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Impacts of lake-derived heavy metal and salinity pollution on soil properties, vegetation structure, and mycorrhizal diversity in the Oum Ghellaz Wetland (Algeria)

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Keywords: Wetland, soil and water pollution, metallic indices, mycorrhizal association, dark septate endophytes.

Abstract: The present study investigates the effects of water contamination on wetland soil, flora, and fungal symbiosis in Dayet Oum Ghellaz, northwestern Algeria, focusing on the impact of high salinity and heavy metals. Water samples, rhizospheric soil, and roots from 14 plant species were collected and analyzed in order to assess water and soil quality, plant biodiversity, arbuscular mycorrhizal fungal (AMF) diversity, and the presence of metallic trace elements (MTEs). The findings revealed a high perturbation index (PeI) of the wetland flora (64%), as well as significant water and soil metallic pollution indices exceeding 2.5 and 5, respectively, indicating environmental contamination, particularly with cadmium, nickel, zinc, and lead, and elevated levels of sulphate, inorganic nitrogen, and oxygen demand (DBO5 and DCO). The majority of plant species exhibited high levels of mycorrhization ($F > 50\%$), 50% of the plants exhibited dark septate endophytes (DSE), and two species were found to have ectomycorrhizae. A total of 18 AMF morphospecies were identified, predominantly belonging to the families Glomeraceae and Acaulosporaceae. These results emphasize the detrimental effects of heavy metal pollution on wetland ecosystems, highlighting the necessity for further research and management strategies to mitigate contamination impacts on plant-fungal interactions and overall biodiversity.

Introduction

Wetlands are recognized for their ecological significance and are frequently protected under national and international conservation frameworks (Scott and Jones, 1995; Elarbaoui, and Smiri, 2024). In Algeria, despite limited water resources and climate challenges, several significant wetlands play a crucial role in supporting biodiversity and providing essential ecosystem services (Touitou and Al-Aminb, 2020). These areas are vital for conservation and sustainable management, as they preserve biodiversity, support water resources, and offer habitats for numerous species (Samraoui and De Belair, 1997; Hammana *et al.*, 2024). However, Algerian wetlands face several pollution challenges that can have detrimental effects on their ecosystems and associated species. Industrial activities, such as manufacturing and mining, release various pollutants into the surrounding environment. Untreated or poorly treated industrial wastewater containing heavy metals, chemicals, and other contaminants can pollute wetlands and degrade ecosystems (Ahriz *et al.*, 2010; Sidhoum *et al.*, 2020; Keria *et al.*, 2024).

Additionally, contaminated water can significantly harm wetland soils by altering their physical and biological properties (Zahi *et al.*, 2024). It modifies soil chemistry through nutrient imbalances and the accumulation of toxic elements such as heavy metals, rendering the soil inhospitable for many plants and microorganisms (Demnati *et al.*, 2017).

This contamination also disrupts microbial activity, reducing diversity and abundance while potentially increasing pathogenic microorganisms that can harm flora and fauna (Bouchelouche *et al.*, 2021). Additionally, it affects physical soil properties, leading to changes in texture and structure that reduce porosity and water retention. As a result, plant health may decline, with sensitive species struggling to survive and more tolerant species becoming dominant, thereby altering plant community composition. These changes disrupt essential ecosystem services, such as biodiversity maintenance and water filtration. Furthermore, contaminants can persist in the soil, creating long-term effects that compromise ecosystem health even after the pollution source has been removed (Chalane *et al.*, 2023).

Dayet Oum Ghellaz (Gharabas Lake) is a brackish wetland classified as a protected area by the Algerian Ministry of the Environment. It is a part of a group of 8 wetlands in the city of Oran (western Algeria) that constitute a reserve of international importance and form an important complex for the wintering and migration of birds (Direction Générale des Forêts, 2006; Bahi et al., 2020). Dayet Oum Ghellaz has been affected by pollution from wastewater discharges, which have altered the water microbial flora (Aibeche et al. 2020; Mahi et al. 2021) causing eutrophication and several episodes of fish mortality in June 2019 and August 2020 (El Watan 2019).

Arbuscular mycorrhizal fungi (AMF) colonize the roots of most terrestrial plants, forming a mutually beneficial symbiotic relationship. They develop specialized structures called arbuscules, which facilitate nutrient exchange between the fungus and the host plant. This symbiosis plays a crucial role in plant growth, nutrient uptake, and stress tolerance (Wahab et al., 2023).

Studying the diversity of AMF provides valuable insights into their ecological roles and their potential to enhance plant adaptation to environmental stressors. By identifying and characterizing AMF species associated with plants in stressed environments, it is possible to better understand their potential roles in mitigating the adverse effects of water contamination and salinity on plant health. In addition, investigating the relationship between AMF diversity and water contamination can elucidate interactions between plant-microorganism symbiosis and water quality (Branco et al., 2022).

This research represents the first comprehensive study of mycorrhizal diversity in the Oum Ghellaz Wetland, addressing a critical gap in our understanding of fungal interactions within this unique ecosystem. Unlike the previous studies (Sidhoum et al., 2020 and 2025), which primarily focused on the general ecological impacts of pollution in wetlands, the present study specifically explores the origin of heavy metal and salinity pollution and their direct relationship with plant health and mycorrhizal diversity. By exploring these interconnected factors, this work provides new insights into the resilience and adaptability of plant and mycorrhizal communities.

Therefore, this study aims to characterize the chemical properties of water and assess their influence on the diversity of mycorrhizal fungi, in relation to key soil properties (pH, nutrient content, organic matter, and texture) and the AMF-plant symbiosis community. By integrating water chemistry with soil characteristics and plant-AMF interactions, this study seeks to elucidate how aquatic chemistry and wetland soil conditions shape mycorrhizal diversity and functionality.

Material and methods

Site description

The investigation was conducted at Dayet Oum Ghellaz, located 25 km southeast of the city of Oran (35°36'21" N 0°25'49" W). The lake is approximately 1 km in diameter and covers an area of 300 ha (Figure 1). The regional bioclimate is semi-arid Mediterranean, characterized by mild winters followed by hot, dry summers. Temperatures range from 5.1 °C to 32.9 °C, and the average annual precipitation is 325.7 mm (Bahi et al., 2020).

Sample collections

Water samples were aseptically collected from five locations at the lake surface, at depths of 0.5–1 m and placed in 1L sterile flasks for physicochemical analysis. The flasks were then transported to the laboratory in a cooler. Fourteen plant species representing 11 families were sampled, with five replicates per species.

Soil samples were collected from the rhizosphere at depths of 10 to 30 cm, with approximately 500 g obtained per sample. These samples were used to assess the diversity of arbuscular mycorrhizal fungal spores and to perform physicochemical analyses of the soil. Roots associated with each sampled plant were also collected to quantify their arbuscular mycorrhizal status.

The herbarium specimen's determination

The floristic inventory was conducted using classical references, including the Flora of North Africa (Maire, 1952–1987), the flora of Algeria and southern desert regions (Quézel and Santa, 1962–1963), as well as studies on saline fields and wetland vegetation in the Oran region (Quézel and Simonneau, 1960; Bahi et al., 2020). The perturbation index (PeI), which quantifies therophytization, was calculated according to Loisel and Gamila (1993) as follows:

$$PeI = \frac{[(\text{Number of chamaephytes} + \text{Number of therophytes}) \cdot 100]}{\text{Total number of species}} \quad (1)$$

Water physicochemical analyses

Physicochemical analyses of the water samples collected from the OG station were conducted at the Water and Sewerage Company laboratory (SEOR, Oran) following internationally recognized ISO standard procedures.

Biochemical oxygen demand (BOD₅) and chemical oxygen demand (COD) were determined according to ISO 5815-1/2:2003 and ISO 6060:1989, respectively. Total phosphates, nitrate, nitrite, ammonium, and sulphate were measured by UV-Visible spectrophotometry according to ISO standards (ISO 7890-3:1988; ISO 6777:1984; ISO 7150-1:1984; ISO 6878:2004; ISO 9280:1990). Chlorides were measured by titration (ISO 9297:1989), and total nitrogen was determined using the Kjeldahl method (ISO 5663:1984).

Soil Properties Analyses

Soil samples were air-dried at room temperature and sieved through a 2 mm mesh. Soil pH and electrical conductivity were measured using a 1:2.5 (v/v) soil-to-distilled-water suspension. Organic carbon (OC) was estimated using the Anne method (Mathieu and Pieltain, 2003), and organic matter (OM) was calculated using the equation: OM (%) = OC (%) × 1.72. Calcium carbonate equivalent (CCE) was determined using volumetric calcimetry, based on the reaction of calcium carbonate with strong hydrochloric acid (HCl) (36–38%, d = 1.2 g/mL at 25 °C, Sigma-Aldrich). Active calcium carbonate equivalent (ACCE) was quantified according to Drouineau (1942). Total organic nitrogen was determined using the Kjeldahl's method (Mathieu and Pieltain, 2003).

At the National Institute of Soils, Irrigation and Drainage (Matmar regional laboratory, Ghelizane province, analyses were conducted to determine available phosphorus (P₂O₅) and exchangeable cations (K⁺ and Na⁺), as well as grain size distribution using the Robinson pipette method.

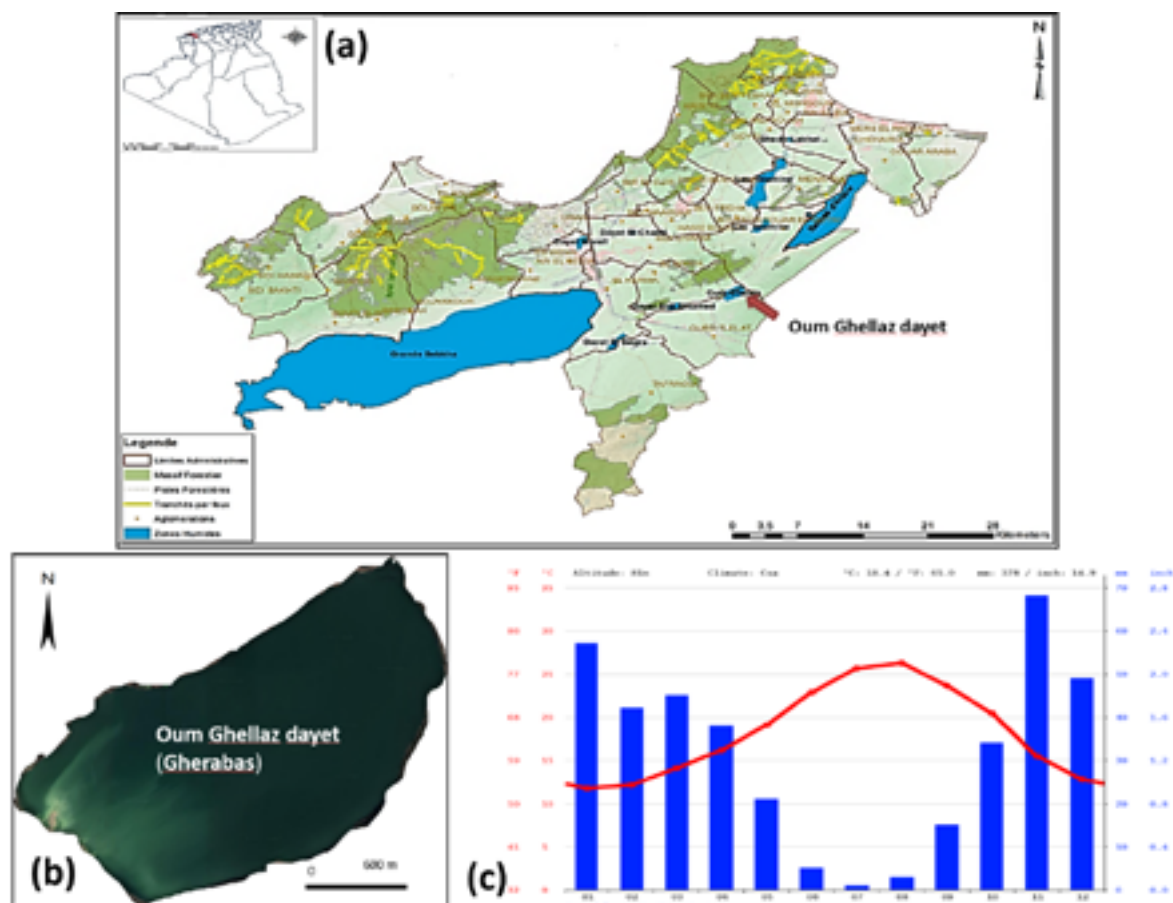


Fig. 1. The localization of Oum Ghellaz dayet: (a). The map of Oran highlights the studied site (Bahi et al., 2020); (b) Oum Ghellaz dayet; (c). Bagnouls and Gausson embrothermic diagram of Oran.

Soil and water metallic pollution index

Trace elements were extracted from 0.5 g of air-dried soil (105 °C) using the aqua regia method (ISO 11466:1995). Soil samples were digested with 2.5 mL of HNO₃ (≥ 65%, d = 1.37–1.41 g/mL at 20 °C, Sigma-Aldrich) and 7.5 mL of HCl (36–38%, d = 1.2 g/mL at 25 °C, Sigma-Aldrich), heated at 100 °C for 2 hours, filtered, and diluted to 25 mL with distilled water. The resulting extracts were stored at 4 °C and analyzed using an atomic absorption flame emission spectrophotometer (AAS Shimadzu AA-7000).

Heavy metal concentrations in lake water were determined using LCK test kits with a colorimetric method. The analyzed metals included chromium (Cr), cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), iron (Fe), and nickel (Ni).

The contamination level was evaluated using the metal index (MI), calculated as the ratio between the measured concentration of each heavy metal and its corresponding European Community standard or Algerian guideline value. An MI > 1 indicates that the sample is contaminated (Tamasi and Cini, 2004).

For both soil and water, the integrated pollution index (IP) was calculated as the mean of the metal index (MI) values obtained for the different metals in each sample:

$$IP = \frac{\sum MI}{n} \quad (n \text{ is the number of analyzed metals}) \quad (2)$$

IP > 1 indicates that the soil or water sample is polluted.

Arbuscular mycorrhization and dark septate endophyte colonization

Fine roots were cleared in a 10% (w/v) KOH solution at 90°C for 1 hour, rinsed three times, acidified in a 10% lactic acid solution for 10 minutes, and stained with 0.1% trypan blue (w/w) in lactophenol (Phillips and Hayman, 1970). AMF colonization was assessed under a light microscope using 30 root fragments of approximately 1 cm each per root system. Mycorrhizal development was evaluated using the method described by Trouvelot et al. (1986) and expressed as mycorrhizal frequency (F%), representing the proportion of root cortex infected by mycorrhiza, mycorrhizal intensity (% M and m%) in colonized root fragments and the entire root system, arbuscular richness (A%) and a%) in colonized root fragments. For dark septate endophytes (DSE), colonization frequency was estimated using the same approach as for mycorrhizal frequency (F %).

Isolation and morphological identification of AM fungal spores

The soil closely adhering to plant roots was collected after manually removing the bulk soil. AM fungal spores were isolated using the wet sieving method (Gerdemann and Nicolson 1963), followed by soil centrifugation (1398 × g for 10 minutes at room temperature) in a 50% sucrose solution and filtration through a 40 μm mesh (Brundrett et al. 1996). Intact spores were grouped based on their morphological similarity

Table 1: Values of soil and water physicochemical parameters and metal indices measured in Oum Ghellaz Dayet (Northwest Algeria)

Parameters	pH	EC (mS/cm)	Organic matter (mg/L)	Organic Carbon (mg/L)	CCE (%)	ACCE	Total Phosphate (mg/L)	Total Nitrogen (mg/L)	K ⁺ (meq/L)	Na ⁺ (meq/L)
Soil	7.87	5.35	20.96	12.16	21.52	7	62.77	0.560	0.4	21
Water	8.11±0.42	6.22±0.74	/	/	/	/	0.635±0.05	17.4±4.60	/	/
parameters	MES (mg/L)	COD (mg/L)	BOD₅ (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Ammonium (mg/L)	Sulphate (mg/L)	Chloride (mg/L)	Oxidability (mg/L)	
soil	/	/	/	/	/	/	/	/	/	/
Water	59.00±1.00	100.79±18.7	29.70±23.86	21.43±0.39	1.51±0.025	3.34±0.15	828.04±40.06	3473.27±649.56	76.64±5.81	
parameters	Pb²⁺	Fe³⁺	Cu²⁺	Zn²⁺	Cd²⁺	Ni²⁺	Cr	PI		
Soil MI	0.67	0.80	0.095	2.49	17.21	10.4	/	5.27		
Water MI	2.29±1.43	2.21±0.44	0.179±0.09	0.23±0.07	2.12±1.11	10.18±7.95	0.98±0.41	2.59		

CE: electrical conductivity; CCE: calcium carbonate equivalent; ACCE: acid cation exchange capacity; MES: suspended matter; COD chemical oxygen demand. BOD₅: Biochemical oxygen demand; MI: metallic index; PI: pollution index.

and observed under a stereomicroscope (Leica EZ4HD), and their diameter was measured. Permanent slides of all spores were prepared using a drop of polyvinyl alcohol/lactic acid/glycerol (PVLG) mixed with Melzer's reagent (1:1, v/v) on a slide. Species identification was based on spore morphological characteristics and confirmed by comparison with published descriptions (Oehl et al. 2011; Redecker et al. 2013).

Statistical analysis

Ecological diversity measures used to describe the structure of AMF communities included spore density (number of spores in 100g of soil), species richness (number of identified AMF species per soil sample), relative abundance (RA), isolation frequency (IF), Shannon's diversity index (H'), evenness (E), Simpson's dominance index (D), and Sorensen's similarity coefficient (C_s) (Simpson 1949). Relative abundance refers to the proportion of individuals of a given species relative to the total community and was calculated using the following formula:

$$RA = \frac{\text{spore number of a species (genus)}}{\text{total number of identified spore samples}} \cdot 100 \quad (3)$$

The Shannon diversity index is a mathematical measure of species diversity in a community. H' value was calculated according to the formula:

$$H' = -\sum P_i \ln P_i \quad (4)$$

where P_i is the relative abundance of each identified species per sampling site and calculated by the following formula:

$$P_i = \frac{n_i}{N} \quad (5)$$

where n_i is the number of spores of a species and N is the total number of all identified spores.

The evenness also called "equitability", refers to homogeneity of the species, $0 < E < 1$, $E = 1$ means that all species have the same frequency. It was calculated according

$$\text{to the formula: } E = \frac{H'}{H'_{\max}} \quad (6)$$

where $H'_{\max}$ is the maximal H' which can be determined by the following formula:

$$H'_{\max} = \ln S \quad (7)$$

where S is the total number of identified species per sampling site.

The Simpson's dominance index (D) measuring the probability that two randomly selected individuals in a community belong to the same species ($0 < D < 1$) was calculated

$$\text{using the formula: } D = \frac{\sum n_i(n_i - 1)}{N(N - 1)} \quad (8)$$

Results

Dayet Oum Ghellaz (OG) soil and water physicochemical analysis

The data on soil and water parameters reveal several insights into their conditions (Table 1). The soil analysis also indicated that the texture is loamy clayey, comprising 42% clay, 31.42% silt, and 26.41% sand.

The OG soil has a pH of 7.87, indicating near-neutral conditions that are generally favorable for most crops.

In contrast, OG water exhibits a slightly alkaline pH of 8.35 ± 0.15 , which may affect nutrient availability, particularly micronutrients. The electrical conductivity (EC) of the OG soil is 5.35 mS/cm, indicating moderate salinity, while OG water shows a higher EC of 6.85 ± 0.05 mS/cm, suggesting potential salinity stress. Soil organic matter and organic carbon levels are relatively high, at 20.96% and 12.16% respectively, indicating substantial organic content that may support soil fertility. However, soil nitrogen content is moderate (0.560 mg/L), whereas water shows a higher concentration (17.4 mg/L). The calcium carbonate equivalent (CCE) of the OG soil is 21.52%, which may contribute to pH buffering and calcium availability, while the acid cation exchange capacity (ACCE) is relatively low (7), suggesting limited nutrient retention capacity. Phosphorus availability in the soil is high (62.77 ppm), which is important for root development, whereas potassium levels are low (0.4 meq/l). Sodium concentration in the OG soil is elevated (21), indicating potential salinity-related constraints.

Chemical oxygen demand (COD) represents the amount of oxygen by strong chemical oxidants to oxidize organic and inorganic substances in water (Rejsek, 2002). In the OG water, COD is high (100.79 ppm), indicating substantial organic and inorganic load that may influence water quality and soil functioning. Suspended particulate matter (MES) is also notable (59.00 ± 1.00 mg/l), providing insights into microbial activity. The water sample also shows high concentrations of inorganic nitrogen, sulphate, chlorides and elevated oxidability.

The soil and water metal indices ($MI > 1$) indicate that metallic trace element (MTE) concentrations exceed the soil and water quality guidelines, respectively, as defined by AFNOR NFU 44-041 and Executive Decree No.11-125 of 22 March 2011 on water quality, suggesting potential environmental contamination.

In soil, very high pollution levels of cadmium (17.21) and nickel (10.4) indicate significant contamination risks, while zinc (2.49) shows elevated levels that may be associated with anthropogenic sources. Water indices reveal concerning mean values for lead (2.29 ± 1.43) and cadmium (2.12 ± 1.108), indicating potential ecological and health risks. The high variability in nickel concentrations (10.18 ± 7.95) further suggests possible point-source pollution, necessitating immediate monitoring and remediation efforts.

Plant species composition and abundance

The floristic survey conducted at the site identified 25 vascular plant species belonging to 14 families (Table 2). The flora is primarily characterized by the dominance of taxa from the Asteraceae family (28%), followed by Amaranthaceae and Poaceae (12% each), and Solanaceae (8%). The remaining families collectively account for 4% of the species diversity. The table presents a diverse range of plant species categorized by family, their abundance, and biological types. Among the species listed, therophytes are the dominant biological type, representing approximately 60% of the total flora. Hemicryptophytes, including species such as *Beta vulgaris* subsp. *maritima* and *Centaurea* sp., account for about 24%. Phanerophytes, such as *Olea europea* and *Tamarix gallica*, represent around 8% of the species, while chamaephytes make up about 4%, represented by *Suaeda vera*. Geophytes, represented by *Foeniculum vulgare*, also account for about 4%.

Table 2: Abundances of plant species in the Dayet Oum Ghellaz, Algeria. Abundance classes: I: very rare, II: rare, III: occasional, IV: frequent, V: abundant (Bradai et al. 2015).

Family	Taxa	Plant species abundance	Biological type
Amaranthaceae	<i>Beta vulgaris</i> subsp. <i>maritima</i> Arcang (L.)	II	Hemicryptophytes
	<i>Chenopodium album</i> L.	II	Therophytes
	<i>Suaeda vera</i> Forssk. ex J.F. Gmel.	II	Chamaephyte
Apiaceae	<i>Foeniculum vulgare</i> (Mill.) Gaertn.	II	Geophyte
Asteraceae	<i>Centaurea</i> sp.	II	Hemicryptophytes
	<i>Chrysanthemum coronarium</i> L.	II	Therophytes
	<i>Echinops spinosus</i> ssp. <i>eu-spinosus</i> M.	II	Hemicryptophyte
	<i>Onopordon macrocanthum</i> Schousb.	II	Therophytes
	<i>Silybum marianum</i> (L.) Gaertn.	II	Therophytes
	<i>Scolymus hispanicus</i> L.	IV	Therophytes
	<i>Xanthium spinosum</i> L.	II	Therophytes
Brassicaceae	<i>Sinapis arvensis</i>	IV	Therophytes
	<i>Raphanus raphanistrum</i>	III	Therophytes
Caryophyllaceae	<i>Vaccaria hispanica</i>	III	Therophytes
Convolvulaceae	<i>Convolvulus althaeoides</i> subsp. <i>elegantissimus</i> (Mill.) Fiori	II	Hemicryptophytes
Juncaceae	<i>Juncus acutus</i> L.	V	Hemicryptophytes
Oleaceae	<i>Olea europea</i> L.	II	Phanerophytes
Papaveraceae	<i>Papaver rhoeas</i> L.	II	Therophytes
Plantaginaceae	<i>Plantago lagopus</i> L.	II	Hemicryptophytes / Therophytes
Poaceae	<i>Avena sativa</i>	II	Therophytes
	<i>Hordeum maritimum</i> With subsp. <i>maritimum</i>	II	Therophytes
	<i>Hordeum vulgare</i>	III	Therophytes
Primulaceae	<i>Anagallis arvensis</i> subsp. <i>parviflora</i> (Hoff. & Link.) Batt.	III	Therophytes
Solanaceae	<i>Whitania frutescens</i> L.	I	Nanophanerophytes
Tamaricaceae	<i>Tamarix gallica</i>	V	Phanerophytes

Plantago lagopus is classified as both a hemicryptophyte and therophyte. The perturbation index (PeI) for the study area was 64%.

Fungal root endophyte colonization of wetland plant species

In the study, 14.28% of the plant species were identified as non-mycorrhizal. Among the mycorrhizal types, 58.33% were classified as *Arum* type, while 16.66% were categorized as *Paris* type. Additionally, 50% of the plants exhibited dark septate endophytes (DSE), and two species were found to form ectomycorrhizae. Most plant species were highly mycorrhized ($F > 50\%$) (Table 3, Figure 2).

AMF community composition and diversity

From the analyzed rhizospheric soils, 18 morphospecies were identified based on morphological criteria (Table 4). The arbuscular mycorrhizal (AM) community comprised several taxa, including Acaulosporaceae with 9 taxa represented by two genera: *Acaulospora* (8 species) and *Kuklospora*

(1 species); Glomeraceae with 7 taxa, including *Glomus* (3 species), *Funneliformis* (2 species), *Rhizoglossum* (1 species), and *Sclerocystis* (1 species); Archaeosporaceae represented by *Archaeospora* (1 species); and Ambisporaceae represented by *Ambispora* (1 species).

The highest spore abundance was noted for Acaulosporaceae (50.6%), followed by Glomeraceae (30.68%), then Archaeosporaceae (13.97%), and Ambisporaceae (2.18%) (Table 4).

Diversity measurements of AMF communities in Dayet Oum Ghellaz

The ecological parameters provide valuable insights into the diversity and distribution of fungal species within the studied community. The Shannon diversity index ($H' = 2.455$) indicates a relatively high level of diversity. The evenness value ($E = 0.76$), close to 1, reflects a fairly uniform distribution of individuals among species. Additionally, Simpson's dominance index ($D = 0.1$) indicates low dominance, suggesting a low probability that two randomly selected individuals belong to

the same species, which further supports the observed high diversity.

DISCUSSION

This study highlights significant environmental disturbances affecting the Dayet Oum Ghellaz wetland ecosystem, primarily driven by water contamination characterized by elevated salinity and heavy metal concentrations. The high perturbation index (PeI) of 64% recorded in the native flora reflects substantial ecological stress, consistent with the

pollution indices indicating considerable contamination by cadmium, nickel, zinc, and lead. Water quality assessment revealed elevated sulphate concentrations, inorganic nitrogen, and oxygen demand, signifying high oxidative conditions that further threaten biological integrity. Despite these adverse conditions, most plant species maintained high levels of mycorrhizal colonization, with over half hosting dark septate endophytes and some forming ectomycorrhizae, suggesting a degree of symbiotic resilience under pollutant stress. The identification of 18 AMF morphospecies, predominantly

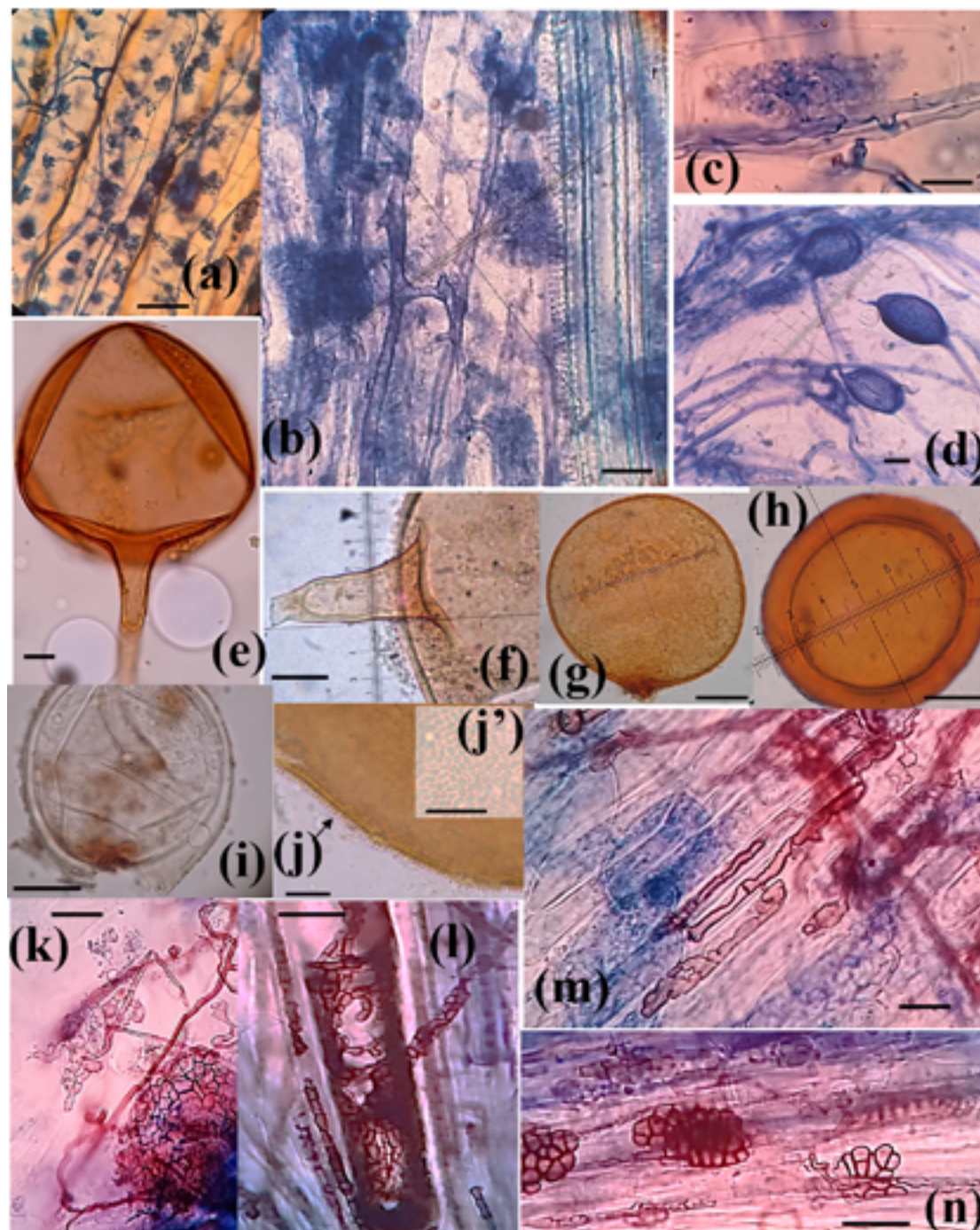


Fig. 2. Fungal diversity in the rhizospheric soil and inside studied plant roots: (a)-(d): AMF mycelium in the roots of investigated plant species. (e)-(j): AMF spores isolated from plant species rhizosphere: *F. mosseae* (e), *F. coronatum* (f); *G. macrocarpum* (g), *A. melea* (h), *Acaulospora* sp.1(i), *Acaulospora spinosa* (j) the arrow indicates spine ornamentations on spore wall (j'). (K)-(n): Brownish thick mycelium aggregates to form a puzzle or Brain-like microsclectrotium, lobed mycelium. Scale bars: (a)=100 μ m; (b), (f), (h), (i), (l), (m), (n)=20 μ m; (c)=10; (d), (e), (g), (j), (k), =50 μ m.

Table 3: Fungal root endophyte colonization of wetland plant species

Family	Plant Species	AM type	F%	M%	m%	a%	A%	F% DSE
Amaranthaceae	<i>Beta vulgaris</i> subsp. <i>Mari-tima</i> Arcang (L.)	I	24.24	16.7	68.88	19.82	16.67	03.33
Apiaceae	<i>Foeniculum vulgare</i>	P	82.5	43.75	53.05	84.47	36.96	2
Asteraceae	<i>Silybum marianum</i> (L.) Gaertn.	A	75.86	28.07	37	96.52	27.09	0
	<i>Centaurea</i> sp.	A	96.67	35.97	37.21	93.9	33.56	0
	<i>Xanthium spinosum</i> L.	A	60	23.23	38.71	89.93	20.89	44.44
Caryophyllaceae	<i>Vaccaria hispanica</i>	-	17.85	0.32	1.8	0	0	0
Juncaceae	<i>Juncus acutus</i> L.	NM	0					50
Malvaceae	<i>Malva</i> sp.	A	77	14.65	18.92	67.42	9.87	0
Plantaginaceae	<i>Plantago lagopus</i> L.	A	73.33	14.37	19.59	85.27	12.25	0
Oleaceae	<i>Olea europea</i> L.	A	55.26	11.47	20.76	55.32	6.35	47.36
Poaceae	<i>Hordeum vulgare</i>	A	52.5	8.18	15.57	79.27	6.48	12.5
	<i>Avena sativa</i>	I	72.22	59.78	82.77	97.49	58.23	0
Primulaceae	<i>Anagalis caerulea</i>	P	58	12.36	21.31	76.97	9.51	0
Tamaricaceae	<i>Tamarix gallica</i>	NM	0	0	0	0	0	73.33
AM type: Arbuscular mycorrhizal type; F%: mycorrhizal frequency; M%, m%: mycorrhizal intensity of colonized root fragments and the entire root system; A%: arbuscular richness; a %: arbuscular richness of colonized root fragments; F%DSE: mycorrhizal frequency of Dark Septate Endophytes colonization. I: intermediary P: Paris-type; A: Arum-type; - : not determined; NM: Non-mycorrhizal.								

belonging to Glomeraceae and Acaulosporaceae, highlights the diversity of fungal symbionts present. These findings emphasize the profound impact of heavy metal pollution on wetland biodiversity and plant-fungal interactions, emphasizing the urgent need for targeted environmental management and further research to develop mitigation strategies and preserve ecosystem functionality.

Soil and water pollution index values exceeding 1 clearly indicate that Dayet Oum Ghellaz is a contaminated site. Similar contamination patterns have been documented in nearby wetlands, including Dayet Morsli and Telamine Lake, as well as in the water of Oum Ghellaz (Aibeche et al., 2020; Sidhoum et al., 2020). These studies reveal the presence of heavy metals such as lead (Pb), cadmium (Cd), copper (Cu), and chromium (Cr), largely associated with industrial activities in the region. Additionally, studies by Belabed et al. (2017) and Louhi et al. (2012) report high concentrations of metallic trace elements in soils surrounding the El-Hadjar steel complex, emphasizing the widespread nature of soil contamination driven by industrial emissions and anthropogenic pressures.

The elevated pollution index of lead in water (2.29 ± 1.43), alongside measurable soil levels (0.67), suggests potential leaching from contaminated soils as a primary pathway affecting water quality, highlighting associated health risks linked to lead exposure. Cadmium presents a contrasting pattern, with high soil concentrations (17.21), but relatively lower water levels (2.12), indicating limited mobility and restricted transfer from soil to water. This persistence in soil raises concerns regarding long-term ecological accumulation. In contrast, zinc exhibits a similar pattern, with higher soil levels (2.49) and minimal presence in water (0.23), suggesting limited mobility likely influenced by soil physiochemical conditions. Copper, however, shows relatively higher levels in water than in soil,

implying active anthropogenic contributions, possibly from agricultural runoff or industrial discharges, warranting further investigation.

Overall, the distribution patterns of these metals reflect diverse sources and pathways of pollution. Lead likely originates from atmospheric deposition and historical use of lead-contained products, with subsequent mobilization via rainfall and runoff. Iron appears naturally abundant but may also be influenced by mineral weathering or industrial processes (Taylor and Konhauser, 2011; Mizerna et al. 2025). Copper and zinc are likely associated with both agricultural practices and industrial activities, with zinc showing limited mobility despite elevated soil levels (Xue et al. 2000; Moliszewska et al. 2025). Cadmium and nickel, both associated with industrial processes such as mining and refining, demonstrate variable distribution between soil and water, emphasizing the need for comprehensive environmental monitoring (Fishbein, 1981; Wolska et al. 2024). These findings emphasize the importance of integrated environmental management strategies that address both soil and water quality, aiming to reduce health risks and preserve ecological integrity.

The high prevalence of therophytes (annual plants) observed in this study, representing a substantial proportion of the flora, indicates a marked level of therophytization driven by environmental conditions such as seasonal flooding (Hammada et al. 2004; Miara et al., 2016). This dominance of therophytes, reflected by a high perturbation index, signals a concerning shift in plant communities linked to human-induced stressors. This ecological pattern, characterized by the prevalence of annual species adapted to disturbed and open habitats, is often linked to ecosystem degradation processes, including overgrazing and subsequent biodiversity loss (Grime 1977; Barbero et al. 1989a and b). The elevated perturbation

index further underscores anthropogenic pressure negatively affecting the vegetation and reflecting broader environmental degradation (Touarfia et al. 2021).

The data suggest potential pollution in the water sample, with a notable presence of organic matter that may contribute to oxygen depletion, thereby affecting aquatic organisms. Elevated nitrate concentrations, together with increased nitrite and ammonium levels, indicate ongoing nitrogen transformations and possible toxicological risks. High sulfate and chloride levels may originate from industrial discharges or urban runoff, further impacting water quality. The oxidability value indicates a significant oxidation capacity, likely due to organic pollutants, highlighting the importance of continued monitoring and management of the water body (Wu et al. 2023).

Additionally, the lake is bordered by agricultural land, where runoff and rainfall can carry nitrates from fertilizers into the aquatic system. Nitrate commonly originates from fertilizer application, manure decomposition, or septic effluents, and represents the final stage of nitrogen oxidation following organic matter decomposition (Hakeem et al 2017). Agricultural activities are widely recognized as a major source of nitrate pollution, contributing to the deterioration of water quality (Lawniczak et al. 2016). Excess nitrate can stimulate excessive growth of algae and aquatic plants, leading to eutrophication. This process can threaten biodiversity and disrupt the ecological balance of the aquatic ecosystem (Tiwari and Pal, 2022).

High levels of chloride (Cl⁻) in wetlands typically originate from agricultural runoff, road de-icing practices, industrial discharges, and natural geological sources. Elevated chloride concentrations can lead to increased salinity, which may stress freshwater organisms, reduce biodiversity by favoring salt-

tolerant species, and impair plant growth by interfering with nutrient uptake. Such shifts in species composition can disrupt ecosystem functions, including food webs and nutrient cycling, ultimately compromising wetland ecological integrity and the associated flora and fauna (Hill and Sadowski, 2016).

When sulfate is abundant, sulfate-reducing bacteria (SRB) utilize it during organic matter decomposition, producing sulfide, which contributes to nutrient cycling and organic matter breakdown. This process can alter wetland redox potential and influence other microbial communities. However, excessively high sulfate levels may generate toxic hydrogen sulfide, posing risks to aquatic organisms and disrupting ecosystem balance. Therefore, maintaining appropriate sulfate levels is crucial for preserving wetland ecological functioning (Lamers et al., 1998).

In wetlands, sulfate and iron interactions are particularly important under anaerobic conditions, where sulfate-reducing bacteria produce sulfide, influencing iron mobility in iron-oxide-rich environments. Sulfate mainly originates from the dissolution of minerals such as gypsum and pyrite (Ghazali and Zaid, 2013), but anthropogenic inputs, including industrial discharges (Osibanjo et al., 2011) and chemical fertilizer application (Iounes et al., 2016) also contribute. Certain microorganisms can oxidize hydrogen sulfide back to sulfate, thereby further regulating sulfur cycling (Belghiti et al., 2013). Under reducing conditions, sulfate reduction may promote the conversion of ferric iron to soluble ferrous iron, increasing its mobility and availability. Conversely, excessive sulfide production can lead to the precipitation of iron as iron sulfides, removing it from the water column and altering its biological functions. Both elevated sulfate and iron can significantly modify wetland geochemistry, impacting plant growth and microbial community structure. Consequently,

Table 4: The spore density and relative abundance (RA) of AMF species isolated in the study site:

Amf family	AMF species	Relative abundance (%)
Acaulosporaceae	<i>Acaulospora spinosa</i>	2.18
	<i>Acaulospora thomii</i>	13.97
	<i>Acaulospora melea</i>	14.59
	<i>Acaulospora tortuosa</i>	1.2
	<i>Acaulospora</i> aff. <i>excavata</i>	6.55
	<i>Acaulospora</i> cf. <i>morrowae</i>	9.6
	<i>Acaulospora</i> sp1.	2.15
	<i>Acaulospora</i> sp.2	1.31
	<i>Kuklospora spinosa</i>	1.5
Ambisporaceae	<i>Ambispora reticulata</i>	2.3
Archaeosporaceae	<i>Archaeospora myriocarpa</i>	13.97
Glomeraceae	<i>Funneliformis coronatum</i>	3.62
	<i>Funneliformis mosseae</i> .	10.9
	<i>Glomus achrum</i>	1.74
	<i>Glomus nanolumen</i>	1.31
	<i>Glomus</i> sp.	1.9
	<i>Rhizoglomus intraradices</i>	10.91
	<i>Sclerocystis</i> sp.	0.3
Unidentified		4.45
Species Richness		18
Spore density in 100g of soil		1145

careful monitoring is essential, as imbalances may contribute to toxicity risks, biodiversity loss, and altered ecosystem functioning.

The high prevalence of dark septate endophytes (DSE), observed in this study at over 50%, aligns with previous research emphasizing their dominance in stressed ecosystems such as wetlands (Kumar and Muthukumar, 2014). The findings also reveal a positive correlation between DSE colonization and elevated soil concentrations of chromium (Cr) and copper (Cu), suggesting that these fungi may infect plant roots under metal-enriched conditions or that host plants may utilize DSE as a strategy to mitigate abiotic stress in polluted conditions. DSE have been reported to facilitate the accumulation of specific trace elements and promote plant growth (Li et al. 2024). Although the functional role of melanin in these symbioses is not yet fully understood, it appears to contribute to stress tolerance in both fungi and their plant hosts (Cordero and Casadevall, 2017; Hidayat, 2019). Moreover, DSE have been shown to enhance host tolerance to extreme temperatures and heavy metals, potentially through melanin production (Santos et al., 2021; Yu et al., 2021). According to these authors, DSE tend to be more resilient under adverse environmental conditions than mycorrhizal fungi, either due to being more competitive in disturbed or moderately polluted soils or superior adaptation to environmental stressors.

The species richness observed in this region is similar to that typically found in wet environments but lower than values reported by Wang et al. (2011) and Marins et al. (2009), which ranged from 23 to 27 morphotypes. According to Moore et al. (2016), arbuscular mycorrhizal (AM) fungal diversity in wetlands is quite high and comparable to that observed in many terrestrial ecosystems. The Shannon diversity index ($H = 2.46$) indicates a relatively high AMF diversity in this wetland compared with several similar environments. For example, lower values have been reported in the Abras de Mantequilla wetlands (Ecuador), ranging from approximately 1.78 to 1.90 (Prieto-Benavides et al. 2025), while assessments of fungal communities in other neotropical settings, such as mangrove sediments, show peak diversity values around 2.17. Although Shannon index values typically range from about 1.5 to 3.5, the value of 2.46 suggests a moderately high and relatively well-distributed fungal community compared with more disturbed systems, where values may fall between approximately 0.74 and 2.06 (Masebo et al. 2023).

The prevalence of Acaulosporaceae and Glomeraceae has been widely documented in studies of wetland ecosystems (Miller and Bever, 1999; Sun et al., 2016; Yang et al., 2015; Ban et al., 2017; Sidhoum et al., 2020; Sidhoum et al., 2025). This dominance is largely attributed to the ability of Glomeraceae to form anastomoses between hyphal networks, facilitating the reconnection and maintenance of fungal mycelial structures (de la Providencia et al., 2005). Additionally, these fungi can propagate via mycelial fragments, mycorrhizal root pieces, and spore germination (Yang et al., 2015). In stressed soils, the most prevalent arbuscular mycorrhizal fungal (AMF) species include *Acaulospora thomii*, *Acaulospora melea*, *Archaeospora myriocarpa*, *Rhizoglossus intraradices*, and *Funneliformis mosseae* (Xavier Martins and Rodrigues, 2020; Lumibao et al., 2024), which is consistent with the patterns observed in this study. However, further research is necessary

to determine the relative influence of host plant identity and environmental factors, such as climate and soil physiochemical properties, on AMF community composition. These fungi play a key role in enhancing plant resilience to environmental stressors, especially in ecosystems affected by high salinity and elevated heavy metal concentrations.

Conclusion

In conclusion, this study highlights the significant impact of water contamination, particularly with heavy metals and high salinity, on the Dayet Oum Ghellaz wetland ecosystem. The high perturbation index and elevated pollution levels demonstrate substantial environmental stress, which potential consequences for biodiversity and ecological stability of the area. Despite these challenges, the persistence of diverse mycorrhizal fungi and plant-fungal symbioses suggests a degree of resilience within belowground biological interactions.

Ethical approval: This study does not involve any research conducted on human participants or animals by any of the authors.

Competing interests: The authors declare no conflicts of interests.

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