Natural Resources for Human Health

eISSN: 2583-1194

Original Research

Received 20 June 2026
Revised 13 August 2026
Accepted 15 September 2026

Natural Resources for Human Health 2026, Vol. 6(5):1041–1065

KEYWORDS:
Glyphosate biodegradation, Soil bacteria, Bioremediation, Enrichment culture, Abiotic tolerance, Pseudomonasaceae, Herbicide-contaminated soil

Growth-based Screening and Abiotic Tolerance Profiling of Glyphosate-Tolerant Bacteria Isolated from Pesticide-Exposed Agricultural Soils in Mostaganem, Algeria

Aicha Chadli[1], Fouad Mekhalef Benhafsa[2], Nour El Houda Neggaz[3]

[1] Biotechnology applied laboratory to agriculture and environmental preservation, higher school of agronomy, Ex- Hall of technology kharoubba, Mostaganem (27000), Algeria
[2] Centre de Recherche Scientifique et Technique en Analyses Physico-chimiques (CRAPC). Zone Industrielle, BP 384 Bou-Ismail, Tipaza, Algeria. Unité de Recherche en Analyses Physico-Chimiques des Milieux Fluides et Sols – (URAPC-MFS/ CRAPC), 11, Chemin Doudou Mokhtar, Ben Aknoun – Alger, Algeria. Laboratory of Advanced Materials and Physicochemistry for the Environment and Health (MAPES), Djillali Liabes University, P.B. 89 Sidi Bel Abbes 22000, Algeria.
[3] Biotechnology applied laboratory to agriculture and environmental preservation, higher school of agronomy, Ex- Hall of technology kharoubba, Mostaganem (27000), Algeria).
Corresponding Author: achadli@esa-mosta.dz

ABSTRACT: Glyphosate, which is called N-phosphonomethylglycine is the commonly used herbicide all over the world including in Algeria. It stays in the soil used for farming. Causes problems for the environment and for biotechnology. These problems are connected to soil pollution. What happens to a main breakdown product of glyphosate, which is called aminomethylphosphonic acid or AMPA. Native bacteria found in the soil that can use glyphosate as a source of carbon are very interesting because they might help clean up the soil. However we do not know enough about the types of these bacteria and how well they can handle glyphosate in the farming areas of Algeria and North Africa. Soil samples were taken in May 2023 from two farming areas, in Mostaganem, Algeria, These samples differ in herbicide history. One sample was treated times with glyphosate specifically with Roundup Ultra Plus. The other sample was treated with metribuzin and, with linuron. Researchers counted all microflora as well as those that are tolerant to glyphosate. They used dilution and plating techniques to do the counts. The cells were plated on Plate Count Agar. The cells were also plated on a salt medium that used glyphosate as the only carbon source. Thirty purified isolates were screened for growth at OD_{660} over 120 hours, and effects of pH, temperature, NaCl concentration, glyphosate concentration, and glucose co-supplementation were evaluated. Glyphosate degrading microflora reached 6.632±0.01 Log CFU/g in treated soil, which's ten times higher than in untreated soils. All thirty isolates were able to grow on glyphosate with peak growth happening between 48 and 120 hours. 80% Of the isolates preferred an alkaline pH environment. Eighteen isolates showed growth at 30°C. The bacteria could still grow in conditions with 5% NaCl. 5 g/L glyphosate. Glucose helped boost growth in every isolate. Twelve of the selected isolates belonged to families such as *Pseudomonasaceae*, *Aeromonadaceae*, *Chromobacteriaceae*, *Rhizobiaceae* and *Vibrionaceae*. These results show that Mostaganem agricultural soils contain a variety of bacterial species that can handle glyphosate. They also show tolerance to different environmental stresses. This makes them good candidates, for bioremediation studies. Before these

strains can be used in real field situations it is necessary to confirm glyphosate removal using chemical methods. Molecular identification must also be done to know what species we are working with.

1. INTRODUCTION

Global agricultural systems face mounting pressure from population growth, rising food demand, climate change, biodiversity loss, and the degradation of natural resources (FAO, 2020). Agricultural intensification, and with it a heavy and growing reliance on chemical inputs such as herbicides, fungicides, and insecticides, has become difficult to avoid under these constraints (Yesguer, 2015; Aubertot et al., 2005). Among these compounds, glyphosate (N-phosphonomethylglycine) is today one of the most extensively used herbicides in the world, marketed under numerous commercial formulations, of which Roundup is the best known. It is a non-selective, systemic herbicide that acts by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme of the shikimate pathway that is essential for the biosynthesis of aromatic amino acids in plants, bacteria, and fungi (Mesnage & Antoniou, 2023). From an environmental standpoint, glyphosate is highly soluble in water and shows a strong affinity for the organic and mineral fractions of soil. This property reduces its mobility toward groundwater but increases its residence time in the topsoil, favoring the accumulation of its main degradation product, aminomethylphosphonic acid (AMPA), which is considered more persistent, and potentially more ecotoxic, than the parent molecule (Silva et al., 2018; Bento et al., 2022). Microbial biodegradation constitutes the principal route of glyphosate dissipation in the environment, carried out by a wide range of microorganisms – bacteria, fungi, and actinobacteria – capable of using glyphosate as a source of carbon, nitrogen, or phosphorus (Feng et al., 2022). Two major metabolic pathways have been described: the AMPA pathway, catalyzed by glyphosate oxidoreductase, which yields AMPA and glyoxylate; and the sarcosine pathway, mediated by a carbon-phosphorus (C-P) lyase, which generates sarcosine and inorganic phosphate without producing AMPA and is therefore generally regarded as more environmentally favorable (Wang et al., 2021).

A wide taxonomic diversity of glyphosate-degrading bacteria has been reported, spanning Gramnegative genera such as *Pseudomonas*, *Rhizobium*, *Agrobacterium*, and *Stenotrophomonas*, and Gram-positive genera such as *Bacillus*, *Arthrobacter*, and *Paenibacillus*. Among these, *Stenotrophomonas acidaminiphila* Y4B has demonstrated a degradation capacity of 98% within 72 hours (Li et al., 2022), while other genera such as *Flavobacterium*, *Geobacillus*, *Enterobacter*, and *Alcaligenes* have also been reported to degrade this herbicide (Obojska et al., 2002; Balthazor & Hallas, 1986). Biodegradation efficiency is governed by several interacting abiotic factors, including an optimal temperature range of 25–35°C, a slightly alkaline pH (6.5-8), the availability of readily assimilable co-substitutes such as glucose or citrate, and the presence of oxygen, since most of the bacteria involved are strict aerobes. Commercial formulations containing surfactants, however, can inhibit microbial growth and reduce overall degradation efficiency (Wang et al., 2021; Chaparro et al., 2023).

In Algeria like else glyphosate is used a lot, in cereal crops market gardens and tree farms. Glyphosate is applied times and this raises the danger that soil, surface water and groundwater get contaminated. Glyphosate can also change the makeup and work of the life in soil. Glyphosate has been shown to either boost or slow breathing but the effect depends on how much glyphosate is used what kind of soil it is and how long the microbes have been exposed to glyphosate (Haney et al., 2000; Veiga et al., 2001). When soil contains phosphate glyphosate is harder to break down because the microbes cannot use it as a phosphorus source (Kishore & Jacob 1987). Scientists have looked at ways glyphosate could disappear through chemical reactions but those methods usually do not work well in nature. Therefore microbial breakdown remains the most hopeful way to remove glyphosate from farm fields (Stenrod et al., 2003; Forlani et al., 1999).

Despite this abundant international literature, comparatively little work has systematically characterized indigenous glyphosate-degrading bacteria from Algerian agricultural soils under multiple, deliberately varied abiotic conditions representative of a Mediterranean agroecosystem. This gap is especially important because glyphosate and other herbicides like metribuzin and linuron are widely used in market-gardening systems as noted by Yesguer in 2015. It's also relevant because plants in these areas may develop strains that adapt to conditions-such, as high temperatures, salinity and pH levels-that are typical of Mediterranean and semi-arid soils. Understanding how these plants respond to stressors could help improve weed management strategies in these regions. The present study addresses this gap by combining an enrichment-based isolation strategy with a systematic, multi-factorial evaluation of abiotic tolerance among thirty glyphosate-tolerant isolates recovered from pesticide-exposed soils near Mostaganem, Algeria.

The specific objectives of the present study were to: (i) enumerate total and glyphosate-degrading soil microflora from two agricultural sites differing in herbicide-exposure history; (ii) isolate and phenotypically characterize glyphosate-tolerant bacterial strains recovered by selective enrichment; (iii) evaluate the growth kinetics of these isolates in glyphosate-supplemented minimal medium over 120 hours; (iv) determine the effect of initial pH, incubation temperature, salinity (NaCl), glyphosate concentration, and glucose co-substrate addition on isolate growth; and (v) identify, at the genus and, where possible, species level, the twelve most tolerant isolates using standard biochemical methods (API 20NE), in order to propose candidate strains for future bioremediation applications.

2. LITERATURE REVIEW AND THEORETICAL FRAMEWORK

2.1. Environmental fate and ecotoxicological relevance of glyphosate

Glyphosate has been sold over the world since the 1970s. It is still the used herbicide in todays farming. The way it works is by stopping EPSPS in the shikimate pathway. This makes it deadly, to types of plants. In theory it does not hurt animals because animals do not have this pathway. Because the shikimate pathway is also present in many bacteria, fungi, and protists, however, glyphosate and its formulated products can exert measurable effects on non-target soil microbial communities, sometimes stimulating and sometimes inhibiting microbial biomass and respiration depending on the dose applied, soil type, and prior exposure history (Haney et al., 2000; Busse et al., 2001; Stratton & Stewart, 1992).

Once in soil, glyphosate binds strongly to iron and aluminum oxides and to organic matter, a property that limits its leaching to groundwater but extends its residence time, particularly in fine-textured soils (Bento et al., 2022). Its principal degradation product, AMPA, is frequently detected in agricultural soils across Europe and elsewhere and is generally considered more persistent than the parent molecule, with a slower dissipation rate and a comparable or greater potential for ecotoxicity (Silva et al., 2018; Wirenlehr et al., 1997). Under simulated rainfall and across a range of soil textures, both glyphosate and AMPA have been shown to persist for extended periods, reinforcing the case for microbial mineralization as the environmentally preferable removal pathway (Bento et al., 2022).

2.2. Metabolic pathways of microbial glyphosate degradation

Two enzymatic routes lead the research on glyphosate catabolism. These two enzymatic routes are the discussed paths, in studies focused on bacterial glyphosate catabolism. The first, catalyzed by glyphosate oxidoreductase, cleaves the C-N bond of the glyphosate molecule to yield AMPA and glyoxylate; AMPA is subsequently degraded only slowly, and its accumulation in culture supernatants or in soil is often used as an indirect marker of incomplete mineralization via this route (Kishore & Jacob, 1987; Feng et al., 2022). The second route proceeds via a carbon-phosphorus (C-P) lyase enzyme complex, encoded in many bacteria by the phn or related organophosphonate-degrading (opd) gene clusters, which cleaves the C-P bond directly, releasing sarcosine and inorganic phosphate; sarcosine is further oxidized to glycine and formaldehyde by sarcosine oxidase (Wang et al., 2021; Wacket et al., 1987).

Because the sarcosine pathway avoids AMPA formation altogether, strains that preferentially rely on this route are of particular biotechnological interest for bioremediation, since they in principle eliminate the risk of substituting one persistent organophosphonate residue for another. Both pathways can allow glyphosate to serve as a source of carbon, nitrogen, or phosphorus, and the relative importance of each element as a selective driver depends heavily on the nutritional composition of the growth medium: under phosphorus-limited conditions bacteria tend to favor phosphonate-cleaving (C-P lyase) activity, whereas under phosphorus-replete but carbon- or nitrogen-limited conditions, the AMPA pathway may predominate (Kertesz et al., 1994; Quinn et al., 1988, 1989). The genes encoding C-P lyase activity are frequently carried on mobile genetic elements, which is consistent with reports of horizontal transfer of glyphosate-degrading capacity among unrelated soil bacterial taxa (Chaparro et al., 2023).

2.3. Taxonomic diversity of glyphosate-degrading bacteria

A wide taxonomic range of bacteria has been reported to degrade glyphosate. Among Gramnegative genera, Pseudomonas has been studied most extensively since the original description of Pseudomonas sp. strain PG2982 by Kishore and Jacob (1987), and has since been repeatedly recovered from glyphosate-contaminated soils worldwide (Obojska et al., 2002; Tolbot et al., 1984). Members of the Rhizobiaceae, including Agrobacterium and Rhizobium species, have also been shown to degrade glyphosate efficiently (Liu et al., 1991), and Rhizobium sp. strain GX19 was recently characterized for its aerobic glyphosate metabolic pathway (Wang et al., 2023). Among Gram-positive and actinobacterial genera, Arthrobacter sp. GLP-1

was among the earliest strains shown to use glyphosate as a sole source of phosphorus and nitrogen (Pipke et al., 1987; Pike & Amrhein, 1988), and Bacillus species, including Bacillus subtilis strain Bs-15, have demonstrated tolerance to very high glyphosate concentrations of up to 40 g/L (Yu et al., 2015).

More recently, immobilized Stenotrophomonas acidaminiphila Y4B has been shown to degrade more than 90% of glyphosate within 72 hours (Li et al., 2022), and bacterial consortia enriched directly from contaminated soils have achieved degradation of up to 400 mg/L of glyphosate within a few days of incubation (Li et al., 2024). This wide and growing variety of species probably shows that different kinds of bacteria have developed ways to break down materials because of the pressure from their environment and that the genes used for breaking down materials have spread between different types of bacteria in the soil through things, like plasmids and transposons which can move around easily (Chaparro et al., 2023).

2.4. Abiotic determinants of biodegradation efficiency

The efficiency of glyphosate biodegradation by soil bacteria is governed by several interacting abiotic variables. Temperature strongly influences both microbial growth rate and enzyme kinetics; Moorman (1994) and Walker et al. (1992) reported that, within the range normally encountered under field conditions, pesticide degradation rates in soil generally increase with temperature, and Walker et al. (1992) further identified soil temperature as the single most important environmental factor governing pesticide degradation rates.

Soil pH modulates both the bioavailability of glyphosate, which is more soluble and less strongly adsorbed to soil colloids at alkaline pH, and the expression of organophosphonate-degrading (opd) genes, which have been reported to occur in higher copy numbers in alkaline soils (Singh et al., 2003). Salinity can also shape degradation capacity: bacterial strains isolated from saline environments, such as Saharan soils near Biskra or the waters of Sebkhet Sefioune (Ouargla, southeastern Algeria), have shown effective degradation of glyphosate and other pesticides despite substantial salt concentrations, suggesting that halotolerance and xenobiotic-degradation capacity are not mutually exclusive traits in these environments (Benslama, 2014; Sebihi & Merabti, 2019; Benabbes et al., 2020). Finally, the availability of an easily assimilable co-substrate, such as glucose, has repeatedly been shown to enhance the biodegradation of xenobiotic compounds through a co-metabolic effect that increases overall microbial metabolic activity without necessarily being itself the primary carbon source targeted by the degradative enzyme system (Horwath, 1972; Kumar & Philip, 2006; Guha et al., 1997; Mallick et al., 1999).

2.5. Rationale for the present study

Taken together, the literature reviewed above establishes that glyphosate biodegradation is carried out by a taxonomically diverse assemblage of bacteria, that at least two distinct metabolic pathways can be mobilized depending on nutrient status, and that abiotic factors such as temperature, pH, salinity, and co-substrate availability can each substantially modulate degradation efficiency. However, comparatively few studies have characterized indigenous glyphosate-tolerant bacterial communities from Algerian agricultural soils, and fewer still have done so under a systematically varied, multi-factorial set of abiotic conditions spanning the ranges of temperature, pH, and salinity relevant to Mediterranean and semi-arid agroecosystems.

In Mostaganem there is a gap that matters a lot. Mostaganem is a place where people grow market gardens and trees. They use glyphosate a lot. They also use herbicides like metribuzin and linuron. Local strains from these soils can be tolerant to things like heat, high pH and some salt. This matters when we think about bioremediation, in the Mediterranean. So we set up a study to examine thirty bacterial isolates from two pesticide-exposed farms, near Mostaganem. We want to find glyphosate-tolerant isolates that grow fast can handle many stresses and are diverse so they can help with future bioremediation.

3. MATERIALS AND METHODS

3.1. Study sites and soil sampling

We collected soil samples from two sites in the wilaya of Mostaganem, Algeria. These sites were chosen because they have different histories of herbicide use. The first site, called Site 1 is in the garden of the École Supérieure d'Agronomie de Mostaganem at the Hall de Technologie, where glyphosate-based treatments-specifically Roundup Ultra Plus-have been applied times at the coordinates 35°57'05.8"N 0°05'57.9"E. The second site, called Site 2 is an agricultural plot owned by SARL AGROMOSTA, in the Mesra area. This plot is used for market gardening, growing potatoes and carrots. It has

received applications of the herbicides metribuzin and linuron (coordinates: 35°50'10.3"N 0°10'31.7"E; orientation 30°N). The contrasting agronomic and phytosanitary histories of these two sites provided a context, for isolating autochthonous bacterial strains that may be able to degrade a wide variety of herbicides, including glyphosate.

Three composite soil samples. Soil samples 1, 2 and 3. Were analyzed. Soil sample 1 came from the glyphosate treated site, Site 1 and that matches the later finding of a higher density of glyphosate-degrading microflora. Samples 2 and 3 came from Site 2, treated with metribuzin and linuron. Soil sampling took place in spring, May 2023 a time we chose because it was neither too hot nor too dry so that the resident microbial community stayed intact. For each site, a representative composite sample was obtained by combining five elementary surface samples (0-20 cm depth, approximately 500 g each), collected after removal of surface organic debris, distributed along two crossed diagonals covering an area of one are (100 m^2), using a manual soil auger, following the method described by Simonart (1957). Samples were homogenized in clean containers, transferred into sterile plastic bags, and immediately placed in an isothermal cooler maintained at 4°C using ice packs for transport to the laboratory.

Figure 1: Satellite location of the two soil sampling sites in Mostaganem, Algeria: Site 1 (Higher school of agronomy garden, glyphosate-treated) and Site 2 (SARL AGROMOSTA plot, Mesra, treated with metribuzin and linuron).

Source: *Original data from the study.*

3.2. Soil microbiological analyses

Prior to microbiological analysis, each soil sample was ground manually in a porcelain mortar to break up aggregates and successively sieved through 5 mm and 2 mm mesh screens to remove coarse particles and homogenize particle size. The herbicide used for all enrichment assays was glyphosate, commercialized as Roundup Ultra Plus.

Total mesophilic microflora was enumerated by the suspension-dilution method described by Rapilly (1968) and Marchal and Bourdon (1982). For each sample, 10 g of soil was aseptically suspended in 90 mL of sterile 0.85% physiological saline (NaCl 8.5 g/L, peptone 0.5 g/L) and mechanically agitated for 30 minutes. The stock suspension was serially diluted (10^{-1} to 10^{-8}), and 0.1 mL of each dilution was spread onto Plate Count Agar (PCA: tryptone 5 g/L, yeast extract 2.5 g/L, glucose 1 g/L, agar 15 g/L; pH 7.0). Plates were incubated at 30°C for 24-48 h and colonies counted and expressed as colony-forming units per gram of dry soil (CFU/g).

For enrichment and enumeration of glyphosate-degrading microflora, 10 g of soil was introduced into 95 mL of sterile solid minimal medium (MMS: $FeSO_4 \cdot 7H_2O$ 0.013 g/L, $CaCl_2 \cdot 2H_2O$ 0.013 g/L, $MgSO_4 \cdot 7H_2O$ 0.25 g/L, Na_2HPO_4 7.5 g/L, KH_2PO_4 5 g/L, NH_4NO_3 5 g/L, yeast extract 0.6 g/L, agar 15 g/L; pH 7.0), with Roundup Ultra Plus added aseptically after filter-sterilization (0.22 µm) at a rate equivalent to a final glyphosate concentration of 1 g/L. Cultures were incubated at 30°C under rotary agitation (150 rpm) for 24 h to favor selection of glyphosate-utilizing bacteria, then serially diluted (10^{-1} to 10^{-8}) and plated onto solid MMS with glyphosate (1 g/L); plates were incubated at 30°C for 24-96 h before colony enumeration.

3.3. Isolation, purification, and morphological characterization

Only plates yielding between 30 and 300 CFU/mL were retained for isolation. From these, three to five colonies were randomly selected per sample and purified by successive subculturing on nutrient agar (NA: meat extract 1 g/L, yeast extract 2 g/L, peptone 5 g/L, NaCl 5 g/L, agar 15 g/L; pH 7.0) until pure isolates were obtained, following Jans et al. (2012). Colony morphology (form, elevation, margin, color, surface texture) was recorded on NA and on the differential media King A and King B, following the classification scheme of Bergey (1984). Gram staining was performed following Laprent and Laprent (1990) to assess cell shape, arrangement, sporulation, and Gram reaction. Oxidase and catalase activity were determined following Marchal and Bourdon (1982). Purified strains were stored at -20°C in nutrient broth supplemented with 30% (v/v) glycerol, following Hassanshahian et al. (2014).

3.4. Growth kinetics in glyphosate-supplemented medium

Bacterial inocula were prepared by culturing isolates in nutrient broth for 72 h at 30°C under rotary agitation (150 rpm); cells were then harvested by centrifugation (4000 rpm, 5 min), washed twice in sterile 0.9% saline, and resuspended to a density equivalent to 0.5 McFarland units ($OD_{660} \approx 0.18$), following Anwar et al. (2009). Glyphosate degradation kinetics were then studied by inoculating 36 mL of sterile MMS broth containing 1 g/L glyphosate with 4 mL of standardized inoculum, incubating at 30°C under agitation (150 rpm) for five days, and measuring optical density at 660 nm (OD_{660}) every 24 h as an indirect indicator of bacterial growth and glyphosate utilization.

3.5. Optimization of culture conditions

The effect of initial pH on growth was evaluated in 20 mL of MMS broth (1 g/L glyphosate) adjusted to pH 5, 6, 7, 8, 9, or 10 with sterile buffers, inoculated with 5% (v/v) of an 18- 24 h culture, and incubated at 30°C for 24 h (150 rpm), following Singh et al. (2003). The effect of temperature was evaluated identically at pH 7 across incubation temperatures of 25, 30, 37, 40, and 45°C. Tolerance to salinity was assessed in MMS broth (pH 7, 1 g/L glyphosate) supplemented with NaCl at 1%, 2%, 3%, 4%, or 5%, incubated at 30°C for 24 h. Tolerance to glyphosate concentration was assessed in MMS broth (pH 7) containing 1, 2, 3, 4, or 5 g/L glyphosate, incubated at 30°C for 24 h, following Yu et al. (2015). Finally, the effect of glucose co-amendment was tested by adding 1 g/L glucose to the glyphosate-supplemented medium and comparing growth to glyphosate-only controls.

3.6. Biochemical identification of selected isolates

Based on their tolerance to environmental stressors and to elevated glyphosate concentrations, twelve isolates (B1, B3, B4, B7, B9, M3, M5, M9, D1, D4, D7, and D10) were selected from the thirty screened strains for biochemical identification using the API 20NE gallery (BioMérieux, France), performed and interpreted according to the manufacturer's recommendations and following Tortora et al. (2003). Identification was achieved at the genus level and, for most isolates, at the presumptive species level, with taxonomic affiliation to family assigned according to the classification of Bergey (1984).

3.7. Data presentation and statistical considerations

To evaluate the effectiveness of the treatments, all results are expressed as mean ± standard deviation from three independent determinations (triplicates). Statistical significance of the data was evaluated using analysis of variance (ANOVA). Differences were considered statistically significant for p-values between 0,01 and 0,05 ($0,01 < p < 0,05$). All statistical analyses and data processing were performed using Statbox (version 6.40).

4. RESULTS

4.1. Enumeration of total and glyphosate-degrading soil microflora

Enumeration of total and glyphosate-tolerant soil microflora revealed considerable microbial density across the three analyzed soil samples (Table 1). Total mesophilic microflora ranged from 7.117±0.01 to 7.361±0.02 Log CFU/g dry soil across the three samples, indicating an overall high level of background microbial activity. Regarding glyphosate-degrading microflora specifically, the lowest densities were recorded in the two soil samples not directly treated with this herbicide: 4.350±0.01 Log CFU/g (sample 3) and 4.474±0.01 Log CFU/g (sample 2). By contrast, sample 1, originating from soil that had received repeated glyphosate applications, showed a markedly higher density of glyphosate-degrading bacteria, reaching 6.632±0.01

Log CFU/g – approximately one order of magnitude above the other two samples – consistent with a history-dependent enrichment of glyphosate-tolerant populations at this site.

Table 1: Enumeration of total microflora and glyphosate-degrading microflora in the three soil samples.

Soil sample	Total microflora (Log CFU/g dry soil)	Glyphosate-degrading microflora (Log CFU/g dry soil)	Source
Sample 1 (glyphosate-treated, Site 1)	7.361± 0.02	6.632± 0.01	Original data from the study
Sample 2 (metribuzin/linuron-treated, Site 2)	7.117± 0.01	4.474± 0.01	Original data from the study
Sample 3 (metribuzin/linuron-treated, Site 2)	7.120± 0.02	4.350± 0.01	Original data from the study

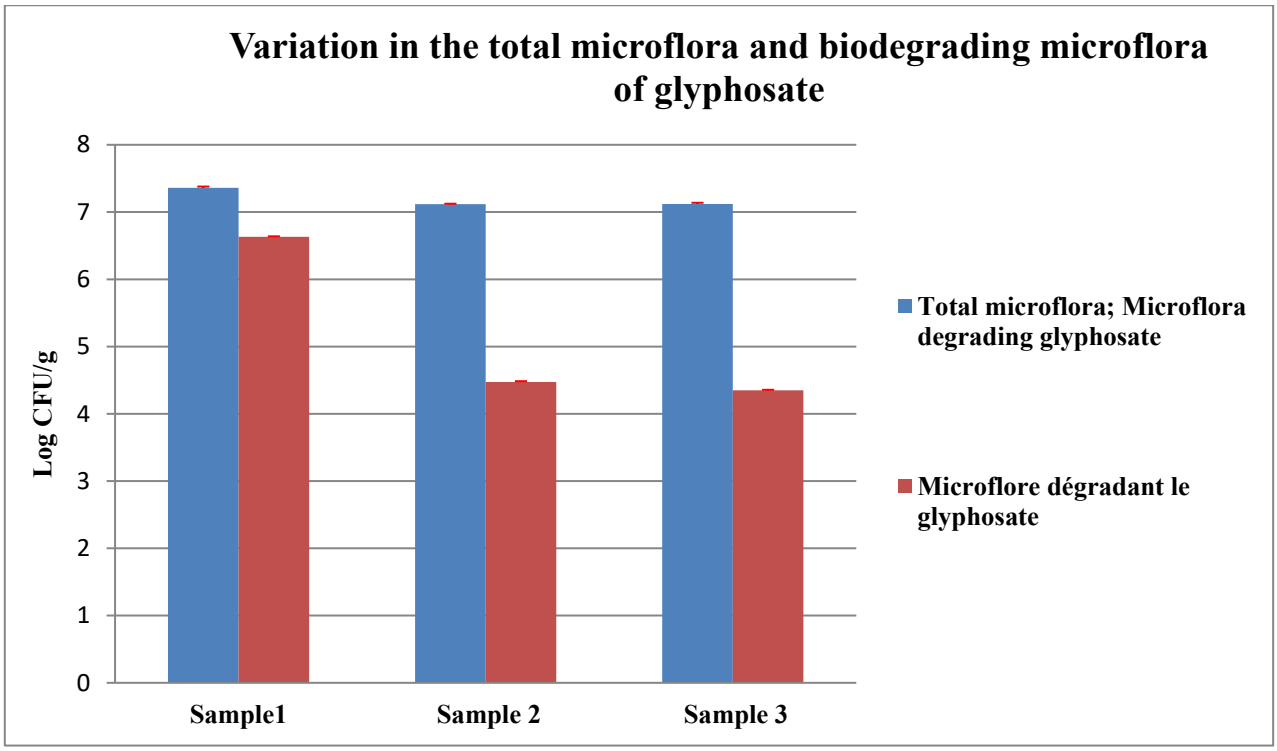

Figure 2: Enumeration of total microflora and glyphosate-degrading microflora (log CFU/g dry soil) in the three soil samples. X-axis: soil sample; Y-axis: log CFU/g dry soil.

***Source**: Original data from the study*

4.2. Isolation and morphological/biochemical characterization

Following enrichment of the three soil samples in glyphosate-supplemented MMS, thirty bacterial strains were isolated in total.

Macroscopic examination of colonies on nutrient agar revealed diameters of 1-3 mm, colors ranging from orange to beige and white, predominantly rounded shapes, convex or flat elevation, regular or irregular margins, and translucent to opaque appearance, with smooth, viscous-to-creamy colony surfaces. We first performed Gram staining on these isolated glyphosate-tolerant strains – this is the most fundamental classification method in bacterial detection, which can divide bacteria into two major categories: Gram-positive and Gram-negative. The test results showed that 40% of all strains were Gram-positive, and the remaining 60% were Gram-negative. In terms of quantity alone, Gram-negative bacteria accounted for the vast majority

among this batch of glyphosate-resistant strains. Even if the two types of bacteria were further split by morphology, the total sum still exactly matched this proportion: of the 40% that were Gram-positive, 26.66% were spore-forming *bacilli*, and 13.33% were spherical cocci; of the 60% that were Gram-negative, 43.33% were thin rod-shaped bacilli, and 16.66% were spherical *cocci*.

In addition to classification by staining, we also conducted biochemical-level screening. We first tested for oxidase – if a bacteria carries this substance, the test will return a positive result. The results showed that approximately half of the Gram-negative strains tested positive for oxidase; among Gram-positive strains, this proportion was even higher, with two-thirds yielding positive results. We tested a total of 30 strains in this study, and we also measured the activity of another enzyme: catalase. Regardless of whether a strain was Gram-positive or Gram-negative, the activity of this enzyme could be detected in about one-third of all strains.

4.3. Glyphosate biodegradation kinetics

All thirty isolates grew in MMS containing glyphosate (1 g/L) as the sole carbon source, confirming broad tolerance and biodegradation potential across the isolate collection, but growth kinetics varied considerably among strains (Table 2; Figures 3–5). Six isolates (B2, B5, B8, B9, M8, and M9) reached peak OD_{660} after 48 h; three isolates (M1, D4, and D7) peaked after 72 h; fifteen isolates (B3, B4, B7, M2, M3, M4, M5, M6, M7, M10, D1, D3, D5, D6, and D10) peaked after 96 h; and six isolates (B1, B6, B10, D2, D8, and D9) required the full 120 h to reach maximum growth. Among the fastest-growing strains, isolate M9 was particularly notable, reaching the stationary phase within 48 h at a peak OD_{660} of 0.946.

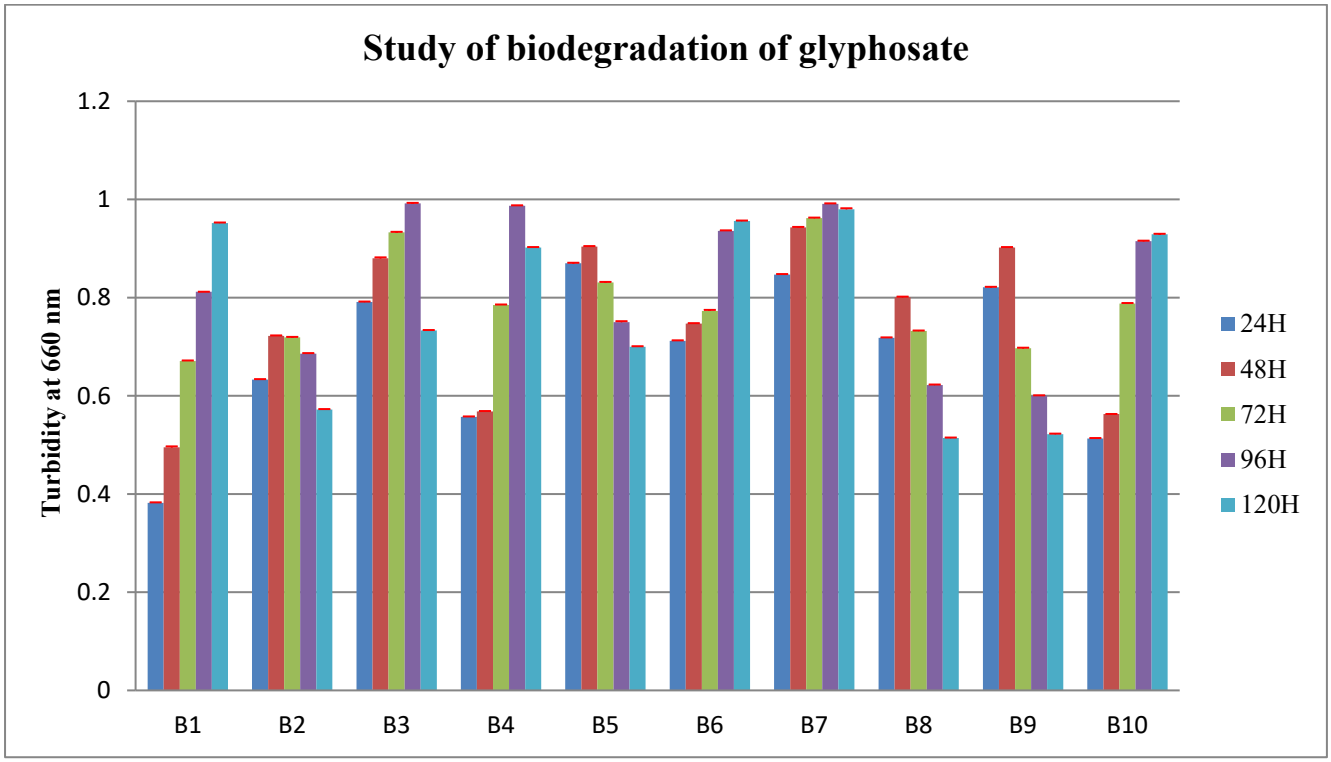

Figure 3: Glyphosate biodegradation kinetics (OD_{660} over 120 h) of the ten isolates of series B (B1-B10) in MMS broth containing 1 g/L glyphosate. [X-axis: incubation time (24-120 h); Y-axis: OD_{660}].

***Source**: Original data from the study.*

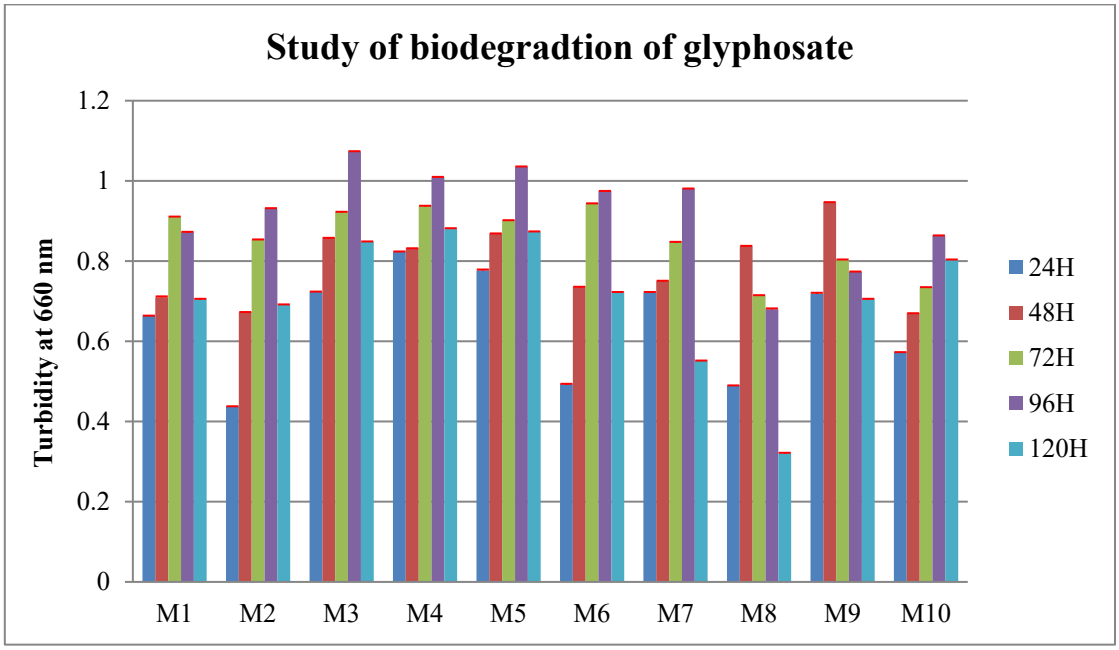

Figure 4: Glyphosate biodegradation kinetics (OD_{660} over 120 h) of the ten isolates of series M (M1- M10) in MMS broth containing 1 g/L glyphosate. [X-axis: incubation time (24-120 h); Y-axis: OD_{660}].

Source: *Original data from the study.*

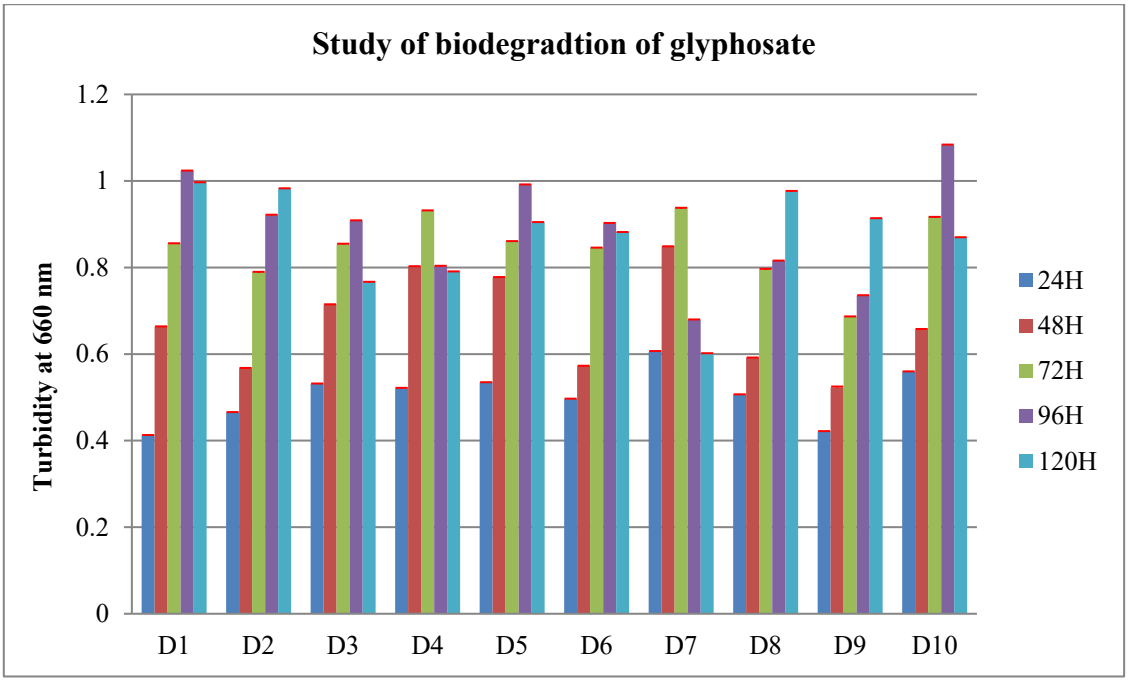

Figure 5: Glyphosate biodegradation kinetics (OD_{660} over 120 h) of the ten isolates of series D (D1-D10) in MMS broth containing 1 g/L glyphosate. [X-axis: incubation time (24-120 h); Y-axis: OD_{660}].

Source: *Original data from the study.*

Table 2: Time to reach maximum growth (OD_{660}) in glyphosate-supplemented MMS broth (1 g/L) among the thirty isolates.

Time to peak growth	Number of isolates	Strains	Source
48 h	6	B2, B5, B8, B9, M8, M9	Original data from the study
72 h	3	M1, D4, D7	Original data from the study
96 h	15	B3, B4, B7, M2, M3, M4, M5, M6, M7, M10, D1, D3, D5, D6, D10	Original data from the study
120 h	6	B1, B6, B10, D2, D8, D9	Original data from the study

4.4. Effect of pH on bacterial growth

Approximately 80% of the thirty isolates achieved their greatest growth under alkaline pH conditions (7-10), while growth of the remaining ~20% decreased when the initial pH fell below 7 (Figures 6–8). The overall highest growth peak recorded across the entire pH screen was that of strain B7 at pH 10 (OD_{660} = 0.999), whereas strain M6 recorded the lowest growth rate observed in this assay, at pH 8 (OD_{660} = 0.521).

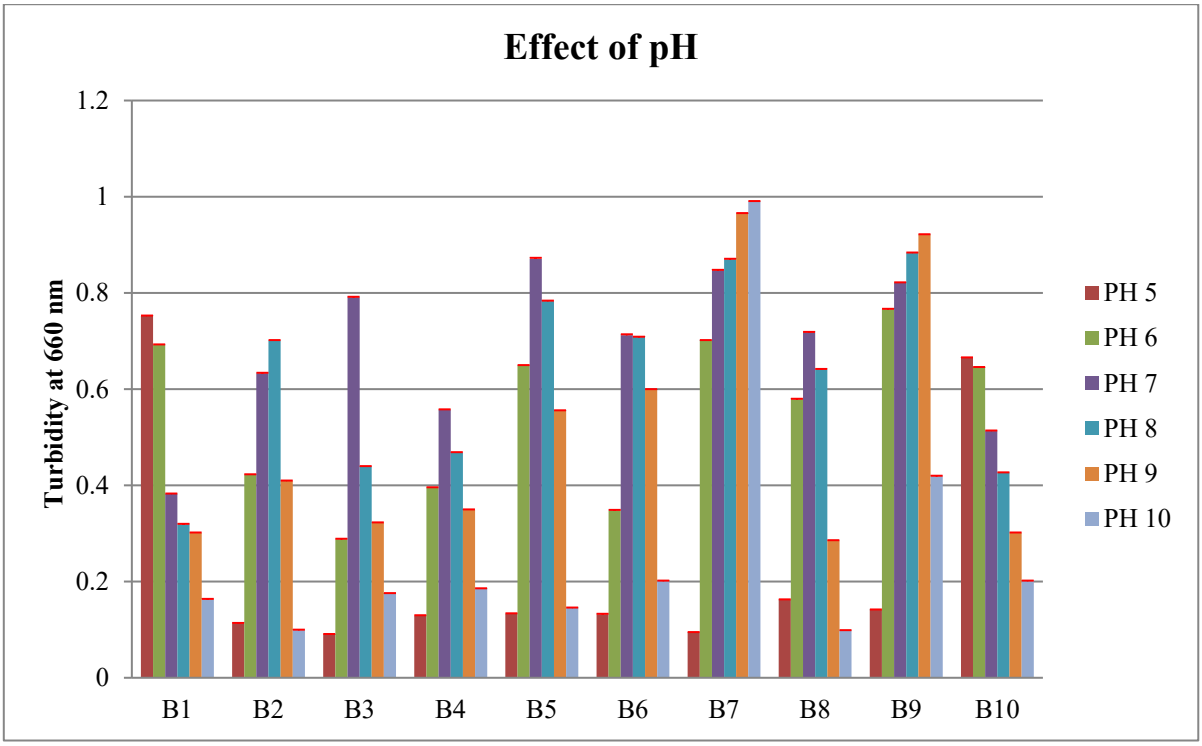

Figure 6: Effect of initial pH (5-10) on the growth (OD_{660}) of series B isolates in glyphosate supplemented medium.

***Source**: Original data from the study.*

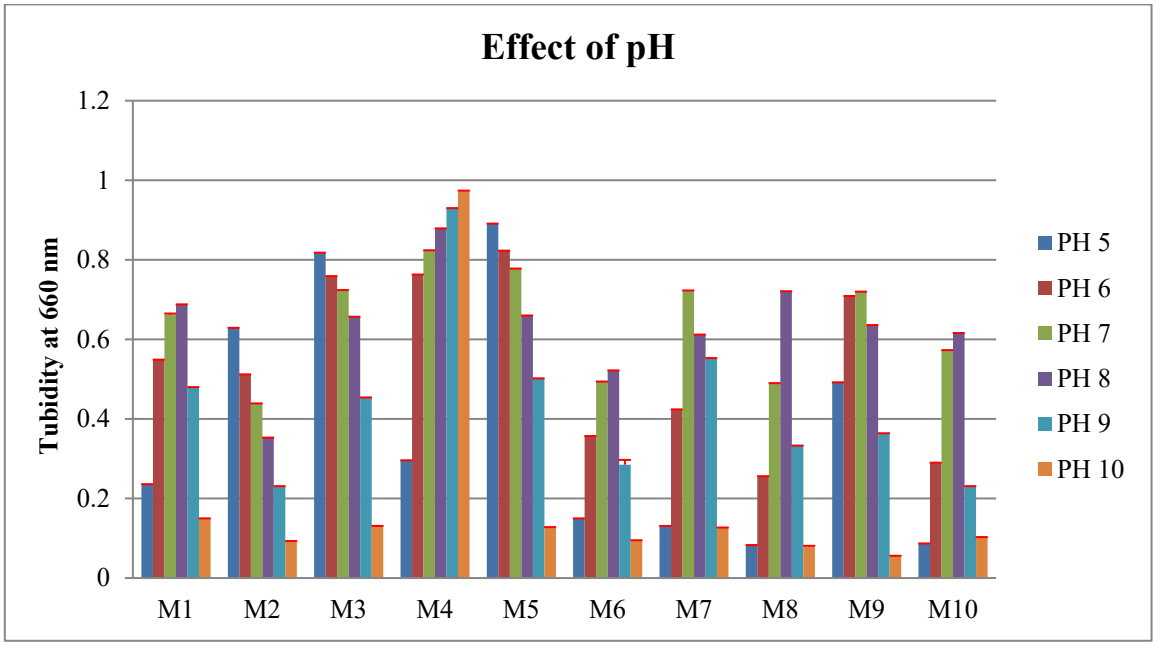

Figure 7: Effect of initial pH (5-10) on the growth (OD_{660}) of series M isolates in glyphosate supplemented medium.

Source: *Original data from the study.*

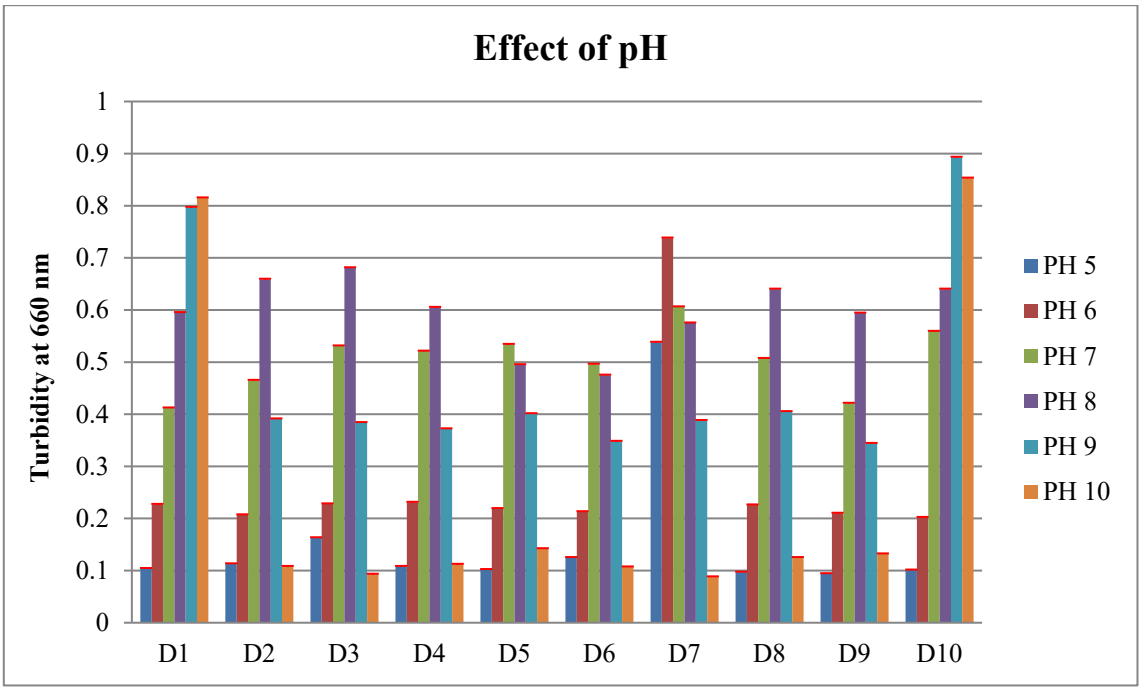

Figure 8: Effect of initial pH (5-10) on the growth (OD_{660}) of series D isolates in glyphosatesupplemented medium.

Source: *Original data from the study.*

4.5. Effect of temperature on bacterial growth

All thirty isolates grew in glyphosate-supplemented medium (1 g/L) across the entire tested temperature range (Figures 9–11). Optimal growth was recorded at 25°C for 3 of 30 isolates, at 30°C for 18 of 30, at 37°C for 5 of 30, and at 40°C or 45°C (combined) for 3 of 30. Strain B3 achieved the highest overall growth peak recorded in this assay (OD_{660} = 0.955) at 40°C,

while strain B6 achieved its own best growth at 25°C (OD_{660} = 0.781), a comparatively unusual result suggestive of a psychrotolerant phenotype for this particular isolate.

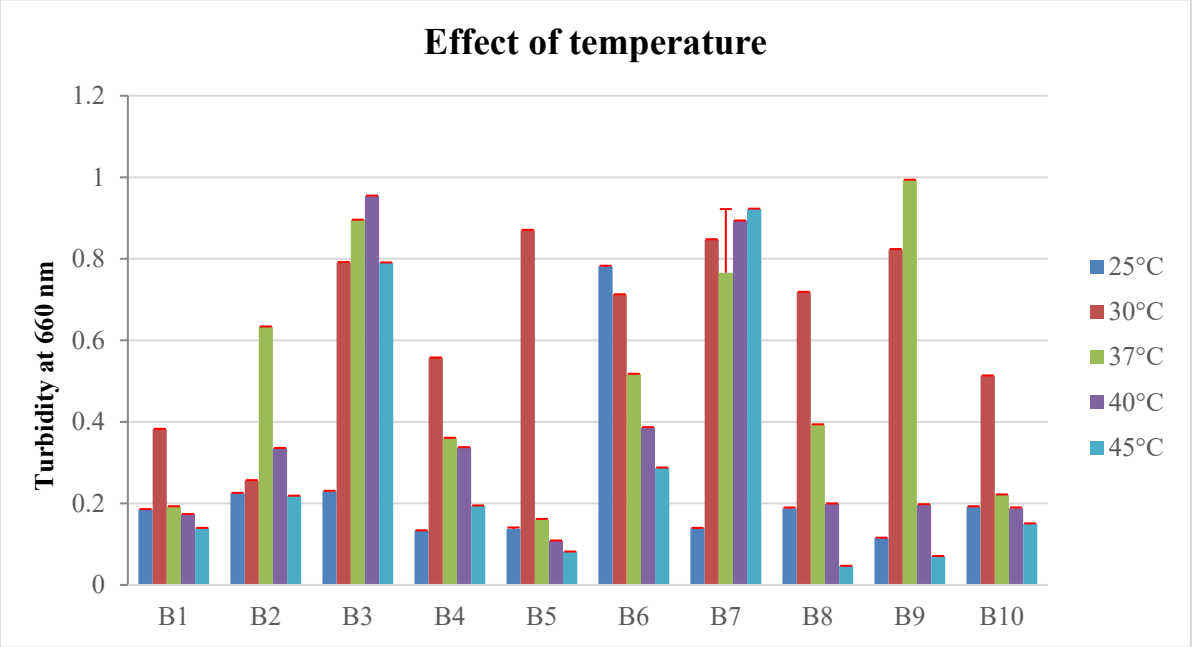

Figure 9: Effect of temperature (25–45°C) on the growth (OD_{660}) of series B isolates in glyphosate supplemented medium.

***Source**: Original data from the study.*

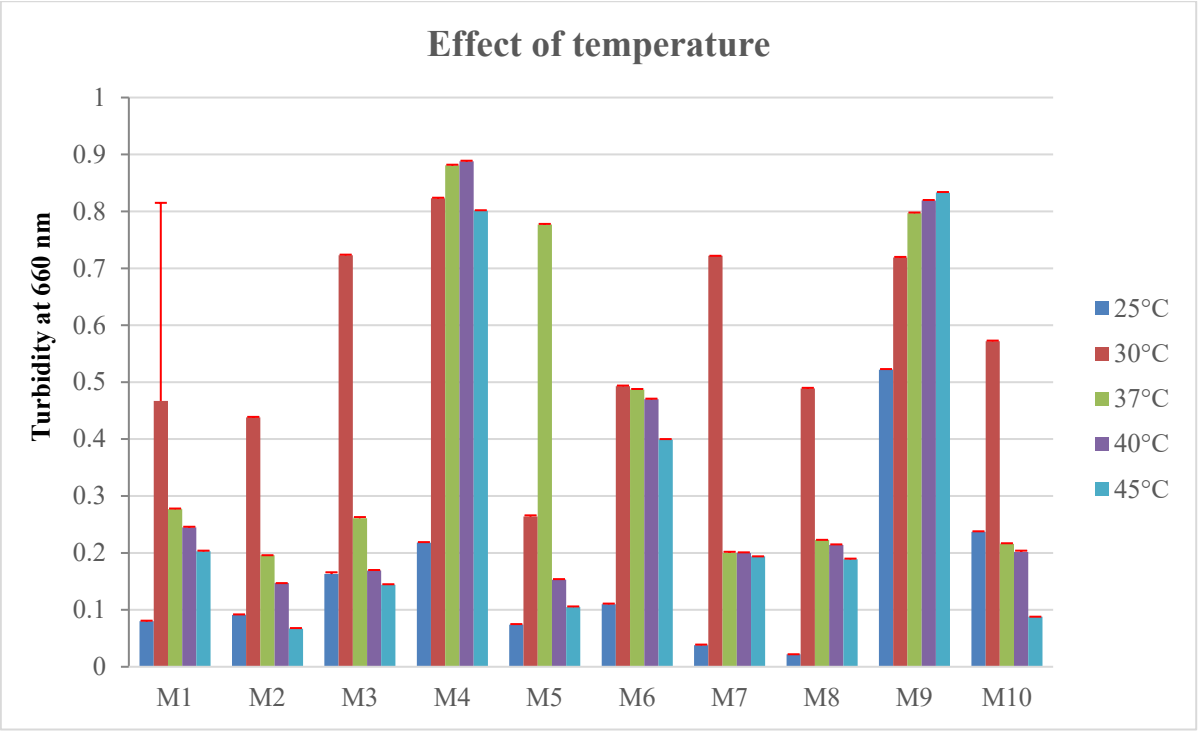

Figure 10: Effect of temperature (25–45°C) on the growth (OD_{660}) of series M isolates in glyphosate supplemented medium.

***Source**: Original data from the study.*

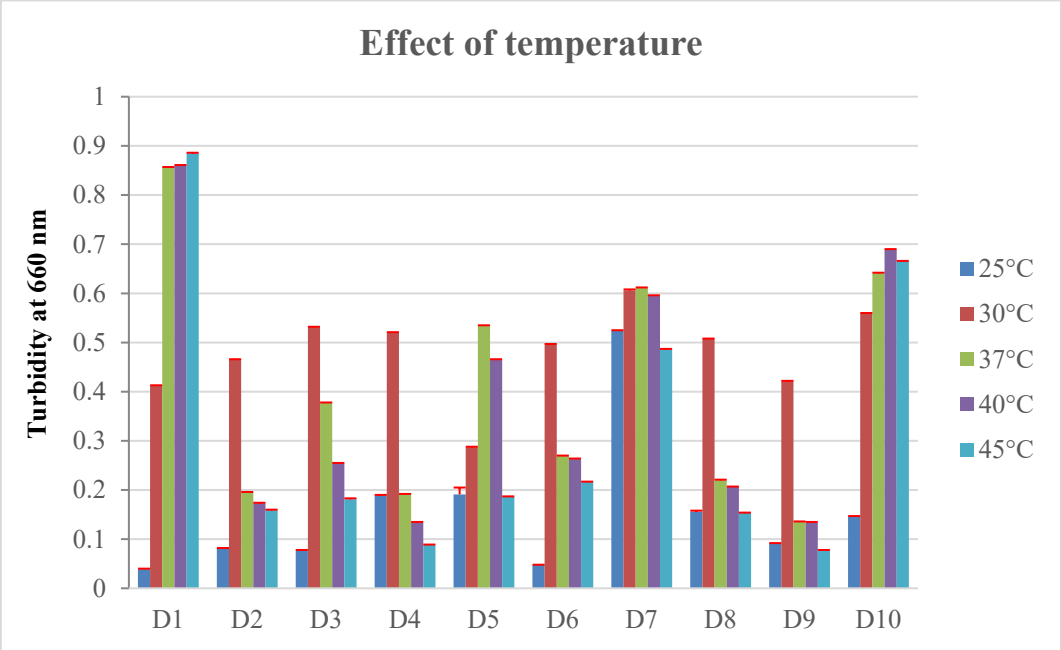

Figure 11: Effect of temperature (25-45°C) on the growth (OD_{660}) of series D isolates in glyphosate supplemented medium.

***Source**: Original data from the study.*

4.6. Tolerance to NaCl

All thirty isolates degraded glyphosate (1 g/L) across the full range of NaCl concentrations tested (Figures 12–14). Optimal growth occurred at 1% NaCl for 9 of 30 isolates, at 2% for 8 of 30, at 3% for 5 of 30, and at 4% or 5% (combined) for 4 of 30. The single highest growth peak recorded in this assay was that of strain D9 (OD_{660} = 0.991) at 3% NaCl. Strains B3, M4, D1, and D10 were each able to grow at the maximum tested concentration of 5% NaCl, with strain M4 reaching OD_{660} = 0.981 under this condition.

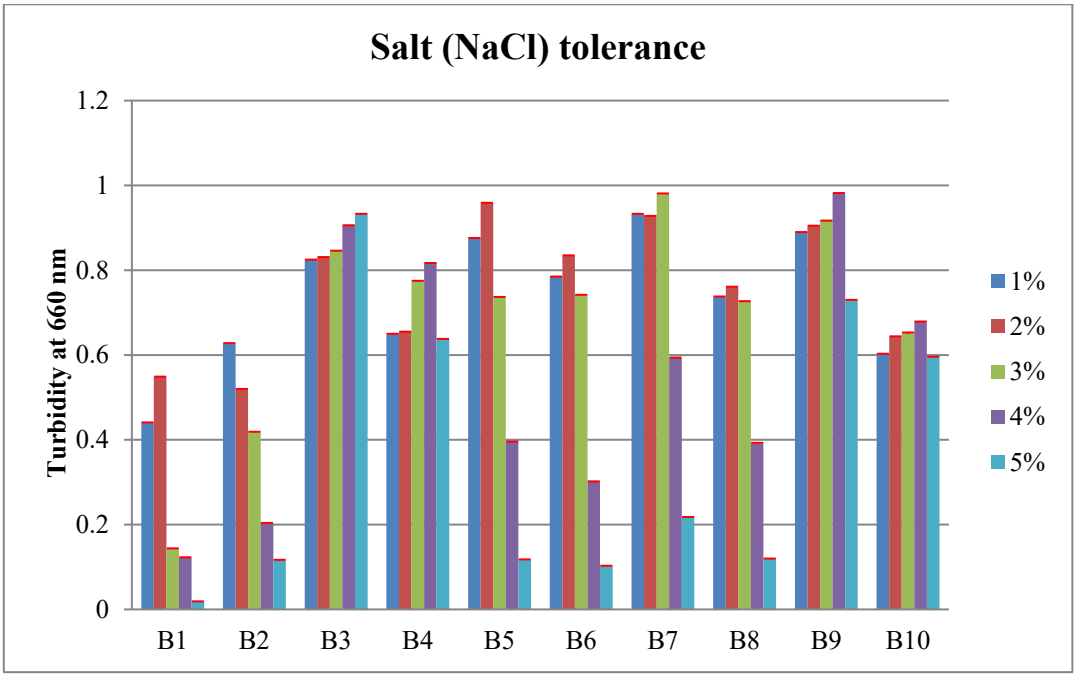

Figure 12: Effect of NaCl concentration (1-5%) on the growth (OD_{660}) of series B isolates in glyphosate supplemented medium.

***Source**: Original data from the study.*

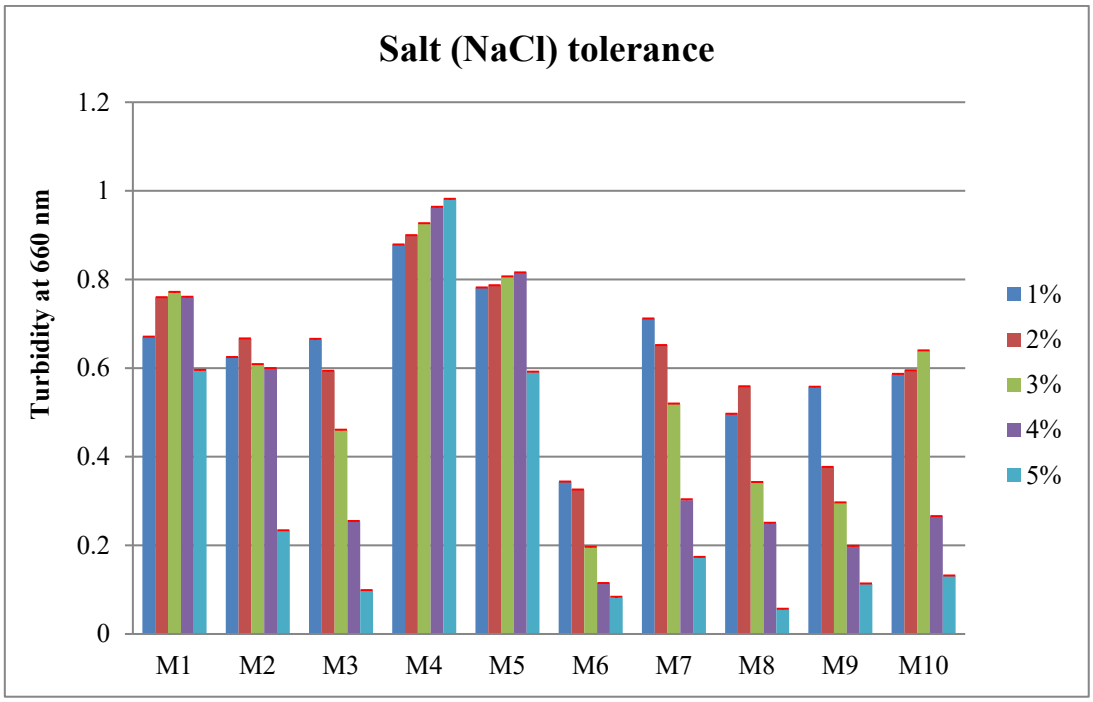

Figure 13: Effect of NaCl concentration (1–5%) on the growth (OD_{660}) of series M isolates in glyphosate supplemented medium.

***Source**: Original data from the study.*

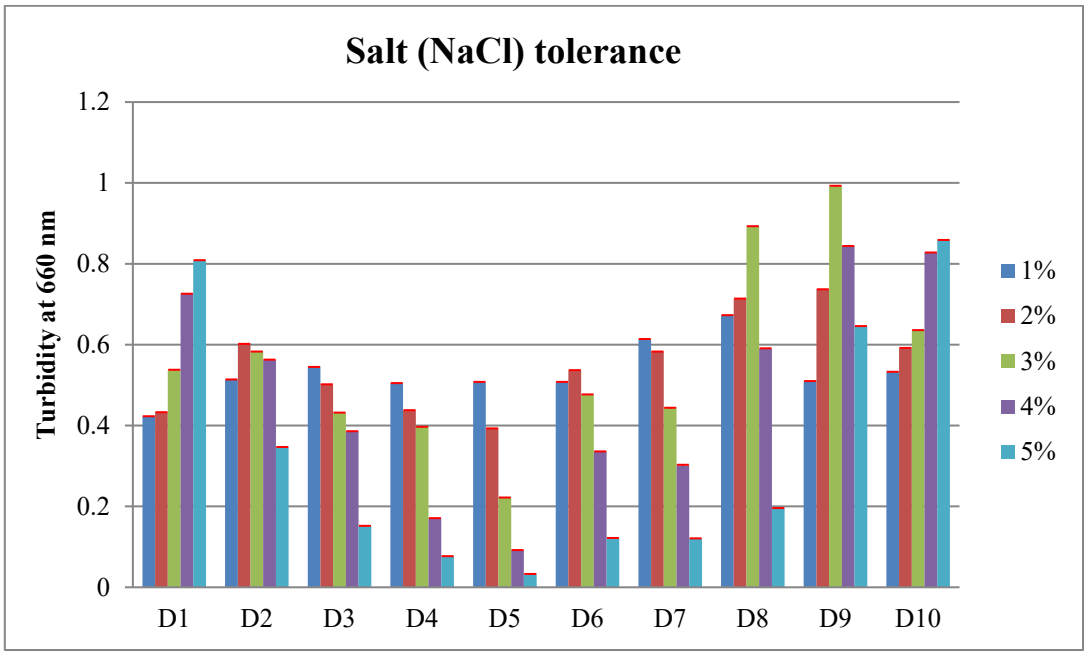

Figure 14: Effect of NaCl concentration (1-5%) on the growth (OD_{660}) of series D isolates in glyphosate supplemented medium.

***Source**: Original data from the study.*

4.7. Tolerance to elevated glyphosate concentrations

An increase in growth relative to lower concentrations was observed for 47% of isolates at an initial glyphosate concentration of 1 g/L; as glyphosate concentration increased further, growth of most strains declined moderately, indicating a degree of concentration-dependent inhibition (Figures 15–17). The most pronounced growth at an intermediate concentration was recorded for strain B7 at 3 g/L, whereas strain D10 displayed the opposite pattern, with growth increasing progressively to reach its own maximum at the highest tested concentration of 5 g/L (OD_{660} = 0.831). Critically, no isolate showed complete growth inhibition at any tested glyphosate concentration up to 5 g/L, indicating that the collection as a whole tolerates glyphosate concentrations well above typical field application residues.

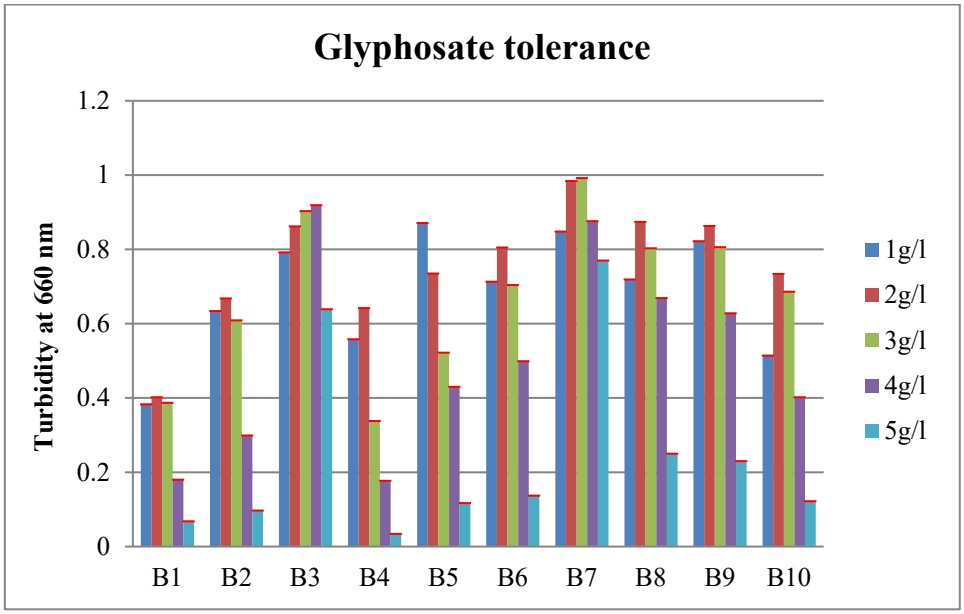

Figure 15: Effect of glyphosate concentration (1-5 g/L) on the growth (OD_{660}) of series B isolates.

***Source**: Original data from the study.*

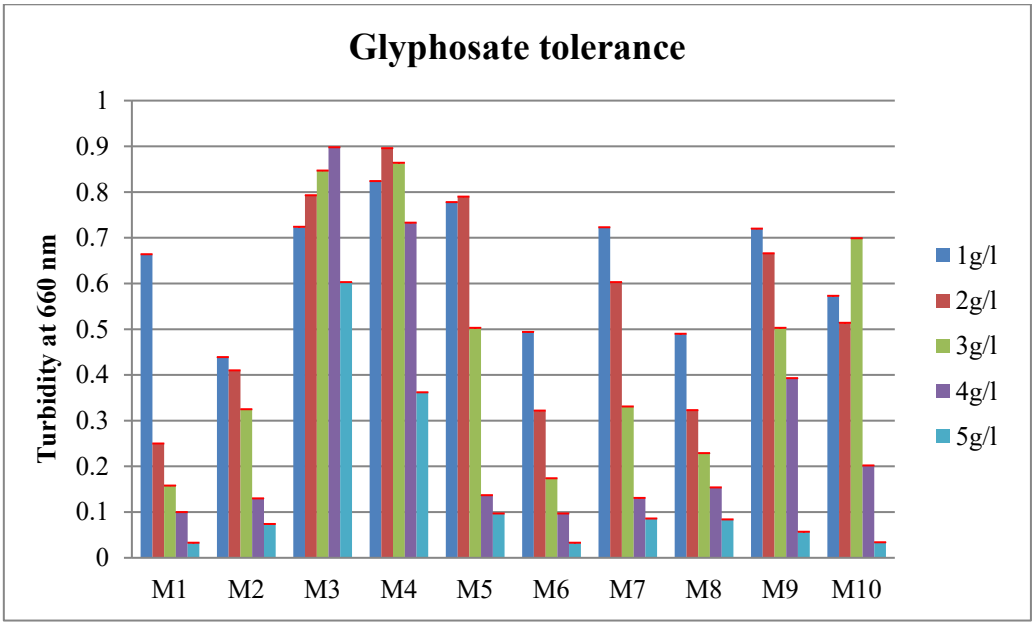

Figure 16: Effect of glyphosate concentration (1-5 g/L) on the growth (OD_{660}) of series M isolates.

***Source**: Original data from the study.*

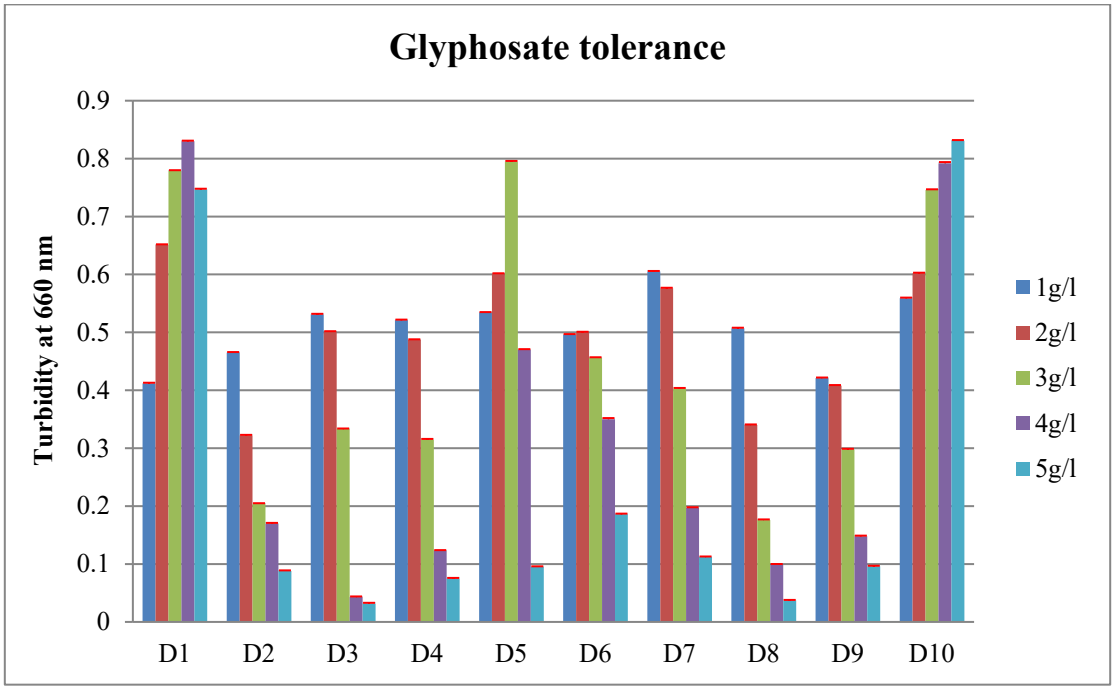

Figure 17: Effect of glyphosate concentration (1-5 g/L) on the growth (OD_{660}) of series D isolates.

***Source**: Original data from the study.*

4.8. Effect of glucose co-amendment

Addition of glucose (1 g/L) to the glyphosate-containing medium significantly increased growth in every one of the thirty isolates tested (Figures 18–20). The highest growth recorded under glucose co-amendment was that of strain B3 (OD_{660} = 1.430), and the lowest was that of strain D4 (OD_{660} = 0.434); notably, even this minimum value represented a net increase relative to the corresponding glyphosate-only condition for strain D4, underscoring the consistency of the co-metabolic growth stimulation across the entire isolate collection.

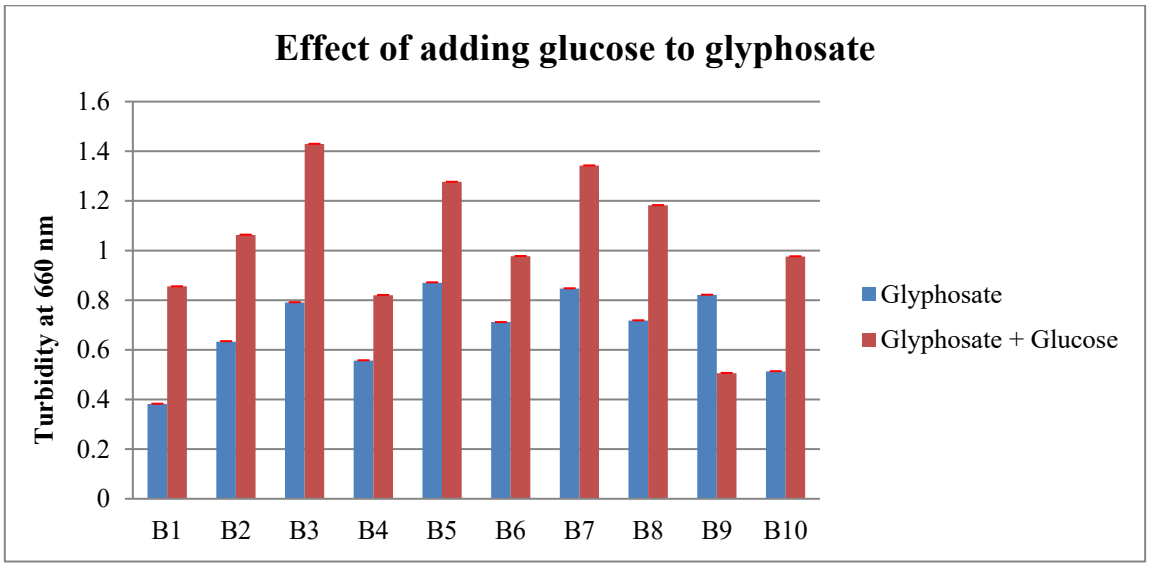

Figure 18: Effect of glucose co-amendment (1 g/L) on the growth (OD_{660}) of series B isolates in glyphosate-supplemented medium (glyphosate only vs. glyphosate + glucose).

***Source**: Original data from the study.*

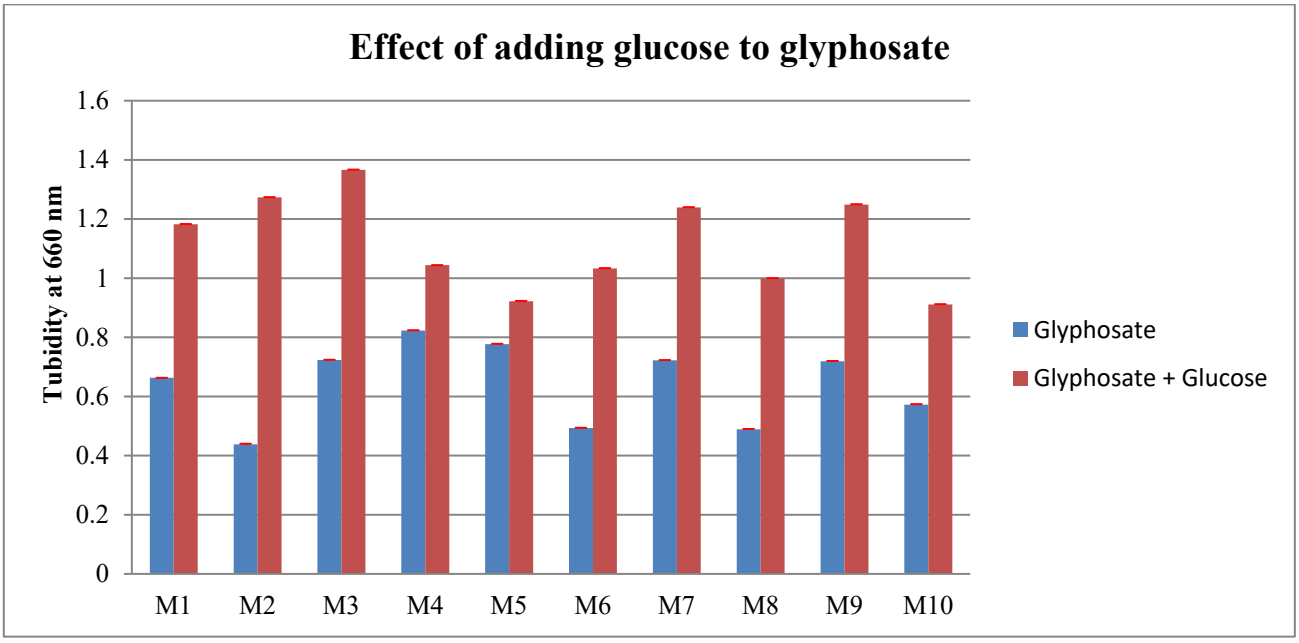

Figure 19: Effect of glucose co-amendment (1 g/L) on the growth (OD_{660}) of series M isolates in glyphosate-supplemented medium (glyphosate only vs. glyphosate + glucose).

Source: *Original data from the study.*

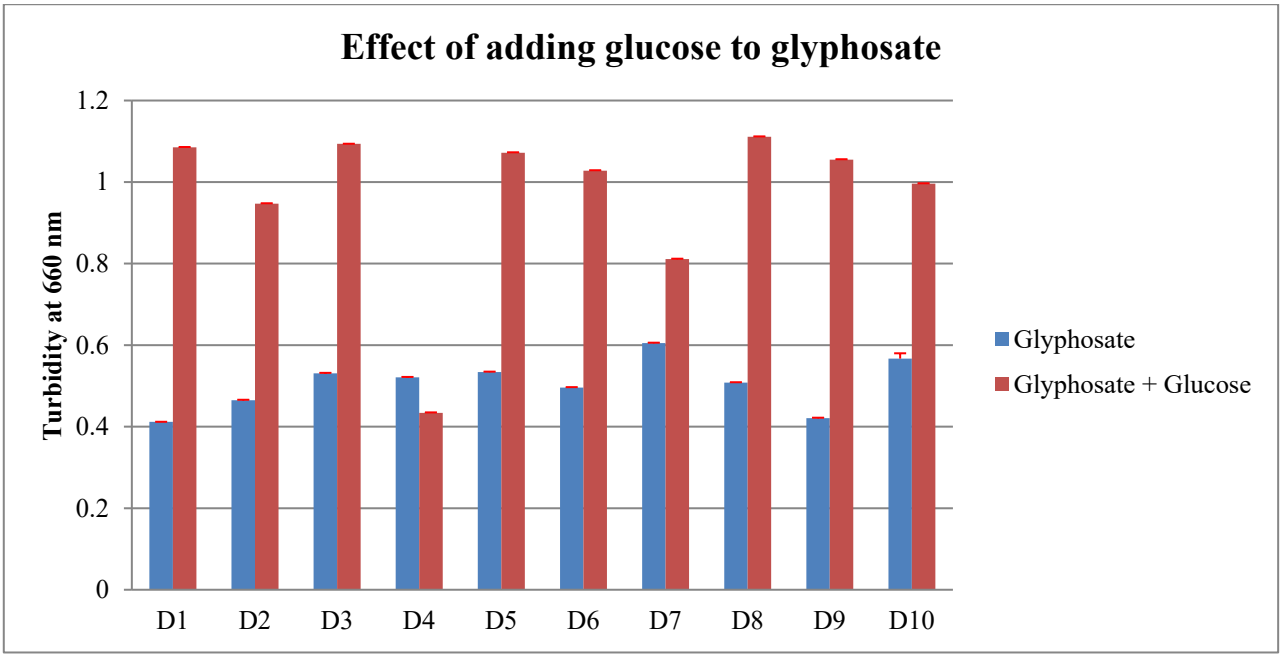

Figure 20: Effect of glucose co-amendment (1 g/L) on the growth (OD_{660}) of series D isolates in glyphosate-supplemented medium (glyphosate only vs. glyphosate + glucose).

Source: *Original data from the study.*

4.9. Biochemical identification of the twelve selected isolates

On the basis of their combined tolerance to abiotic stressors and to elevated glyphosate concentrations, twelve isolates were selected for biochemical identification with the API 20NE gallery: B1, B3, B4, B7, B9, M3, M5, M9, D1, D4, D7, and D10 (Table 3). Identification was achieved at the genus level. According to the classification of Bergey (1984), the twelve isolates were affiliated with five bacterial families – *Pseudomonasaceae*, *Aeromonadaceae*, *Chromobacteriaceae*, *Rhizobiaceae*, and *Vibrionaceae*. All twelve selected strains showed enhanced growth upon glucose addition (Table 3).

Table 3: Optimal abiotic conditions and taxonomic identification of the twelve selected glyphosate degrading strains.

Strain	Time to peak (h)	Optimal T (°C)	Optimal pH	Optimal NaCl (%)	Optimal glyphosate (g/L)	Identified genera
B1	120	30	5	2	2	*Rhizobiaceae*
B3	96	40	7	5	4	*Pseudomonasaceae*
B4	96	30	7	4	2	*Pseudomonasaceae*
B7	96	45	10	3	3	*Aeromonadaceae*
B9	48	37	9	4	2	*Pseudomonasaceae*
M3	96	30	5	1	4	*Pseudomonasaceae*
M5	96	30	5	4	2	*Chromobacteriaceae*
M9	48	45	7	1	1	*Pseudomonasaceae*
D1	96	45	10	5	4	*Aeromonadaceae*
D4	72	30	8	1	1	*Pseudomonasaceae*
D7	72	37	6	1	1	*Vibrionaceae*
D10	96	40	9	5	4	*Aeromonadaceae*

Note: All twelve strains showed improved growth upon glucose addition (1 g/L).

***Source for all tables**: Original data from the study.*

5. DISCUSSION

The present study confirms that agricultural soils of the Mostaganem region host a taxonomically diverse and physiologically robust community of glyphosate-tolerant bacteria capable of using this herbicide as a sole carbon source across a wide range of environmental conditions. Three converging lines of evidence support this conclusion: the markedly higher density of glyphosate-degrading microflora in soil under repeated glyphosate application relative to non-treated soils; the ability of all thirty isolates to grow on glyphosate-supplemented minimal medium; and the broad abiotic tolerance range (pH 5-10, 25-45°C, up to 5% NaCl, and up to 5 g/L glyphosate) recorded among the isolates.

The elevated density of glyphosate-degrading microflora at the glyphosate-treated site (6.632±0.01 Log CFU/g), relative to the two sites without direct glyphosate history (4.350±0.02 and 4.474±0.01 Log CFU/g), is consistent with the well-established principle that repeated pesticide exposure exerts selective pressure that enriches for tolerant and degradative microbial populations (Barriuso et al., 1996; Soulas, 1993). This pattern parallels the findings of Li et al. (2024), who showed that a glyphosate-degrading consortium enriched from contaminated soil could achieve substantial degradation within a short incubation period, and is broadly compatible with reports that glyphosate exposure can stimulate microbial biomass and respiration under certain conditions, even though prolonged exposure may, in other contexts, reduce functional soil diversity

(Haney et al., 2000; Zhang, Wang, Zhang, Li, & Wang, 2024). The detection of glyphosate-degrading bacteria, albeit at lower density, in soils without a direct glyphosate history suggests either background exposure via drift or run-off from neighboring treated plots, or the dissemination of catabolic capacity through horizontally transferable genetic elements, as proposed by Chaparro et al. (2023).

The predominance of Gram-negative bacteria (60%) among the isolates, and their identification as members of the *Pseudomonasaceae*, *Aeromonadaceae*, *Chromobacteriaceae*, *Rhizobiaceae*, and *Vibrionaceae*, is in line with earlier reports that Gram-negative bacteria constitute the majority of pesticide-transforming soil microorganisms (Topp et al., 2000). The genus *Pseudomonas* was particularly well represented among the twelve identified, consistent with the historically central role of this genus in glyphosate catabolism, first documented for *Pseudomonas sp.* strain PG2982 by Kishore and Jacob (1987) and repeatedly confirmed across numerous glyphosate-contaminated environments worldwide (Obojska et al., 2002; Tolbot et al., 1984). The recovery of strain B1 corroborates earlier reports that the *Rhizobiaceae* harbor effective glyphosate degraders (Liu et al., 1991) and is consistent with the more recent characterization of *Rhizobium sp.* GX19 as an aerobic glyphosate-metabolizing strain (Wang, Zhang, & Feng, 2023). The recovery of strains belonging to the *Aeromonadaceae* and *Vibrionaceae* families – less classically associated with glyphosate degradation in the reviewed literature – suggests that the specific agroecological, and possibly moderately saline, character of the Mostaganem sites may select for a somewhat different taxonomic assemblage than that typically reported in temperate, non-saline agricultural soils elsewhere.

Recent studies from North Africa, the Middle East, and other Mediterranean and saline agroecosystems provide useful points of comparison. Algerian saline environments — including Saharan soils near Biskra and the brackish waters of Sebkhet Sefioune in Ouargla — have repeatedly yielded pesticide- and glyphosate-degrading bacteria despite substantial ambient salinity (Benslama, 2014; Sebihi & Merabti, 2019; Benabbes et al., 2020), a pattern that is directly echoed by the present isolates, several of which (D9, M4, B3, D1, and D10) tolerated NaCl concentrations of up to 5%. Reports of a newly characterized *Rhizobium sp.* GX19 from an aerobic agricultural setting (Wang, Zhang, & Feng, 2023) and of consortium-level degradation exceeding 400 mg/L glyphosate within days (Li et al., 2024) similarly point to a broader Mediterranean and North African trend of recovering robust, multiply-tolerant glyphosate-degrading taxa from agricultural soils subject to recurrent herbicide pressure, alongside continuing evidence that glyphosate and AMPA can alter soil microbial community composition and enzymatic activity in paddy and other intensively managed systems (Zhang, Wang, Xu, & Liu, 2024).

The observed growth kinetics, with strains reaching maximal optical density between 48 and 120 hours, are consistent with an induction phase preceding full expression of glyphosate-catabolic enzyme systems, as discussed by Chaparro et al. (2023). Strain M9, which reached the stationary phase within 48 h, is a particularly promising candidate for rapid bioremediation applications; given its short lag phase, it is tempting to hypothesize that M9 might preferentially employ the sarcosine (C-P lyase) pathway, which is generally regarded as more metabolically direct and ecologically favorable because it avoids AMPA accumulation (Feng et al., 2022). It must be stressed, however, that the present dataset does not include direct biochemical confirmation of either the AMPA or the sarcosine pathway – for instance via HPLC or LC-MS/MS quantification of glyphosate, AMPA, or sarcosine, or via targeted amplification of opd/C-P lyase gene clusters – and this pathway attribution therefore remains a hypothesis for future molecular and analytical testing rather than a demonstrated result. Likewise, while *Stenotrophomonas acidaminiphila* Y4B has been reported to degrade more than 90% of glyphosate within 72 hours (Li et al., 2022), no equivalent quantitative degradation percentage can be, or is, reported for the present isolates in the absence of analytical quantification of residual glyphosate.

The consistent preference of most isolates for alkaline pH (7-10) aligns with the mechanistic explanation proposed by Singh et al. (2003), who linked elevated soil pH to a greater abundance of organophosphonate-degrading (opd) genes, and is compatible with the greater aqueous solubility and reduced soil adsorption of glyphosate under alkaline conditions, which would be expected to increase its bioavailability to bacterial cells. The minority of isolates favoring more acidic conditions mirrors the pH range reported for *Ochrobactrum anthropi* by Shushkova et al. (2012). The broad thermal tolerance recorded – with the majority of isolates (18/30) optimal at 30°C but a subset performing well up to 45°C – is consistent with the mesophilic character expected of most soil bacteria, while the atypical case of strain B6, which grew best at 25°C, illustrates the physiological heterogeneity that can exist even within a single enrichment series, compatible with a psychrotolerant phenotype as discussed by Moorman (1994) and Walker et al. (1992) regarding temperature-dependent pesticide degradation in soil.

The absence of complete growth inhibition at glyphosate concentrations up to 5 g/L – well above typical field application rates – indicates a substantial safety margin for practical bioremediation use at contaminated or spill sites, where residual concentrations can exceed recommended agricultural doses. This tolerance is nonetheless more modest than that reported for *Bacillus subtilis* strain Bs-15, which tolerated up to 40 g/L glyphosate although its own growth optimum was likewise 1 g/L (Yu et al., 2015), suggesting scope for further strain improvement or bioprocess optimization to extend the practical concentration range over which the Mostaganem isolates remain fully functional. The pronounced growth stimulation observed upon glucose addition, consistent with Kumar and Philip (2006) and with the co-metabolic framework described by Horwath (1972), Guha et al. (1997), and Mallick et al. (1999), has direct practical implications for bioremediation design: field- or reactor-based strategies employing these strains may benefit substantially from co-amendment with an easily assimilable carbon source, particularly during the early stages of bioaugmentation, when indigenous glyphosate concentrations alone may be insufficient to sustain rapid bacterial growth.

These findings carry practical relevance for the design of glyphosate bioremediation strategies at multiple scales. For in situ applications – for example, treatment of contaminated agricultural plots or spill sites without soil excavation – the broad pH, temperature, and salinity tolerance of the Mostaganem isolates suggests that they could remain metabolically active across the seasonal and spatial heterogeneity typical of Mediterranean field soils, particularly if applied together with a co-substrate such as glucose to stimulate co-metabolic activity (biostimulation).

For ex situ applications, such as slurry-phase or fixed-bed bioreactors treating contaminated soil or wastewater, the rapid growth kinetics of strains such as M9 (peak growth at 48 h) and the high glyphosate tolerance of strains such as D10 (growth optimum at 5 g/L) make these isolates attractive candidates for bioaugmentation of engineered systems, where inoculum density and reactor residence time can be more tightly controlled than under field conditions. More broadly, the taxonomic diversity of the twelve selected strains – spanning five families – suggests that a multi-strain consortium, rather than a single isolate, may offer the most robust and versatile basis for either biostimulation of resident soil microflora or bioaugmentation with exogenous, well-characterized degraders, since different strains appear to occupy at least partially distinct optima for pH, temperature, and salinity.

Taken together, these results position the twelve selected strains – particularly B3, B7, D10, M9 and D4 – as strong candidates for further development, given their combination of rapid growth, broad pH and temperature tolerance, salinity tolerance, and high glyphosate tolerance. The principal scientific contribution of this work lies in documenting, for an Algerian Mediterranean agroecosystem, a taxonomically diverse assemblage of glyphosate-tolerant bacteria with tolerance profiles extending beyond those typically required for standard agricultural exposure scenarios, thereby providing an empirical basis for subsequent applied bioremediation research in North African soils.

6. CONCLUSION

The results obtained in this study leave little doubt as to the contamination of agricultural soils in the Mostaganem region by pesticides, a finding that makes this environment of clear interest for the search for bacteria capable of degrading these organic micropollutants. This observation is fully consistent with the microbiological analysis, which revealed the presence of bacterial strains belonging to five families: *Pseudomonasaceae*, *Aeromonadaceae*, *Chromobacteriaceae*, *Rhizobiaceae*, and *Vibrionaceae*. The study further enabled the growth kinetics of thirty microbial strains, isolated from three soil samples, to be monitored in a medium containing 1 g/L glyphosate, together with the influence of temperature, pH, and NaCl concentration, tolerance to elevated glyphosate concentrations, and the effect of glucose addition as a second carbon source.

Overall, growth kinetics revealed that all isolated bacterial strains were able to grow on glyphosate over different time periods, with maximal growth generally occurring at temperatures of 30–37°C and, for some strains, up to 45°C. Growth of most isolates was favored at alkaline pH ranging from 7 to 10, and the strains were able to tolerate up to 5% NaCl. Growth of the bacterial isolates was not inhibited following exposure to glyphosate concentrations of up to 5 g/L, and glyphosate-supported growth was markedly more pronounced in the presence of glucose as a second carbon source.

In conclusion, this work enabled the selection of twelve of the best-performing strains, which may be considered conditional candidates for future application in bioremediation, with a view toward their use as biocatalysts in the detoxification of glyphosate-contaminated environments. Confirming and extending these findings – through analytical quantification of glyphosate removal and molecular confirmation of strain identity – represents the logical next step toward translating these phenotypic results into validated bioremediation tools.

7. LIMITATIONS AND FUTURE RESEARCH

This study has several limitations that should be explicitly acknowledged.

• **First**, bacterial growth (optical density at 660 nm) was used throughout as an indirect proxy for glyphosate biodegradation; no analytical quantification of residual glyphosate or of its degradation products (AMPA, sarcosine) was performed by HPLC, LC-MS/MS, or an equivalent method. Actual removal efficiencies, degradation rate constants (e.g., first-order halflives), and pathway assignment (AMPA versus sarcosine route) therefore cannot be reported and should not be inferred from growth data alone.

• **Second**, biochemical identification using the API 20NE gallery allowed genus-level, but molecular confirmation by 16S rRNA gene sequencing and phylogenetic analysis was not performed in the present dataset and would be required to confirm taxonomic assignments with certainty, as recommended by Chaparro et al. (2023) and Zhang, Wang, Zhang, Li, and Wang (2024).

Finally, all degradation assays were conducted at laboratory scale in liquid MMS broth, and further work under real soil, mesocosm, or field conditions is required to validate the practical bioremediation performance of these isolates.

Building on these findings, several directions for future research are proposed:

(i) molecular confirmation of isolate identity by 16S rRNA gene sequencing, ideally complemented by whole-genome sequencing of the most promising strains to screen for known glyphosatecatabolic gene clusters (opd, C-P lyase operons);

(ii) analytical quantification of glyphosate, AMPA, and sarcosine by HPLC or LC-MS/MS to establish true degradation kinetics and pathway usage;

(iii) evaluation of consortium-level (multi-strain) degradation performance and of biostimulation versus bioaugmentation strategies under simulated soil or reactor conditions;

(iv) field- or mesocosm-scale pilot trials to assess the practical bioremediation potential of the most tolerant isolates identified here.

REFERENCES

[1] Ali-Haimoud, D. (1980). Contribution à l'étude des sols alfatiers: fixation d'azote symbiotique, effet du paillage sur cette activité [Unpublished thesis]. USTHB, Alger, Algérie.

[2] Anwar, S., Liaquat, F., Khan, Q. M., Khalid, Z. M., & Iqbal, S. (2009). Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1. Journal of Hazardous Materials, 168 (1), 400–405. https://doi.org/10.1016/j. jhazmat.2009.02.059

[3] Aubertot, J. N., Barbier, J. M., Carpentier, A., Gril, J. J., Guichard, L., Lucas, P. H., Savary, S., & Voltz, M. (2005). Pesticides, agriculture et environnement. Éditions Quae.

[4] Audus, L. J. (1964). Herbicide behavior in the soil: II. Interactions with soil microorganisms. In L. J. Audus (Ed.), The physiology and biochemistry of herbicides (pp. 163–206). Academic Press.

[5] Balthazor, T. M., & Hallas, L. E. (1986). Glyphosate-degrading microorganisms from industrial activated sludge. Applied and Environmental Microbiology, 51 (2), 432–434. https://doi.org/10.1128/aem.51.2.432-434.1986

[6] Barriuso, E., Calvet, R., Schiavone, M., & Soulas, G. (1996). Les pesticides et les polluants organiques des sols: Transformation et dissipation. Étude et Gestion des Sols, 3 (4), 279–296.

[7] Benabbes, R., Sanchez-Ballester, N. M., Bataille, B., Leclercq, L., Shawqi, T., & Soulairol, I. (2020). Structure–properties relationship in the evaluation of alginic acid functionality for tableting. Pharmaceutical Science & Technology, 21 (3), 94. https://doi.org/10.1208/s12249-020-1633-3

[8] Benslama, O. (2014). Isolement et caractérisation des bactéries capables de dégrader l'herbicide glyphosate et optimisation des conditions de culture pour une dégradation plus efficace [Unpublished master's thesis]. Université des Frères Mentouri 1, Constantine, Algérie.

[9] Bento, C. P. M., Yang, X., Gort, G., Xue, S., & Ritsema, C. J. (2022). Persistence of glyphosate and AMPA in soils of different textures under simulated rainfall. Science of the Total Environment, 825, 153999. https://doi.org/10.1016/j.scitotenv.2022.153999

[10] Bergey, R. (1984). Bergey's manual of systematic bacteriology (9th ed.). Williams & Wilkins.

[11] Busse, M. D., Ratcliff, A. W., Shestak, C. J., & Powers, R. F. (2001). Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology and Biochemistry, 33 (12–13), 1777–1789. https://doi.org/10.1016/S0038-0717(01)00103-1

[12] Chaparro, J. M., Zhang, R., & Vivanco, J. M. (2023). Impact of glyphosate-based herbicides on soil microbiota and plant health: A review. Journal of Applied Microbiology, 134 (5), lxad008. https://doi.org/10.1093/jambio/lxad008

[13] Chaussod, R. (1994). La qualité biologique des sols: Évaluation et implications. INRA Éditions.

[14] Chaussod, R., Breuil, M. C., Nouaim, R., & Lévêque, J. (1999). La fertilité des sols viticoles: indicateurs microbiologiques (pp. 15–22). INRA Éditions.

[15] Cheah, U., Kirkwood, R., & Lum, K. (1998). Degradation of four commonly used pesticides in Malaysian agricultural soils. Journal of Agricultural and Food Chemistry, 46 (3), 1217– 1223. https://doi.org/10.1021/jf9705214

[16] Devers-Lamrani, M., Pesce, S., & Rouard, N. (2014). Impact écotoxicologique de pesticides sur les communautés microbiennes naturelles responsables de la biodégradation de pesticides dans les sédiments de rivière. In Protection des cultures et santé environnementale: héritages et conceptions nouvelles (pp. 45–58). Quae.

[17] Dick, R. E., & Quinn, J. P. (1995). Glyphosate-degrading isolates from environmental samples: Occurrence and pathways of degradation. Applied Microbiology and Biotechnology, 43 (3), 545–550. https://doi.org/10.1007/BF00218460

[18] Dommergues, Y., & Mangenot, F. (1970). Ecologie microbienne du sol. Masson et Cie.

[19] FAO, IFAD, UNICEF, WFP, & WHO. (2020). The state of food security and nutrition in the world 2020. FAO. https://doi.org/10.4060/ca9692en

[20] Feng, Y., Zhan, H., Yu, P., & Chen, S. (2022). Biodegradation pathways and regulatory mechanisms of glyphosate-degrading microbes: A review. Critical Reviews in Biotechnology, 42 (1), 83–98. https://doi.org/10.1080/07388551.2021.1992283

[21] Forlani, G., Mangiagalli, A., Nielsen, E., & Suardi, C. M. (1999). Degradation of the phosphonate herbicide glyphosate in soil: Evidence for a possible involvement of unculturable microorganisms. Soil Biology & Biochemistry, 31 (7), 991–997. https://doi.org/10.1016/S0038-0717(99)00010-3

[22] Fu, G. M., Li, R. Y., Hu, M., Yuan, X. Q., Li, B., Wang, F. X., Liu, C. M., & Wan, Y. (2016). Utilisation des herbicides en forêt et gestion durable. Ministère de l'Agriculture et de la Pêche.

[23] Grayston, S. J., Wang, S., Campbell, C. D., & Edwards, A. C. (1998). Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry, 30 (3), 369–378. https://doi.org/10.1016/S0038-0717(97)00124-7

[24] Guha, A., Kumari, B., & Roy, M. K. (1997). Possible involvement of plasmid in degradation of malathion and chlorpyrifos by *Micrococcus sp*. Folia Microbiologica, 42 (6), 574–576. https://doi.org/10.1007/BF02815469

[25] Haney, R. L., Senseman, S. A., & Hons, F. M. (2000). Effect of Roundup Ultra on microbial activity and biomass from selected soils. Journal of Environmental Quality, 29 (3), 774–780. https://doi.org/10.2134/jeq2000.00472425002900030024x

[26] Hassanshahian, M., Zeynalipour, M. S., & Musa, F. H. (2014). Isolation and characterization of crude oil degrading bacteria from the Persian Gulf (Khorramshahr provenance). Marine Pollution Bulletin, 82 (1–2), 39–44. https://doi.org/10.1016/j.marpolbul.2014.03.027

[27] Hocinat, A., & Ali-Khodja, H. (2018). Biodégradation de quelques composés organiques volatils et certains pesticides par des actinomycètes provenant d'un sol agricole et de boues activées [Unpublished doctoral dissertation]. Université de Constantine, Algérie.

[28] Horwath, R. S. (1972). Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriological Reviews, 36 (2), 146–155. https://doi.org/10.1128/br.36.2. 146-155.1972

[29] Jans, H., Huo, Q., Bethard, S., Vulic, I., & Moens, M. F. (2012). Gold nanoparticle-enabled biological and chemical detection and analysis. Chemical Society Reviews, 41 (7), 2849–2866. https://doi.org/10.1039/C1CS15280G

[30] Kertesz, M. A., Cook, A. M., & Leisinger, T. (1994). Microbial metabolism of sulfur- and phosphorus-containing xenobiotics. FEMS Microbiology Reviews, 15 (2–3), 195–215. https://doi.org/10.1111/j.1574-6976.1994.tb00136.x

[31] Kishore, G. M., & Jacob, G. S. (1987). Degradation of glyphosate by *Pseudomonas sp.* PG2982 via a sarcosine intermediate. Journal of Biological Chemistry, 262 (25), 12164– 12168.

[32] Krzysko-Lupicka, T., & Orlik, A. (1997). The use of glyphosate as the sole source of phosphorus or carbon for the selection of soil-borne fungal strains capable of degrading this herbicide. Chemosphere, 34 (12), 2601–2605. https://doi.org/10.1016/S0045-6535(97)00104-X

[33] Kumar, M., & Philip, L. (2006). Enrichment and isolation of a mixed bacterial culture for complete mineralization of endosulfan. Journal of Environmental Science and Health, Part B, 41 (1), 81–96. https://doi.org/10.1080/03601230500234529

[34] Larpent, J. P., & Larpent, G. M. (1990). Mémento technique de microbiologie (2nd ed.). Lavoisier.

[35] Li, J., Chen, W.-J., Zhang, W., Hu, D., Zhang, Y., Lin, Z., Zhang, X., & Wang, W. (2022). Effects of immobilized *Stenotrophomonas acidaminiphila* Y4B on glyphosate degradation. Journal of Agricultural and Food Chemistry, 70 (43), 13945–13958. https://doi.org/10. 1021/acs.jafc.2c05612

[36] Li, Y., Wang, S., Zhang, J., & Liu, X. (2024). Characterization of a glyphosate-degrading bacterial consortium enriched from contaminated soil and its degradation performance in microcosm studies. Environmental Pollution, 332, 122003. https://doi.org/10.1016/j. envpol.2023.122003

[37] Liu, C. M., McLean, P. A., Sookdeo, C. C., & Cannon, F. C. (1991). Degradation of the herbicide glyphosate by members of the family *Rhizobiaceae*. Applied and Environmental Microbiology, 57 (6), 1799–1804. https://doi.org/10.1128/aem.57.6.1799-1804.1991

[38] Maillard, E. (2014). Transport and degradation of pesticides in wetland systems: A downscaling approach [Unpublished doctoral dissertation]. Université de Strasbourg.

[39] Mallick, B. K., Banerji, A., Shakil, N. A., & Sethunathan, N. N. (1999). Bacterial degradation of chlorpyrifos in pure culture and in soil. Bulletin of Environmental Contamination and Toxicology, 62 (1), 48–54. https://doi.org/10.1007/s001289900841

[40] Marchal, N., & Bourdon, J. L. (1982). Les milieux de cultures pour l'isolement et l'identification biochimique des bactéries. Doin Éditeurs.

[41] Mazella, N., Tran-Thi Nhu, T., Delest, B., & Delmas, F. (2009). Développement et validation d'une méthode permettant le dosage du glyphosate et de l'AMPA dans les eaux de surface par HPLC-ESI-MS/MS [Conference presentation]. XXIXe Congrès du Groupe Français des Pesticides, Toulouse, France.

[42] Mesnage, R., & Antoniou, M. N. (2023). Glyphosate, oxidative stress and neurotoxicity: A mechanistic review of human and animal studies. Chemico-Biological Interactions, 369, 110313. https://doi.org/10.1016/j.cbi.2023.110313

[43] Moneke, A. N., Okpala, G. N., & Anyanwu, C. U. (2010). Biodegradation of glyphosate herbicide in vitro using bacterial isolates from four rice fields. African Journal of Biotechnology, 9 (26), 4067–4074.

[44] Moorman, T. B. (1994). Pesticide degradation by soil microorganisms: Environmental, ecological and management effects. Advances in Soil Science, 33, 121–165.

[45] Müller, B. (2021). Glyphosate: une histoire d'amour. Mondes toxiques, monde commun. Éditions de la Sorbonne.

[46] Obojska, A., Lejczak, B., & Kafarski, P. (2002). Isolation and characterization of a glyphosate-degrading *Pseudomonas sp.* Applied Microbiology and Biotechnology, 58 (6), 771– 776. https://doi.org/10.1007/s00253-002-0945-6

[47] Pike, R., & Amrhein, N. (1988). Isolation and characterization of a mutant of *Arthrobacter sp.* strain GLP-1 which utilizes the herbicide glyphosate as its sole source of phosphorus and nitrogen. Applied and Environmental Microbiology, 54 (11), 2868–2870. https://doi.org/10.1128/aem.54.11.2868-2870.1988

[48] Pipke, R., Amrhein, N., Jacob, G. S., Kishore, G. M., & Schaefer, J. (1987). Metabolism of glyphosate in an *Arthrobacter sp.* GLP-1. European Journal of Biochemistry, 165 (2), 267–273. https://doi.org/10.1111/j.1432-1033.1987.tb11434.x

[49] Quinn, J. P., Peden, J. M. M., & Dick, R. E. (1988). Glyphosate tolerance and utilization by the microflora of soils treated with the herbicide. Applied Microbiology and Biotechnology, 29 (5), 511–516. https://doi.org/10.1007/BF00269074

[50] Quinn, J. P., Peden, J. M. M., & Dick, R. E. (1989). Carbon–phosphorus bond cleavage by gram-positive and gram-negative soil bacteria. Applied Microbiology and Biotechnology, 31 (3), 283–287. https://doi.org/10.1007/BF00258411

[51] Rapilly, F. (1968). Les techniques de mycologie en pathologie végétale. Annales des Épiphyties, 19, 1–102.

[52] Roslychy, E. B. (1982). Glyphosate and the response of the soil microbiota. Soil Biology and Biochemistry, 14 (2), 87–92. https://doi.org/10.1016/0038-0717(82)90079-3

[53] Rueppel, M. L., Brightwell, B. B., Schaefer, J., & Marvel, J. T. (1977). Metabolism and degradation of glyphosate in soil and water. Journal of Agricultural and Food Chemistry, 25 (3), 517–528. https://doi.org/10.1021/jf60211a018

[54] Santos, A., & Flores, M. (1995). Effects of glyphosate on nitrogen fixation of free-living heterotrophic bacteria. Letters in Applied Microbiology, 20 (6), 349–352. https://doi.org/10.1111/j.1472-765X.1995.tb01318.x

[55] Sebihi, A., & Merabti, D. (2019). Étude de la biodégradation du glyphosate par des bactéries isolées des eaux de Sebkhet Sefioune – Ouargla (Sud-Est de l'Algérie) [Unpublished master's thesis]. Université Kasdi Merbah, Ouargla, Algérie.

[56] Shushkova, T. V., Ermakova, I. T., Sviridov, A. V., & Leontievsky, A. A. (2012). Biodegradation of glyphosate by soil bacteria: Optimization of cultivation and the method for active biomass storage. Microbiology, 81 (1), 44–50. https://doi.org/10.1134/S0026261712010146

[57] Simonart, P. (1957). Symposium sur les méthodes d'étude microbiologique du sol. Institut National pour l'Étude Agronomique du Congo Belge.

[58] Singh, B. K., Walker, A., Morgan, J. A. W., & Wright, D. J. (2003). Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Applied and Environmental Microbiology, 69 (9), 5198–5206. https://doi.org/10.1128/AEM.69.9. 5198-5206.2003

[59] Soulas, G. (1985). La dégradation des pesticides dans le sol: Aspects microbiens et cinétiques. Science du Sol, 23 (3), 203–214.

[60] Soulas, G. (1993). Evidence for the existence of different physiological groups in the microbial community responsible for 2,4-D mineralization in soil. Soil Biology and Biochemistry, 25 (4), 443–449. https://doi.org/10.1016/0038-0717(93)90070-N

[61] Stenrod, M., Charnay, M. P., Benoit, P., Eklo, O. M., & Barriuso, E. (2003). Degradation of glyphosate in sandy soils as affected by temperature and soil characteristics [Technical report]. INRA.

[62] Stratton, G. W., & Stewart, K. E. (1992). Glyphosate effects on microbial biomass in a coniferous forest soil. Environmental Toxicology and Water Quality, 7 (3), 223–236. https://doi.org/10.1002/tox.2530070303

[63] Tang, M., & You, M. (2011). Isolation, identification and characterization of a novel triazophos-degrading *Bacillus sp.* (TAP-1). Microbiological Research, 167 (5), 299–305. https://doi.org/10.1016/j.micres.2011.10.004

[64] Tolbot, H. W., Johnson, L. M., & Munnecke, D. M. (1984). Glyphosate utilization by *Pseudomonas sp.* and *Alcaligenes sp.* isolated from environmental sources. Current Microbiology, 10 (5), 255–259. https://doi.org/10.1007/BF01577138

[65] Topp, E., Zhu, H., Nour, S. M., Houot, S., Lewis, M., & Cuppels, D. (2000). Characterization of an atrazine-degrading *Pseudaminobacter sp.* isolated from Canadian and French soil. Applied and Environmental Microbiology, 66 (7), 2773–2782. https://doi.org/10.1128/AEM.66.7.2773-2782.2000

[66] Tortora, G. J., Funke, B. R., & Case, C. L. (2003). Introduction à la microbiologie (pp. 157–355). Renouveau Pédagogique.

[67] Trotter, D. M., Wong, M. P., & Kent, R. A. (1990). Canadian water quality guidelines for glyphosate. Environment Canada, Inland Waters Directorate, Water Quality Branch.

[68] Tsuioshi, Y., Kremer, R., & Paulo, R. (2009). Glyphosate interactions with physiology, nutrition, and diseases of plants: Threat to agricultural sustainability. European Journal of Agronomy, 31 (3), 111–113. https://doi.org/10.1016/j.eja.2009.07.001

[69] Veiga, F., Zapata, J. M., Marcos, M. L. F., & Alvarez, E. (2001). Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain. Science of the Total Environment, 271 (1–3), 135–144. https://doi.org/10.1016/S0048-9697(00)00838-6

[70] Wacket, L. P., Shames, S. L., Venditti, C. P., & Walsh, C. T. (1987). Bacterial carbon– phosphorus lyase: Products, rates, and regulation of phosphonic and phosphinic acid metabolism. Journal of Bacteriology, 169 (2), 710–717. https://doi.org/10.1128/jb.169. 2.710-717.1987

[71] Walker, A., Welch, S. J., & Roberts, S. J. (1992). Soil temperature as a factor influencing pesticide degradation rates in soil. Pesticide Science, 35 (4), 305–311.

[72] Wang, J., Zhang, C., Zhang, S., & Yang, X. (2021). Biodegradation of glyphosate by microorganisms: A review. Frontiers in Microbiology, 12, 667970. https://doi.org/10.3389/fmicb.2021.667970

[73] Wang, Y., Zhang, H., & Feng, H. (2023). Biodegradation of glyphosate by a newly isolated *Rhizobium sp.* GX19 in aerobic conditions and its metabolic pathway. Environmental Technology & Innovation, 29, 103127. https://doi.org/10.1016/j.eti.2022.103127

[74] Wardle, D. A., & Parkinson, D. (1992). The influence of the herbicide glyphosate on interspecific interactions between four fungal species. Mycological Research, 96 (3), 180–186. https://doi.org/10.1016/S0953-7562(09)80928-3

[75] Wirenlehr, S., Komoba, D., & Gläbgen, W. E. (1997). Mineralization of [14C] glyphosate and its plant-associated residues in arable soils originating from different farming systems. Pesticide Science, 51 (4), 436–442.

[76] Worthing, C. R., & Hance, R. J. (2000). The pesticide manual (12th ed.). British Crop Protection Council.

[77] Yesguer, R. (2015). L'usage des pesticides en agriculture en Algérie: État des lieux et perspectives. Revue des Bio-ressources, 5 (2), 35–45.

[78] Yu, X. M., Yu, T., Yin, G. H., Dong, Q., An, M., Wang, H., & Ai, C. (2015). Glyphosate biodegradation and potential soil bioremediation by *Bacillus subtilis* strain Bs-15. Genetics and Molecular Research, 14 (4), 14717–14730. https://doi.org/10.4238/2015.November. 18.22

[79] Zhang, L., Wang, W., Xu, J., & Liu, M. (2024). Comparative effects of glyphosate and AMPA on microbial communities and soil enzyme activities in paddy fields. Journal of Hazardous Materials, 452, 131304. https://doi.org/10.1016/j.jhazmat.2023.131304

[80] Zhang, W., Wang, H., Zhang, T., Li, J., & Wang, X. (2024). Microbial activity and community composition associated with glyphosate degradation in soil. Environmental Pollution, 334, 122120. https://doi.org/10.1016/j.envpol.2023.122120