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ADVANCES IN PLANT-MICROBE MEDIATED REMEDIATION OF GLYPHOSATE

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Abstract

Glyphosate (GLA) has long been the primary herbicide used to control both annual and perennial weeds. However, its extensive application has resulted in widespread persistence in agroecosystems, posing risks to both biotic and abiotic components of the environment. To address these impacts, various remediation strategies—such as adsorption, photocatalytic degradation, and microbial degradation—have been investigated. Among these, microbial degradation, particularly rhizodegradation, stands out for its efficiency, versatility, and eco-friendly nature. Rhizodegradation relies on diverse microorganisms that metabolize GLA, using its carbon, nitrogen, and phosphorus as nutrient sources. This review consolidates current understanding of microbial GLA degradation, emphasizing the role of GLA-degrading microorganisms in remediating contaminated environments. It explores the mechanisms by which microbes break down GLA and the interactions that facilitate its detoxification. Additionally, the review assesses the practicality and effectiveness of employing GLA-degrading microbes in bioremediation across various ecological contexts. By detailing microbial degradation pathways and their environmental significance, this article provides a solid foundation for developing sustainable strategies to manage GLA contamination and mitigate its ecological consequences.

INTRODUCTION

Glyphosate: a potent herbicide

Glyphosate (GLA) is a broad-spectrum post-emergent herbicide used in agriculture (Schütte *et al.*, 2017). Due to its low production cost, it is widely used for weed eradication worldwide. As weeds severely limit the production of crops by up to 29% and reduce their yield by 47%. Historically, mechanical weeding has been the main form of weeding, and it can effectively control weeds and provide favorable conditions for crop growth. However, mechanical weeding increases the risk of soil erosion, reduces soil organic matter, and affects the physical, chemical, and biological properties of soil. In addition, repeated mechanical weeding practices increases labor and energy costs, thereby reducing farmer's income (Mitchell *et al.*, 2016). That is why, the use of herbicides increased to overcome these problems without compromising farmers' economic benefits.

It is one of the most used herbicides in agriculture since the 1990s, and its use has increased rapidly (Benbrook, 2016). It is estimated that between 1994 - 2014, the use of GLA has increased by nearly 15 times globally (Benbrook, 2016), indicating that the application of GLA active ingredients in the global farmland is 0.53 kg/ha including United States which is 1.0 kg/ha. Between 1996 to 2011, use of herbicides in the developed countries like United

States increased by 239 million kg, of which GLA was the main component (Benbrook, 2016). In Australia, herbicides used increased by 30% between 2002 and 2012, having cost US$ 700 million to US$ 1.1 billion, respectively (Yang *et al.*, 2018). It is estimated that GLA being a part of approximately 50% of all pesticides used in the United States and 40% globally (USEPA, 2011).

Among Asia-pacific countries, China and India are the main users of GLA but there is shifting going on in European Union due to regulatory issues. In other continents i.e., Africa, South Africa are the main user of GLA. According to the African Biodiversity Center (ABC), 12 to 20 million liters increased has been seen in the use of GLA from 2008 to 2012 as the imports of GLA increased by 177%. As the use of GLA results in high yield and give economic benefits to farmers, its use is increased with the passage of time. But cultivation of genetically modified

Initial production and its applicability

Glyphosate was first synthesized in 1950 (Franz *et al.*, 1997). Commercially, it was approved in 1975 for commercial use as herbicide for maize and many other crops grown on farms, orchards, and vineyards (Richmond, 2018). In early 1970s, Monsanto improved the quality in the present formulation of GLA herbicide. It was registered and used for the first time in the United States in 1974 (Gill *et al.*, 2017), and was accepted widely as its usage was increased from 10,000 to 80,000 tons/year in 1992 and 2007, respectively and still expanding (Coupe *et al.*, 2012). In 2017 1.35 million tons GLA was used for different field purposes (Van Bruggen *et al.*, 2018).

Due to its adsorption behavior with soil minerals and clays, it is immobile in the soil to leach down in ground water. But its biologically converted product (aminomethyl phosphoric acid (AMPA) is unstable (Rose *et al.*, 2016). During biodegradation, GLA-oxidoreductase degrades the C-N bond to produce AMPA and glyoxylic acid. During this cycle, intermediates of sarcosine and glycine are formed by C-P lyase and sarcosine oxidase enzymes (Hove-Jensen *et al.*, 2014). Glyphosate application significantly affects microbial biomass and activity, soil nutrient status and availability by interfering with microbial activity and seed germination (Nguyen *et al.*, 2016). It affects the health of humans and other organisms by entering the food chain, and its negative impact ranges from stimulation to cancer risk (Van Bruggen *et al.*, 2018) (Table 1).

Glyphosate contamination

Glyphosate is the most used herbicide and considered as non-toxic. But its excessive use in farmland pollutes the

Table 1: Chemical and physical c

General Name	IUPAC name	MW (g/mol)	Chemical formula	Solubility in water (g/L)	Log P (at 25 °C)	Density (g/cm^3)
Glyphosate	2-(phosphonomethylamino) Acetic acid; propan-2-amine	228.185	C6H17N2O5P	12	− 5.4	1.7
Aminomethylphosph onic Acid	-	111.04	CH6NO3P	50	0.4	1.6
Sarcosine	*N*-methylglycine	89.093	C3H7NO2	89.09	− 2.8	1.093
Glyoxylate	Glyoxylic acid	74.035	C2H2O3	224	− 0.07	1.384

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Formylphosphonate	Formylphosphonic Acid	110.005	CH_3O_4P	24.8	− 1.8	1.79
Methylamine	Méthanamine	31.057	CH_5N	100	− 0.57	0.693
Glycine	2-Aminoacetic acid	75.066	$C_2H_5NO_2$	249.9	− 3.2	1.61

haracteristics of glyphosate and its metabolites
(Singh *et al.*, 2020)

crops in combination with GLA is not beneficial as approximately 85% of genetically modified maize and soybean varieties are resistant to GLA.soil and water. GLA residues can be found in soils which are frequently supplied with GLA. In addition to the soil contamination, water resources and food is also

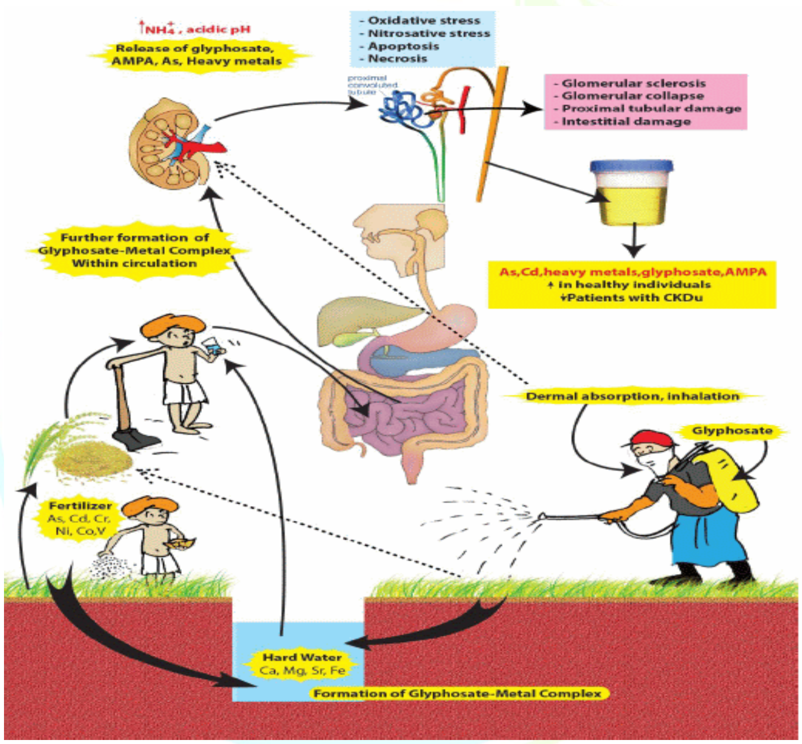

Figure 1: Effects of glyphosate application on human health. (Jayasumana *et al.*, 2014)

contaminated and can cause severe toxicological effects (Figure 1). The adverse effects on single-celled organisms to multicellular organisms have been observed from many experiments. For example, GLA reduces the rate of photosynthesis of Euglena, limits the radial growth of mycorrhizal fungal species and proliferation of certain bacteria in the rhizosphere microbial community. In multicellular organisms, GLA can pose serious threats such as genotoxicity, cytotoxicity, nuclear aberrations, hormone destruction, chromosomal aberrations to DNA damage (Tarazona *et al.*, 2017; Gill *et al.*, 2018).

The rhizospheric soils of maize and soybean are main source of bacteria, proteobacteria, acidobacteria, and actinomycetes in varying amounts. But after the application of GLA, the relative abundance of these organisms recorded to be decreased (Newman *et al.*, 2016). The floating aquatic plant *Ludwigia peploides* is widely distributed in American rivers. Functional analysis of its differentially expressed genes revealed the destruction of several key biological processes, such as energy metabolism and Ca^{2+} homeostasis, cell signaling and endoplasmic reticulum stress response due to the mixing of agricultural runoff water from GLA applied fields (Pérez *et al.*, 2017). Glyphosate concentrations in freshwater mussels (*Mytilus galloprovincialis*) were also recorded higher (10 - 1000 μg/L) after their exposure to GLA for 7, 14, and 21 days. Several biomarkers (antioxidant enzyme and acetylcholinesterase activity) were also affected by higher GLA concentration, highlighting the potential risks of GLA to aquatic invertebrates (Matozzo *et al.*, 2018). The US Environmental Protection Agency (US-EPA) has classified the GLA as "actually non-toxic and non-irritant." However, the Food and Agriculture Organization (FAO) reported that glyphosate and its main metabolite, AMPA have potential toxicological significance due to residues remaining in the food chain. FAO further pointed out that if the daily consumption does not exceed 1 mg/kg body mass, the dietary risks of GLA and AMPA are non-toxic (Bai and Ogbourne, 2016; Nicolopoulou-Stamati *et al.*, 2016).

Remediation of glyphosate: possible approaches and mechanisms

Bioremediation

Bioremediation is the use of living entities for the remediation of pollutants. It could be done using microbes or plants. But, before their use as bioremediation agents, they should have the ability to decompose pollutants rapidly without producing or affecting the environment. (Ghosal *et al.*, 2016) Bioremediation standards include that no hazardous by-products must be formed, inhibitory compounds must be inattentive, pollutants must be bioavailable, environments must be augmented and also maintained microbial biomass and their activities. The diverse technologies used for bioremediation be contingent on three elementary principles, namely the adaptability of pollutants to biotransformation, the availability of pollutants to microorganisms, and the chance to optimize biological activity.

In situ bioremediation

In-situ bioremediation attempts to remediate the polluted environment at the polluted spot and avoid the transportation of pollutants to other places. It a cheap and ideal choice, as the interference caused at the application site is minimal. Chemotaxis is significant for in-situ bioremediation studies as microbes with chemotactic capabilities can migrate into areas comprising toxins. Ballarini *et al.*, (2014) found that improving the chemotactic behaviour of bacteria can enhance the effectiveness of in- situ bioremediation. If pesticides are used, they can be processed in-situ using numerous media. One of the finest examples is the use of molds as degrading agent for pesticides. In research laboratory analysis, white rot fungi have been found to be able to degrade pesticides 45-75% more effectively than control samples (Magan *et al.*, 2010).

Bioventing

Bioventing is a practice, in which oxygen and micro-nutrients are injected into the polluted sites to sustain and accelerate bioremediation (Shanahan, 2004). Nitrogen and phosphorus are two common nutrients which are added for this purpose. But it is difficult to operate this method to every soil type. Different soil factors disturb this technique like fine textured soil (such as clay) has less absorptivity, which precludes the diffusion of oxygen and micro-nutrients from the organisms through the soil and it becomes challenging to regulate the moisture level of fine-textured soil. Moreover, fine-textured soil is easily lost from water- saturated soil conditions, preventing oxygen from reaching soil microorganisms throughout the contaminated area (Ahmadpour *et al.*, 2012). To overcome this problem, biological ventilation is used for proper supply of oxygen to clayey soils which drives air across the well into the soil (Behera, 2014; Jónsson and Davíðsdóttir, 2016).

Biosparging

Biosparging involves injection of air below the groundwater level under pressure to increase the oxygen concentration in the groundwater to increase biodegradation rate of pollutants through native bacteria. The convenience and difficult to installing tiny diameter air injection spots make the system design and construction quite flexible. Before installation of the system, the degree and type of pollution along with soil attributes must be assessed to evaluate the suitability. These methods are not appropriate for composites that are too volatile. Bio-injection promotes mutually aerobic biodegradation. This technique is usually utilized for the restoration of hydrocarbon and gasoline polluted areas (Marchand *et al.*, 2010).

Bioaugmentation

When microorganisms are introduced into polluted locations to augment degradation, the procedure is called bioaugmentation. But the disadvantages associated to this technique are, a) the familiarized microbes rarely contest with native microbes, making it impossible to develop and maintain useful population levels: b) most soils that have been exposed to biodegradable waste for a long time contain effectively degradable microorganism. Bioaugmentation is usually combined with bio-stimulation, in which sufficient water, nutrients, and oxygen are also introduced into the contaminated site to enhance the activity of the introduced microbial degraders or to promote metabolism. The concept of bio-stimulation is to increase the degradation potential of contaminated substrates by adding micro-nutrients or reducing bioremediation limiting factors (Khan *et al.*, 2013; Xu *et al.*, 2014; Gkorezis *et al.*, 2016).

Ex-situ bioremediation Landfarming

Landfarming includes digging of contaminated soil and sieving it via mechanical separation. The contaminated soil is then placed on clean soil in layers, and natural processes degrade the contaminants. Sometimes, concrete or clay films are used to conceal the contaminated soil layer. Oxygen can also be added to accelerate the process. Crushed limestone or agricultural lime can also be used to adjust the pH of the soil (maintain it near 7.0). Tillage is practiced in pesticides contaminated soils for landfarming purposes (Chattopadhyay and Chattopadhyay, 2015). Since the 1950s, huge quantities of pesticides used in all around the world to control pest. Based on the soil analysis and environmental surveys, FAO has developed site-specific remediation plans. Remediation is usually established on the cost of nutrients supply which are supplied to microbes for degradation of contaminants (Morillo and Villaverde, 2017).

Biopiling

Biopiling method is comprised of a treatment bed, polluted soil pile, aeration facility, water and nutrient supply and a leachate collection system. Heat, humidity, micro-nutrients, pH, and oxygen are the factors that control this process (Kuppusamy *et al.*, 2016).

Composting

In composting process, microorganisms are used for the degradation of organic wastes and pollutants at a high temperature of 55 - 65°C. During the composting, heat is produced which leads to an increase in the degradation ability of the microbes and their metabolic activity. There are more microbial populations in compost than in soil. General steps in composting are, excavation and screening of contaminated soil to eliminate large stones and garbage, its transportation to the composting facility and addition of amendments (alfalfa, wheat straw, fertilizer, farming waste and wood chips) as supplementary carbon source. Then the contaminated soil and the amendments are covered into piles. During the composting process, humidity, temperature, pH and explosive intensity are continuously monitored (Shao *et al.*, 2009).

Bioreactors

Bioreactors use the contaminated soil which is interspersed with water and micro-nutrients, and the blended material is stirred through a systematic bioreactor to invigorate the act of microbes. This approach is more suitable for clayey soils and is usually a fast procedure. Numerous types of bioreactors can be used, such as bio-slurry reactors, fermentation tanks, fictitious bed reactors and many closed structures, which should be used if the prospective hazards of discharge are exceptionally serious. In bioreactor, GLA degradation was most effective in the medium with pH 7.0 and aeration rate was 10-60% of air saturation supplemented with glutamate and ammonium chloride as resource of nitrogen and carbon, respectively. Due to the adaption of microbial cells and induction of the relevant enzymatic system, the microbial culture grown in the presence of GLA exhibited 1.5-2-fold higher efficiency of GLA degradation (Shushkova *et* *al.*, 2012; Roy *et al.*, 2014; Agnello *et al.*, 2016; Morillo and Villaverde, 2017)

Electro dialysis

Electrodialysis is a method that uses flux and a unique semi- permeable electric charge-based membrane. The cation- permeable membrane and the anion-permeable membrane have flow channels among them, and conductors are alternately situated on each side of the membrane. The electrode attracts counter ions via the membrane to remove it from the wastewater. Recently, this method has been used to remove pesticides from sewage (Mikhaylin and Bazinet, 2016).

Phytoremediation

Plant-aided bioremediation has been used for more than 300 years, and has become an eco-friendly approach for remediating various soil contaminants. It is a cheap, and efficient technique that can be utilized in the field. Most importantly, its monitoring is very easy process. Valuable products may also be recycled and reused. In fact, it is the usage of the distinctive and discriminating functions of plant roots, as well as uptake, conversion, volatilization, and rhizospheric degradation, which are crucial practices used in the procedure of phytoremediation. In this process, the plant provides a favourable micro-environment around its roots, which is conducive to the degradation of pollutants. Not only rhizobacteria but also endophytic microbes are implicated in the degradation of toxic pollutants in the soil environment (Liu *et al.*, 2017).

Endogenous microbes are generally non-pathogenic and naturally occur in the inner tissues of plants. They can stimulate the growth of plants by generating a variety of natural products and promote the biodegradation of soil contaminants. The use of bacterial endophytes to reduce the level of toxic herbicide residues in crops. (Jha *et al.*, 2015; John and Shaike, 2015) revealed that the inoculated pea plants (*Pisum sativum*) with poplar endophytes have ability to degrade 2,4-dichlorophenoxy acetic acid (2,4-D).

There has been a lot of research on the use of phytoremediation to remediate pesticides from the natural environment. Plant roots soak up pesticides on the surface, and dead roots add up organic matter to the soil, which can improve the adsorption of pesticides on organic matter in soil that may undergo bacterial transformation (Morillo and Villaverde, 2017). According to preliminary research on Kochia sp. of plant degradation of trifluralin, metolachlor and atrazine increased in the contaminated soil. Deep-rooted of poplar has also successfully reclaimed atrazine contaminated soil and groundwater. Majsztrik *et al.* (2017) studied the phytoremediation potential of the aquatic plant *Lemna minor*, which can eliminate GLA and isoproturon from the contaminated water. Using biotechnological tools can make phytoremediation more efficient. Bacteria (endophytic bacteria or rhizosphere) can be engineered via gene allocation to degrade hazardous pollutants existing in the environment. Though, genetic engineering of endogenous, rhizobacteria and genetically modified plants looks a favourable method for remediating polluted sites. The phytoremediation of pesticides has been thoroughly researched using the traditional plants. Transgenic plants manufactured to metabolize pesticides and prolonged contaminants can be utilized for plants to phytoremediate in soil and water (Mathew *et al.*, 2017).

Rhizoremediation

Rhizospheric microorganisms are found near roots and involved in plant growth promotion are known as plant growth promoting rhizobacteria (PGPR). These microorganisms play an important role in recycling plant nutrients, maintaining soil structure, detoxifying toxic chemicals and controlling plant pests. On the other side, root secretions give nutrients for rhizosphere microorganisms, thereby boosting the action of microorganisms in the rhizosphere, thereby stimulating the growth of plants and reducing the toxicity of metal in plants. PGPR and arbuscular mycorrhizal fungi (AMF) that promote plant growth has received much attention worldwide. The presence of contaminants in the soil tends to naturally choose organisms (such as bacteria, yeast, and fungi) that like the compound as a resource of food and vitality (Ma *et al.*, 2011).

Rhizoremediation uses the root exudates and naturally occurring rhizospheric microorganisms or certain microorganisms separated by enrichment methods for degradation of pollutants. This technique has great remediation potential. The reason behind it is the stimulation of microbes through root exudates. The success of rhizosphere restoration depends on many factors such as climate, soil conditions, suitable plant species, and related rhizosphere microorganisms (Figure 2). In some cases, radical application can have a direct impact on the concentration of pollutants during implanting. In additional cases, it may take numerous seasons for plants to interact deeply with contaminated areas. In addition, this may differ on whether the plant itself is unambiguously or ramblingly intricate in the remediation of pollutants (Fatima, 2019).

Root exudates such as sugar, alcohol, and organic acids are used by the microbes to enhance microbial growth and pollutant remediation activity. Some of these compounds can also serve as chemotactic signals for microorganisms. The plant roots also loosen the soil and transport water to

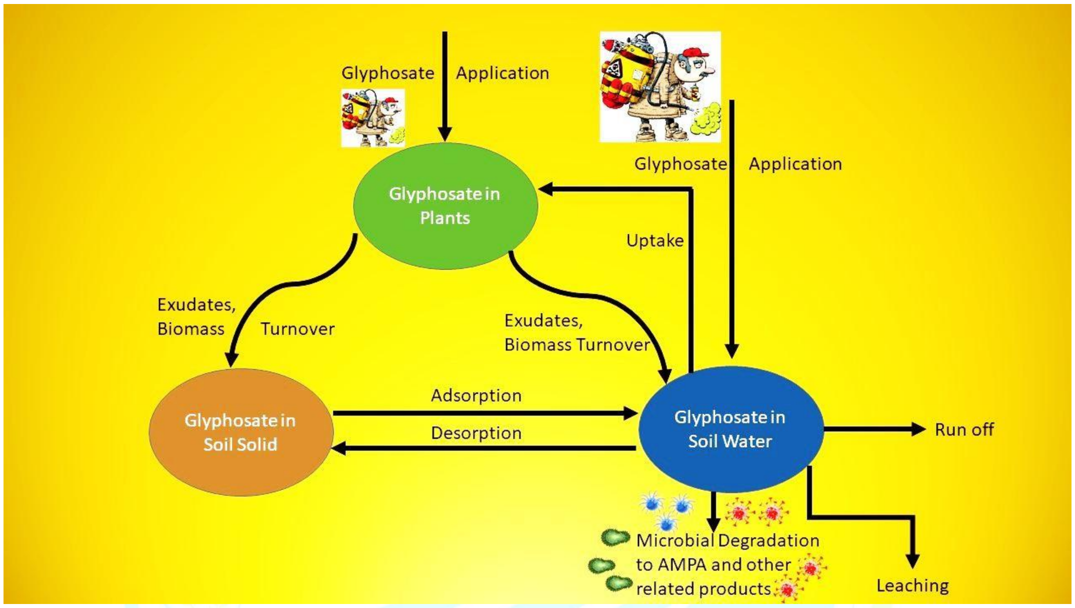

Figure 2: Potential events, takes place after glyphosate application

the rhizosphere, which further enhances the activity of microorganisms.

The degradation, metabolism, or mineralization rate of pollutants in the soil varies on the biological activity of PGPR and AMF in the soil, which is mainly derivative from the enzyme and proteins of soil bacteria. However, the decomposition of contaminants is generally restricted by the accessibility of electron acceptors or donors, co-metabolites, plant vitamins, in-organic nutrients, hormones, pH and

Remediation of glyphosate using rhizospheric bacteria

Glyphosate tolerant PGPR (*Pseudomonas aeruginosa* and *Bacillus cereus*) used in the remediation of GLA contaminated soil. Soil samples were spiked with GLA at

3.1 mg/ml, 7.2 mg/ml and 14.4 mg/ml, and then inoculated with *Pseudomonas aeruginosa* and *Bacillus cereus* Readings were noted by using the gas chromatography-mass

Table 2: Microbial species involved in degradation water

Microbial species	Intermediates	Geographical location	Source	References
Achromobacter sp.	Sacrosine	Russia	Soil	(Vandermaesen *et*

MPS 12 A				*al*., 2016)
Achromobacter sp. 16 kg	-	Russia	Soil	(Ermakova *et al*., 2017)
Aspergillus niger	Nigeria	AMPA and sarcosine	Soil	(Singh *et al*., 2019)
Aspergillus oryzae A-F02	China	AMPA and methylamine	Soil	(Fu *et al*., 2017)
A. section Flavi and A. niger	Argentina	-	-	(Carranza *et al*., 2017)
Bacillus subtilis	India	AMPA and methylamine	Soil	(Singh *et al*., 2019)
Bacillus cereus CB4	China	AMPA, glyoxylate, sarcosine, glycine and formaldehyde	Soil	(Fan *et al*., 2012)
Comamonas odontotermitis P2	Pakistan		Soil	(FIRDOUS *et al*., 2017)
Fusarium oxysporum	Nigeria	AMPA and sarcosine	Soil	(Singh *et al*., 2019)
Geobacillus caldoxylosilyticus T20	UK	AMPA	-	(Obojska *et al*., 2002)
Ochrobactrum anthropi GDOS	Iran	AMPA	Soil	(Hadi *et al*., 2013)
Ochrobactrum anthropi GPK 3	Russia	-	Soil	(Ermakova *et al*., 2017)
Pseudomonas pseudomallei	USA	AMPA	Soil	(Peñaloza-Vazquez *et al*., 1995)
Rhizobium leguminosarum	India	AMPA and methylamine	Soil	(Singh *et al*., 2019)
Rhizobium meliloti 1021	Massachusetts, USA	Sarcosine	Mutation of the wild strain	(Kremer and Means, 2009)
Streptomyces sp.	India	AMPA and methylamine	Soil	(Singh *et al*., 2019)
Penicillium notatum	Poland	AMPA	Mutation of the wild type	(Bujacz *et al*., 1995)
Ochrobactrum anthropi GPK 3 *Achromobacter* sp. 16 kg	Russia	-	Soil	(Ermakova *et al*., 2017)
Trichoderma viridae	Nigeria	AMPA and sarcosine	Soil	(Sidhu *et al*., 2019)
Trichoderma viride Strain FRP 3	Indonesia		Soil	(Arfarita *et al*., 2016)

spectrometry before and after inoculation of the GLA bacteria. The spectrum of the contamination level after extraction was taken through acetonitrile (GC-MS). The bacterium showed significant ability to degrade GLA at 3.1 mg/ml GLA concentration, control, *Pseudomonas aeruginosa, Bacillus cereus* and consortia showed a degradation percentage of recorded 49%, 76.11, 85.8, and 75.8%, respectively, while at a concentration of 7.2 mg/ml. Below, the mixed culture of *Pseudomonas aeruginosa, and Bacillus cereus*, isolate 1, isolate 2 and control degradation percentage were 84.9, 72.7, 66.4% and 39.2%, respectively. The isolates also showed a significant degradation rate (separately and consortia) at a concentration of 14.4 mg/ml. GC-MS results showed that the degradation products changed significantly compared with the control. This study shows that large amounts of GLA are degraded by *Pseudomonas aeruginosa* and *Bacillus cereus*. Therefore, they may have great potential in the bioremediation of GLA-contaminated soil (Ezaka *et al.*, 2018) (Table 2).

Concluding remarks and future prospects

Undue extensive use of GLA also plays an important role to achieve highest crop yield and swift agricultural development. Though, due to its exhaustive use, GLA contamination has appeared as an imperative issue. The chronic negative effects of GLA on the environment should entice considerable interest to remove GLA residues from the contaminated environments. In Recent times, numerous methods such as phytoremediation, in-situ and ex-situ techniques could be used to efficiently degrade GLA. Consequently, GLA degrading microbes having effective degradation and bioremediation capabilities in GLA contaminated environments are deemed as the most encouraging approach. Different strains of microbes have been considered to degrade GLA by utilizing GLA as sole phosphorus (P), carbon (C) or nitrogen (N) source. Though, genetic and biochemical characteristics of highly effective degrading enzymes have yet not been appropriately investigated. Most of the GLA degrading microbes in which complete degradation path has been clearly understood should be utilized. The most ubiquitous GLA degradation pathway in microbial strains is the cleavage of C-N bond and change into AMPA which is either further decomposed or excreted to the environment. Consequently, before the substantial use of GLA degrading microbes for bioremediation, comprehensive foundation work should be achieved.

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