

# Fate of herbicide residues in soil- Australian context: insights towards mechanism, aspects, and recent advancements

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## Research Article

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# Abstract

Herbicides are chemical compounds that are toxic to weed plants. Modern agriculture relies heavily on herbicides for the control of weeds to maximize yield in crops. It is estimated that herbicide usage in the Australian grains industry increased by more than 30% from 2002 to 2018, approximately \$1.80 billion. The increased popularity of herbicides in farming systems has not only raised concerns about their negative impacts on the environment, human health, and agricultural sustainability due to the rapid evolution of herbicide resistance, but also raised questions about their fate in soil. Due to excessive use of herbicides, there is great concern about contamination which can lead to soil and water pollution, reduced biodiversity, and depression in soil heterotrophic bacteria (including denitrifying bacteria) and fungi. Moreover, understanding the fate of herbicides in soil is a prerequisite for the precise assessment of its behaviour and potential environmental risk. This review illustrates a brief overview of the present status of herbicide residues in Australian farming systems with a detailed understanding of the transport and degradation processes of herbicides in soil. Furthermore, this review also encompasses microbial degradation, mechanisms, factors, and microorganisms associated with degradation and recent advancement in microbial degradation of herbicides.

## Full Text

### Introduction

Weeds are classified as an important biological constraint to food production and one of the major yield reducing factors (FAO, 2018). Weeds compete with crops for available resources, such as nutrient, light, water, and space, posing a great threat to sustainable crop production. As weed control is critical in respect to increased crop production, significant approaches should be taken to check both the active weed population and the soil seedbank (Graziani et al., 2012). Weed control includes several techniques to destroy or suppress weed populations for minimizing competition in crop field. These techniques attempt to maintain a balance between costs involved for weed control and yield loss. Weed control is generally labour intensive considering the variety of options available to eradicate or destroy alien species from the desired field. Considering all the weed control techniques available, chemical weed control is widely accepted among farming communities due to labour insensitivity (Parish, 1990). Chemicals that are used to control, suppress, or kill plants or to severely interrupt their normal growth processes are called herbicides (Beste, 1983). Herbicides provide quick control and when used appropriately, increase efficiency, reduce horsepower and energy requirements (Zimdahl, 2018). As for example, the introduction of 2,4-D in the sugarcane industry, a single knapsack herbicide sprayer was more effective and efficient than 15 labours weeding with hoe (Smith et al., 2011). Atrazine allowed corn cultivation possible and profitable in some parts of the world where it was not possible before (Heri et al., 2011). In addition, application of atrazine increased four-folds of the land area that farmers could grow and manage in USA (Heri et al., 2011). Undoubtedly, the rapid development and adoption of herbicides significantly contributed to global food production, whereas increasing resistance and non-target toxicity of herbicides has subsequently become a global concern.

## **Herbicides in Australian agriculture**

The Australian farming system has undergone a massive revolution over the past 25 years with the adoption of conservation tillage, including minimal or zero till, which has reduced cultivation practices for weed control (Congreve & Cameron, 2014). Minimal or zero till refers to sowing of seeds with minimum soil disturbance, allowing reduced evaporation and increasing yields. This revolution has led the foundation of modern technologies in crop production, reducing cultivation practices and options available for weed control. However, reduced cultivation practices favours weed population which ultimately increases dependence on chemical weed control (Allmaras et al., 1998). Moreover, Llewellyn et al. (2012) reported that the reduced price of the predominant herbicide, glyphosate, was also responsible for the rapid adoption of zero tillage among 78% farmers in 2008. These factors contributed significantly to the increased adoption of herbicides as a sole medium for weed control (D'Emden et al., 2006). Research revealed that herbicide application saves water over tillage practices, allowing 27 mm of extra water in the soil profile and increasing grain yields by 15-25% (Wylie, 2008). Statistics showed that herbicide usage in Australian farming systems is gradually increasing every year (Figure 1).

At present, Australian farmers spend more than 1 billion dollar per year, which is approximately more than 50% of the annual pesticide expenditure. The increased popularity of herbicides in farming systems has raised concerns about their negative impacts on the environment, human health, and agricultural sustainability due to the rapid evolution of herbicide resistance. In Australia, herbicide resistant weed populations are prevalent compared to major grain producing countries (Llewellyn & Powles, 2001). Adaptation and evolution of herbicide resistance has enabled scientists and growers to radically rethink about existing weed management approach due to the lack of diversity into their weed control programs. Increased use of pre-emergent herbicides could bring diversity in the weed control program. It is estimated that the use of pre-emergent selective herbicides in Australian winter broad acre crops has risen from less than 1 million ha to approximately 7 million ha in a decade (D'Emden et al., 2006). The increased use of herbicide compounds in agricultural fields leads to the accumulation of herbicide residues in the environment which deserves attention and requires appropriate management strategies. Development of an efficient and sustainable remediation technique is essential for safe crop production and environmental cleanliness.

## **History and significance of herbicide residues in Australian soil**

Herbicides are applied to soil to control unwanted vegetation that can interfere with the growth and development of commercial crops. Now, persistence is considered the period of time an herbicide remains active in the soil. In general, herbicide persistence is expected until the end of crop harvest but not beyond that. However, existing soil conditions, herbicide chemical structure as well as application method determine the persistence of herbicides in the soil (Eleftherohorinos, 1987; Webster & Shaw, 1996). Long term persistence of herbicides may lead to soil and ground water contamination (Juhler et al., 2001), affect biodiversity and decrease soil heterotrophic bacteria (including denitrifying bacteria) and fungi (Song et al., 2013). Some herbicides can remain in soil for weeks, months or even years. This is

advantageous in regards of long term weed control. Herbicides applied in minimal or zero till systems tend to leave a greater concentration of herbicide near the surface zone at the end of the cropping season (Curran, 2016). This portion of herbicide that remains in the soil after use is referred to herbicide residues. Residues could occur in higher concentrations than expected which may affect subsequent crops (Yu et al., 2015). Their persistence can affect sensitive crop species by the residual activity of the herbicide in subsequent years, limiting planting options for farmers.

Regular monitoring on pesticide residues in Australian soils has been carried out across the country over the years. As a result, the Australian government banned the use of organochlorine pesticides (OCs) in late 1970 followed by significant evidence suggesting their persistence in soil (Kookana et al., 1998). A study identified residues of chlordane, heptachlor, dieldrin and DDT present in 18.40, 18.80, 39.00 and 39.60% of samples in Western Australian soils (EPAWA 1989). Harris (1987) reported dieldrin persistence in New South Wales with an average concentration of 0.12 mg kg<sup>-1</sup> soil. Soil contamination of DDT has been reported in thousands of cattle dip sites in New South Wales with massive concentrations ranging from 10000 – 100000 mg kg<sup>-1</sup> soil (Barzi et al. 1996). Compared to the OCs, modern herbicides and their persistence in soil did not receive much attention; therefore, herbicide persistence in soil is not fully understood in Australia. To better understand and manage herbicide residues in the soil, Grains Research and Development Corporation (GRDC) co-invested in a five-year project to conduct a National field Survey of herbicide residues. The soil survey of different crop fields from around Australia prior to sowing in 2015 and 2016 detected residues of 23 chemicals, with glyphosate and AMPA being most frequently detected, followed by trifluralin, 2,4- D, diflufenican, atrazine etc (Rose et al., 2022).

From last two decades, the increased use of glyphosate worldwide led to a decent number of research focussing the potential persistence of glyphosate in soil (Benbrook, 2016). Following the world trend, glyphosate (15%) was the most frequently applied herbicides, followed by trifluralin (10%), MCPA (9%), paraquat (7%) and triasulfuron (7%) in Western Australian cropping systems from 2010 to 2014 (Harries et al., 2020). According to (Rose et al., 2022), Glyphosate and its primary metabolite, AMPA were frequently detected over two years (67 and 93% of samples, respectively), with median concentrations of 0.22 mg kg<sup>-1</sup> and 0.31 mg kg<sup>-1</sup>, respectively in 2016. Maximum concentrations of glyphosate detected in topsoils worldwide is comparatively higher than Australian concentrations detected; <1.50 mg kg<sup>-1</sup> in Argentina (Aparicio et al., 2013) and around 2.00 mg kg<sup>-1</sup> in EU (Silva et al., 2018). Contributing to this variety of results from various geographic locations, several analytical procedures have been used to reflect fast and reliable quantification of the contaminant, with variable limits of detection, quantification and accuracy may influence the results (Martins-Gomes et al., 2022). Another possible explanation is majority of glyphosate applied as preemergence application and summer fallow spraying in Australian farming systems (Harries et al., 2020) compared to the greater use of glyphosate-resistant crops in other parts of the world (Martins-Gomes et al., 2022). Moreover, prolonged drought conditions along with strong adsorption, would facilitate persistence of glyphosate and AMPA in Australian agricultural soils due to low microbial activity (Borggaard and Gimsing, 2008).

Trifluralin was also frequently detected (>50% sampling frequency) both in 2015 and 2016 across Australia but the maximum residue concentration ( $5.35 \text{ mg kg}^{-1}$ ) in 2016 was extremely higher than  $0.59 \text{ mg kg}^{-1}$  in 2015 (Rose et al., 2022). Trifluralin is widely used in Australian farming systems to control grassy weeds. It is well known for long persistence in soil having half-life of (35 to 375 days) under field conditions (Lewis et al., 2016); while 5.80 to 26.74 days under laboratory conditions (Chowdhury et al., 2021). Moreover, trifluralin has been reported to have a carryover potential of 9-24% from one season to next in Australian farming conditions (Jolley and Johnstone, 1994); with possibilities up to 90% carryover under prolonged drought conditions (Johnstone et al., 1998). This agrees with (Chowdhury et al., 2021) who concluded that trifluralin dissipation in soil may be addressed as a function of soil temperature and moisture.

Among others, diflufenican was detected frequently but at lower concentrations over the two years survey across Australian crop fields (Rose et al., 2022). It is also a frequently used preemergence herbicide with long half-life of 224 to 621 d in soil (Lewis et al., 2016) like as trifluralin. The higher detection rate of diflufenican reflects to its strong retention in soil reported by (Hvězdová et al., 2018) and frequency of use in Australian farming systems. According to Pelosi et al. (2021), diflufenican was one of the most frequently detected pesticides in France, with a median concentration of  $0.14 \text{ mg kg}^{-1}$  in crop soil. However, no carryover issues were observed with continuous diflufenican application for four years in central and northern Italy possibly due to rapid microbial degradation (Conte et al., 1998). Although other herbicides such as 2,4-D was frequently detected (94% sampling frequency) in 2016 around Australian soils but at lower concentrations mainly due to its common use in summer fallow and winter season (Rose et al., 2022). This is normally non persistent in nature when microbial activity is sufficient (Dehghani et al., 2014). In addition, atrazine residues were also detected in higher concentrations of New South Wales and South Australian field soils compared to other regions (Van Zwieten et al., 2016).

Pesticides are commonly applied in combinations, with approximately 70% of crop fields worldwide (i.e., about  $8.31 \text{ million km}^2$ ) have multiple pesticide residues in the topsoil region (Tang & Maggi, 2021). Rose et al. (2022) found that herbicide residue mixtures were prevalent in arable Australian cropping soils, with an average of 6–7 different herbicide residues per soil sample; in respect to an average of 6.3 herbicide applications per year to each field in Western Australian cropping systems (Harries et al., 2020). Compared to that, Riedo et al. (2021) observed a median of 9 herbicide residues in arable soils under no-till or conventional management in Switzerland. Most crop fields in Europe have been reported to have higher pesticide residues than in Africa, South and Southeast Asia, and Australia (Tang & Maggi, 2021). Moreover, the agro-climatic conditions of Europe, USA and Latin America are not comparable with the Australian context. Herbicide persistence in soil is directly related to the dissipation behaviour of the specific herbicidal compound where chemical, environmental and soil properties play an important role in determining their fate (Chowdhury et al., 2021). Therefore, understanding the fate of herbicides in the soil is a prerequisite for the accurate assessment of their behaviour and potential environmental risk (Gianelli et al., 2014).

## **Fate of herbicides in soil**

Herbicide behaviour in soil is a complex process that varies according to soil type, soil pH, soil moisture, aeration, organic matter, and temperature (Sinha et al., 2012). The presence of herbicides in surface and groundwater is of great importance considering the potential impact on human health and the environment (Papadakis et al., 2015). Herbicides can move vertically to contaminate groundwater via leaching or laterally to intrude into surface water. Residual herbicides may also be toxic to sensitive plant and animal species or enter the food chain due to accumulation in grains and crops (Chowdhury et al., 2020; Singh et al., 2014). Soil has several scavenging techniques comprising chemical, physical and biological processes to minimise the adverse effects of herbicides (Gao et al., 2013; Gu et al., 2012; Jiang et al., 2017; Wang et al., 2014). After application, a major fraction of the applied herbicides may be absorbed by or sorbed to soil particles or decomposed through biotic or abiotic means.

Biotic degradation, or biodegradation, may be defined as degradation of complex herbicide molecules into simple, often more water soluble and less toxic inorganic molecules by enzymatic degradation due to the activity of beneficial microorganisms (Porto et al., 2011; Wood, 2008). Abiotic degradation refers to chemical transformations involved in removal of the herbicides such as photodegradation and hydrolysis. Other processes include volatilization from soil and plants, surface runoff, plant uptake and leaching due to gravitational force.

## **1. Volatilisation**

Volatilisation is the loss of applied pesticides from plant, soil, and water surface in vapour form. Loss due to volatilisation can be as high as 90% of the amount applied for some highly volatile compounds (Taylor & Spencer, 1990), whereas others have been considered as relatively low due to inherent low vapour pressure (Helling, 2005). Many pesticides can be transported far from their initial site of application and may subject to leaching and runoff (Taylor & Spencer, 1990). The consistent presence of atrazine in rainwater, despite of low vapour pressure indicates loss due to volatilisation and vapour drift (Miller et al., 2000). Physiochemical properties of the compound coupled with existing climatic conditions determine the extent of volatilisation (Kookana et al., 1998). Herbicide volatilisation is higher in sandy soils compared to others. Gerritse et al. (1991) calculated the loss of organochlorines through volatilisation in laboratory experiments and found that approximately 90-98% loss occurred from sandy soil within a week; whereas 9-63% loss from a silt loam soil. Volatilisation loss was reported higher in hot and dry weather when compared to cooler weather conditions. Finlayson and Silburn (1996) observed that half of the endosulfan applied to dry soil was lost through volatilisation in dry hot weather. Whereas volatilisation did not occur when the pesticide was applied under cooler conditions. Method of pesticide application also plays a critical role in determining the volatilisation loss. For example, soil-applied pesticides are less susceptible to volatilisation as compared to the foliar-applied ones, and similarly soil incorporated pesticides are less prone to volatilisation losses than the surface-applied ones (Taylor & Spencer, 1990). The extent of volatilisation loss is maximum during application and immediate after application. Trifluralin is highly volatile, however, soil incorporation immediately after application strongly abated trifluralin volatilisation loss (Bedos et al., 2006).

## 2. Leaching

Herbicide movement in soils followed by rainfall or irrigation is often beneficial when root uptake is necessary for weed control. Leaching reduces losses by volatilization and photodegradation, depending on the herbicide chemical properties. Thus, limited leaching may increase soil persistence. Deeper migration reduces the residual herbicide in the upper vadose zone (extends from the top of the ground surface to the water table), and so could lessen persistence in crop production. Such leached chemicals no longer contribute to weed control and may contaminate groundwater or surface water via lateral discharge. Since microbial activity is much lower in the subsurface horizons and in groundwater compared to the vadose zone, herbicide persistence generally is much longer once it moves below the vadose zone. It is believed that low temperature and absence of degrading microorganisms are responsible for the increased persistence of atrazine under vadose zone (Radosevich et al., 1996). The persistence of atrazine in groundwater is quite long (Klint et al., 1993), undoubtedly it is the most detected pesticides in water samples in Australia (Schult, 2012) and USA (Rosecrans & Musgrove, 2020).

Leaching potential has long been predicted based on herbicide and soil characteristics by using various laboratory methods such as adsorption, soil leaching column, and soil thin-layer chromatography tests. Rapid degradation greatly reduces the potential loss by leaching. For example, the herbicide florasulam has very high potential mobility; 68-92% leached through a soil column with the rate of degradation (DT), often expressed as  $DT_{50}$  and  $DT_{90}$  values, were recorded 2-10 and 16-34 days, respectively. It was judged unlikely to contaminate groundwater (Vencill, 2002). Most herbicide leaching occurs during mass flow of water through the soil matrix, ensuring ample exposure of chemical to soil and soil biota surfaces.

## 3. Abiotic degradation

### Photodegradation

Photodegradation is one of the primary abiotic degradation processes that occurs only in the presence of light. Photodegradation can be often termed as photolysis which may be affected by various environmental factors including soil temperature, moisture, soil type, pH and humic substances (Wang et al., 2014). Verhoeven (1996) defined photodegradation as “the photochemical transformation of a molecule into lower molecular weight fragments, usually in an oxidation process”. Photodegradation of herbicides involves breakdown of organic matter through some organic reactions with the absorption of light occurring generally in surface soils, surface water and in the atmosphere (King et al., 2012). Hydroxylation or decarboxylation (direct photodegradation) and indirect photodegradation by the production of reactive radical species are the major types of organic reactions taking place during photolysis (Fantke & Juraske, 2013). Reactive radical species produced by those oxidation process can degrade persistent compounds in soil (Lutze et al., 2015).

Direct photodegradation may be defined as the chemical transformation of a molecule by fragmentation, to and from electron transfer or intramolecular transformation after absorbing radiation (Schwarzenbach et al., 2003). Conversely, photosensitisers absorb radiation and transfer energy to the herbicides from

their excited state during indirect photodegradation, which is followed by several processes as for direct photodegradation.

Several factors have been identified to influence herbicide photodegradation of surface and groundwater including chemical properties, land topography, soil characteristics, weather, and agricultural operations (Konstantinou et al., 2001) listed in Table 1.

**Table 1.** Factors responsible for exposure to solar radiation and photodegradation (King et al., 2012).



<b>Factor</b>	<b>Potential influence</b>	<b>References</b>
Ozone	Possible slight increase with stratospheric ozone thinning. Potential decreases with high tropospheric ozone.	(Smith et al., 2010)
Latitude	Generally negative relationship. High latitudes susceptible during summer months due to ozone thinning.	(Brandt et al., 2010; Moody et al., 2001; Pancotto et al., 2003)
Season	In grasslands, highest rates during summer in grasslands if seasonally dry, but rates may be higher in spring in areas with summer monsoons.  In temperate deciduous forests, highest directly before defoliation in spring or after senescence in autumn.  In tropical deciduous forests, highest during dry season.	(Brandt et al., 2010; Henry et al., 2008; Rutledge et al., 2010)
Elevation	Most likely positive relationship due to higher proportion of short-wave radiation and higher total irradiance at high elevations.  May be negative relationship in areas where cloud, canopy, or snow cover increases with elevation to the point where litter is shaded.	(Blumthaler et al., 1997)
Cloud cover	Most likely negative relationship.  Modest cloud cover can increase diffuse radiation and potentially increase rates on mostly sunny days	(Madronich et al., 1998)
Leaf area index	Generally negative relationship, but especially so with broadleaf architecture.	(Rozema et al., 1999)
Canopy architecture	Higher rates with vertically distributed structure (e.g., grasslands) than horizontally distributed structure (e.g., broadleaf forests).	(Rozema et al., 1999)
Landscape patchiness	Higher rates in open areas versus under shrubs or trees.	(Köchy & Wilson, 1997)
Evenness	Rates per unit mass potentially greater with increased evenness.	(Mlambo & Mwenje, 2010; Throop & Archer, 2007)
Soil reflectivity	Sandy soils may increase albedo and lead to increased rates in adjacent litter.	(Rozema et al., 1999)
Snow	No photodegradation when buried.  Potential increase in photodegradation in standing dead if surrounded by snow due to albedo.	No reference found
Soil cover/burial	Decreased rates with increasing soil burial.	(Barnes et al., 2012; Brandt et al., 2010; Throop & Archer, 2007)

Photodegradation of the soil surface is quite different from the aquatic system because soil contains a minimum fraction (<5%) of organic matter and a major fraction (>95%) of minerals. Organic and mineral content are the most important parameters affecting herbicide degradation on the soil surface. Mineral particles occupy the major share of the soil particles in which mainly crystalline and non-crystalline amorphous minerals dominate with an array of hydroxyl groups (Parlar, 1990). Various soil clay minerals containing iron associated with the production of reactive radicals, for example, the hydroxyl radicals which may influence the photodegradation of herbicides in soil (Katagi, 2004; Mantzos et al., 2017; Sleiman et al., 2017). In photodegradation of herbicides, light penetration is limited to a layer of 0.1 to 0.5 mm of soil (Hebert & Miller, 1990; Miller et al., 1989). The degradation rate of metazachlor and quizalofop-p-ethyl herbicides were quick under sunlight irradiation compared to dark conditions (Mantzos et al., 2017). Frank et al. (2002) reported significant differences in half-lives of chemicals in various soil depths. Katagi (2004) found the evaluated depth for direct and indirect photolysis are 0.23 and 0.28 mm, respectively for lab conditions whereas 0.32 and 0.62 mm in the field conditions for most of the herbicides. Ismail et al. (2015) observed the reduction of deltamethrin half-life was higher in presence of light compared to dark. Zhang et al. (2010) found a positive trend between soil depth and half-life of pyrene increasing from 19.80 to 37.46 d as soil depth was increased from 1 to 4 mm. Temperature may have very little or no effect on the herbicide photodegradation. According to Rering et al. (2016), temperature did not significantly influence the photochemical degradation of imazosulfuron.

## Hydrolysis

Hydrolysis is the chemical breakdown of a molecule with addition of  $H_2O$ ,  $H_3O^+$ , and  $OH^-$  i.e., water molecules. It is one of the major abiotic degradation processes taking place under certain circumstances, such as within groundwater or due to low microbial activity in soil (Wolfe et al., 1990). Hydrolysis rate in soil may be different than in water as soil organic matter content (Stevenson, 1994), clay content (Yaron, 1978), pH (Muller et al., 2007) and temperature (Getzin, 1981) were found to influence hydrolysis of herbicides. Hydrolytic reactions are mainly pH dependant and can be mediated either chemically or biologically (Kookana et al., 1998). Karpuzcu et al. (2013) found the average rate of chlorpyrifos hydrolysis was  $0.02 \mu\text{mol/g/day}$  at pH 7.2 and  $30^\circ\text{C}$ . On the other hand, an increasing rate of hydrolysis has been observed under acidic conditions for azimsulfuron (Boschin et al., 2007), metsulfuron-methyl and most of the sulfonylurea herbicides (Morrica et al., 2001). Hydrolysis of dimethyl disulphide was faster in neutral or mid-alkaline compared to acidic solutions under constant temperature conditions (Han et al., 2017). However, some exceptions exist regarding the dependency of hydrolysis of some pesticides on soil pH. Shabtai and Mishael (2017); Zhang and Pehkonen (1999) reported that rapid hydrolysis of diazinon in both acidic and alkaline conditions followed half-lives of 0.5, 171 and 6 days at a pH concentration of 3.1, 7.3 and 10.4. It is best to study the hydrolysis of herbicides in a pH range which exists in the field soil, aquifers, and environment to know the fate of these chemicals.

Soil organic matter, clay content and type have strong influence on the hydrolysis of herbicides. Liao et al. (2017) reported that abiotic degradation of methyl parathion was significantly related to the natural organic matter and solution pH. Another study reported that higher concentrations of dissolved organic

matter (DOM) resulted in significant reduction on the rate of chlorpyrifos hydrolysis (Adams et al., 2016). The influence of clay mineral content in degradation of herbicides are also investigated by several researchers (Shabeer et al., 2014). Baglieri et al. (2013) stated that the catalytic activity of clay minerals was mainly responsible for the adsorption of triclopyr, whereas the rate of the reaction depends on the type of clay, exchangeable cation, and the state of hydration. Moreover, hydrolysis allows us to understand the possibility of surface and underground water contamination through the indiscriminate use of herbicides. Basically, when these chemicals leached into the deeper layer due to gravitational force where microbial activity is limited then abiotic degradation is the main process that ultimately determine the fate of herbicides. Among the environmental factors, pH and temperature are most important factors that influence hydrolysis of herbicides. Several studies indicated that pH and temperature regulate the rate of hydrolysis for most of the sulfonylurea herbicides, i.e., pyrazosulfuron ethyl (Singh & Singh, 2013; Zheng et al., 2008), halosulfuron methyl (Grey et al., 2018; Zheng et al., 2008), prosulfuron, primisulfuron methyl, rimsulfuron, and thifensulfuron methyl (Dinelli et al., 1997), rimsulfuron, sulfosulfuron, nicosulfuron, prosulfuron, ethametsulfuron-methyl and metsulfuron-methyl (de Lafontaine et al., 2014), sulfosulfuron, nicosulfuron and rimsulfuron (Cessna et al., 2015).

## **Oxidation and reduction**

Variations of oxidation number in a molecule are referred as oxidation and reduction; where increase represents oxidation and decrease represents reduction. Alternatively, loss or gain of electrons in a molecule can be denoted as oxidation and reduction reactions. Pesticides can only be oxidized or reduced in the soil upon presence of a chemical with adequate redox potential.

Oxidative mechanisms in soils are governed by both oxidative enzymes (Dec & Bollag, 2000) and abiotic catalysts such as metal oxides. However, manganese oxides and hydroxides are major contributors due to their reactivity and frequency in soils (Li et al., 2003). MnOOH and MnO<sub>2</sub> are capable to oxidize a variety of organic contaminants such as phenol (Lin et al., 2009), aniline (Laha & Luthy, 1990) or triazine (Shin & Cheney, 2004).

## **4. Biotic degradation**

### **Microbial degradation**

Microorganism is a broad term that includes bacteria, fungi, archaea, protists, and viruses, typically representing only 0.1% of the total volume of soil. However, they are involved in some major remediation processes that recycle the waste and pollutants in the environment (Torstensson, 1988). Microorganisms are present in soil regardless of the textural classes and types but in different densities. For example, bacteria may be present in between 10<sup>2</sup> to 10<sup>6</sup> per gram soil (Delgado-Baquerizo et al., 2018) and fungal hyphae may also exist as some many thousands of metres per gram soil. Thus, the total biomass of microorganisms in soil could be several tonnes per hectare (Torstensson, 1988). Microorganisms were reported to play a vital role in waste decomposition (Schneider et al., 2010), regulation of plant growth (Hayat et al., 2010), nutrient cycling (Van Der Heijden et al., 2008) and degradation of various dangerous

contaminants and pesticides (Pino & Peñuela, 2011; Zhao et al., 2009). Recent studies suggested the need for rapid exploration of novel microorganisms, their diversity, and innovative ecological functions for the development of bioremediation strategies (Graham et al., 2016; Hua et al., 2015; Martiny et al., 2015; Prosser et al., 2007).

The microbial distribution in soil is regulated by several biotic and abiotic factors. Modification in environmental conditions may alter the equilibrium distribution of the microbial population. This may be the possible reason for the differences in adaptability of microbial populations in different geographical locations (Verma et al., 2014). For instance, the abundance, composition, diversity, and enzymatic activity of microorganisms present in the rhizosphere can be expressed as a subset of overall soil microbial community, which is influenced by the localised physiochemical properties of the soil (Marschner et al., 2004), that may be different from the bulk soil (Foster, 1986). This is reflected where plant root exudates have been reported to shape the composition and abundance of the rhizosphere microbial community (Wu et al., 2017).

### **Microorganisms involved in microbial degradation of herbicide**

The removal of pollutants from the soil by various activities of microorganisms is often referred to by several terms, bioremediation, biodegradation, biomineralization, bioaccumulation, biotransformation, or co-metabolism (Finley et al., 2010; Park et al., 2003; Shakoory et al., 2000). In agricultural context, the over-reliance of chemical compounds leads to the accumulation of toxic compounds in environment which needs to be removed by any means. In this regard, a special group of microorganisms are reported to do this task by enzymatic transformation into non-toxic compounds, are of special importance (Wang et al., 2005; Wood, 2008). Plants, animals, and fungi (Eukaryota) typically remediate pollutants and contaminants through accidental metabolism by broad-spectrum enzymes. Some bacterial extracellular enzymes can decompose ring-based compounds into simple compounds for transport across the cellular membrane for metabolism (Fenner et al., 2013). The differences in degradation are due to the sensitivity of chemical products among the eukaryota. For example, the application of organophosphate ester hampers the nervous system of insects but has no effect on microbes. One hypothesis would be that it could be used as a source of carbon and phosphorous if proper metabolizing enzyme accommodates in that microorganism (Fenner et al., 2013). Bacteria predominates the microbial community regardless of the soil depth as they can utilize alternative electron acceptors in such oxygen deficit conditions (Boopathy, 2000). Moreover, bacteria have the greatest capability to produce new metabolic pathways by the evolution of new enzymes for rapid metabolism (Copley, 2009). Bacteria can transfer clusters of genes evolved in a bacterium to other organisms by cell-to-cell contact, which is known as horizontal gene transfer (Emamalipour et al., 2020). This approach allows the development of a protection system against toxic pollutants due to the continuous exposure to various environmental stress and to take advantage of a broader variety of carbon compounds (Nayak et al., 2018; Parsek et al., 1995; Verma et al., 2014). This is more common among bacteria but also possible between other organisms, where bacteria serve as donor while fungi, plants, and animals serve as recipients (Garcia-Vallvé et al., 2000; Rancurel et al., 2017). There is significant evidence of the generation of new bioremediation pathways within the

microbial community by the transmission of genes responsible for biodegradation (Zhao et al., 2017). Nguyen et al. (2018) reported the intra-field evolution and inter-field exchange of 2,4-D catabolic plasmids and genes within a restrained local environment. Bacterial strains engaged in bioremediation processes have been isolated from different locations of the world. These include strains from *Bacillus* (Eissa et al., 2014), *Pseudomonas* (Lakshmi et al., 2008), *Arthobacter* (Evy et al., 2012), *Ralstonia* (Hay & Focht, 2000), *Rhodococcus* (Park et al., 2003), *Alcaligenes* (Yang et al., 2005), *Nocardiopsis* (Pravin et al., 2012) *Micrococcus* and *Lactobacillus* (Azizi, 2011) and *Acetobacter* (Shakoori et al., 2000). These organisms are highly adaptive in nature and have the capability to degrade a wide range of toxic compounds with the evolution of mutants potentially leading to new metabolic pathways (Suenaga et al., 2001). Laemmli et al. (2000) identified a 2,4-D degrading gene cluster, *tfdII* located on plasmid pJP4 of *Ralstonia eutropha*. Various species within *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Cytophaga*, *Actinobacter*, *Moraxella* and *Klebsiella* have been reported to have such types of plasmids (Sayler et al., 1990).

Although microbial degradation of pesticides greatly focused on bacteria, various fungal strains belonging to different genera including *Aspergillus* (Mohamed et al., 2011), *Trichoderma* (Sene et al., 2010), *Penicillium* (Peng et al., 2012), *Fusarium* (Sene et al., 2010), *Streptomyces* (Mohamed et al., 2011), *Phanerochaete* (Chirnside et al., 2011), *Rhizopus* (Sene et al., 2010), *Trametes* (Bastos & Magan, 2009), *Lentinus* (Nwachukwu & Osuji, 2007) and *Mortierella* (Badawi et al., 2009) have also been reported to degrade a wide range of pesticides. Fungi mediated bioremediation has been reported to be appropriate due to their extended mycelial networks, low specificity to catabolic enzymes and independency towards utilizing organic compounds as growth substrate (Harms et al., 2011). Fungal degradation of pesticides is also regulated by several environmental factors including soil moisture (Bastos & Magan, 2009), temperature (Yang et al., 2011; Yu et al., 2011), pH (Yu et al., 2011), aeration (Hussain et al., 2007) and composition of the medium (Kataoka et al., 2010). Identification and characterization of pesticide degrading fungal strains is a prerequisite for the better understanding of fungal bioremediation. Literature shows that fungal strains have been identified capable of degrading various pesticides including alachlor (Chirnside et al., 2011), pendimethalin (Yu et al., 2011), bensulfuron-methyl (Yu et al., 2011), atrazine (Sene et al., 2010), chlorophenol (Zouari et al., 2002), simazine (Fragoero & Magan, 2008), trifluralin (Fragoero & Magan, 2008), metsulfuron-methyl (He et al., 2006), chlorsulfuron (Boschin et al., 2003), isoproturon (Badawi et al., 2009), diuron (Badawi et al., 2009), linuron (Badawi et al., 2009), glyphosate (Arfarita et al., 2011), metolachlor (Munoz et al., 2011), lindane (Quintero et al., 2008), methyl-parathion (Marinho et al., 2011), endosulfan (Hussain et al., 2007), dichloro diphenyl trichloro ethane (DDT) (Thomas & Gohil, 2011), heptachlor (Xiao et al., 2011), acetamiprid (Wang et al., 2012) and dieldrin (Fragoero & Magan, 2008).

Apart from the isolation and characterization of fungal strains capable of degrading a wide range of pollutants, still there are limitations constraining their wider application. Research suggests that fungi mediated degradation is a slow process and often complete removal of the pollutants is not possible (Sasek & Cajthaml, 2005). This might be due to the time required for the adaptation of the fungal strain in a contaminated environment (Kulshreshtha et al., 2014). Moreover, variations in climatic conditions also play a dominating role in this context. Metabolic process and mechanisms governed by biodegradation

processes need to be addressed under variable environmental conditions which ultimately contribute to fungal biodegradation in site specific conditions. The changes in environmental conditions will affect the physiology of the fungal species ultimately affecting the degradability of the pesticides. Another important aspect regarding fungal biodegradation is that incomplete degradation of the pollutants may lead to the possibility of increased metabolite toxicity compared to the parent pollutant compound (Boopathy, 2000). Accidental accumulation of those metabolites in the environmental components may have serious consequences (Badawi et al., 2009; Xiao et al., 2011).

### Mechanisms involved in microbial degradation of herbicide

Soil microbes are an indispensable part of the ecosystem, maintaining biogeochemical cycles through novel transformations in the biosphere (Whitman et al., 1998). As a part of the transformation process, various organic and inorganic compounds deposited in soil are converted to simple compounds through a variety of metabolic pathways adopted by specific microorganism or groups of microorganisms. Under aerobic conditions, herbicides are primarily converted to CO<sub>2</sub> due to oxidation, but other chemicals may also form. Microorganisms require energy for the various metabolic activities they perform within the soil, and they mainly rely on the organic compounds as a source of energy. The question remains about how microorganisms develop their ability to degrade herbicide compounds. Since microbes catabolize herbicide compounds for assimilation as energy source, their interaction with the herbicidal compounds is significant (Table 2). Catabolic metabolism is mainly dependant on the suitable chemical structure of herbicidal compounds to be utilised as an energy source by the microorganisms. In this regard, selection of the degrading microorganism is the determining factor whether the herbicide compound will be degraded or not.

**Table 2.** Mechanisms involved in herbicide degradation (Torstensson, 1988).

Degradation mechanism	Outcome
1. Direct decomposition of herbicides through adaptation where herbicide compounds serve as energy sources (catabolism).	Repeated application of same herbicide results in faster degradation. May also arise some serious consequences like persistence of some specific herbicides ex. Phenoxy acids, EPTC (S-Ethyl dipropylthiocarbamate), TCA (Trichloroacetic acid), dalapon.
2. Accidental transformation through peripheral metabolic process (co-metabolism).	All herbicides may be degraded by this mechanism.
3. General activities by microorganisms such as, modification of pH, production of different free radicals and other reactive compounds.	Degradation of herbicides due to the influence of microorganisms on biological and non-biological reactions.

In the case of incidental transformation, herbicide degradation rate depends on the availability of other carbon sources which implies that metabolism rate can be altered by the amount of herbicides or carbon sources. This type of metabolism is more common where the amount of herbicide is comparatively lower than the other available carbon sources. Horvath and Alexander (1970) reported an approach of degradation of stable and non-degrading chemicals by increasing the concentration of the primary substrate for the degradation of chlorinated pesticides. Under the above circumstances, the consequence of adaptation and co-metabolism ultimately determines the microbial degradation of herbicides. So, these two phenomena are of primary interest in determining the mechanisms behind microbial activities in soil. These two phenomena are further described below.

### ***Adaptation***

Microbial degradation of herbicide depends on the frequency of herbicide application in soil. Repeated application of the same herbicide in the same field results in increased microbial degradation, suggesting adaptation because of selection (Fang et al., 2015). As a result of the enhanced degradation of herbicides in soil, soil applied herbicides are losing their efficacy (Zablotowicz et al., 2006) and microorganisms are hereby accounted for undermining the effectivity of herbicide compounds. There is some controversy regarding the rapid degradation of several herbicides by soil microorganisms. This rapid breakdown of herbicide has been reported to be disadvantageous by several researchers whereas others have described it as an uncommon phenomenon having little impact on agriculture (Fox, 1983).

There is a range of opinion available to describe how microorganisms build up their capacity to degrade a certain herbicide. According to Kaufman et al. (1983), a specific signal derived from the applied herbicide or other chemicals is responsible for the microbial adaptation to specific herbicides. Some chemicals may act as a motivator in enzyme secretion which further degrades other chemicals. The phenylurea hydrolase-encoding genes *puhA* and *puhB* were identified in the linuron-degrading actinomycetes *Arthrobacter globiformis* D47 (Turnbull et al., 2001) and *Mycobacterium brisbanense* JK1 (Khurana et al., 2009), respectively. Again, it is not mandatory for the herbicide compound to be substrates for the metabolism process governed by enzyme secretion. Traditional culture-based laboratory investigations mainly concentrated on the monoculture of substrates, but the complete degradation of herbicides is faster and more efficient in microbial consortia rather than single microorganism (Kumar et al., 2021). Regarding this, continuous investigations not only revealed the involvement of microbial communities in the remediation of toxicants in the soil but also broaden the possibility to study the interaction between different microbial species (Torstensson, 1988). These innovative studies further laid the foundation of exploring the adaptation mechanism behind herbicide selectivity of the microorganisms in stress conditions. Herbicide degradation usually shows an initial lag phase where no degradation occurs, followed by a sharp decrease in the concentration (Figure 2). The period between herbicide application and initiation of biodegradation is termed as the acclimation period where basically no significant degradation is observed. Zhao et al. (2018) observed a prolonged lag phase followed by higher concentrations of atrazine application, however repeated application of atrazine resulted in faster degradation with decreased half-life (Fang et al., 2015).

This may be due to the multiplication of herbicide degrading organisms during the first application to such a level that increased the degradation rate of herbicide at later applications. Other proposals identified genetic alterations taking place within the microorganism for enzyme synthesis as the main reason for the initial time lapse (Torstensson, 1988). Alterations are mainly due to changes in chromosomal or extra-chromosomal DNA sequences. A specific type of extra-chromosomal DNA, commonly known as plasmid, has been identified to be responsible for the degradation of herbicides (Laemmli et al., 2000). These special types of DNA are smaller than bacterial chromosome and have been reported to bear specific genetic information for biodegradation of herbicides which may be absent in chromosomal genes (Verma et al., 2014). Plasmids are capable of intercellular movement in some microbial communities and provide a pathway for the transfer of the biodegrading genes to other members of the bacterial community (De Souza et al., 1998). Manipulation and transportation of these genes from one organism to other members of the microbial community in such conditions has opened a new horizon in the context of bioremediation.

### ***Co-metabolism***

Co-metabolism is an accidental degradation of an herbicide by an enzyme or co-factor associated with the degradation of another compound (USEPA, 2000). The energy derived at this process is neither sufficient to support microbial growth nor activate relevant enzymes involved in the degradation process (Tran et al., 2013). Co-metabolism is mainly dependant on the substrate metabolism of other compounds. This type of biodegradation is highly sophisticated as only the microorganisms capable to degrade the concerned contaminant are accelerated (Hazen, 2010). This process can be accelerated at low very concentrations particularly to an undetectable limit i.e., parts per trillion, which is the most important advantage (Hazen, 2010). For example, methanotrophs (prokaryotes that metabolize methane for their sole carbon and energy source) have been reported to produce an enzyme called methane monooxygenase, which is capable of oxidizing over 300 compounds (Hazen, 2010). In contrast, some evidence suggest that metabolites produced from this specific type of metabolic pathway may act as inhibitors of microbial degradation (Powell et al., 2011). Microbial co-metabolism may be the effective approach to remove various types of toxic pollutants from soil (Tran et al., 2013). According to Torstensson (1988), majority of the herbicides used in agriculture may be degraded by co-metabolism.

Research suggests that no lag phase has been observed in co-metabolic pathways (Moreira et al., 2012). Moreover, adaptation is absent in co-metabolism which makes it prominent that repeated application of herbicides did not affect co-metabolic degradation at all. Synthetic chemicals which are not degraded by individual microbial species may be mineralized further via the co-metabolic transformations governed by the combined activity of several microbial species. Co-metabolism of herbicides generally occurs in slow rate due to the lower populations of co-metabolizing microorganisms which will not increase in respect to the chemicals applied (Janke & Fritsche, 1985). Even, a single microorganism can co-metabolize a pollutant completely (Juhász & Naidu, 2000). These co-metabolizing microorganisms can be considered as a good option for the development bioremediation strategies.



## Factors affecting microbial degradation of herbicide

### 1. Temperature

Temperature plays a major role in the ecological distribution of microorganisms interlinked with the metabolic activities and degradation of herbicides in soil (Robador et al., 2016). Davidson and Janssens (2006) demonstrated the rapid increase of soil microbial respiration with temperature rise. The increased microbial activity could accelerate degradation of pollutants such as herbicides, and the rate of degradation was faster in warmer regions than in the cooler parts due to increased microbial activity with less seasonal variation throughout the year (Racke et al., 1999). In laboratory conditions, generally the effect of temperature variations on herbicide degradation is of minimum attention and 25 °C has been used as a standard temperature (Racke et al., 1999). Wang and Xie (2012) showed that the optimum temperature range for atrazine degradation was 20-40 °C. Low temperature induces accumulation of toxic pollutants in the environment (Ma et al., 2011). James et al. (1999) reported that triasulfuron may persist in soils at low temperature. In contrary, Levy et al. (2007) blamed the dry and hot weather in summer of 2003 in Germany for the accumulation of isoproturon in soil due to drastic changes in soil microbial community structure and function. Rapid degradation of clopyralid was observed by Tomco et al. (2016), possibly due to high temperature (14.4 and 16.9 °C) in Alaskan soils. Temperature regulates enzymatic activities required for various biochemical processes in soil, yet very little information is available related to sensitivity of enzymatic activities to varying temperatures in the environment (Trasar-Cepeda et al., 2007). According to Wallenstein et al. (2010), enzyme activation mainly depends on the physical and chemical interactions with soil clay, minerals and organic matter. Studies related to temperature effect on specific enzyme activation for the degradation of herbicide compounds found that higher or lower than the optimum temperature will slow down the degradation process. The optimum temperature for herbicide degradation may vary between chemicals but will generally be in the range of 20-30 °C (Jordan, 1990). Dong and Sun (2016) reported that atrazine residue concentration decreased with increasing temperature, increasing degradation rate and half-life by 3-4 times from shifting temperature 5 °C to 35 °C. Degradation of florasulam was strongly influenced by temperature with half-life ranging from 1.0-8.5 days at 20-25 °C to 6.5-85 days at 5 °C (Krieger et al., 2000).

### 2. Soil moisture

Soil microorganisms require moisture for their growth and metabolism. There is a direct relationship between soil microbial activity and moisture content; a decrease in moisture content reduces microbial activity, and rewetting causes a large and rapid increase in activity (Speight & El-Gendy, 2018). Therefore, degradation process is slow in dry soils and generally increases with increasing moisture content (Dong & Sun, 2016). This may be due to the low microbial activity prevailing under extreme dry conditions (Miles et al., 1984). Since moisture content has significant impact on soil microbial activities, herbicide degradation would be expected to be faster in wet soils. Generally, moisture contents between 50-80% field capacity (FC) levels are considered optimal for microbial activity (Morgan & Atlas, 1989). Atrazine

degradation was reported to be 3-4 times higher when soil moisture content was increased from 5% to 20% (Dong & Sun, 2016).

Whereas extreme soil moisture conditions are considered unfavourable for microbial growth and metabolism process, fungal and bacterial oxidative enzymes for degradation are inhibited at low O<sub>2</sub> levels in saturated soils. Excess moisture can accelerate anaerobic transformation of herbicides by reducing the oxygen level, which could hamper the transformation of herbicides (Schroll et al., 2006). Alternatively, soil moisture may not necessarily have any significant effect on the transformation of some herbicides. For example, the half-life of rimsulfuron was reported to be 22.5 and 24.5 days under aerobic and anaerobic conditions, respectively (Schneiders et al., 1993). Some herbicides are reported to be accumulated under anaerobic conditions, e.g., clopyralid (Zhao et al., 2011) whereas others breakdown rapidly, e.g., atrazine (Pal et al., 2006).

### **3. Soil pH**

Soil pH has substantial effects on the reactivity, activity, and persistence of applied herbicides in soil, specifically at extreme pH conditions such as less than 4.5 or higher than 7.5 (Monaco et al., 2002). The basic principle is that herbicide degradation is dependent on the charge of the herbicide molecules and herbicides bearing a positive charge will have a strong affinity to the negatively charged soil clay particles. Whereas herbicides bearing a negative charge will be repelled by soil colloids and exposed to transformation (Ross & Lembi, 1999). Again, soil pH has a major influence not only on the growth and activity of microorganisms but specifically on the growth of microbes responsible for herbicide transformation (Raeder et al., 2015). Optimization of pH in soil is a difficult task depending on the soil type as variation in the soil pH is comparatively less than in water. In addition, enzymes have an operational pH range and changes to pH cause inhibition due to denaturation. As most microbial species survive in the pH range of 4.5-7.5 (Msimbira & Smith, 2020), optimization of pH is critical in soil experiments in regards of biodegradation of herbicides.

Tariq et al. (2003) observed highest degradation of HCH isomers ( $\alpha$  and  $\gamma$ ) in soil slurry with an initial pH of 9.0. Accelerated biodegradation of endosulfan was reported through optimization of pH to 8.0 (Arshad et al., 2008). Optimum condition for biodegradation of pesticides varies with compounds and organisms, but the degradation rate is slow at acidic pH compared to alkaline and neutral pH conditions because acidic pH increases stability of various chemical groups (Reid et al., 2000). Another possible reason may be the reduced activity of bacteria or enzyme involved in the biodegradation process under low pH (Roberts, 1998).

### **4. Soil organic matter**

Although microorganisms represent only 1 to 8% of the soil organic carbon (Roder et al., 1988), they are responsible for the maintenance of C, N and P cycles and other physio-chemical activities in soil through decomposition, mineralisation, and immobilization processes (Sarathchandra et al., 1988). Increase in mineralisation of the herbicides may contribute to the reduction of organic matter content of soil. Low

organic matter content of soil may result in slower microbial degradation of trifluralin with high adsorption capacities and as a result less trifluralin available for degradation in soil (Tiryaki et al., 2004). To boost microbial activity in soil, organic matter content in soil should be replenished (Perucci et al., 2000). According to Burns (1975), at least 1.0% of organic matter should present in soil to ensure the activity of indigenous microorganisms that can involve in the transformation of toxic compounds in soil. To increase the organic matter content in soil, application of various organic amendments such as sawdust, municipal waste compost and synthetic biological waste are frequently practiced in different countries (Palma et al., 2002; Said-Pullicino et al., 2004; Vorkamp et al., 2002). Organic amendments e.g., sawdust have higher C:N ratios than compost, resulting in increased microbial activity as microorganisms require carbon and nitrogen as a nutrient for growth and reproduction (Zhang et al., 2021). Addition of organic amendments in the soil has recently gained increasing interests (Scotti et al., 2015), which facilitates development and functioning of terrestrial ecosystems (Izquierdo & Bedmar, 2008). However, this could lead to a change in the fate and behaviour of herbicides applied in the same soil (García-Jaramillo et al., 2016).

Organic amendment addition will either slow down the microbial degradation process through adsorption (Doyle et al., 1978) or accelerate the remediation process by increasing the microbial metabolic activity (Hance, 1973). Although, herbicide sorption is reported to increase with the addition of organic amendments in soil, dissolved organic matter (DOM) content is also enriched which gradually influences sorption and movement of herbicides in soil (Cox et al., 2001). Marín-Benito et al. (2018) agrees with this statement as they concluded that soil amendment with green compost not only increased half-life (DT<sub>50</sub>) of triasulfuron in soil due to rapid adsorption by soil particles but also accelerated persistence by blocking leaching into the soil.

## **5. Herbicide structural properties and concentration**

Physical and chemical properties of herbicide mainly determine its possibility of biodegradation in the environment. Addition of polar groups such as, OH, COOH, and NH<sub>2</sub> on the phenyl ring makes the herbicidal compound more susceptible to microbial activity (Chowdhury et al., 2008). On the other hand, Cork and Krueger (1991) revealed substituents like halogen and alkyl groups make compounds resistant to microbial degradation. In addition, water solubility and adsorptivity of the herbicide compound are important factors under consideration in this context. Solubility and adsorptivity are inversely related in many herbicide compounds. Herbicides which are likely soluble in water are more prompt to microbial degradation than those which are generally insoluble in water. Chlorinated hydrocarbons such as DDT, pentalene and dieldrin are insoluble in water, sorbed tightly to soil particles and thus are relatively unavailable for microbial degradation (Chowdhury et al., 2008). Again, there is some exception with glyphosate and paraquat which are highly water soluble but adsorbed to soil particles tightly (Williams et al., 2014). Several physical, chemical, and structural parameters that determine the possibility of degradation are listed in Table 3.

**Table 3.** Effect of physical, chemical, and structural properties on the degradability of herbicides (Boettcher et al., 2001).

Properties	Degradation	
	Rapid	Slow
Solubility in water	Soluble	Insoluble
Size	Relatively small	Relatively large
Functional group substitutions	Few	More
Rapid reduction	In oxidized environment	In reduced environment
Rapid oxidation	In reduced environment	In oxidized environment
Origin	Biologically	Either man made or synthetic
Aliphatics	Up to 10 C-chains, straight chains. Aromatic compounds with one or two nuclei	High molecular weight alkanes, branched chains, polyaromatic hydrocarbons
Substitutions on organic molecules	Alcohols, aldehydes, acids, esters, amides, amino acids	Alkanes, olefins, ethers, ketones, dicarboxylic acids, nitriles, amines, chloroalkanes
Substitution position	p-position, o- or p- di-substituted phenols	m- or o- position, m- di-substituted phenols

## 6. Dissolved organic matter (DOM)

Dissolved organic matter (DOM) is the fundamental portion of organic matter having the ability to dissolve in field conditions, and which plays a major role in transportation of pollutants in soil (Kalbitz et al., 2000). Photosynthesis is the primary driver of DOM production in soil which includes organic litter and humus substances accumulated through pedogenesis (Guggenberger et al., 1994; McDowell & Likens, 1988). Soil microbial communities are the substantial agent contributing to the formation of DOM. Guggenberger et al. (1994) investigated DOM structure and fractionation and revealed that DOM may be predominately of microbial origin. Solubility and mobility of various organic compounds and metals are enhanced by DOM (Blaser, 1994; Marschner, 1999; Piccolo, 1994; Zsolnay, 1996) followed by accelerated biodegradation of organic compounds (Raulund-Rasmussen et al., 1998). Previous studies have shown that even a small fraction of DOM can significantly influence the dissipation of various organic compounds, especially DDT and some polychlorobiphenyls (PCBs) (Caron et al., 1985; Hassett & Anderson, 1979). Whereas contradictory results were also reported on the behaviour of the fate of cationic pesticides in soil and water which may be due to the differences in experimental conditions (Barriuso et al., 1992; Klaus et al., 1998; Seol & Lee, 2000). Most studies focussed on the behavioural

pattern of herbicides in water bodies while very little is known about their interaction in soil in presence of DOM (Said-Pullicino et al., 2004). Adsorption and desorption behaviour of atrazine, dimefuron and carbetamide herbicides was influenced by the nature of DOM as per observations of Barriuso et al. (1992). They identified a positive relation between soil adsorption capacity and soil organic carbon content which led them to conclude physio-chemical properties of DOM i.e., pH, organic carbon content and conductivity had a strong influence on the sorption behaviour of herbicides. Pre-treatment with DOM solution increased soil adsorption of less soluble atrazine and dimefuron. This increased adsorption may be due to the increased soil carbon content that contributed adsorption of some organic compounds from DOM solution. On the other hand, Pennington et al. (1991) observed that DOM extracted from different soil samples had no significant interaction with the tested herbicides i.e., alachlor, bromacil and metribuzin which may be due to the variation in physio-chemical characteristics of herbicide compounds.

### **Approaches used to study microbial degradation of herbicide**

Recent improvement in microbiology allows us to use various molecular and proteomic approaches to investigate specific microbial catabolic pathways for the biodegradation of herbicides (Ghosal et al., 2016). As mentioned earlier, some bacteria are capable to produce extracellular enzymes to metabolize complex organic and inorganic compounds to obtain energy and carbon as a part of their assimilation process. Catabolic genes play an important role in shaping the genetic foundation of herbicide biodegradation, with subsequent identification of these genes permitting application of molecular technology to investigate their function (Widada et al., 2002). Microbial genes which are known to degrade herbicides are listed in Table 4. Generally, those catabolic genes are situated on chromosomes, however several have been located to plasmids.

**Table 4.** Isolated bacterial and fungal genes with host organisms (Ortiz-Hernández et al., 2013; Singh & Walker, 2006).

Gene name	Host	Enzyme
<b>Bacterial gene</b>		
<i>opdA</i>	<i>Agrobacterium radiobacter</i>	Organophosphorus hydrolase
<i>opd</i>	<i>Pseudomonas diminuta</i>	Organophosphorus hydrolase
<i>adpB</i>	<i>Nocardia</i> sp.	Aryldialkylphosphatase
<i>Phn</i>	<i>Bacillus cereus</i>	Phosphonate
<i>ophB</i>	<i>Burkholderia</i> sp. JBA3	Organophosphorus hydrolase
<i>Imh</i>	<i>Arthrobacter</i> sp. scl-2	ND
<i>Mpd</i>	<i>Ochrobactrum</i> sp. Yw28 and <i>Rhizobium radiobacter</i>	ND
<i>opdE</i>	<i>Enterobacter</i> sp.	Organophosphorus hydrolase
<i>opaA</i>	<i>Alteromonas</i> spp.	Organophosphorus acid anhydrolase
<i>pepA</i>	<i>Escherichia coli</i>	Amino peptidase A
<i>hocA</i>	<i>Pseudomonas montelli</i>	ND
<i>pehA</i>	<i>Burkholderia caryophilli</i>	Phosphonate monoesterase
<i>ophC2</i>	<i>Stenotrophomonas</i> sp. SMSP-1	ND
<i>OpdB</i>	<i>Lactobacillus brevis</i>	Organophosphorus hydrolase
<i>Oph</i>	<i>Arthrobacter</i> sp.	ND
<i>Mph</i>	<i>Arthrobacter</i> sp. L1	Methyl parathion hydrolase
<i>MphB</i>	<i>Burkholderia cepacia</i>	Methyl parathion hydrolase
<b>Fungal gene</b>		
<i>A-opd</i>	<i>Aspargillus niger</i>	ND
<i>P-opd</i>	<i>Penicillium lilacinum</i>	ND

ND= not determined

Researchers are placing more emphasis on the sequencing of whole genomes from a wide range of microbial populations in the soil to investigate novel genes and degradative elements responsible for the degradation of pesticides. This has provided new insights into the identification of herbicide degrading genes from both culturable and non-culturable microorganisms and provided an increased understanding

of innovative metabolic pathways under various environmental conditions, which is essential for the successful implementation of bioremediation techniques. Li et al. (2007) identified several microbial enzymes, such as organophosphorus hydrolase isolated from bacteria, capable of hydrolysing organophosphate pesticides and utilizing this as a source of carbon. The identified gene (*opd* gene, homologue to *mpd* gene) is highly preserved within plasmid containing 996 nucleotides and is responsible for organophosphorus hydrolases (OPH). Hydroxylation of methyl-parathion was accelerated through the secretion of a specific enzyme methyl-parathion hydrolase. Cui et al. (2001) isolated the gene responsible for hydroxylation of methyl-parathion from the bacterial strains of *Achrobacter*, *Ochrobactrum*, *Pseudaminobacter* and *Achrobacter* by comparing the same gene belonging to *Pleisomonas* sp. To date more than 300 genes have been isolated from various culturable bacterial strains worldwide engaged in biodegradation of aromatic compounds (Bhatt et al., 2019). More and more emphasis has been given to DNA and RNA quantification to identify the number of potential biodegrading genes. It is believed that a positive correlation may exist between the relative abundance of biodegrading genes and their ability to degrade contaminants in the environment. Quantitative studies related to DNA and RNA can significantly promote biodegradation of herbicides by identifying the bulk of genes associated in bioremediation. This can allow the manipulation of the environment to promote the increase in the numbers of organisms involved in biodegradation. Repeated application of atrazine resulted in the increase of the microbial population responsible for herbicide degradation (Fang et al., 2015; Yale et al., 2017). Similar results were observed in case of herbicide MCPA (2-methyl-4-chlorophenoxyacetic acid) (Bælum et al., 2008) and glyphosate (Lancaster et al., 2010). Perhaps potential research using DNA and RNA approaches in the identification of biodegrading genes might result in novel understanding related to the management of biodegradation which could lead to regulation of extent and rate of biodegradation (Lovley, 2003).

The absence of degrading microorganism could make the scenario difficult; favouring the herbicide compound persist in soil longer than usual. However, recent research approaches pointed out the possibility of developing genetically modified microorganisms for herbicide degradation (Hussain et al., 2018; Verma & Jaiswal, 2016). These genetically modified super microorganisms may degrade the herbicide faster than the usual. Although adaptability of the microorganisms in the contaminated site may be an issue which could lead this strategy ill-fated. Moreover, the potential risk associated with genetically modified microorganisms to open environment raised common safety concerns and legislative issues (Hussain et al., 2018).

## Conclusion

In Australia, herbicides applied in minimal or zero till systems tend to maintain a greater concentration of herbicide near the surface zone at the end of the cropping season, which may result in higher residual concentrations, affecting crops subsequently sown. Currently, there is no option available to combat this problem other than using crop rotation to avoid incompatible crop-herbicide combinations which includes routine rotation of fallow and pre-emergent herbicides, reliable record keeping helping identify potential residue issues, and use of tolerant crops or crop cultivars in rotations after dry seasons. Little is known

about the environmental fate of herbicides in Australian soil. Persistence of herbicides in soil could potentially affect sensitive crops in rotation, investigation on critical concentrations at which level causing damage to sensitive following crops could help farmers in selecting crop rotation strategies. In addition, environmental factors are known to have a crucial effect on the persistence of herbicides in soil. Again, persistence of herbicides in soil is directly related with the presence or absence of degrading microorganisms in soil, the shift in microbial community structure and diversity upon herbicide exposure could generate valuable information about potential groups of microorganisms benefitted by herbicide application.

Despite the widespread use of herbicides and consequently their undesirable presence in the environment, microbial degradation pathways of herbicides and their genetic bases remain poorly understood. Enzymes form a critical aspect in the degradation process as the degradation of the herbicide compound is governed through this. Since enzymatic degradation of herbicides poses as a promising approach, necessity of extensive research regarding identification of degradable enzymes should be given utmost priority. Microbial communities possess greater genetic and metabolic diversity compared to a single strain in the degradation process. Moreover, the genetic expression and efficiency of the metabolic pathways are largely determined by the native environmental factors. So, priority investigations should be carried out for the identification and isolation of target genes considering native environmental factors so that the generated data could be applied in the actual field conditions for the successful removal of herbicide residues in soil.

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### *Conflicts of interest*

The authors declare that there are no known conflicts of interest with this review article.

### *Authors' contributions*

Imtiaz Faruk Chowdhury: Conceptualization, Methodology, Writing – original draft, Funding acquisition. Gregory S. Doran: Conceptualization, Methodology, Supervision, Writing – review & editing. Benjamin J. Stodart: Conceptualization, Methodology, Supervision, Writing – review & editing. Chengrong Chen: Supervision, Writing – review & editing. Hanwen Wu: Conceptualization, Methodology, Supervision, Writing – review & editing.

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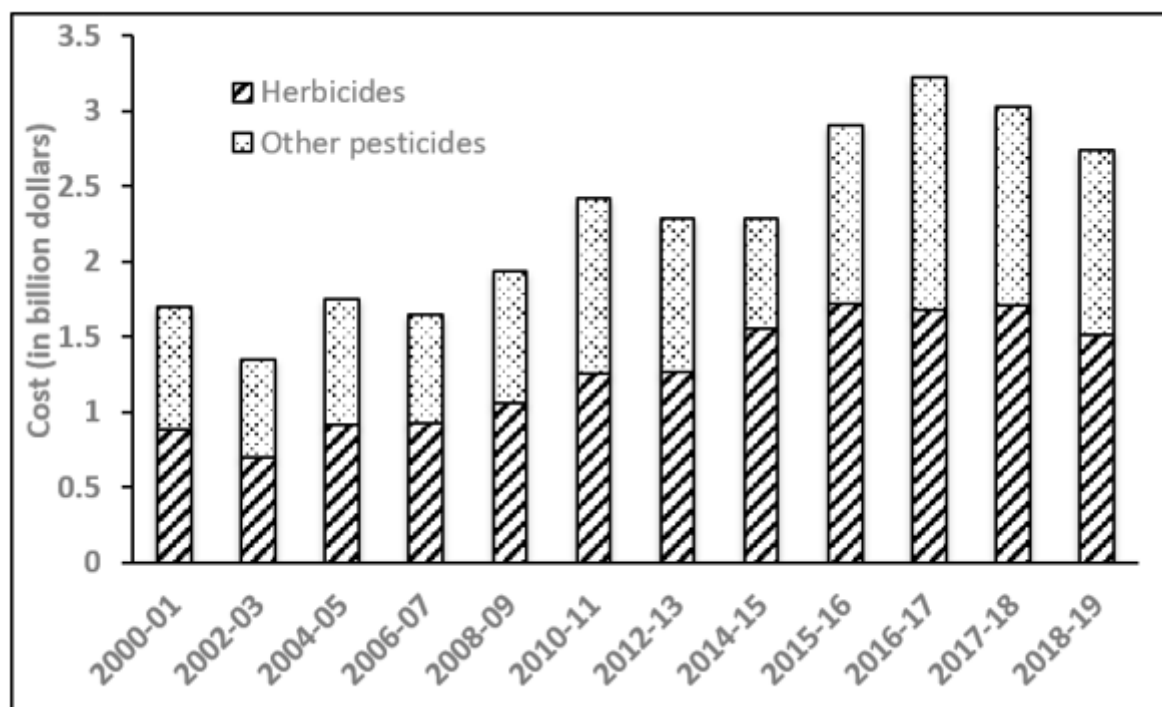


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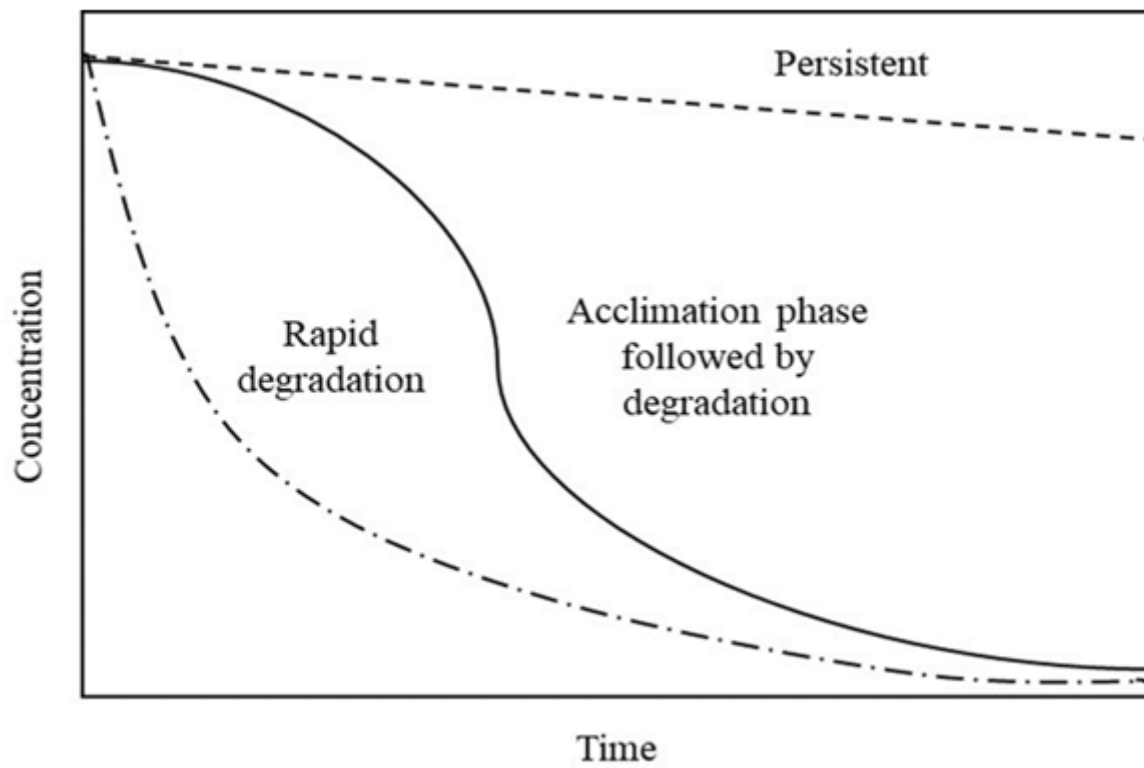
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## Figures



**Figure 1**

Year wise breakdown of herbicide cost in Australian agriculture over total pesticide expenditure, expressed in billion dollars (APVMA, 2021).



**Figure 2**

Degradation of herbicides in soil over period of time (Boettcher et al., 2001).