EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2024, 25(5), 154–166
https://doi.org/10.12912/27197050/185252
ISSN 2719-7050, License CC-BY 4.0

Received: 2024.01.07
Accepted: 2024.02.27
Published: 2024.04.01

The Effect of Heavy Metal Speciation on Arbuscular Mycorrhizal Fungi Associated with *Phoenix dactylifera* L. Growing in Moroccan Urban and Peri-Urban Environments

Mohamed Radi[1], Karim Anzid[2], Abdelilah Meddich[3], Ali Boularbah[4], Mohamed Hafidi[1], Ouahmane Lahcen[1*]

[1] Laboratory of Microbial Biotechnologies, Agrosciences, and Environment, Labeled Research Unit-CNRST N°4, Faculty of Science Semlalia, Cadi Ayyad University, Marrakesh, Morocco

[2] Pharmacology, Neurobiology, Anthropobiology and Environment Laboratory, Department of Biology, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakesh, Morocco

[3] Laboratory of Agro-Food, Biotechnologies and Valorization of Plant Bioresources, Faculty of Sciences Semlalia, Cadi Ayyad University, PO Box 2390, Marrakesh 40000, Morocco

[4] Laboratoire d'Aliments, Environnement et Santé, Département de Biologie, Faculté des Sciences et Techniques-Marrakech, Morocco

* Corresponding author's e-mail: l.ouahmane@uca.ac.ma

ABSTRACT

Impacts of metal pollution on arbuscular mycorrhizal fungi (AMF) in rhizopheric soils of date palms in urban and peri-urban areas were studied. The objective was to assess the impact of rhizospheric soil contamination. Various chemical species of heavy metals on the AMF spore density associated with date palms were evaluated**.** A collection of date palm rhizospheric soil samples from eight sites including three boulevards, three gardens and two distinct areas of the Marrakesh palm grove was under study. These samples were used for counting endomycorrhizal fungal spores, for estimating mycorrhization state of root system and for physico-chemical analyses. A five-stage sequential extraction scheme was used to evaluate the fractionation of some heavy metals like lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn) and iron (Fe). Pearson's correlation coefficients between AMF spore's density and metal species were determined and a multiple linear regression was tested to predict AMF spore density from the chemical species content of soil. A mycorrhizal dependency of date palm was indicated, since a high frequency from 61 to 98% and a middle intensity from 10 to 47% of root colonization by AMF were recorded. The spore density from all sites was found in increasing order of boulevards, garden then palm groves. A significant correlation between AMF spore density and some metal species rhizospheric soil content was recorded; negative for sulfide-bound lead (r = -0.81) and zinc (r = -0.70) and for exchangeable fraction copper (Cu), (r = -0.79) whereas it is positive for exchangeable fraction zinc (r = 0.70). AMF spore density predictions from sulfide-bound Zinc and exchangeable fraction copper exhibited a good fit, with higher R^2 value (0.91, p = 0.002). Since Date palm has a mycotrophic nature, the sustainability of the microbial populations associated with their roots might be ensured by modifying some chemical forms of heavy metals like sulfide-bound zinc and exchangeable fraction copper.

Keywords: *Phoenix dactylifera* L., arbuscular mycorrhizal fungi, heavy metal speciation.

INTRODUCTION

In urban areas, soil is the main support of human activity. It is used as a support for constructions and infrastructure (buildings, roads, pipes, cables, etc.), a substrate for plants, a support for industrial, agricultural or domestic activity, a receptacle for the rejection and burial of waste, as well as an intermediary controlling the quality of atmospheric air and water (Craul 1999; Morel et al., 1999). These activities induce changes in soil and, in some cases, production of new soils from

various wastes and by-products of human activities (William et al., 1997; Wong et al., 2006). Human activities are also responsible for different organic or metal pollutants that contaminate the soil (Wong et al., 2006; Ge et al., 2000; Schwartz et al., 2001; Banat et al., 2004; El Khalil et al., 2008). The metallic trace elements (MTE) are part of these pollutants and are highly toxic non-degradable elements as well as continually added to the soils by various activities: in agriculture by the application of sewage sludge or in the metallurgical industry (Thimy et al., 2009). The concentration and type of heavy metals influence phytotoxicity, and in some cases plant growth may be reduced (Shah et al., 2007; Salt 2009; Singh et al., 2003). The metals in soils can be found in soil solution, on exchange sites, occluded into soil oxide material, incorporated into organic plant litter or in the lattice structure of primary and secondary soil minerals (Cottenie and Verloo 1984; Rao et al., 2007). The speciation of heavy metals exerts strong influences on the mobility, bioavailability and toxicity of heavy metals in contaminated soils (Ure and Davidson 2002; Hass and Fine 2010). Due to its physico-chemical and biological composition, soil constitutes an environment for the growth and development of many living organisms (Calvet et al., 2003). Mycorrhizal fungi are a major component of soil microflora and have major roles in the biogeochemical cycles of the elements and in plant mineral nutrition. In polluted sites, the fungal population is confronted with the effects of metal micro-pollutants leading to the reduction of its biodiversity and its functioning (Lyval et al., 1997; Cairney 1999; Entry et al., 2002) since metals directly influence the germination of mycorrhizal fungal spores (Lyval et al., 1994). The roots of more than 80% of vascular plant species are present or likely to develop mycorrhizal structures that improve the uptake and transfer of essential elements to the plant (Duponnois et al., 2012). Thus, any decline in fungal diversity may lead to the reduction of plant biodiversity (Van der Heijden et al., 1998). In Morocco, palm groves are experiencing several problems of degradation under the action of anthropogenic activities and climate change (Oihabi et al., 1991; Jaiti et al., 2008; Meddich et al., 2015). Date palm occupies a large area of 44 000 ha with about 4.4 million feet or an average density of 100 feet/ha (Oihabi et al., 1991). These areas have regressed by 70% since the beginning of the 20th century. In their study, El Khalil et al. (2008) have reported that urban and peri-urban soils in Marrakesh may contain significant levels of technic materials that are potential vectors of metallic trace elements. These technic inputs could be the source of physical, chemical and biological changes that can affect the soil structure and its functioning (Craul 1999; Wong et al., 2006; Sánchez et al., 2002) as well as cause its degradation and transformation into anthropized soil (Craul 1999; Schwartz et al., 2001). The study of the diversity and abundance of mycorrhizal fungi in the Kettara site north of Marrakesh has shown that this diversity is strongly affected by soil pollution (El Faiz et al., 2015). Similarly, Bennisse et al. (2004) showed that in the site Draâ Sfar Marrakesh, bacterial abundance is highly influenced by the metal pollution in this site and that bacterial diversity is not affected by this pollution. The main objective of the current study was to assess the impact of rhizospheric soil contamination via various chemical species of heavy metals on the AMF spore density associated with date palms in different areas of the city of Marrakesh.

MATERIALS AND METHODS

Soil and roots sampling

The study area is located in Marrakesh, the top tourist destination of Morocco. The choice of sites has considered the date palm state and the anthropogenic activity Figure 1. Both of the soil and the plant root systems were collected at the same time from the rhizosphere of date palm in six urban and two peri-urban areas, see Figure 1. In each area, samples were collected at 15 sampling points, around the date palm trunk at a depth of 10 to 40 cm and homogenized to obtain a representative sample for each area. Each soil sample was composed of root fragments of at least five adult trees randomly selected and the distance between two samples was at least 1 m. The soils were then dried in the open air at room temperature and then sieved to 2 mm, avoiding any type of contamination between the samples to perform physico-chemical analyses, heavy metal contents and enumeration of AMF spores.

Physico-chemical analyses of soils

Soil pH was measured with a pH meter using a mixture of 10 g soil and 20 ml distilled water.

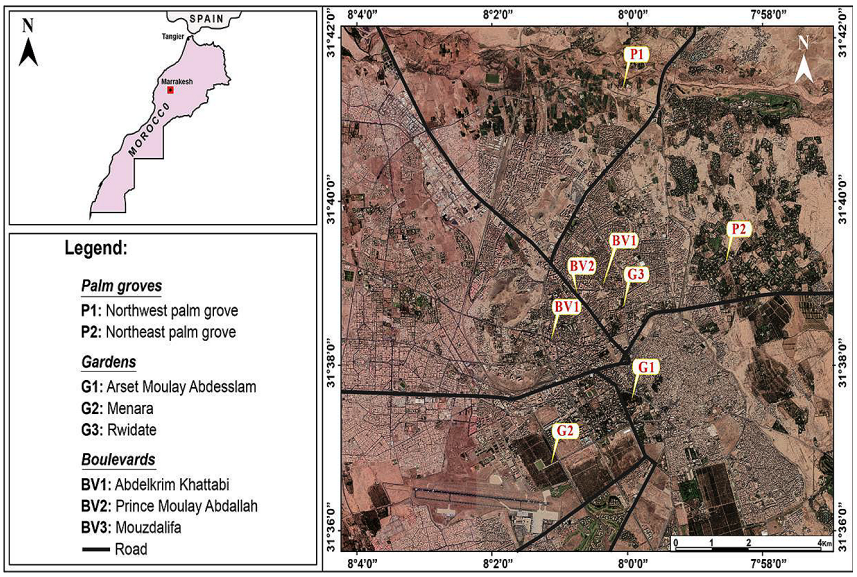

Figure 1. Locations of the study sites in Marrakesh Morocco. The P1 location is characterized with agricultural activities based on irrigation with waste water of associated crops. The P2 location contains wild palm trees and no agricultural activities. The gardens G1, G2 and G3 benefits from constant irrigation and maintenance. The boulevards BV1, BV2 and BV3, are characterized by no irrigation or maintenance but with anthropogenic activities (traffic, wastes, etc.). Coordinates are from Google Earth, 2020.

Soil electrical conductivity was determined on 1/5 soil-water extract with a conductivity meter (Crison instrument, Barcelona, Spain) by a standard procedure and expressed as $\mu s \cdot cm^{-1}$ (Rodier et al., 1984). The total carbonate calcium content ($CaCO_3$) of soils was quantified by an acid (HCl) dissolution followed by measurement of the volume of CO_2 evolved using a Bernard Calcimeter. The total organic carbon, the major component of soil organic matter was determined using Anne's method based on potassium dichromate as described by Aubert (1978). Total Kjeldahl nitrogen was determined by distillation, after mineralization by concentrated sulfuric acid in the presence of the selenium catalyst (Rodier et al., 1984). The assimilable phosphorus was evaluated using the colorimetric method (molybdenum blue) with a spectrophotometer according to the Olsen method.

Chemical speciation of heavy metals in soils

Total metal concentrations were determined after soil mineralization according to the AFNOR NF X 31–151 standard. One gram of each sample was calcined at 550 °C for four hours and sustained an attack by hydrofluoric acid (5 ml). After evaporation of the acid, the residue obtained was solubilized with a mixture 1:3 of HCl/HNO_3. The chemical speciation of metals in soils was determined according to the protocol described by Sposito et al. (1982). Five-stage sequential extraction scheme was used to evaluate the fractionation of lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn) and iron (Fe) in soils. The experimental conditions adopted were potassium nitrate (KNO_3) for the exchangeable fraction to be extracted; water (H_2O) for soluble forms; sodium hydroxide (NaOH) for organic-matter-bound forms; EDTA for carbonate-bound and HNO_3 for sulfide-bound forms, see Table 1. The supernatant of each fraction was filtered on Whatman filters (0.45 µm) and acidified with nitric acid 1% as well as analyzed by flame atomic absorption spectrometry at the regional Laboratory of the National Drinking Water Agency of Marrakesh.

Isolation of mycorrhizal fungi and mycorrhization state

Infectivity parameters of mycorrhizal fungi

Examination of the mycorrhization state of the root system was carried out using the method described by Trouvelot et al. (1986). For AMF colonization assessment, the roots were cut into 1 cm long segments, cleared in KOH solution (10% w/v, at 90 °C) for 45 min, acidified with lactic acid for 10 min at room temperature, stained with trypan blue solution (0.01%, w/v) for 20 min at 90 °C and finally bleached with lactic acid – glycerin

(1:1 by volume) (Phillips and Hayman 1970). For each sample, 100 randomly selected root segments were used. The roots were mounted on microscope slides (10 segments per slide) and were examined one by one under a compound microscope (BX51, Olympus Co. Tokyo, 400 × magnification) to determine if any AMF structures were present and to estimate the proportion of the root segment colonized by AMF divided by the total number of root segments examined. The amount of AMF was estimated according to (Brundrett 1981) from absent (class 0) to very high (class 5): 1) trace of mycorrhizal infection; 2) less than 10% of fungal infection; 3) about 11% to 50% of fungal infection; 4) about 51% to 90% of fungal infection; 5) over 91% of fungal infection. These scores were used to calculate:

- mycorrhizal frequency (F%), which reflects the extent of fungal colonization and was calculated as:

$$F = 100 \times (N - n_0)/N \quad (1)$$

where: N is the total number of observed segments and n_0 is the number of segments without mycorrhizae.

- mycorrhizal intensity (M%), which expresses the per cent of root length colonized by fungi and was calculated as:

$$M = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1)/N \quad (2)$$

where: n_5, n_4, n_3, n_2 and n_1 are, respectively, the numbers of segments scored 5, 4, 3, 2 and 1.

Isolation of mycorrhizal fungi spores

As described by Gerdeman and Nicolson (1963), fungal spore extraction was carried out from the rhizospheric soil of each site. One hundred grams of dry soil was wet sieved on 800 to 50 µm mesh sieves. The soil retained by the last two sieves (50 and 200 µm) is recovered for spore extraction (Brundrett 1996). Eight grams of sieved soil are suspended in 8 ml of distilled water. After centrifugation (for 5 min at 2000 rpm), the supernatant is eliminated as it contains the light debris including the dead spores. The pellet is resuspended in 8 ml of the sucrose solution (60%) and centrifuged for 10 min at 2000 rpm. The spore-containing supernatant is filtered under vacuum on Whatman filters (0.45 µm). The spores retained by the filter are recovered using a fine brush and stored in tubes containing distilled water until they are used in the enumeration. Spores were counted on lined filter paper using a microscope (BX51. Olympus Co. Tokyo) at 100×magnification. The richness of any form of fungal spores was calculated per 100 g of dry soil.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS statistical software for Windows version 10.0.5 (SPSS 1999). To test distributions of soil physico-chemical proprieties, spore density and metal fractions between the three study sites (palm groves, boulevards and garden), the one-way ANOVA test for multiple group comparisons was used. The Tukey HSD test as a post-hoc was used for multiple means comparisons. This a posteriori stepwise test compares pairs of means, controlling the comparison-wise alpha error at a defined level. Pearson's correlation coefficients between mycorrhizal fungi spore density and metal species were determined. Stepwise multiple linear regression analyses were used to determine relationship between spore density (independent variable) and metal fractions (dependent variables); a model was developed for each combination of metal species and density of mycorrhizal spores. All treatments were carried out in triplicate, and the values are given as means ± standard errors. Differences were considered to be significant when the P value was less than or equal to 0.05.

RESULTS

Physico-chemical characterization of soils

Here are the physico-chemical properties of the soil samples that were collected: Table 2. The range of pH values is 7.7 to 8.6, which is quite alkaline. From one site to another, the soil electrical conductivity (EC) varies greatly; however, Boulevard B2 had the highest EC at 2406 µs·cm^{-1}, while Garden 3 had the lowest EC, which did not surpass 300 µs·cm^{-1}.

The other sites showed intermediate values. Concerning the calcium carbonate content, the analyses showed that the lowest values were recorded in garden 3 and palm grove 2 which are 12.91 mg·g^{-1} and 55.32 mg·g^{-1}, respectively. In turn, a relatively high content was recorded at sites P1, B1, B2, B3 and J1. A potential

explanation for this accumulation could involve the higher anthropogenic activity. There were also small differences in the amounts of total organic carbon which were slightly higher at the Boulevard 1 site at 2.3% than at the others sites. The lowest value, 0.39%, was from Garden soils. The carbon-to-nitrogen ratio was significantly lower at Palm grove and Boulevard 2 soils than in other sites. The levels obtained for total phosphorus and available phosphorus vary from one site to another. Thus, the maximum values recorded for the total phosphorus are noted at Garden 2 site with 123.31 mg/g, while for available phosphorus the maximum values were recorded at Boulevard 2 site with 0.40 $mg \cdot g^{-1}$. For the other sites, both forms of phosphorus generally remain weak, especially at Garden 3 site with 0.06 $mg \cdot g^{-1}$. Total nitrogen values ranged from 0.01% in Garden soil to 0.19% and 0.20% respectively in Boulevard and Palm grove soil.

Mycorrhizal status and spores counting

The microscopic observations have shown that all examined roots of date palm were intensively colonized by mycelia and vesicles. The root colonization model clearly belongs to the Arum type (data not shown). Mycorrhizal status depended significantly on the sampling site, see Figure 2. Indeed, the mycorrhizal infection frequencies and intensities range from an average of 61 in P2 to 98% in G3 and 10 in P2 to 48% in P1 respectively. The lowest mycorrhizal intensities were recorded in soil with the same total organic carbon content in soil which recorded 10% in P2 and 18% in G1 respectively. Maximum similarity of mycorrhizal frequency was recorded between all sites except for the site P2 61% characterized by the highest power of hydrogen (pH = 8.6). Among the eight studied sites Figure 3, the rhizospheric soil from palm groves had maximum spore density between 113 and 80 spores per 100 g dry soil in P1 and P2, respectively, while boulevards had the least mean spore density and had recorded 13 spores per 100 g soil. The spore density of the three gardens ranged between 37 and 38 spores per 100 g dry soil. Spore density from several sites was found in increasing order of boulevards, garden then palm groves.

Determination of the total content of metals and evaluation of their speciation.

The average contents of metallic trace elements in the soil samples calculated on 3 samples per station are presented in Table 1:

- Pb – the average contents obtained are around 77.33 $mg \cdot kg^{-1}$. The maximum value is recorded at the boulevards level which is 137.53 $mg \cdot kg^{-1}$.
- Cd – the limit values for Cd contents range between 0 and 9.153 $mg \cdot kg^{-1}$. The highest average values are recorded at the boulevards.
- Cu – the average Cu contents in the different stations vary between 0.6 and 61.33 $mg \cdot kg^{-1}$ with a slight increase in the average value at the boulevard stations.

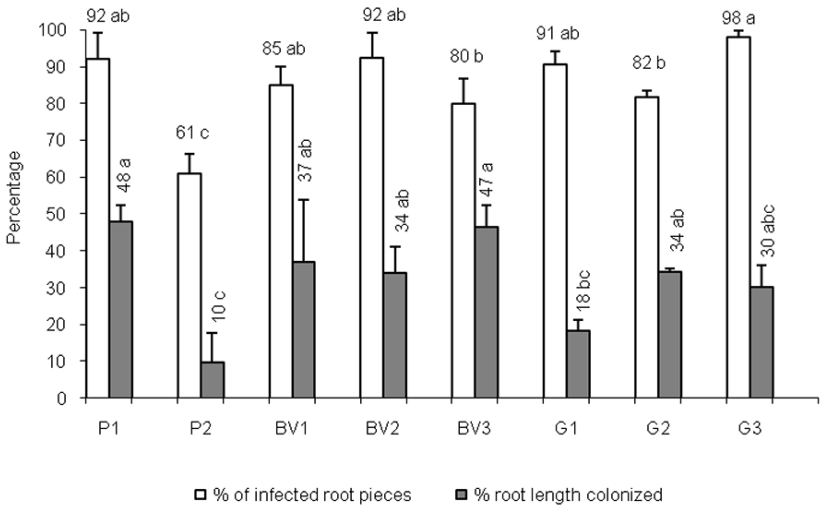

Figure 2. The percentages of infected root pieces and the percentages of colonized root length in different study sites

Table 1. Mean spore density (per 100 g dry soil) and metal species content between sites.

Specification	Study area		Palm groves	Boulevards	Gardens	F	p-value
	Spore density		97.0 ^{a}	38.3 ^{b}	17.1 ^{c}	34.7	0.001 **
Metals forms	Lead	Pb_KNO_3	07.8 ^{b}	07.7 ^{b}	33.6 ^{a}	167.3	0.000 ***
		Pb_H_2O	15.2 ^{b}	27.6 ^{a}	08.1 ^{c}	85.5	0.000 ***
		Pb_NaOH	07.4 ^{ab}	00.0 ^{bc}	02.5 ^{bc}	4.53	0.075 ^{ns}
		Pb_EDTA	25.9 ^{a}	37.0 ^{a}	41.9 ^{a}	0.94	0.450 ^{ns}
		Pb_HNO_3	04.1 ^{c}	19.6 ^{a}	12.3 ^{b}	9.64	0.019 *
		Pb_Residual	07.0 ^{b}	56.7 ^{a}	06.1 ^{b}	45.1	0.001 **
	Cadmium	Cd_KNO_3	2.6 ^{a}	3.5 ^{a}	3.0 ^{a}	1.2	0.351 ^{ns}
		Cd_H_2O	3.1 ^{a}	2.3 ^{b}	1.9 ^{ab}	10.1	0.017 *
		Cd_NaOH	0.0 ^{b}	1.6 ^{a}	0.0 ^{b}	1288.1	0.000 *
		Cd_EDTA	0.9 ^{a}	0.9 ^{a}	0.9 ^{a}	0.51	0.628 ^{ns}
		Cd_HNO_3	0.9 ^{b}	1.4 ^{a}	0.7 ^{b}	6.95	0.036 *
		Cd_Residual	0.8 ^{b}	1.6 ^{a}	0.5 ^{b}	73.6	0.000 ***
	Copper	Cu_KNO_3	09.1 ^{b}	33.8 ^{a}	38.4 ^{a}	14.7	0.008 **
		Cu_H_2O	52.3 ^{a}	61.3 ^{a}	49.6 ^{a}	3.7	0.105 ^{ns}
		Cu_NaOH	2.6 ^{ab}	0.60 ^{b}	05.4 ^{a}	10.4	0.017 *
		Cu_EDTA	06.0 ^{a}	3.46 ^{a}	06.6 ^{a}	1.0	0.433 ^{ns}
		Cu_HNO_3	03.4 ^{a}	02.7 ^{a}	03.0 ^{a}	1.4	0.339 ^{ns}
		Cu_Residual	03.7 ^{a}	04.1 ^{a}	02.5 ^{a}	2.3	0.200 ^{ns}
	Zinc	Zn_KNO3	38.9 ^{a}	15.0 ^{b}	06.0 ^{c}	17.7	0.005 **
		Zn_H_2O	21.1 ^{b}	59.7 ^{a}	07.2 ^{c}	5.7	0.051 *
		Zn_NaOH	01.6 ^{b}	6.3 ^{ab}	20.3 ^{a}	79.5	0.000 ***
		Zn_EDTA	11.1 ^{ab}	03.6 ^{b}	23.2 ^{a}	9.1	0.022 *
		Zn_HNO_3	11.0 ^{b}	21.0 ^{a}	12.6 ^{b}	19.8	0.004 ***
		Zn_Residual	09.5 ^{b}	11.8 ^{a}	12.6 ^{a}	1.0	0.428 ^{ns}
	Iron	Fe_KNO_3	30.0 ^{b}	57.2 ^{a}	19.7 ^{b}	8.9	0.023 *
		Fe_H_2O	111.5 ^{b}	202.9 ^{a}	183.5 ^{a}	0.7	0.551 ^{ns}
		Fe_NaOH	04.0 ^{b}	15.5 ^{a}	15.3 ^{a}	0.6	0.580 ^{ns}
		Fe_EDTA	196.8 ^{b}	115.1 ^{c}	364.0 ^{a}	1.6	0.290 ^{ns}
		Fe_HNO_3	2274 ^{a}	1872.6 ^{a}	1840 ^{a}	0.5	0.644 ^{ns}
		Fe_Residual	111.5 ^{b}	2666.6 ^{a}	3050.6 ^{a}	0.3	0.753 ^{ns}

Note: Mean values within the same row followed by different letters (a, b, c) are significantly different (*) using Tukey HSD test as a post-hoc test when ANOVA indicated significant differences ($p<0.05$). Means followed by the same letter did not differ significantly. (ns – not significant *p <0.05, **p <0.01, ***p<0.001).

- Zn: the highest average levels are obtained at the boulevards with a maximum limit value of 123.33 mg·kg^{-1}.
- Fe: unlike the other elements studied and whatever the station iron has high values.

The Gardens have recorded the lowest average values of the total ETMs content studied. The analysis of the ETMs of the different sites showed significant contents of Pb, Cd, Cu, Zn and Fe in total form X-MLT, followed by the soluble form X-H_2O, then the exchangeable form X-KNO_3 whereas the other forms remained lower. For the total form X-MLT, the comparison between the contents of the existing ETM in the soil samples of each site showed very high contents at the level of the boulevards compared to the other sites. Similarly, the mobile forms X-KNO_3 and X-H_2O of Pb, Cd, Cu, Zn, and Fe also showed higher contents at the boulevards, except for Pb-KNO_3 and Cu-KNO_3 which showed important values at garden level. On the other hand, the contents are very high for the Zn-KNO_3 form in the palm groves. For the other organic fractions X-NaOH, carbonates X-EDTA, sulfide X-HNO_3 and residuals, the distribution of ETMs showed low grades in all the sites, especially

Table 2. Pearson correlation coefficients between spore density and soil heavy metal species

	Metal species	X-KNO_3	X-H_2O	X-NaOH	X-EDTA	X-HNO_3	X-Residual
Spore density	Lead	- 0.153^{ns}	- 0.439^{ns}	N/A	N/A	- 0.817*	- 0.656^{ns}
	Cadmium	N/A	0.541^{ns}	- 0.663^{ns}	N/A	- 0.460^{ns}	- 0.528^{ns}
	Copper	- 0.799*	N/A	0.099^{ns}	N/A	N/A	N/A
	Zinc	0.704*	- 0.451^{ns}	- 0.397^{ns}	0.040^{ns}	- 0.700*	N/A
	Iron	- 0.372^{ns}	N/A	N/A	N/A	N/A	N/A

Note: X – Pb, Cd, Cu, Zn, and Fe. *, ns – statistically significant at the probability level 0.05 and non-significant respectively. N/A – non-applicable.

in Cd-NaOH which takes zero values at the level of gardens and palm groves (Table 1). The contents of Pb-H_2O, Pb-HNO_3, Pb-MLT, and Pb-Residual in the rhizospheric soils of the boulevards are significantly higher compared to the two other sites. For Cadmium, significant differences were observed for the distribution of the concentrations of the five forms, namely those of Cd-H_2O, Cd-HNO_3, Cd-MLT, Cd-NaOH, and Cd residual, indeed the rhizospheric soils of the boulevards are more contaminated than the other sites. With the exception of Cd-H_2O, the concentration of the other four forms is equal between the palm groves and the prospected gardens. In turn, for Copper, three forms present significant differences, namely those of Cu-KNO_3, Cu–MLT, and Cu-NaOH. Also, the rhizospheric soils of the boulevards are more contaminated than the other sites. Furthermore, significant differences were observed for the distribution of concentrations of all forms of zinc. Likewise, the rhizospheric soils of the boulevards are contaminated compared to other sites. On the other hand, only the contents of rhizospheric soils in Fe-HNO_3 and in Fe-MLT are not significantly different in the studied sites. The other forms have significant differences, indeed the rhizospheric soils of the boulevards are always more contaminated than the other sites.

Influence of heavy metal fraction contents on Mycorrhizal spore density

Table 2 shows the correlation coefficients between heavy metal fractions content and spore density. The results show that spore density is negatively and significantly correlated with the sulfide bound lead (Pb-HNO_3: r = -0.817; p = 0.001), sulfide bound zinc (Zn-HNO_3: r = -0.723; p = 0.021) and copper exchangeable form soil content (Cu-KNO_3: r = -0.782, p = 0.022). However, the obtained results indicate a significant positive correlation in spore density with zinc exchangeable form soil content (Zn-KNO_3: r = 0.650, p = 0.041). No correlations were found for other chemical forms (Table 2). The linear regression equation explains the variability observed in the abundance of spores considering the contribution of the predictor variables (Pb-HNO_3 or Pb-MLT or Pb-NaOH). The results suggest that 85.5% of the spore abundance is explained by the concentration of Pb-HNO_3 and that 64.6% of the spore abundance is explained by the concentration of Pb-MLT. It can be concluded that the rhizospheric contents of Pb-HNO_3 and Pb-MLT contribute significantly ($p < 0.001$ and $p < 0.05$) to the density of spores (Figure 2). Similarly, for copper, the linear regression equation explains the variability observed in the abundance of spores taking into account the contribution of the predictor variables (Cu-KNO_3 or Cu-MLT). The results suggest that 61.1% of the abundance of spores is explained by the concentration of Cu-KNO_3 and 60.9% of this abundance is explained by the concentration of Cu-MLT. The rhizospheric contents of Cu-KNO_3 and Cu-MLT contribute significantly ($p < 0.05$) the density of the spores (Figure 2). For zinc, the linear regression equation explains the variability observed in the abundance of spores taking into account the contribution of the predictor variables (Zn-HNO_3 or Zn-KNO_3 or Zn Residual). The results suggest that 49.0% of the spore abundance is explained by the concentration of Zn-HNO_3 and 49.5% of the spore abundance is explained by the concentration of Zn-KNO_3. The Zn-Residual form does not have a significant correlation; however, Zn-MLT has a low correlation with the abundance of spores. It can be concluded that the rhizospheric contents of Zn-MLT and Zn-HNO_3 contribute significantly ($p < 0.05$) the abundance of spores (Table 2). Figure 4 shows a scatter plot of the multiple linear regression of the predicted mean number of spores per 100 g of dry soil as a function of metal species content. This

multiple linear regression model was developed to predict AMF spore density using four metal species, and an R2 level of 0.91 (p = 0.01) was achieved, with a standard error of ±12%. Thus, predictions of spore density from sulfide-bound zinc and the exchangeable fraction of copper showed a good fit, with a higher R2 value (0.91).Salinity measures the total amount of soluble salts (minerals) in the soil and it is an important consideration for management of healthy soil. Basically, too much salt makes it harder for crops to pull water and nutrients into their roots, and at very high levels it can stunt root growth. The values shown in the figure are the mean values of root samples from each study site assessed three times. Error bars indicate the standard error of mean: P – palm groves, BV – boulevards, G – gardens. The data labeled with different letters are significantly different ($p < 0.05$) using the Tukey HSD test. The values shown in the figure are the mean values of spores from each study site assessed three times. The data labeled with different letters are significantly different ($p < 0.05$) using the Tukey HSD test.

DISCUSSION

The pH soil affects mineral nutrient soil quality and much of microorganism activity. In the current study, the pH of soil samples has shown a

Table 3. Soil physicochemical properties

Sites	pH	EC ($\mu S.cm^{-1}$)	Ca CO_3 (mg g^{-1})	TOC (%)	C/N ratio	PO_4^{-3} (mg g^{-1})	TP (mg g^{-1})	TN (%)
P1	8.0 ± 0.0 ^{c}	753 ± 24.6 ^{ab}	21.3 ± 0.0 ^{a}	1.92 ± 0.1 ^{b}	88.2	0.13 ± 0.0 ^{ef}	73.84 ± 5.2 ^{b}	0.18
P2	8.6 ± 0.0 ^{a}	1126 ± 6.0 ^{ab}	5.5 ± 0.0 ^{f}	1.86 ± 0.1 ^{cd}	45.0	0.27 ± 0.0 ^{cde}	37.13 ± 2.6 ^{c}	0.14
BV1	7.7 ± 0.0 ^{e}	1127 ± 9.0 ^{ab}	18.8 ± 0.1 ^{c}	2.05 ± 0.0 ^{a}	53.3	0.37 ± 0.0 ^{ab}	62.04 ± 0.4 ^{b}	0.20
BV2	7.8 ± 0.0 ^{e}	2420 ± 64.3 ^{a}	19.9 ± 0.1 ^{b}	1.94 ± 0.0 ^{b}	26.2	0.40 ± 0.0 ^{a}	77.30 ± 9.9 ^{b}	0.19
BV3	7.9 ± 0.0 ^{cd}	1112 ± 62 ^{ab}	12.2 ± 0.3 ^{e}	1.78 ± 0.0 ^{d}	50.0	0.24 ± 0.0 ^{de}	71.89 ± 4.3 ^{b}	0.03
G1	7.9 ± 0.0 ^{c}	1233 ± 122 ^{ab}	18.9 ± 0.2 ^{c}	1.86 ± 0.0 ^{cd}	41.8	0.33 ± 0.0 ^{abc}	59.98 ± 0.9 ^{b}	0.02
G2	8.3 ± 0.0 ^{b}	518 ± 17.6 ^{ab}	13.4 ± 0.0 ^{d}	1.82 ± 0.0 ^{d}	45.1	0.27 ± 0.0 ^{bdc}	122.4 ± 9.4 ^{a}	0.02
G3	8.3 ± 0.0 ^{b}	225 ± 1.0 ^{b}	1.3 ± 0.0 ^{g}	1.66 ± 0.0 ^{e}	2.5	0.06 ± 0.0 ^{f}	45.42 ± 1.2 ^{c}	0.01

Note: P – palm groves, BV – boulevards, G – gardens. Values are mean ± standard errors of one triplicate. Different letters a, b, c, d, e, f and g indicate significant differences between study sites according to the Tukey HSD post-hoc test ($p < 0.05$). Means followed by the same letters did not differ significantly. EC – electric conductivity; $CaCO_3$ – total carbonate calcium content; TOC – total organic carbon; C:N ratio – carbon to nitrogen ratio; PO_4^{3-} – available phosphorus; TP – total phosphorus; TN – total nitrogen.

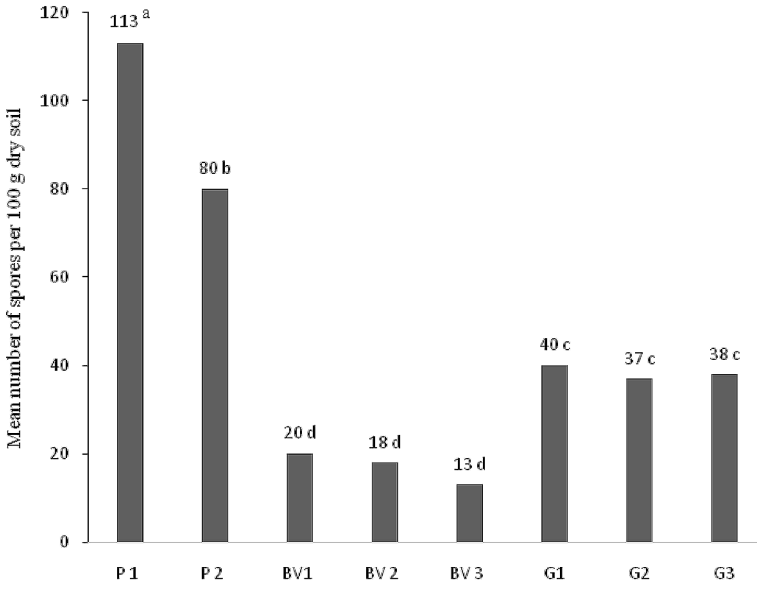

Figure 3. The mean number of spores per 100 g dry soil by studied sites: P – palm groves, BV – boulevards, G – gardens

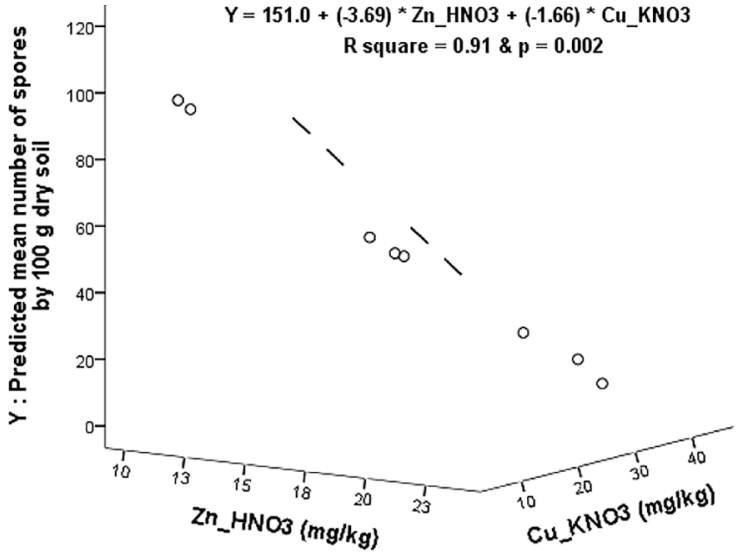

Figure 4. Scatter plots of the multiple linear regression of the predicted mean number of spores per 100 g dry soil based on the content of metal species

variation from 7.7 to 8.6, which indicated soils basic nature (pH value above 7.5). Except for Palm grove 2 and Garden 2 and 3, the pH of soil in all other sites was within the optimal range for the development of fungi 5.5–8.0 (Zorpas et al., 2003). Several factors that disturb the EC value of the soil include temperature, amount of fertilizers, salinity, moisture level, irrigation and types of soil (Hawkins et al., 2017). Experimental results showed that the soil EC is directly related to the nutrient concentration and inversely related to the depth of the soil (Othaman et al., 2019). The conductivity study of soil samples has shown variation in conductivity values between 225 $\mu s \cdot cm^{-1}$ to 2420 $\mu s \cdot cm^{-1}$, these values suggest a non-saline to very slightly saline soil. In boulevard BV2 site, soils have significant high level of salinity compared to other sites, which may increase their electrical conductivity. At salinity above to 2000 $\mu s \cdot cm^{-1}$, roots of larger plants may have difficulty accessing nutrients and water and above to 4000 $\mu s \cdot cm^{-1}$, roots are physically damaged and may die. In their study, Chebaane et al. (2020), soils in different date palm plantations presented a high salinity rate up to 7600 $\mu s \cdot cm^{-1}$. The free $CaCO_3$ ranged from 1.3 to 21.3%. Table 3 indicates that these soils are moderate to high calcareous in nature. $CaCO_3$ was found highest in Palm grove P1 followed by Boulevard BV2 and was comparatively lowest in P2 and G3. The soil total organic carbon was found low to moderately high range (1.66 to 2.05%). Soil organic carbon is a measurable component of soil organic matter. Organic matter makes up only 2–10% of the mass of most soils and plays an important role in the physical, chemical and biological function of agricultural soils. The mobility of mineral elements is strongly influenced by soil parameters in particular pH, mineral element and organic matter content (Leyval et al., 1994; Radi et al., 2021). According to Tremel-Schaub and Feix (2005), the mineral elements enter the constitution of the solid phase of the soil. They can be linked weakly to the soil particles by electrostatic bonds (exchangeable forms). They can also be relatively strongly linked to the soil by ionic molecular type bonds (organic complex) or forms organo-metallic complexes (inorganic speciation). While some species are more labile than others (Cd and Zn), others are very stable (Cu and Pb) and easily form organic complexes with Fulvic acids (Prasad 1999). The increase in the content of one species of the same metal may induce the increase in the intensity of soil contamination. The presence of one heavy metal may affect the availability of another in the soil and hence plant. In other words, antagonistic and synergistic behaviors exist among heavy metals (Chibuike and Obiora 2014). With the exception of Pb-EDTA. the five lead species differ significantly according to their rhizosphere content (Pb-H_2O, Pb-HNO_3 and Pb-Residual). In contrast, five forms of Cadmium differ significantly from those of Cd-H_2O, Cd-HNO_3, Cd-MLT, Cd-NaOH, and Cd-Residual. In fact, with the exception of Cd-H_2O. the concentration of other forms remains equal between palm

groves and gardens. For copper, three forms show significant differences, namely those of Cu-KNO_3, Cu-MLT, and Cu-Residual. Similarly, significant differences were observed in the distribution of concentrations of all forms of zinc. On the other hand, only the soil content rhizosphere in Fe-KNO_3 differs significantly according to the study sites. Other forms have no significant difference. In fact, the contents of these forms are higher in the rhizosphere soils of the boulevards than in the other sites of the palm groves and gardens. Heavy metals accumulate in living organisms and have short and long-term toxic effects (Salt 2009). In addition, the toxicity of an element is related to its chemical form (to its speciation) as well as environmental factors (Babich 1980). Among the organisms that are affected by heavy metals are mycorrhizal fungi (Ouatiki et al., 2022; Raklami et al., 2021). The latter colonize the cortex of the roots and develop an extraradicular mycelium. Their role is decisive in increasing the interface between plants and the surrounding soil and the transfer of mineral elements (P and N) as well as toxic metals, such as (Cu and Zn). Mycorrhizal fungi are also susceptible to metal pollution (Leyval et al., 1997; Cairney 1999; Entry et al., 2002). A decline in fungal diversity can reduce plant biodiversity in a terrestrial ecosystem (Van der Heijden et al., 1998). El Faiz et al. (2015) showed that inoculation with isolated mycorrhizal fungi from mining soils resulted in a significant increase in the growth of the *Cana indica* seedlings by contribution to control plants. In fact. the presence of fungi in the rhizosphere of the mining soils had induced a promoter effect of plant growth and resistance to toxicity. (Ouatiki et al., 2022). The abundance of spores and the infectious potential of the mycorrhizal fungus vis-à-vis the roots of the date palm are affected by water stress (Meddich et al., 2021; Raklami et al., 2021) and by metal pollution (Leyval 1995). A weak mycorrhizal symbiosis has been found in the Palm Grove P2 and Garden 1. This result can be explained by the rarity in these sites of mycorrhizal fungi. In turn, other sites have a significant symbiosis. In contrast, the number of spores is very low in the boulevards and important in the gardens and very important in the palm groves. Besides, the increase in ETM content is also noted in the boulevards. On the other hand, palm groves and gardens have low levels. ETM may indeed have toxic effects on Macs (Gadd 1993) leading to a decrease in spore density and the infectious potential of mycorrhizal fungi (Leyval 1995). Recent studies of the effects of heavy metal pollution on colonization and mycorrhizal function, the adaptive mechanisms of AMF to polluted soils have been detailed by several authors (Leyval, et al., 2002; Meharg et al., 2003; Gohre et al., 2006; Hildebrandt et al., 2007). Indeed, the conducted study is based on the correlation between the concentration of the heavy metals species and the abundance of spores. Thus, generally when the content of heavy metals increases the abundance of spores decreases. This is the case for boulevards, where the obtained results showed a low abundance of spores and a high ETM content. However, the increase in this abundance is noted in the soils of gardens and palm groves. which are characterized by a low level of ETM. This can be explained by the great pollution that exists at the level of the boulevards acting directly on the production and abundance of spores and on the specific richness (Ortega-Laro et al., 2007; Mohamed Radi et al., 2021). The results of the study of the correlations between the concentration of seven forms of heavy metals and the abundance of spores vary according to the shape and content of the metal in the soil. In fact, two forms of Lead (Pb-HNO_3: r = 0817; p = 0.013 and Pb-MLT: r = -0817; p = 0.013) were negatively correlated with spore abundance. Thus, when the content of these two forms of lead increases, the abundance of spores decreases, as ETM may indeed have toxic effects on AMF (Gadd. 1993; Tahiri et al., 2021). In study sites. two forms of copper were negatively correlated with spore abundance (Cu-KNO_3: r = -0799; p = 0.017) and (Cu-MLT: r = -0810; p = 0.015). Both forms have a direct and detrimental effect on spore abundance. Similar studies also noted that Cu enters the cytosol. Preferentially accumulates in fungal vacuoles and spores (Gonzalez-Guerrero et al., 2008). Further work on preferential accumulation of Cu in spores has also recently been demonstrated by the microscopic detection of some blue-green spores in the extra-radical mycelium of *G. intraradices* (Ferrol et al., 2009). Excess Cu is transferred to vacuoles, where it can be stored from the cytosol and to specific fungal structures (spores and vesicles) and then, it would alter these structures because it limits core metabolic functions (Ferrol et al., 2009). For zinc, a single form (Zn_ HNO_3: r = -0700; p = 0053) was negatively correlated with spore abundance. The same results were obtained by Boyle and Paul (1988), which showed a negative correlation between zinc concentration and root colonization by AMF in industrial sludge-treated soils. However, regardless of the site

studied, the results obtained for cadmium and iron show that the abundance of spores is not significantly correlated with these metals. Unlike the other studied elements (Pb, Cu, Zn), iron has very high levels in soil samples. In contrast. cadmium known for its high toxicity and its facility to be bioaccumulated in organisms is less concentrated in these samples. These two elements were not correlated with spore abundance. Similar results were observed by Leyval (1994). He showed that the percentage of germination of the AMF spores was not negatively correlated with the total metal concentration in soils or with cadmium available and evaluated by chemical extraction. Heavy metals can reduce and eliminate root colonization by AMF, as well as germination of their spores. The total number of spores, the size and the diversity of mycorrhizal populations decreased significantly in the soil with increased concentration of heavy metals (Del Val et al., 1999; Radi et al., 2014). The scatter plot of the multiple linear regression shows that AMF spore density is significantly correlated with sulfide-bound zinc and the exchangeable fraction of copper showed a good fit, with a higher R2 value (0.91). Thus, higher concentrations of these fractions may reduce spore density. Gonzalez-Guerrero (2005) studied the morphogenetic response of *Glomus intraradices* hyphae using carrot roots in the presence of several Cu concentrations. He observed that Cu induced significant changes in fungal morphogenesis, especially at the cytoplasmic wall and reduction of sporulation. In contrast, at low Cu concentrations, he found an increase in hyphal length, while at higher concentrations in this element, the growth of the extra – radical - mycelium was localized and severely limited. Similar results were obtained for the extra-radical mycelium of *G. intraradices* in response to excess Cd, Pb, Zn, and low pH (Bago et al., 2004; Pawlowska et al., 2004). Other studies suggest that fungi may, in some cases, slow down the transfer of trace elements from the soil to the plant, especially when soil trace element levels are elevated on a polluted site. Mycelium may decrease the uptake of metals in root tissues (by cell wall binding, polyphosphate grains, organic acids, and metallothionein -type peptides in mycelium) (Prasad 1999). However, the decrease in the toxicity of heavy metals by colonization of MA fungi may vary to a large extent, depending on the concentration of metal in the soil. symbiosis and plant growth conditions (Leyval et al., 1997; Weissenhorn et al., 1995; Hildebrandt et al., 1999). Leyval et al. (1994) showed that the spores isolated from polluted soils gained a tolerance highlighted under conditions where metals are more readily available. However, the mechanisms involved in this increased tolerance to heavy metals in polluted soils remain to be elucidated and the role of mycorrhizae formed by these fungi on the transfer of metals to plants.

CONCLUSIONS

The objective of this study was to study the impact of anthropogenic activities via metallic pollution on the variability of Arbuscular Mycorrhizal Fungi (AMF) associated with date palm, including AMF infectivity and spore density in the urban and peri-urban soils of Marrakesh. The study of the speciation of heavy metals (Cd, Cu, Fe, Pb, and Zn) in soil samples shows that their contents vary depending on the sampling site and the chemical form of each metal. For example, heavy metal content generally increases in boulevards by refilling at other sites studied. The low frequencies and intensities of mycorrhization recorded in the boulevards can be explained by diffuse pollution of heavy metals, mineral elements and micropollutants. The latter have a detrimental effect on mycorrhizal fungi with a decrease in spore abundance and the infectious potential of soils. This decline in fungal diversity may have negative consequences for the growth of date palm plants.

REFERENCES

1. Aubert G. 1978. Méthodes d'analyse des Sols Edition C.R.D.P. Marseille. France.
2. Babich H., Stotzky G. 1980. Environmental factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. Crit Rev Microbiol. (2), 99–145.
3. Bago B., Cano C., Azco´n-Aguilar C. 2004. Differential morphogenesis of the extraradical mycelium of an arbuscular mycorrhizal fungus grown monoxenically on spatially heterogeneous culture media. Mycologia, 96, 452–462.
4. Banat I.M., Marchant R., Rahman T.J. 2004. *Geobacillus debilis* sp. nov. a novel obligately thermophilic bacterium isolated from a cool soil environment. and reassignment of Bacillus pallidus to *Geobacillus pallidus* comb. Int. J Syst. Evol. Microbiol. 54, 2197–2201.
5. Bennisse R., Labat M., ElAsli A., Brhada F.,

Chandad F., Lorquin J., Pol Liegbott P., Hibti M., Qatibi A. 2004. Rhizosphere bacterial populations of metallophyte plants in heavy metal-contaminated soils from mining areas in semiarid climate. World Journal of Microbiology & Biotechnology, 20, 759–766.

6. Boyle M., Paul E.A. 1988. Vesicular-arbuscular Mycorrhizal associations with barley on sewage amended plots. Soil Biol.Biochem. 20, 945–948.
7. Brundrett M. Bougher N., Dell B., Grove T., Malajczuk N. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32.
8. Cairney J.W.G., Meharg A.A. 1999. Influences of anthropogenic pollution on mycorrhizal fungal communities. Environmental Pollution, 106, 169–182.
9. Calvet R. 2003. Constituants organiques dans le sol. Propriétés et fonctions. Tom 1: Constitution et structure. Phénomènes aux interfaces. Dunod. 159–218.
10. Craul. P.J. 1999. Urban soils: applications and practices. (books.google.com). Canada.
11. Del val C., Barea J.M., Azcon-aguilar C. 1999. Diversity of Arbuscular Mycorrhizal Fungus populations in heavy metal contaminated soils. Appl. Environ. Microbiol. 65, 718–723.
12. Duponnois R., Hafidi M., Wahbi S., Sanon A., Galiana A., Baudoin E., Sanguin H., Bâ A, Prin Y., Bally R. 2012. La symbiose mycorhizienne et la fertilité des sols dans les zones arides: un outil biologique sous-exploité dans la gestion des terres de la zone sahélo-saharienne. IRD 349-367.
13. El Faiz A., Duponnois R., Winterton P., Ouhammou A., Meddich A., Boularbah A., Hafidi M. 2015. Effect of Different Amendments on Growing of *Canna indica* L. Inoculated with AMF on Mining Substrate. International Journal of Phytoremediation, 17, 503–513.
14. El Khalil H., El Hamiani O., Bitton G. 2008. Heavy Metal Contamination From Mining Sites In South Morocco: Monitoring Metal Content And Toxicity Of Soil Runoff And Groundwater. Environnemental Monitoring and Assessment, 136, 147–160.
15. Entry J.A., Rygiewicz P.T., Watrud L.S., Donnelly P.K. 2002. Influence of adverse soilconditions on the formation and function of Arbuscular mycorrhizas. Advances in Environmental Research, 7, 123–138.
16. Ferrol N., Gonzalez-Guerrero M., Valderas A., Benabdellah K., Azco´n-Aguilar C. 2009. Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. Phytochemistry Reviews, 3, 551–559.
17. Gadd G.M. 1993. Interactions of fungi with toxic metals. New Phytologis,t 124, 25–60.
18. Ge S.S., Hang C.C., Zhang T. 2000. Adaptive neural network control for strict-feedback nonlinear systems using backstepping. Journal of Automatica, 3(6), 1835–1846.
19. Gerdeman J.W., Nicolson T.H. 1963. Spore of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46, 235–244.
20. Gohre V., Paszkowski U. 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremédiation. Planta, 223, 1115–1122.
21. Gonzalez-Guerrero M., 2005. Estudio de los mecanismos implicados en la homeostasis de metales pesados en el hongoformador de micorrizas arbusculares *Glomus intraradices*. Dissertation University of Granada.
22. Gonzalez-Guerrero M., Melville L.H., Ferrol N., Lott J.N.A, Azcón-Aguilar C., Peterson R.L. 2008. Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the Arbuscular mycorrhizal fungus *Glomus intraradices*. Canadian Journal of Microbiology, 54, 103–110.
23. Hildebrandt U., Kaldorf M., Bothe. H., 1999. The zinc violet and its colonization by arbuscular mycorrhizal fungi. Journal of Plant Physiology 154 709-717.
24. Hildebrandt U., Regvar M., Bothe H., 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry, 68, 139–146.
25. Jaiti F., Kassami M., Meddich A., El Hadrami I. 2008. Effect of Arbuscular Mycorrhization on the Accumulation of Hydroxycinnamic Acid Derivatives in Date Palm Seedlings challenged with *Fusarium oxysporum f. sp. Albedinis*. Journal of Phytopathology, 156, 11–12.
26. Leyval C., Joner E.J., del Val C. 2002. Potential of Arbuscular mycorrhizal fungi for bioremediation. In: S. Gianinazzi. H. Schu¨epp, J.M. Barea, K. Haselwandter. Mycorrhizal technology in agriculture. Birkha user. Basel. 175–186.
27. Leyval C., Singh B.R., Joner E.J. 1995. Occurence and infectivity of arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties. Water. Air and Soil Pollution, 84, 203–216.
28. Leyval C., Turnau K., Haselwandter K. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological. ecological and applied aspects. Mycorrhiza, 7, 139–153.
29. Leyval C., Weissenhorn I., Glashoff A., Berthelin J. 1994. Influence des métaux lourds sur la germination des spores de champignons endomycorhiziens a arhuscules dans les sols. Journal Acta Botanica Gallica: Botany Letters, 141, 523–528.
30. Meddich A., Oihabi A., Jaiti F., Bourzik W., Hafidi M. 2015. Role of Arbuscular Mycorrhizal Fungi on vascular wilt and drought tolerance in date palm (*Phoenix dactylifera* L.). Canadian Journal of Botany, 93(6), 369–377. https://doi.org/10.1139/cjb-2014-0249.
31. Meddich A., Oihabi. A., Abbas Y., Bizid E. 2000. Rôle

des champignons mycorhiziens à arbuscules de zones arides dans la résistance du trèfle (*Trifolium alexandrinum* L.) au déficit hydrique. Agronomie, 20, 283–295.

32. Meharg A. 2003. The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. Mycol Res. 107, 1253–1265.
33. Morel Y.R. 1999. Barouki. Repression of gene expression by oxidative stress INSERM U490. Centre universitaire des Saints-Pères. 45 rue des Saints-Pères. 75006 Paris. France.
34. Ouatiki E., Midhat L., Tounsi A., Amir S., Aziz F., Radi M., Ouahmane L. 2022. The association between Pinus halepensis and the Ectomycorrhizal fungus *Scleroderma* enhanced the phytoremediation of a polymetal-contaminated soil. International Journal of Environmental Science and Technology, 0123456789. https://doi.org/10.1007/s13762-022-03993-4
35. Oihabi A. 1991. Effet des endomycorhizes V.A sur la croissance et la nutrition minérale du palmier dattier. Thèse de Doctorat d'Etat. Univ. Cadi Ayyad Marrakech. Maroc/Univ. Bourgogne Dijon. France.
36. Ortega-Laro M.P., Siebe C., Estrada A., Webster R. 2007. Mycorrhizal inoculum potential of Arbuscular mycorrhizal fungi in soils irrigated with wastewater for various lengths of time as affected by heavy metals and available. P. App. Soil Eco. 37, 129–138.
37. Pawlowska. T.E., Charvat I. 2004. Heavy-metal stress and developmental patterns of Arbuscular mycorrhizal fungi. Appl Environ Microbiol. 70, 6643–6649.
38. Phillips J.M., Hayman D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55, 158–161.
39. Prasad M.N.V., Hagemeyer J. 1999. Heavy metal stress in plants. From molecules to ecosystems. Springer, 401
40. Radi M., Hamdali H., Meddich A., Ouahmane L., Hafidi M. 2014. Le potentiel mycorhizogène des sols urbains en zones semi-arides et la tolérance du Palmier dattier (*Phoenix dactylifera* L.) au déficit hydrique. Journal of Materials and Environmental Science, 5(6), 1957–1967.
41. Radi M., Meddich A., Ouatiki E., Ouahmane L., Hafidi M., Ben Salem A. 2021. Effects of Urbanization on the Degradation of the Palm Grove Around the City of Marrakech. February, 323–337. https://doi.org/10.4018/978-1-7998-7512-3.ch016
42. Raklami A., El Gharmali A., Ait Rahou Y., Oufdou K., Meddich A. 2021. Compost and mycorrhizae application as a technique to alleviate Cd and Zn stress in Medicago sativa. International Journal of Phytoremediation, 23(2), 190–201. https://doi.org/10.1080/15226514.2020.1803206
43. Raklami A., Tahiri A., Bechtaoui N., Abdelhay E.G., Pajuelo E., Baslam,M., Meddich A., Oufdou K. 2021. Restoring the plant productivity of heavy metal-contaminated soil using phosphate sludge, marble waste, and beneficial microorganisms. Journal of Environmental Sciences (China), 99, 210–221. https://doi.org/10.1016/j.jes.2020.06.032
44. Rodier K.R.1984. L'analyse de l'eau. Eaux naturelles. Eaux résiduaires. Eau de mer. Dunod1365 p.
45. Salt D.E, Smith R.D., Raskin I. 1998. Phytoremediation. Plant Physiol. Plant Mol. Biol, 49, 643–668. in thèse Thimy D H 2009: impacts des métaux lourds sur l'interaction plante/ ver de terre/ microflore tellurique 169p.
46. Sánchez M., Soriano M., Delgado G., Delgado R. 2002. Soil Quality in Mediterranean Mountain Environments. Published in Soil Sci. Soc. Am. J. 66, 948–958.
47. Schwartz. R., Ting. C.S., King. J. 2001.Whole proteome PI values correlate with subcellular localizations of proteins for organisms within the three domains of life. Genome Res. 11, 703–9.
48. Shah K., Nongkynrih J.M., 2007. Metal hyperaccumulation and bioremediation. Biologia Plantarum, 51, 618–634.
49. Singh O.V., Labana S., Pandey G., Budhiraja R., Jain R.K. 2003 Phytoremediation: an overview of metallic ion decontamination from soil. Appl. Microbiol. Biotechnol. 61, 405–412.
50. Sposito G., Lund L.J., Chang A. 1982. Trace metal chemistry in arid- zone field soils amended with sewage sludge. I. Fractionation of Ni. Cu. Zn. Cd. and Pb in solid phases. Soil sci. Soc. Am. J. 46, 260–264.
51. Thimy D.H. 2009. Impacts des métaux lourds sur l'interaction plante/ ver de terre/ microflore tellurique. 169.
52. Tremel-Schaub A., Feix I. 2005. Contamination des sols transferts des sols vers les plantes. 422.
53. Trouvelot A., Kough J.L., Gianinazzi-Pearson. 1986. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthode d'estimation ayant une signification fonctionnelle mychorrhizae: Physiology and genetics. ESM Dijon
54. Van der Heijden M.G.A., Moutoglis P., Streitwolf-Engel R., Boller T., Wiemken A,. Sanders I.R. 1998. Mycorrhizal fungal diversity determines plant biodiversity. ecosystem variability and productivity. Nature, 396, 7–75.
55. Weissenhorn I., Leyval C., 1995. Root colonization of maize by a Cd-sensitive and a Cd tolerant *Glomus mosseae* and cadmium uptake in sand culture. Plant and Soil, 175, 233–238.
56. William R.E., Pouyat R.V. 1997. The genesis. classification and mapping of soils in urban areas. Journal of Urban Ecosystems, 217–228.
57. Wong L.T., Mui K.W., Hui P.S. 2006. A statistical model for characterizing common air pollutants in air. journal of Atmospheric Environment, 404246-404257.