.00	50.50±6.70	80.81±5.10	47.00±3.30	100.89±7.20
Zinc	90.00	Wt	300.00±23.00	191.90±21.00	296.00±20.70	181.35±8.80	265.00±14.70	149.61±8.60	273.50±21.10	131.77±8.50
		Cun	110.00±12.00	100.00±11.00	89.50±6.30	69.60±6.70	98.00±5.70	87.23±9.40	104.50±8.30	81.38±5.20
		Ucs	49.00±3.90	50.70±7.00	46.50±3.30	39.82±4.70	48.00±5.30	30.30±2.30	38.00±3.70	40.36±4.60

M: Mibladen; Z: Zaïda; Avg.: Average; Wt: waste treatment; Cun: clay uncovering; Ucs: unpolluted control soil

Table 3 pollution indices of the substrates

	Pollution index (PI)	
	Mibladen	Zaïda
Waste treatment	5.76	7.54
Clay uncovering	1.27	1.18
Unpolluted control soil	0.65	0.71

have a significant effect on the MTEs concentrations, contrary to fertilizers, which have a non-significant effect.

Generally, the decrease in MTEs content in rhizospheric versus non-rhizospheric substrates could be explained by the consumption of these elements by *L. multiflorum*.

3.3 Effectsof MTEson root and shoot biomass of plant ryegrass

Biomass production is an indicator of a plant's tolerance to different types of substrates. In contact with contaminated soils, plants react differently depending on the variety chosen. In this study, we found that Italian ryegrass can grow on substrates contaminated with MTEs (Figure 2).

When comparing the biomass of harvested plants of the studied substrates with and without added fertilizers, it was reported that the addition of fertilizers increases both root and shoot biomass in all substrates. For the Mibladen experiment, the reduction in root and the shoot biomass for each treatment was reported in the order of $B_{Ucs} > B_{Wt} > B_{Cun}$. Similar findings were reported for the Zaïda experiment when chemical fertilizer was added, while the biomass order (for root and shoot) for the rest of the results under the Zaïda experiment was variable. According to statistical analysis, significant effects of substrates on root and shoot biomass were found in the Mibladen experiment, while the Zaïda experiment showed a non-significant effect. Regarding the fertilizer's effect on the ryegrass biomass, no significant effect was recorded for both experiments but the application of fertilizers increased the biomass

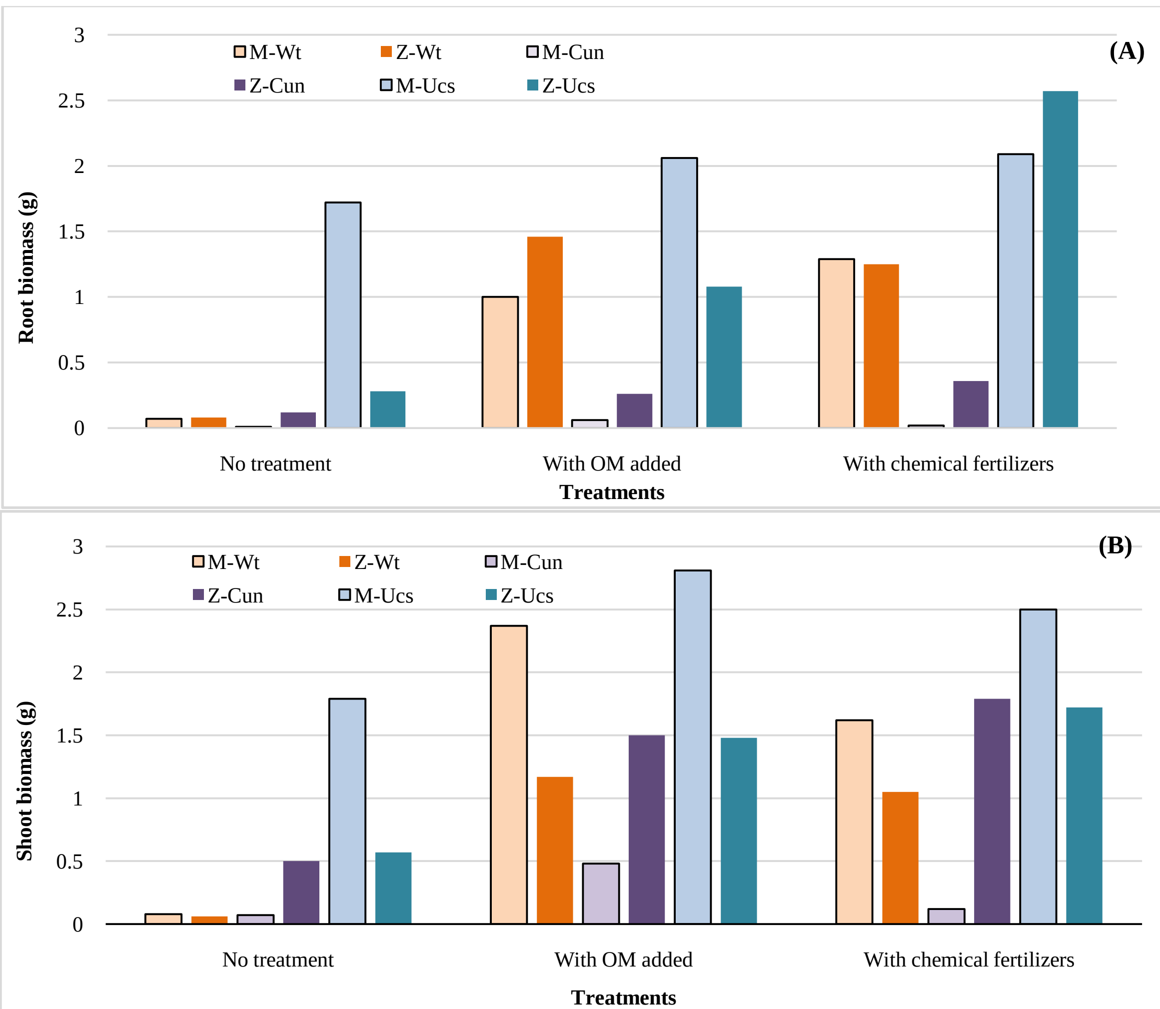

Figure 2 Plant biomass in studied substrates and under different treatments for both experiments; (A) Root biomass, (B) Shoot biomass; M: Mibladen, Z: Zaïda; Wt: waste treatment, Cun: clay uncovering, Ucs: unpolluted control soil

of below- and above-ground parts of the plant. This means that type of substrate affects the biomass of plant organs with and without fertilizers. The difference in these biomass values between substrate types is due to the nutrient contents and organic matter available to the Italian ryegrass plant in each substrate and the structure and texture of each substrate.

3.4 Phytoremediation of MTEs by Italian ryegrass

3.4.1 MTEs content in ryegrass plant organs

The MTEs concentrations in the Italian ryegrass cultivated in the substrates of the contaminated mining sites were measured to

Table 4 MTEs contents in ryegrass roots with treatment effects for both experiments

MTEs (mg.kg^{-1})		No treatment		With OM treatment		With chemical Fertilizers treatment	
		M	Z	M	Z	M	Z
		Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Cadmium	Wt	6.80±0.68	0.03±0.00	8.60±0.77	1.95±0.18	8.70±0.61	1.91±0.11
	Cun	7.00±0.63	0.11±0.01	7.80±0.86	0.11±0.01	6.80±0.34	0.31±0.02
	Ucs	7.90±0.47	0.10±0.01	7.25±0.36	0.62±0.06	7.45±0.60	4.73±0.33
Copper	Wt	27.00±2.97	0.10±0.01	65.50±6.55	0.00±0.00	94.00±4.70	7.63±0.53
	Cun	28.00±1.68	0.53±0.03	16.00±0.80	0.32±0.02	28.00±2.52	0.99±0.10
	Ucs	27.00±1.89	0.56±0.03	36.00±2.88	0.19±0.02	36.50±2.19	1.26±0.15
Lead	Wt	343.00±27.44	0.39±0.02	1570.00±109.90	53.63±5.36	1575.00±110.25	587.66±58.77
	Cun	49.00±4.41	3.05±0.34	100.00±9.00	3.20±0.22	49.00±4.41	5.34±0.32
	Ucs	101.50±10.15	0.56±0.04	66.00±3.96	9.37±0.56	75.00±3.75	157.77±12.62
Zinc	Wt	120.00±8.40	0.49±0.04	830.00±83.00	53.63±2.68	730.00±73.00	232.78±20.95
	Cun	79.00±7.90	3.05±0.31	530.00±63.60	0.29±0.02	79.00±5.53	5.34±0.53
	Ucs	95.00±4.13	0.67±0.05	110.00±9.90	54.64±4.37	174.00±17.40	389.17±35.03

OM: organic matter; Avg.: Average; M: Mibladen, Z: Zaïda; Wt: waste treatment, Cun: clay uncovering, Ucs: unpolluted control soil.

Table 5 MTEs contents in the above-ground part of ryegrass with treatment effects for both experiments

MTEs (mg.kg^{-1})		No treatment		With OM treatment		With chemical Fertilizers treatment	
		M	Z	M	Z	M	Z
		Avg. ±SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Cadmium	Wt	6.20±0.43	0.31±0.02	7.55±0.45	0.70±0.05	8.15±0.49	0.55±0.03
	Cun	7.00±0.56	0.05±0.00	7.70±0.77	0.93±0.08	7.10±0.43	1.03±0.07
	Ucs	6.85±0.41	0.85±0.04	6.80±0.34	1.06±0.05	7.30±0.66	1.24±0.10
Copper	Wt	18.00±1.98	0.52±0.05	27.50±2.75	1.00±0.08	41.50±2.08	1.09±0.08
	Cun	18.00±1.08	2.00±0.12	40.00±2.00	4.64±0.42	23.00±2.07	5.89±0.41
	Ucs	15.50±1.09	1.13±0.06	23.50±1.88	1.77±0.12	18.00±1.62	3.56±0.32
Lead	Wt	120.00±12.00	0.52±0.04	180.00±12.60	12.00±1.20	168.50±11.80	8.72±0.87
	Cun	81.00±7.29	13.00±11.70	87.00±7.83	4.64±0.32	109.00±10.90	76.57±8.42
	Ucs	62.00±6.20	2.26±0.16	78.00±4.68	5.32±0.32	61.50±3.08	5.33±0.32
Zinc	Wt	131.00±11.79	2.08±0.19	410.00±36.90	18.00±1.62	450.00±45.00	29.43±2.65
	Cun	350.00±42.00	9.00±0.90	300.00±33.00	11.60±0.70	250.00±22.50	23.56±2.12
	Ucs	94.50±9.45	5.65±0.40	72.00±5.76	14.18±1.13	84.50±6.76	14.22±1.28

OM: organic matter; Avg.: Average; M: Mibladen, Z: Zaïda; Wt: waste treatment, Cun: clay uncovering, Ucs: unpolluted control soil.

evaluate the capacity of this plant to absorb these elements by their organs. The MTEs content in the root and the above-ground parts are summarized in tables 4 and 5 respectively.

From these tables, it can be seen that very high levels of MTEs were found in both parts of the plant grown at the Mibladen site compared to the Zaïda site. For the Mibladen site, the MTEs ranged from 6.80 to 1575.00 mg.kg^{-1} for below-ground ryegrass parts and from 6.20 to 450.00 mg.kg^{-1} for the above-ground parts. For the Zaïda site these elements ranged from 0.00 to 587.66 mg.kg^{-1} for the below-ground parts and from 0.05 to 76.57 mg.kg^{-1} for above-ground ryegrass parts. In addition, in the Mibladen substrates for the remaining treatments, most of the MTEs contents are higher in the below-ground parts compared to the above-ground parts of *L. multiflorum*. A contrary finding was observed in the substrates of the Zaïda site without fertilizers. Some MTEs concentrations are reversed in favour of the roots with the application of fertilizers, especially in the case of chemical fertilizers.

In general, it was reported that Cd, Pb, Zn, and Cu accumulations in the Italian ryegrass organs depend on the type of MTEs, soil characteristics, plant species, and climatic conditions. To evaluate their accumulation and their transfer to each ryegrass plant organ, the calculation of phytoremediation indices is necessary.

3.4.2 Bioaccumulation and translocation factors

The BAF and TF values of MTEs in the study areas are shown in Tables 6 and 7 respectively. Comparing the effect of the three treatments and the type of contaminated substrates, similar findings are noticed for the Zn and Cu elements. Indeed, for these elements, the highest BAF were found in the substrates of clay uncovering without fertilizers for the Mibladen site (3.911 and 2.571 for Zn and Cu respectively) whereas, in the Zaïda site, these elements were found to be high in waste treatment substrates when chemical fertilizers were applied (0.290 and 0.482 for Zn and Cu respectively). For the Mibladen experiment, the highest BAF was found in the unpolluted control soils with chemical fertilizers added (4.286 and 2.224 for Cu and Zn respectively). In the Zaïda experiment, the BAF was highest in the control soils when OM was added (0.468 for Zn). Regardless of the studied site, the highest BAFs were for Cd (1.283 with OM added and 1.010 with chemical fertilizers for the Zaïda site) and Pb elements (0.505 and 0.279 in the Mibladen and Zaïda sites respectively when chemical fertilizers were applied) recorded in clay uncovering substrates when fertilizers were added. Regarding the highest concentrations of these last toxic elements in the unpolluted control soils, they were recorded for the Pb with OM added (1.545 and 0.066 in Mibladen and Zaïda respectively), and for Cd without fertilizers (2.740) in Mibladen site and when chemical fertilizers were applied (0.409) in Zaïda site.

The huge difference in the concentrations of the MTEs accumulated by the plant can be attributed to the MTEs content in substrates. Previous studies reported that a plant species can be considered as an accumulator and hyperaccumulator for MTEs when BAF and BAF/TF are greater than one respectively (Samsuri et al. 2019; Laghlimi et al. 2022). In addition, Samsuri et al. (2019), reported that plants can be tolerant when they can grow well in the presence of high concentrations of toxic metals. This can lead to the immobilization of MTEs in the soils.

Table 6 Bioaccumulation factor in ryegrass plant grown on contaminated substrates with treatment effects for both experiments

		No treatment		With OM added		With chemical Fertilizers	
		M	Z	M	Z	M	Z
Cadmium	Wt	1.04	0.09	1.09	0.28	0.98	0.22
	Cun	1.04	0.03	1.28	0.64	1.17	1.01
	Ucs	2.74	0.28	1.84	0.35	1.83	0.41
Copper	Wt	1.00	0.02	1.12	0.03	1.41	0.05
	Cun	2.57	0.11	2.11	0.27	1.18	0.48
	Ucs	2.82	0.11	2.24	0.18	4.29	0.35
Lead	Wt	0.07	0.00	0.11	0.00	0.02	0.00
	Cun	0.39	0.04	0.41	0.01	0.50	0.28
	Ucs	1.31	0.02	1.54	0.07	1.31	0.05
Zinc	Wt	0.44	0.01	1.55	0.12	1.65	0.22
	Cun	3.91	0.13	3.06	0.13	2.39	0.29
	Ucs	2.03	0.14	1.50	0.47	2.22	035

M: Mibladen; Z: Zaïda; Wt: waste treatment; Cun: clay uncovering; Ucs: unpolluted control soil.

Table 7 Translocation factor in ryegrass plant grown on contaminated substrates with treatment effects for both experiments

		No treatment		With OM added		WithchemicalFertilizers	
		M	Z	M	Z	M	Z
Cadmium	Wt	0.91	10.33	0.88	0.36	0.94	0.29
	Cun	1.00	0.45	0.99	8.45	1.04	3.32
	Ucs	0.87	8.50	0.94	1.71	0.98	0.26
Copper	Wt	0.67	5.20	0.42	-	0.44	0.14
	Cun	0.64	3.77	2.50	14.50	0.82	5.95
	Ucs	0.57	2.02	0.65	9.32	0.49	2.83
Lead	Wt	0.35	1.33	0.11	0.22	0.11	0.01
	Cun	1.65	4.26	0.87	1.45	2.22	14.34
	Ucs	0.61	4.04	1.18	0.57	0.82	0.03
Zinc	Wt	1.09	4.24	0.49	0.34	0.62	0.13
	Cun	4.43	2.95	0.57	40.00	3.16	4.41
	Ucs	0.99	8.43	0.65	0.26	0.49	0.04

M: Mibladen; Z: Zaïda; Wt: waste treatment; Cun: clay uncovering; Ucs: unpolluted control soil

Phytostabilization aims to stabilize pollutants chemically and physically by reducing bioavailable MTEs from sand preventing erosion (El Aafi 2016).

In the Mibladen experiment, *L. Multiflorum* can be classified as a hyperaccumulator plant especially in the clay uncovering substrates without fertilizers for Zn (3.911) and Cu (2.571) and Cd (1.283) with OM added. The *L. Multiflorum* had a low BAF value for Pb (BAF < 1) in the same experiment for all the treatments and contaminated substrates of the Zaïda experiment.

Regarding the highest TF, it was reported very high (TF>>1) in clay uncovering for the Zaïda experiment (40.00; 14.50, and 8.46 when OM was added for Zn; Cu, and Cd respectively and 14.34 for Pb with chemical fertilizers). Generally, for the contaminated substrates and treatments effect, most of the TFs are higher than one for the Zaïda experiment, while in the case of the Mibladen site TF value was lower than 1 for most of the results.

4 Conclusion

Phytoremediation allows the treatment and stabilization of mine tailings. The findings of this study showed that the Zaïda and Mibladen areas are highly contaminated with MTEs. This is because they were abandoned without any pollution control or rehabilitation. These MTEs accumulate with time and will seriously affect the food chain. From these results, it can be concluded that *L. Multiflorum* is more suitable for Zn, Cu, and Cd phytoextraction from the soils of Mibladen than those of Zaïda, especially in clay uncovering versus waste treatment. Meanwhile, this plant grown under clay uncovering with OM added is shown as a better phytotranslocator for the three MTEs (Zn > Cu > Cd) in the Zaïda experiment than in the Mibladen experiment. Further, the overall observation of BAF and TF of Pb indicated that *L. Multiflorum* is more tolerant and less of a phytotranslocator for this metal in Mibladen than Zaïda, especially in clay uncovering with chemical fertilizers. The phytoremediation results indicated the ability of *L. Multiflorum* to tolerate, hyperaccumulate, and translocate MTEs in the mining soils of Haut Moulouya (Morocco). This ryegrass plant can be used as a phytoremediator plant to establish an ecological cover in this area by accumulating or preventing the migration of Zn, Cu, Cd, and Pb with the slope and their diffusion in the neighboring agriculture areas.

Acknowledgments

The authors are thankful to the MCGPINRA ICARDA project for financial support of part of this study.

Conflict of Interest

The authors attest that there is no conflict of interest regarding the publication of this manuscript.

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