

DISCOVERING BIOREMEDIATION MICROORGANISMS IN A TEMPORARY WETLAND OF CENTRAL MEXICO HIGHLANDS USING ENVIRONMENTAL DNA METABARCODING

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ABSTRACT

This study employed environmental DNA (eDNA) analysis through metabarcoding, alongside bright-field microscopy, to assess biodiversity and identify microorganisms with bioremediation potential in the temporary wetlands of Querétaro, Mexico. Environmental DNA provides a non-invasive and innovative method for monitoring various organisms, from microbes to higher taxa. Samples were collected from a temporary wetland in Pedro Escobedo. WilderLab® processed environmental DNA samples, and microscopy (using a UNICO G504 trinocular microscope) was conducted at UAQ's Laboratory of Molecular Genetics and Evolutionary Ecology (100 hours of observation, 90 wet mounts). Our findings revealed 136 Operational Taxonomic Units (OTUs) across 25 phyla, including bacteria, green algae, ciliates, crustaceans, and rotifers. In addition, 32 species and morphospecies of green algae, diatoms, and cyanobacteria were identified microscopically. The literature review highlighted that several identified bacterial and green algae species could degrade organic and inorganic pollutants, e.g., *Exiguobacterium* sp., *Gemmatimonas* sp., *Methylobacterium* sp., *Psychroglaciecola* sp. for arsenic, lead, mercury; *Chryseobacterium* sp. and *Acholeplasma* sp. for radioactive elements such as uranium. The findings contribute to understanding microbial diversity in Querétaro's wetlands and support future research on employing native microorganisms for aquatic ecosystem remediation.

Keywords: eDNA, biodiversity, bioremediating bacteria, Operational Taxonomic Unit, Querétaro state.

1 INTRODUCTION

Mexico's rich topography, soil, climate, and vegetation have contributed to a high diversity of wetland types, covering approximately 114,000 km² of this territory (56 % inland wetlands and 44% coastal wetlands) (Olmsted 1993; Instituto Nacional de Estadística y Geografía [INEGI] 2025). These ecosystems provide critical ecosystem services, including food, water, flood control, groundwater recharge, biogeochemical regulation, and cultural value (Davidson et al. 2019; Wood et al. 2024). Wetlands offer various ecosystem services, including regulating water quality through bioremediation. Therefore, identifying microorganisms with bioremediation potential is key, particularly in the temporary/ephemeral wetlands of Querétaro state, a unique ecosystem (like Californian vernal pools) that is poorly studied (Lobato-de Magalhães et al. 2022).

Microorganisms in wetland sediments can break down various pollutants, such as organic waste, industrial chemicals, hydrocarbons, pesticides, heavy metals, and nanoparticles, through specialized metabolic pathways. These processes are essential for the ecosystem's self-purification and resilience. Environmental DNA (eDNA) analysis through metabarcoding allows large-scale biodiversity assessment to identify and monitor organisms, ranging from microorganisms to higher organisms such as plants and animals. This novel and non-invasive technique has been applied for studying biodiversity (Bird et al. 2024; Blackman et al. 2024; Foulquier et al. 2024). This powerful, non-invasive tool for studying microbial diversity in aquatic systems allows culturable and unculturable species to be detected. This research aimed to identify microbial assemblages of a temporary wetland in Pedro Escobedo, Querétaro, focusing on its bioremediation potential. Understanding microbial diversity and its remediation potential in highland temporary wetlands is crucial for identifying new taxa and underscoring the importance of this unique ecosystem. It will support efforts to protect these threatened ecosystems. To gain deeper insights into these ecosystems and their potential for bioremediation, the following research questions were proposed: (1) What is the diversity (at

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a lower taxonomic level) of microorganisms in the wetland? (2) Are there any microorganisms identified as remediators? and (3) Which microorganisms would be particularly relevant for future remediation studies?

2 MATERIAL AND METHODS

2.1 Study Area and Sampling

The study area was located in a temporary wetland, municipality of Pedro Escobedo, Querétaro (Lat. 20°24'38.2", Long. -100°15'58.6", altitude 2,305 m a.s.l.), covering approximately one hectare of land (Lobato-de Magalhães et al. 2022). The climate in this region is semi-arid and semi-warm, classified as Cwb in the Köppen climate classification; this implies that the region experiences warm, dry summers with maximum temperatures ranging between 25°C and 32°C. Winters are temperate, with temperatures fluctuating between 18°C and 22°C. The rainy season extends from May to September, with July and August being the wettest months, while the dry season lasts from October to April, with December and January being the driest. The average annual precipitation in the region ranges from 500 to 600 mm (INEGI 2025).

The wetland exhibits fluctuations in hydroperiods, being intermittently flooded, with the water level varying seasonally according to rainfall. The site is flooded chiefly from June to September, while from October to December, the water level decreases, resulting in shorter hydroperiods. These fluctuations are the main drivers of community dynamics, and by supporting biota adapted to temporary waters, these wetlands contribute to the diversity of aquatic and facultative plants and animals (Calhoun et al. 2017). The aquatic flora of the temporary wetland consists of species such as *Eleocharis acicularis*, *E. densa*, *Luziolla fluitans*, *Marsilea mollis*, *Najas guadalupensis*, *Nymphoides fallax*, *Sagittaria demersal* (Lobato-de Magalhães and Martínez 2018; Lobato-de Magalhães et al. 2022) (Figure 1).



Figure 1. Highland temporary wetland (2305 m a.s.l.), Pedro Escobedo, Querétaro, Mexico. (Photo: T. Lobato-de Magalhães)

Environmental DNA was measured in water samples and were collected from the temporary wetland at the beginning and end of the rainy season 2024. The recommendations provided by the manufacturer of the Comprehensive Freshwater eDNA Mini Kit WilderLab® for analysis were strictly followed for sample collection, handling, and storage. Genetic material was collected from the equivalent of 1 L of water from 20 random points within the wetland, using a filter while taking necessary precautions to minimize exposure to air, sunlight, and contamination from the collector's DNA. Factors can affect the integrity of the eDNA in the samples and compromise the quality of the results. For shipment to the laboratory, the samples were preserved in collecting filters with the addition of a preservative provided by the manufacturer, allowing for storage for up to six months, ensuring optimal sample preservation until the metabarcoding analysis.

For water samples to be observed under microscopy, three 100 ml samples were collected at three random points distributed throughout the wetland, using sterile bottles and discarding two sample volumes before collecting the final definitive sample. The samples were kept under cold chain conditions, using a cooler with refrigerant bags, and then stored at 4°C. Additionally, there was an *in situ* analysis of the water's physical and chemical parameters at three points within the wetland using a Hanna HI 91130 probe as follow (range) pH 6.23-6.80, dissolved oxygen 3.47-4.83 mg/L, conductivity 75,00-127.0 µS/cm, total dissolved solids 20.50-63.50 ppm, salinity 0.02-0.06 PSU.

2.2 Environmental DNA Metabarcoding

The Comprehensive Freshwater eDNA analysis (WilderLab®, <https://www.wilderlab.co.nz>) was used to identify crustaceans, algae, and other microorganisms. The DNA extraction, sample quality control, barcode library preparation/indexing, sequencing, and bioinformatics analysis were performed by WilderLab®, including noise removal and taxonomic identification. This mass DNA sequencing method uses a specific region of the genome, referred to as a ‘barcode’ or ‘marker’, which enables the identification and classification of species present in a sample, since it is unique to each species. We employed a variant of environmental DNA barcoding called metabarcoding, which amplifies and sequences multiple barcodes simultaneously (Shea et al. 2023).

2.3 Optical Microscopy

For the preparation of permanent slides, the process involved fixing the samples onto the slides using 70% ethanol, as it is less aggressive to cells and minimizes the contraction or distortion that may occur with higher ethanol concentrations. Once the ethanol evaporated, a coverslip was placed, and the sample was sealed with clear varnish. Each slide was labeled with the date, the sampling site, and the name of the student responsible for its preparation. Given the ephemeral nature of the wetland, these permanent slides serve as invaluable tools for documenting the microbial communities of the ecosystem. They also facilitate comparison between microscopic observations and the eDNA analysis obtained.

Water samples were analyzed using bright-field optical microscopy on a UNICO G504T microscope, with the primary goal of describing the taxa present in the wetland by identifying microorganisms such as diatoms, green algae, dinoflagellates, cyanobacteria, amoebae, ciliates, rotifers, cladocerans, copepods, pollen grains, and fungi. Ten observations of each freshwater sample were conducted at 10x and 40x magnification (100 hours of observation, 90 wet mounts). As an integral part of the process, the observations were documented through photographic and video recordings of the identified microorganisms, using a digital AmScope camera mounted on a C-mount and coupled to a

trinocular UNICO G504T optical microscope. This photographic archive was supplemented with written descriptions and the application of taxonomic keys for microorganism identification. Optical microscopy was conducted at the Laboratory of Molecular Genetics and Evolutionary Ecology at UMBA, in the Applied Microbiology Unit of UAQ. Taxonomic guides were used as references for identifying the diversity of the samples, and digital tools such as Algae Database (<https://www.algaebase.org>), iNaturalist (<https://www.inaturalist.org>), and Encyclopedia of Life (<https://eol.org/>) were consulted.

3 RESULTS AND DISCUSSION

3.1 Environmental DNA Metabarcoding

We identified 136 Operational Taxonomic Units (OTUs), distributed across 25 phyla, 37 classes, and 53 orders, including the following: bacteria (73 OTUs), green algae (19 OTUs), ciliates (10 OTUs), crustaceans (9 OTUs), rotifers, cryptomonads, flagellates (5 OTUs apiece), heterokont algae (4 OTUs), diatoms (2 OTUs), fungi, oomycetes, protists (1 OTU apiece) (Figure 2 and 3; Table 1).

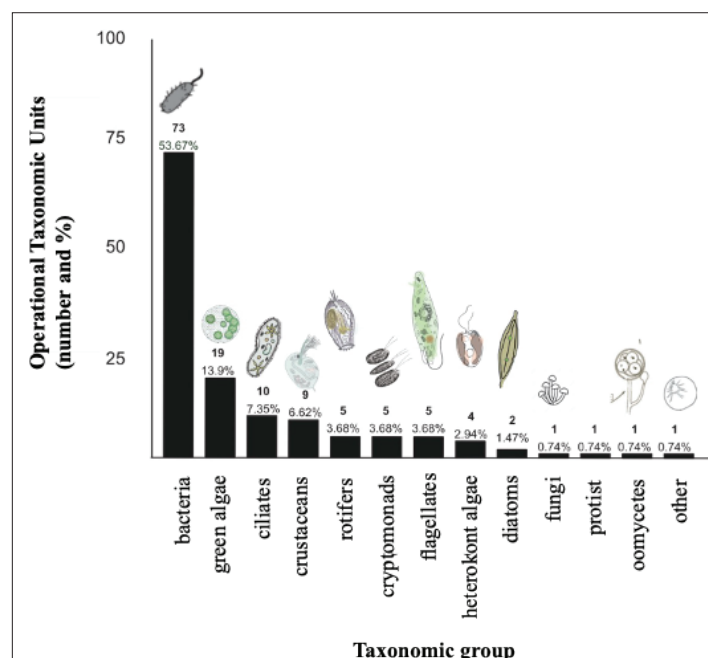


Figure 2. Operational Taxonomic Units richness and percentage by taxonomic group by environmental DNA samples of a highland temporary wetland, Pedro Escobedo, Querétaro, Mexico.

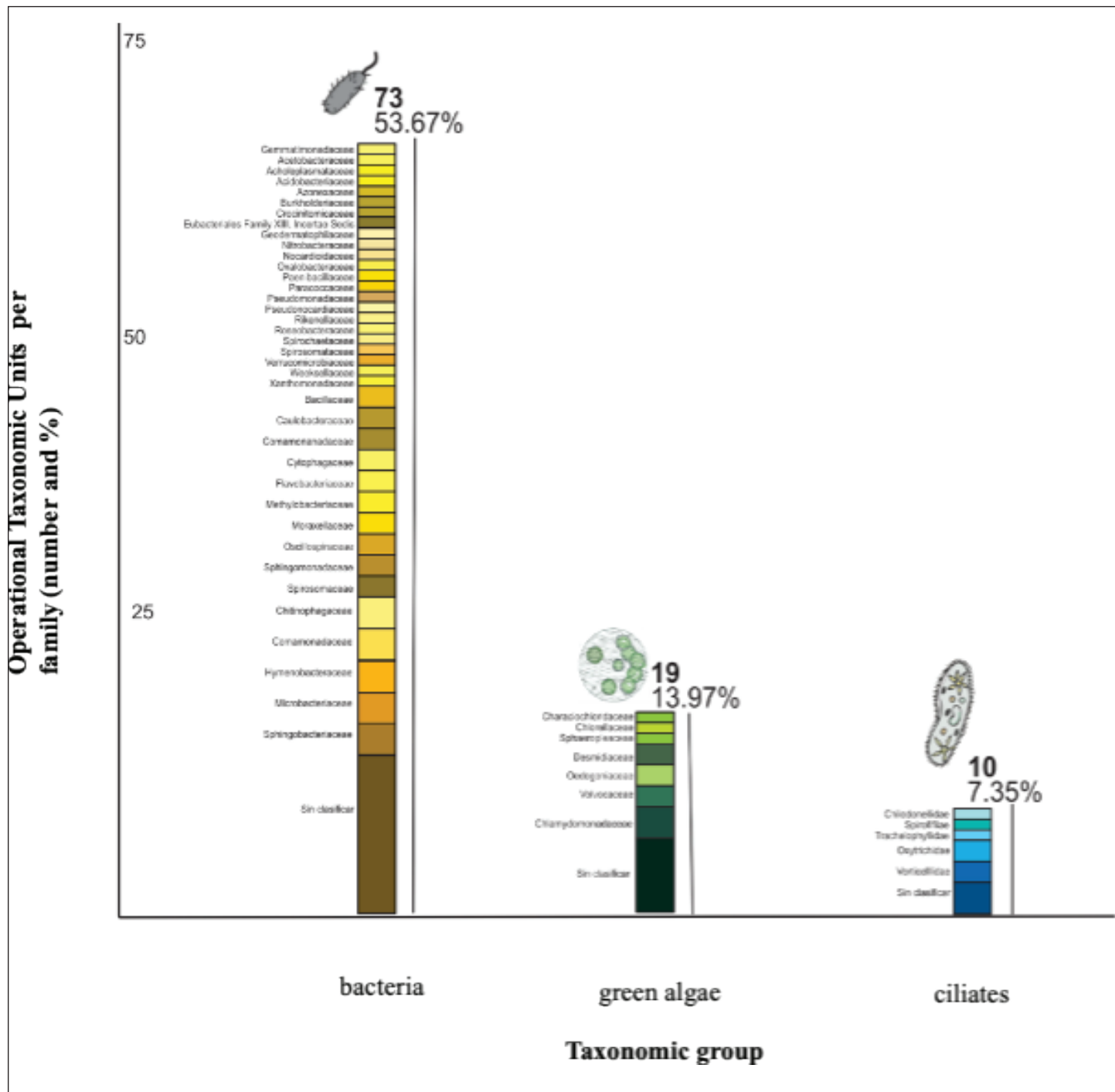


Figure 3. Operational Taxonomic Units richness by the three major taxonomic groups (highlighting major families) by environmental DNA samples of a highland temporary wetland, Pedro Escobedo, Querétaro, Mexico.

Taxonomic group	Families/taxa
Bacteria [73 OTUs, 38 families]	The class Alphaproteobacteria is the richest [14 OTUs], followed by Betaproteobacteria (11), Cytophagia (eight), Actinomycetes (seven), Flavobacteriia, Gammaproteobacteria (five apiece), Bacilli (four), Chitinophagia, Clostridia, Sphingobacteriia (three apiece), other encompassed one OTU. The richest families are Chitinophagaceae, Comamonadaceae, Hymenobacteraceae, Sphingobacteriaceae (three apiece). Taxa that have not been classified into a family (15 OTUs or 21%).
Green algae [19 OTUs, seven families]	The richest family is Chlamydomonadaceae (three species), followed by Desmidiaceae, Oedogoniaceae, and Volvocales (two species apiece). Taxa that have not been classified into a family (seven OTUs or 37%).
Ciliates [ten OTUs, five families]	The richest families are Oxytrichidae and Trachelophyllidae (two OTUs apiece). Taxa that have not been classified into a family (three OTUs or 30%).
Crustaceans [nine OTUs]	The richest families are Daphniidae and Macrotrichidae (two species apiece). Taxa that have not been classified into a family (five OTUs or 44%).
Rotifers [five OTUs]	The families Brachionidae and Flinidae have one OTU apiece. Taxa that have not been classified into a family (three OTUs or 60%).
Cryptomonads [five OTUs]	The families Chroomonadaceae, Cryptomonadaceae, Pyrenomonadaceae have one OTU apiece. Taxa that have not been classified into a family (two OTUs or 40%).
Flagellates [five OTUs]	The family Euglenaceae has three species. Taxa that have not been classified into a family (two OTUs or 40%).
Heterokont algae [four OTUs]	The family Chromulinaceae has one OTU. Taxa that have not been classified into a family (three OTUs or 75%).
Diatoms [two OTUs]	This community is 100% dominated by the family Bacillariaceae.
Fungi [one OTU]	Sporobolomyces sp.
Protist [one OTU]	Order Bigyra
Oomycetes [one OTU]	Family Pythiaceae
Microscopic animal	Family Chaetonotidae

Table 1. Observed taxa in environmental DNA samples of a highland temporary wetland, Pedro Escobedo, Querétaro, Mexico.

3.2 Optical Microscopy

Sixteen taxa of green algae (*Actinastrum* sp., *Gonium sociale*, *Scenedesmus quadricaudata*, *Tetraabaena sociales*, *Tetraedhron* sp., *Volvox* sp. and ten morphospecies), eight diatoms (*Cymbella* sp., *Navicularis* sp, *Nitzschia acicularis*, *Nitzschia* sp., *Synedra* sp., and three morphospecies), five flagellates (*Phacus* sp., *Trachelomonas bacilari*, *T. hispida*, *T. volvocinensis*, and *Strombomonas* sp.), two

cyanobacteria (*Anabaena* sp. and a morphospecies) and one crustacean (*Daphnia* sp.) were observed under microscopic examination, in total 32 species and morphospecies (Fig. 4). From the 32 taxa, six morphospecies of green algae, four diatoms, and two cyanobacteria were only observed by optical microscopy and not detected in the eDNA analysis.

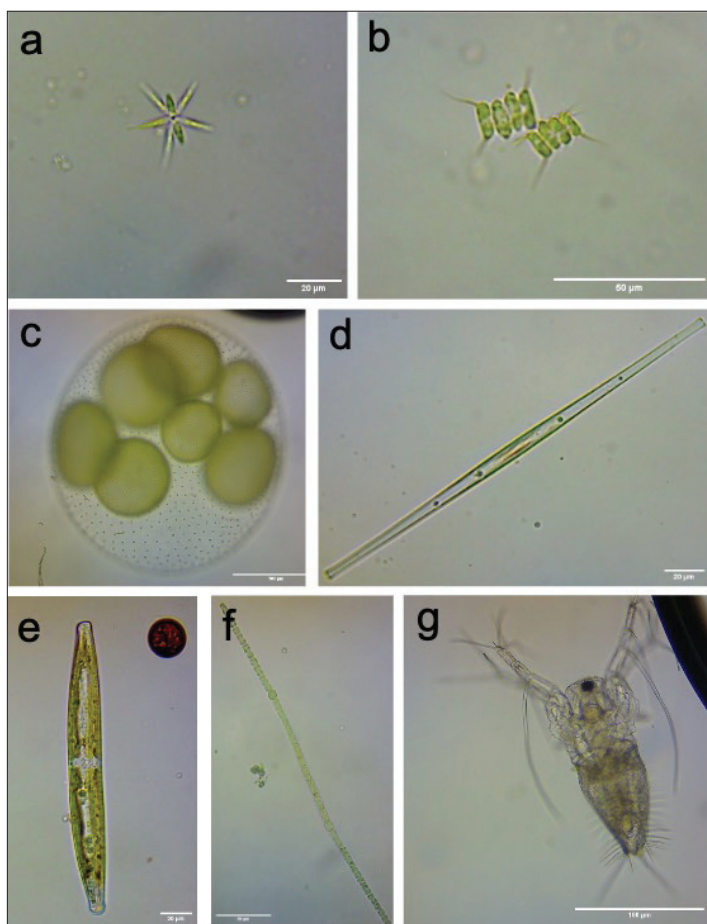


Figure 4. Examples of taxa from a highland temporary wetland [Pedro Escobedo, Querétaro, Mexico] observed via optical microscopy: Green algae (a) *Actinastrum* sp., (b) *Scenedesmus quadricaudata*, (c) *Volvox* sp.; Diatoms (d) *Nitzschia* sp., (e) *Synedra* sp.; Cyanobacteria (f) *Anabaena* sp.; Crustacean (g) *Daphnia* sp. Copyright: M. J. Hernández-de Santos.

3.2 Remediation Application

Wetlands are critical players in regulating and improving water quality, acting as natural filters for the retention, transformation, and recirculation of nutrients, sediments, and pollutants. These ecosystems actively prevent eutrophication and enhance water quality in downstream water bodies (Davidson et al. 2018) due to the diversity of microorganisms inhabiting wetlands, especially bacteria, which colonize a wide range of environments or microhabitats generated by the combination of water, nutrients, soil, sediments, and vegetation (Liu et al. 2020; Kumar et al. 2022). The main phylum of functional bacteria in treatment wetlands to remove pollutants are Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes (Wang et al. 2022).

We conducted a literature review to identify the potential of target taxa to degrade various organic compounds, metals, and radioactive elements. Some of the key families of bacteria identified through the eDNA analysis are the following: Achleplasmataceae, Bacillaceae, Burkholderiaceae, Caulobacteraceae, Chitinophagaceae, Comamonadaceae, Cytophagaceae, Flavobacteriaceae, Gemmatimonadaceae, Geodermatophilaceae, Hymenobacteraceae, Methylobacteriaceae, Microbacteriaceae, Moraxellaceae, Nocardiodaceae, Paenibacillaceae, Pseudomonadaceae, Sphingobacteriaceae, and Sphingomonadaceae (Table 2 and Appendix 1). Li et al. (2022) found that nitrogen enrichment enhances bacterial communities in hydrocarbon-contaminated soils, particularly boosting the PAH-scavenging capacity of Chitinophagaceae, possibly through co-metabolism with fungi. Similarly, Blanco-Enríquez et al. (2018) reported that Chitinophagaceae bacteria can degrade pyrene, a key water pollutant. Révész et al. (2020) identified *Malikia* (Comamonadaceae) as dominant in benzene- and toluene-degrading microbial communities in activated sludge, with their activity linked to oxygenase-encoding genes. Some Comamonadaceae species also possess regulatory systems for low-oxygen environments, and genetic diversity within the family may influence degradation abilities. Wang et al. (2023) showed that *Ramlibacter* can degrade sulfadiazine in ryegrass-planted soil, highlighting root-microbe synergy in rhizosphere bioremediation. Ajibade et al. (2023) also reported *Ramlibacter*'s ability to degrade sulfamethoxazole in wetlands. Lastly, Guo et al. (2020) demonstrated that *Hymenobacter* can break down the pesticide imidacloprid in lake surface waters via hydroxylation.

Family	Remediation use/Species observed in this study with prior research on bioremediation	References*
Bacteria		
Acholeplasmataceae	Species: <i>Acholeplasma</i> sp. Uranium reduction.	Leavitt et al. 2012 Kumar and Chandra 2020
Bacillaceae	Species: <i>Exiguobacterium</i> sp. Degradation of polistireno, PAHs, PGPR, remediation of arsenic, cadmium, copper, mercury, tellurium, chrome.	Parthasarathy et al. 2022 Maroof et al. 2022 Xiao et al. 2024
Burkholderiaceae	Species: <i>Polynucleobacter</i> sp. Degradation of sulfonamides, DeSOx.	Gu et al. 2022 Zhang et al. 2024
Caulobacteraceae	Species: <i>Brevundimonas</i> sp. Degradation of DeSOx, alquibencenos sulfonados (TritonX-100, detergents)	Saravanan et al. 2022 Cortés-Lorenzo et al. 2013 Song et al. 2023
Chitinophagaceae	Species: <i>Segetibacter</i> sp., <i>Sediminibacterium</i> sp. Fenantreno, hydrocarbon-contaminated soils, polycyclic aromatic hydrocarbons (PAHs), pyrene.	An et al. 2007 Blanco-Enríquez et al. 2018 Kariyawasam et al. 2022
Comamonadaceae	Species: <i>Malikia</i> sp., <i>Macromonas</i> sp., <i>Ramlibacter</i> , sp. Benzene and toluene in activated sludge, cDCE and DeSOx in subterranean waters, sulfamethoxazole, pharmaceutical contaminants such as sulfadiazine.	Benedek et al. 2018 Révész et al. 2020 Ajibade et al. 2023 Song et al. 2023 Wang et al. 2023
Cytophagaceae	Species: <i>Spirosoma</i> sp. Degradation of phosphates, resistant to gamma rays.	Lee et al. 2014 Li et al. 2017
Flavobacteriaceae	Species: <i>Flavobacterium</i> sp. Degradation of PCBs, diesel, organophosphates, nylon	Negoro et al. 1994 Ning et al. 2012 Khalid et al. 2021
Gemmatimonadaceae	Species: <i>Gemmatimonas</i> sp. Degradation of phenols, sulfonamides, remediation of copper, zinc, arsenic, lead.	Liang et al. 2020 Kong et al. 2024 Liu et al. 2022 Zhang et al. 2022
Geodermatophilaceae	Species: <i>Blastococcus</i> sp. Remediation of copper, zinc, arsenic, lead.	Sbissi et al. 2025
Hymenobacteraceae	Species: <i>Siccationidurans</i> sp., <i>Hymenobacter</i> sp., <i>Rufibacter</i> sp. Degradation of imidacloprid, an agricultural pesticide that can persist in soils for over a year and eventually move into surface waters or leach into groundwater. Resistant to UV rays.	Srinivasan et al. 2015 Guo et al. 2020 Lee et al. 2021
Methylobacteriaceae	Species: <i>Methylobacterium</i> sp., <i>Psychroglaciecola</i> sp. Remediation of cadmium, chrome, mercury, arsenic, PGPR, organophosphates, PAHs.	De Marco et al. 2004 Onder Erguven et al 2021
Microbacteriaceae	Species: <i>Rhodoluna</i> sp, <i>Curtobacterium</i> sp. Chrome reduction	Gutiérrez et al. 2010
Moraxellaceae	Species: <i>Acinetobacter</i> sp. Remediation of diesel, PAHs, acrylamides, cypermethrin	Dahal et al. 2023 Gur Ozdal and Algur 2022
Nocardiodaceae	Species: <i>Nocardiodes</i> sp. Degradation of DEHP, nitrophenols, pesticides, dyes, explosives, PAHs, dibenzofurans, ibuprofen.	Ebert et al. 2001 Kubota et al. 2005 Zhu et al. 2020 Ma et al. 2023

Family	Remediation use/Species observed in this study with prior research on bioremediation	References*
Paenibacillaceae	Species: <i>Paenibacillus</i> sp. Remediation of copper and zinc.	Govarthanan et al. 2016
Pseudomonadaceae	Species: <i>Pseudomonas</i> sp. Remediation of lead, copper, oxyanions, pesticides, PHAs, phenolic composts, dye.	Gilani et al. 2015 Li et al. 2017 Palanivel et al. 2020 Fakhar et al. 2020 Ivanova et al. 2022 Shah et al. 2025 Vélez et al. 2021
Sphingobacteriaceae	Species: <i>Mucilaginibacter</i> sp., <i>Pedobacter</i> sp. Degradation of PCBs, DeSOx, PGPR, PAHs.	You et al. 2022 Mastny et al. 2021 Vasconcelos et al. 2022 Tabata et al., 2000 Li et al. 2019
Sphingomonadaceae	Species: <i>Sphingomonas</i> sp., <i>Novospingobium</i> sp. Degradation of PGP, PAHs, diesel, remediation of copper, zinc, cadmium.	Chen et al. 2014 Lyu et al. 2014 Rodríguez-Conde et al. 2016 Xu et al. 2018 Asaf et al. 2020 Hu et al. 2023
Spirosomaceae	Species: <i>Arcicella</i> sp. Degradation of polyphenols, nitrogen and phosphates.	Chen et al. 2013 Chai et al. 2017
Weeksellaceae	Species: <i>Chryseobacterium</i> sp. Degradation of pesticides organochlorides (OCPs), DDT, glyphosate, PAHs, remediation of uranium, cadmium, lead, zinc.	Szoboszlay et al. 2008 Gurav and Jadhav 2013 Qu et al. 2015 Jadhav 2016 Majewska et al. 2022 Zhang et al. 2022 Khare and Acharya 2024
Green algae		
Chlamydomonadaceae	Species: <i>Chlamydomonas</i> spp. Degradation of various organic compounds, including the biotransformation of trichlorfon, PAHs such as benz[a]anthracene, polymers like polystyrene, bisphenol A, and pharmaceuticals such as enrofloxacin, carbamazepine, ciprofloxacin, erythromycin, estrone, norfloxacin, ofloxacin, paracetamol, progesterone, roxithromycin, salicylic acid, sulfadiazine, sulfadimethoxine, sulfamethoxydiazine, fametazine, triclocarban, triclosan, trimethoprim, ibuprofen, azithromycin, sulfapyridine, estradiol, and diclofenac.	Zhou et al. 2014 Hom-Díaz et al. 2015, 2022 Xiong et al. 2016 Li et al. 2022 Carbó et al. 2023 Liakh et al. 2023 Seoane et al. 2023
Chlorellaceae	Species: <i>Chlorella</i> sp. Biotransformation or absorption of pesticides (lindane, naphthalene, mirex, DDT, organophosphates, pyrethroids, and oxadiazoles), and removal of nickel, zinc, and lead, degradation of cephalosporins, ceftazidime, cefradine, cefalexin, amoxicillin, azithromycin, enrofloxacin, florfenicol, and levofloxacin.	Avila et al. 2021 Ferreira et al. 2011 Xiong et al. 2021 Aranguren Díaz et al. 2022
Desmidiaceae	Species: <i>Cosmarium</i> sp., <i>Staurastrum</i> sp. Removal of synthetic dyes from wastewater.	Daneshvar et al. 2007 Sudha et al. 2020
Oedogoniaceae	Species: <i>Oedogonium</i> sp. Remediation of inorganic nitrogen and phosphates, bioaccumulation of Ca and Mg.	Oberholster et al. 2018 Lawton et al. 2021

Table 2. Practical remediation applications identified through the literature on the microorganism list discovered in the environmental DNA samples of a highland temporary wetland, Pedro Escobedo, Querétaro, Mexico.* See Appendix 1.

Understanding bacterial diversity in wetlands and its potential for bioremediation has become an increasingly relevant area of research. The analysis of environmental DNA extracted from wetland water samples enables the identification and characterization of microorganisms present in the ecosystem, even those difficult to identify using optical microscopy. This technique has revealed a previously unexplored wealth of bacteria and green algae in the wetland and has provided invaluable information on the diversity and remediation capabilities of its microbial communities. Overall, bacterial species are studied for the degradation of organic and inorganic pollutants, as well as radiation, while green algae are known for degrading various organic compounds, including the biotransformation of trichlorfon, PAHs such as benz(a)anthracene, polymers like polystyrene, bisphenol A, and pharmaceuticals like enrofloxacin, carbamazepine, ciprofloxacin, erythromycin, estrone, norfloxacin, ofloxacin, paracetamol, progesterone, roxithromycin, salicylic acid, sulfadiazine, sulfadimethoxine, sulfamethoxydiazine, fametazine, triclocarban, triclosan, trimethoprim, ibuprofen, azithromycin, sulfapyridine, estradiol, and diclofenac (Table 2 and Appendix 1).

We also highlight for remediation the green algae families: Chlamydomonadaceae, Chlorellaceae, Desmidiaceae, and Oedogoniaceae (Table 2 and Appendix 1). Microalgae from the genus *Chlamydomonas* have demonstrated the ability to degrade a wide range of organic pollutants, including trichlorfon, PAHs like benz(a)anthracene, polystyrene, bisphenol A, and numerous pharmaceuticals such as antibiotics, hormones, and painkillers. Similarly, *Cosmarium* (Desmidiaceae) has shown potential for removing synthetic dyes from wastewater. Within the Oedogoniaceae family, *Oedogonium* species have been reported to remove inorganic nitrogen and phosphates while bioaccumulating minerals such as calcium and magnesium. In the Chlorellaceae family, *Chlorella* species identified through eDNA metabarcoding have been associated with the uptake or degradation of various pesticides (e.g., lindane, DDT) and metals (nickel, zinc, lead), as well as a wide range of antibiotics including cephalosporins and fluoroquinolones.

Therefore, microorganisms present in wetlands are key players in regulating water quality, particularly bacteria (Kumar et al. 2023) and microscopical green algae (Fang et al. 2023).

4 CONCLUSION

Overall, the study recorded 148 microorganism taxa. Environmental DNA has proven to be a powerful technique for assessing the microbial diversity of wetlands. We observed more taxa through environmental analysis (136 OTUS) than optical microscopy (32 species and morphospecies). This was because most of the taxa identified via eDNA included bacteria (73 OTUS), which are not easily visible with optical microscopy techniques. In contrast, 12 taxa identified through optical microscopy were not detected by eDNA analysis.

We found potential remediation uses in the literature for several taxa on our list (e.g., *Exiguobacterium* sp., *Malikia* sp., *Macromonas* sp., *Ramlibacter* sp., *Sphingomonas* sp., *Novospingobium* sp., *Mucilaginibacter* sp., *Pedobacter* sp.) (Table 2 and Appendix 1). Microorganisms from the temporary wetlands of Querétaro state should be included in future research on remediation issues, particularly given the current growth in the industrial sector in this region and its associated environmental challenges.

Our findings would be relevant for identifying potentially new records of microorganisms in Querétaro's freshwater wetlands and compiling a list of selected species for future studies on remediation, using microorganisms associated with aquatic ecosystems. Continued exploration using eDNA techniques is likely to uncover further functional diversity. We are optimistic that using the eDNA method could improve our knowledge and understanding of the environment, benefiting science and society by conserving inland wetlands in the Central Mexico Highlands.

Author Contributions

María José Hernández-de Santos wrote the first draft manuscript; conducted fieldwork, a literature review on remediation, and bright-field microscopy analysis; and prepared graphics. Tatiana Lobato-de Magalhães conceptualized the study, secured funding, translated

the draft manuscript into English, and reviewed the final version.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Declaration of Originality

This is an original work that has not been published before. Images, figures, and quotations included in the article have been properly cited and permission has been granted for any that are not those of the authors.

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