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BIODEGRADATION OF PESTICIDES IN SOIL – A REVIEW

Harshita Singh, Dhruv Singh[1], Ajay Kumar[1], Anita Sharma, Damini Maithani and M.C. Singh[2]

Department of Microbiology
GB Pant University of Agriculture & Technology, Pantnagar-263 145, UK
[1]Department of Plant Protection, CCS University, Meerut-250 004, UP
[2]ICAR-National Bureau of Plant Genetic resources, New Delhi-110 012

Abstract: Modern agriculture relies heavily on herbicides for the control of weeds and ease out to maximize yield in crops. With the development of herbicide-tolerant crops, use of herbicides is increasing around the world that has resulted in severe contamination of the environment. The strategies are now being developed to clean these substances in an economical and eco-friendly manner. An attempt has been made to pool all the available information about microbial degradation of key herbicides, 2,4-dichlorophenoxyacetic acid, atrazine, metolachlor, diuron, glyphosate, pendimethalin and paraquat. Based on the information, it has been found that the inoculums size, amounts of additional co-substrates carbon and nitrogen compounds, organic matter of soil and pH are the major factors that affected the extent and rate of herbicides degradation. Due to excessive use of herbicides, ecosystems are under threat of its pollution. Microbes are the main vehicle for remediation of herbicides, and new discoveries, such as novel biodegradation pathways, multispecies interactions and community-level responses to herbicides addition, are helping us to understand, predict and monitor the fate of herbicides. The information may be useful in developing safer and economic microbiological methods for cleanup of soil and water contaminated with such herbicides.

Keywords: Biodegradation, herbicides, microbes, toxicity.

Introduction

Herbicides are class of chemical compounds that are toxic to plants, especially unwanted ones. Modern agriculture relies heavily on herbicides for the control of weeds and ease out to maximize yield in crops. These compounds have economical benefits to sustain an increasing world population. The development of herbicide-resistant plants has also led to an unexpected increase in the resilience of weeds. Genetically modified crops resistant to herbicides have become so prevalent that resistant weeds are beginning to appear, necessitating new forms of genetic modification. Weeds have become more and more resistant to herbicides, prompting farmers to use a wider variety and larger quantity of herbicides to control them. The introduction of herbicide-tolerant plants at first decreased herbicide use, but afterwards increased its usage and scope. Majority of herbicides are reported to constitute between 40 and 60% of pesticides used for agricultural purpose. Due to excessive use of herbicides, there is great concern about their potential environmental hazard.

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Herbicides contamination can lead to soil and water pollution (Juhler et al., 2001), reduced biodiversity and depression in soil heterotrophic bacteria (including denitrifying bacteria) and fungi (Song et al., 2013). The environmental fate of herbicides is a matter of recent concern provided that only a small fraction of the chemicals reach the target organisms leading to great impacts of residual herbicides in soil and water on human, animal and crop health. Major sources of herbicides contamination appear to be an inadequate management practices specifically involving on-farm handling of herbicides. The chemical properties and quantity of herbicides determine their toxicity and persistence in the environment. Their interaction with targeted and non targeted organisms has extensively damaged the ecosystem through entry into the food chains. Application method of herbicides generally include foliar applied method, soil applied, broadcast and spot. Commonly used herbicides include 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine, metolachlor, diuron, glyphosate, pendimethalin, and paraquat. These herbicides are harmful to organisms even at micro levels. Their uses have resulted in severe contamination of the environment, and strategies are now being developed to clean these substances in an economical and eco-friendly manner. The present review summarizes information on microbial degradation path- ways of these herbicides.

2, 4-Dichlorophenoxyacetic acid (2, 4-D)

2, 4-D is one of the most widely used chlorinated acidic phenoxy herbicide in the world and is an analogue of a growth hormone, auxin. 2,4-D was first synthesized in 1941, marketed commercially and registered for use in the United States in 1944 and 1948, respectively. It has provided economical, selective, effective control of broadleaf weeds in agriculture crops, pastures and forests for the past decades. This is used for post- emergent control of broad-leaf weeds. 2,4-D is formulated as amine salts (mainly dimethyl-amine salt), which are more soluble in water than acid, and ester derivatives (2-ethyhexyl ester), which are readily dissolved in an organic solvent.

Biodegradation of 2, 4-D

Several bacterial strains have been described that are able to use 2,4-D as the sole carbon and energy source. The most commonly cited 2, 4-D degrading genera are *Pseudomonas, Alcaligenes, Ralstonia, Delftia, Arthrobacter* and *Burkholderia*. Degradation of 2,4-D *via* oxidative cleavage of ether bond with subsequent chlorophenol hydroxylation followed by the modified ortho-cleavage pathway of chlor-ocatechols has been demonstrated for most of these isolates. The electron effects, the spatial orientation and the hydrophobic effect of their substituent groups have obvious influence on their degradation pathway. Evans et al. (1971)

isolated two strains of *Pseudomonas* sp. capable of degrading 2,4-D and proposed biodegradation pathway of 2,4-D. Plasmid involvement in the degradation of 2,4-D was first reported by Fisher et al. (1978). They reported that ability to degrade 2,4- D is encoded by a 58-megadalton conjugal plasmid, pJPl. Genes encoding 2,4-D degradation are often located on conjugative plasmids, pEMT1 and pJP4 (Chong & Chang, 2009) but have been found to be also chromosomally located in *Burkholderia* spp (Matheson et al., 1996). Plasmid pEMT1 contains the same degradative genes in an organization similar to that of plasmid pJP4 but does not belong to the same incompatibility group as pJP4. Don & Pemberton (1985) constructed a genetic and biophysical map of pJP4 by using transposon mutagenesis, deletion analysis, gene cloning and restriction analyses. Plasmid pJP4 contains the genes for the degradation of 2, 4-D to chloromaleyl acetic acid, whereas chromosomal genes of the host are necessary for complete mineralization of the compound. The modified ortho cleavage pathway in degradation of 2,4-D is encoded on conjugative plasmids (Poh et al., 2002) which give information on movement of genes by horizontal gene transfer. The genes responsible for 2,4-D degradation pathway (*tfdA, -B, -C,-D, E* and *F*) are localized to the transmissible plasmid, pJP4 is well understood and the enzymes participating in the pathway have been purified and characterized (Evans et al., 1971). The *tfd* genes encoding these enzymes have been localized, cloned and sequenced (Don & Pemberton (1985); Tsutsui et al., 2013). The regulatory mechanisms of *tfd* gene expression have been elucidated (Inoue et al., 2012). Kitagawa et al. (2002) cloned and characterized new family of 2,4-D degradation genes, *cadRABKC*, from *Bradyrhizobium* sp. strain HW13. The *cadR* gene was inferred to encode an AraC/XylS type of transcriptional regulator from its deduced amino acid sequence. The *cadABC* genes were predicted to encode 2,4- D oxygenase subunits from their deduced amino acid sequences. The *cadK* gene was presumed to encode a 2,4-D transport protein from its deduced amino acid sequence that showed 60% identity with the 2,4-D transporter, TfdK, of strain *Ralstonia eutropha* JMP134. Gene bio augmentation with conjugative plasmids-harboring bacteria capable of degrading 2,4-D on the indigenous microbial community has been evaluated by several researchers previously (Inoue et al., 2012; Tsutsui et al., 2013). Hoffmann et al. (2003) reported that the putative genes of the complete 2,4-D degradation pathway are organized in a single genomic unit in alkali tolerant strain *Delftia acidovorans*.

Atrazine

Atrazine, [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], is a selective herbicide belonging to the family of the striazines. Because of its high mobility in soil and its

massive application, atrazine has often been detected in surface and ground waters at concentrations well above the permissible limits (Tappe et al., 2002). The moderate persistent, chemical water solubility and low soil sorption partition coefficient are key factors influencing its potential contamination to aquifers, groundwater and rain water as it normally percolates to groundwater or river via infiltration (Siripattanakul et al., 2009). Atrazine inhibits photosynthesis and its associated noncyclic photophosphorylation is in higher plants (Shelton et al., 1996). All higher plants probably metabolize atrazine by N-dealkylation with some species such as corn and wheat, which contain benzoxazinone, and utilize hydroxylation as well.

Biodegradation of atrazine

Microbial metabolism has been regarded as the most important mechanism of atrazine degradation in soil. A number of microorganisms with different atrazine degradation efficiencies and growth characteristics have been reported. One of the well-known atrazine-degrading bacteria is *Pseudomonas* sp. strain ADP, which can decompose atrazine to NH3 and CO2 by some enzymes (Siripattanakul et al., 2009). Another best characterized strain whose atrazine metabolic track is different from *Pseudomonas* sp. strain ADP is *Arthrobacter aurescens* TC-1. It can metabolize atrazine to cyanuric acid through a degrading pathway catalyzed by TrzN, AtzB and AtzC enzymes. The degradation of atrazine occurs predominantly by biological processes, including N-dealkylation, dechlorination and ring cleavage. Atrazine biodegradation can be initiated by N-dealkylation of the ethyl or isopropyl side chains to produce deethylatrazine (DEA) or deisopropylatrazine (DIA). Dechlorination has been reported as an early step in atrazine metabolism, and two different s-triazine hydrolase enzymes have been characterized. In some microorganisms, complete biodegradation of atrazine to ammonia and CO2 has been obtained. *Pseudomonas* sp. strain ADP might be the best characterized atrazine mineralizing one (Mandelbaum et al.,1993). The genes encoding the three enzymes that are responsible for the conversion of atrazine to cyanuric acid were atzA, B and C. The research on atrazine-degrading microorganisms has been directed to the isolation and characterization of natural occurrence lineages in environments contaminated with this pesticide. For the purpose of potential bioremediation practice, a large variety of atrazine degrading bacteria from diverse genera have been isolated (Rousseaux et al., 2001). Among bacteria, there are reports on atrazine degradation by individual strains such as *Pseudomonas* sp. (Mandelbaum et al., 1995), *Rhodococcus rhodochrous*, *Acinetobacter* spp., *Agrobacterium* sp., *Microbacterium* sp., *Bacillus* sp.,

Micrococcus sp., *Deinococcus* sp. and *D. acidovorans* (Vargha et al., 2005), as well as by species consortia including *Agrobacterium tumefaciens*, *Caulobacter* and *Pseudomonas* sp. ADP is now the most known and the best-characterized atrazine-degrading bacterium (Wackett et al., 2002). However, microorganisms from genus *Arthrobacter* are well known for their strong capacity to degrade atrazine and have been isolated from agricultural and heavily contaminated soils at spill sites and industrial wastewaters from atrazine production plants (Zhou et al., 2012). The atrazine-degrading bacteria generally initiate the degradation through a hydrolytic dechlorination, catalyzed by the enzyme atrazine chlorohydrolase (AtzA), encoded by the atzA gene, followed by two hydrolytic deamination reactions catalyzed by hydroxyatrazine ethylaminohydrolase (AtzB) and N-isopropylammelide isopropylamino- hydrolase (AtzC), encoded by the genes atzB (trzB) e atzC (trzC), respectively (De Souza et al., 1998), which convert atrazine sequentially to cyanuric acid that is then completely mineralized to CO2 and NH3 by other three hydrolysis. In some bacterial strains, the biodegradation of atrazine initiate through N dealkylation of the lateral ethyl and isopropyl chains to DEA and DIA (Kaufman & Blake, 1970). *Pseudomonas sp*. ADP is the best-characterized bacterial strain capable to degrading the herbicide atrazine. The atrazine catabolic pathway in this bacterium contains six enzymatic steps encoded by atzABC and the atzDEF genes. The atzABC genes have been shown to be widespread and plasmid borne in a number of bacteria isolates (De Souza et al., 1998: Rousseaux et al., 2001; Wackett et al., 2002). In *Pseudomonas* sp. ADP, the atzABCDE genes are harbored on the catabolic plasmid pADP-1 (Martinez et al., 2001). In *Pseudomonas* sp. ADP, the atzDEF operon encodes cyanuric acid amidohydrolase (AtzD), biuret amidohydrolase (AtzE) and allophanate hydrolase (AtzF), involved in cleavage of the cyanuric acid to carbon dioxide and ammonia, which is assimilated as a nitrogen source (De Souza et al., 1998). Garcia-Gonzalez et al. (2003) have demonstrated that nitrogen control of atrazine metabolism is functional under soil conditions and may therefore limit the potential of *Pseudomonas* sp. strain ADP for atrazine bioremediation in nitrogen-fertilized agricultural soils. The atzABC genes are constitutively expressed and are not regulated either by induction of atrazine or by repression of other N sources in this strain (Devers et al., 2007). The atzDEF genes are divergently transcribed from AtzR, predicted to encode a transcriptional LysR type regulator (LTTR). A putative LTTR-binding site can in fact be found upstream of atzD gene, thereby suggesting that transcription of the atzDEF operon may be regulated and the protein encoded by the orf99 (AtzR) play a role in this regulation. The atzDEF operon resides in a contiguous cluster

adjacent to the orf99, a potential transcriptional LTTR. Atrazine biodegradation can be positively or natively affected by the external addition of organic carbon sources (Abdelhafid et al., 2000) and is dependent on types of added carbon sources found that the addition of citrate to soils resulted in enhanced atrazine degradation by *Arthrobacter* sp. strain KU001. A good candidate for bio augmentation is the *Arthrobacter* strain DAT1 that has shown high atrazine-degrading efficiency both in liquid cultures and soils (Wang & Xie, 2012). This strain can utilize atrazine as a sole nitrogen source for growth and harbors the atrazine-metabolic genes trzN, atzB and atzC.

Metolachlor

Metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(methoxyprop-2-yl)acetamide) is a selective chloroacetamide herbicide used to control broadleaf and annual grass weeds in corn (*Zea mays* L.), soybean (*Glycine max* L. Merr.), peanut (*Arachis hypogaea* L.) and potato (*Solanum tuberosum* L.). Large quantities of metolachlor (22 million kg of active ingredient) are applied to agricultural fields in the United States, particularly in the Midwest, where most of the corn and soybeans are grown. Metolachlor is a member of the chloroacetanilide herbicide chemical family. Other members include acetochlor, alachlor, butachlor, butenachlor, delachlor, diethatyl, dimethachlor, metazachlor, propachlor, propisochlor, prynachlor, terbuchlor, thenylchlor. When it is absorbed through the roots and shoots, it acts as a growth inhibitor by suppressing synthesis of chlorophyll, proteins, fatty acids and lipids, isoprenoids (including gibberellins) and flavonoids (including anthocyanins).

Biodegradation of metolachlor

A major breakdown pathway of metolachlor in the soil is by both aerobic and anaerobic microorganisms. The transformation by soil microorganisms of metolachlor to its primary degradates: metolachlor ethane sulfonic acid (ESA) and metolachlor oxanilic acid (OA) has been suggested to occur as a result of shifting of chlorine atom of the parent compound by glutathione, followed by the formation of the ESA and OA degradates by different enzymatic pathways (Barbash et al., 1999). Degradation of metolachlor in soil occurs mainly by microbial decomposition (Xu et al., 2008) and photo-degradation. Microbial degradation rates are affected by soil depth, organic carbon and dissolved oxygen concentrations, temperature and size of microbial populations. In case of sandy soils, a half-life of 81 days for anaerobic microbial populations and 67 days for aerobic microbial populations were reported in the laboratory. Photo-degradation occurs only when metolachlor

is present on the soil surface. Fifty percent of surface-applied metolachlor can be degraded in eight days on soil, while only 6% degrades over one month in soils where metolachlor was incorporated into the surface layer.

Diuron

Diuron (3,4-dichlorophenyl)-1,1-dimethylurea) is a systemic substituted wide-spectrum phenylurea herbicide used for weed control in agricultural crops and non crops areas. Non crops areas include along fence lines, pipelines, power lines, railway lines, roads, footpaths; in timber yards and storage areas; and around commercial, industrial and farm buildings, electrical substations and petroleum storage tanks. Diuron is used as an algaecide in ornamental ponds, fountains and aquaria and mildewcide in paints. Diuron is available in wettable powder, granular, flowable, pelleted/tableted, liquid suspension and soluble concentrate formulations. Technical grade (the grade that is usually used for agricultural purposes) diuron is a white, crystalline and odorless solid. Diuron is a systemic substituted phenylurea herbicide. It is easily taken up from soil solution through the root system of plants and rapidly translocated into stems and leaves by the transpiration system, moving mainly via the xylem. Diuron primarily functions by blocking the Hill reaction in photosynthesis process, limiting the production of high energy compounds such as ATP, which is used for several metabolic processes. Diuron binds to the QB-binding site on D1 protein of the photo system-II complex in chloroplast, thus blocking electron transport from QA to QB. This process prevents CO2 fixation and the production of ATP and other high energy compounds, which are needed for plant growth. The inability to re-oxidize QA promotes the formation of triplet state chlorophyll, which interacts with ground state oxygen to form singlet oxygen. Both triplet chlorophyll and singlet oxygen can extract hydrogen from unsaturated lipids, producing a lipid radical and initiating a chain reaction of lipid per-oxidation. Lipids and proteins are attacked and oxidized, resulting in loss of chlorophyll and carotenoids, and in leaky membranes, which cause cells and cell organelles to dry and disintegrate rapidly.

Biodegradation of diuron

Diuron is susceptible to degradation by soil microorganisms, and enriched cultures of aquatic microorganisms from pond water could also degrade diuron to 3,4-dichloroaniline as a major metabolite (Fratila-Apachitei et al., 1999). Bogaerts et al. (2000) studied microbial degradation of diuron and ecotoxicology to investigate its breakdown after application to soils. Quantitative biodegradation assays were executed with fungal strains, showing that diuron was degraded but not entirely. A series of tests were taken out to select the most

efficient fungal strain for diuron degradation. Among the fungal strains, only four strains were able to transform diuron to an extent (up to 50%) after seven days of incubation: *Beauveria bassiana*, *Caenorhabditis elegans*, *Phanerochaete chrysosporium* and *Mordellistena isabellina*. Diuron degradation by the fungal strains led to the formation of two metabolites obtained in different proportions according to the microorganism. For the fungal strains, diuron degradation led to the formation of the demethylated products. The identified metabolites were synthesized in sufficient amounts to confirm their structures and determine their non-target toxicity using four biotests. According to the Microtox test, the metabolites N-(3,4-dichlorophenyl)-N-methylurea and N-3, 4-dichlorophenylurea presented a three times higher toxicity than that of diuron. Dalton et al. (1966) reported that the removal of N-methyl groups eliminates herbicidal activity of diuron. Decomposition is followed by the removal of the urea group, which results in the formation of 3,4-dichloroaniline, ammonia and CO2.

Glyphosate

Glyphosate [N-phosphonomethyl glycine] is an active ingredient of herbicides applied to annual and perennial weeds. Glyphosate inhibits 5-enolpyruvilshikimate-3-phosphate synthase, the enzyme of shikimic acid synthesis that participates in the synthesis of aromatic amino acids. Glyphosate is a broad-spectrum, non-selective systemic herbicide that can be used to control most weeds, both annual and perennial plants, under many varied situations such as agriculture, forestry, orchards, vineyards, industry and no-till cropping systems and has been domesticity classified as an easily degradable herbicide in the past. Although glyphosate is believed to be a relatively safe compound, in our days, reports can be found that glyphosate has negative effects on human health. In addition, nowadays, glyphosate and one of its principal metabolites (aminomethylphosphonic acid) have frequently been detected in ground water. The application of glyphosate results in the yellowing and decay of leaves within 5–10 days caused by the breakdown of aromatic amino acids synthesis. Penaloza-Vazquez et al. (1995) reported that glyphosate remains unchanged in the soil for varying lengths of time, because of its adsorption on clay particles and organic matter present in the soil. This condition makes this herbicide very persistent in soils and sediments.

Biodegradation of glyphosate

Glyphosate is an amphoteric and non-volatile compound, no photo degradation happens and it is stable in air. It is practically insoluble in most of organic solvents, for instance, ethanol, acetone and benzene, because of its high polarity, but it is completely soluble in water. The degradation of glyphosate is slower in soils with a higher adsorption

capacity. Degradation rate was also affected by the particular microbial community of each soil. Microorganisms known to degrade glyphosate include *Pseudomonas* sp., *Arthrobacter atrocyaneus* and *Flavobacterium* sp. The primarily metabolite of glyphosate is aminomethylphosphonic acid, which is non-toxic and degraded by microbes at a somewhat slower rate than the parent compound (Carlisle & Trevors, 1986).

Pendimethalin

Pendimethalin (N-(1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitrobenzenenamine, a dinitroaniline herbicide) is both a preemergence and early post-emergence herbicide, used for control of most annual grasses and many annual broad-leaved weeds in crop fields. Pendimethalin was first registered as a pesticide in the United States in 1972. Pendimethalin has relatively long persistence in soil due to immobilization with low leaching potential or due to hydrophobic nature of pendimethalin adsorb strongly to organic matter and clay minerals. It degrades more rapidly in anaerobic soil than in aerobic soil conditions (Megadi et al., 2010). Its mode of action is inhibition of mitotic cell division in developing root systems.

Biodegradation of pendimethalin

Biodegradation of pendimethalin in soil was reported by several investigators under both aerobic and anaerobic conditions with bacteria and fungi in different types of soils (Zheng & Cooper, 1996). Four metabolites were formed and identified as N-(1-ethylpropyl)-3,4-dicarboxy 2,6-dinitrobenzenamine- N-oxide, N-(1-ethylpropyl)-3,4-dimethoxy-2,6-dinitrobenzenamine and benezimadazole-7-carboxyaldehyde. The reactions involved were monohydrolysis of 2- methyl groups followed by dihydrolysis. Further oxidation of amine groups and hydroxylation of propyl groups produced the above-mentioned metabolites.

Paraquat

Paraquat (1,1-dimethyl-4,4-bipyridinium) is a quaternary nitrogen herbicide widely used for control of broadleaf weed. It is nonselective compound that destroys green plant tissue on contact by disrupting photosynthesis and rupturing cell membranes, which allows water to escape leading to rapid desiccation of foliage. Paraquat has strong affinity to bound to clay minerals and organic matter in the soil.

Biodegradation of paraquat

Paraquat is considered a toxic to soil fungi and bacteria causing a reduction in their population (Sahid et al., 1992). Several bacterial and fungal isolates obtained from soil and waste water can degrade paraquat. The contact herbicide paraquat is used to control weeds in a wide range of crops. When paraquat enters the soil environment, it is rapidly and strongly

bound to clay minerals and organic matter and deactivated. Various studies have used pure cultures of soil micro-organisms to elucidate the degradative pathways of ring-labeled paraquat. These studies established the range of bacteria and fungi able to degrade paraquat (e.g.*Corynebacterium fascians* Dows, *Lipomyces starkeyi* Loo and Rij, *Aspergillus niger* van Teigh, *Penicillium*. These studies established the range of bacteria and fungi able to degrade paraquat (e.g. *Corynebacterium fascians* Dows, *Lipomyces starkeyi* Loo and Rij, *Aspergillus niger* van Teigh and *Penicillium frequentans* West, *Fusarium* sp and *Pseudomonas* sp) and that conditions in soil solution are conducive to the degradation of paraquat. In majority of the cases, however, paraquat degradation was shown to be extremely variable and evidence of only one degradation product other than CO2 has been reported, the N-methyl betaine of isonicotinic acid (Funderburk & Bozarth, 1967). The latter product has, however, been shown to be a major intermediate in the photolytic degradation of paraquat (Slade, 1965).

Factors effecting microbial degradation of herbicides

Based on the information, it has been found that the inoculums size, amounts of additional co-substrates carbon and nitrogen compounds, organic matter of soil and pH are the major factors that affected the extent and rate of herbicides degradation. Agricultural soils are rich in nitrogen due to routine fertilization, and most atrazine-degrading bacteria use atrazine as a nitrogen source. However, Yang et al. (2010) reported members of *Klebsiella* sp. A1 and *Comamonas* sp. A2 capable of degrading atrazine and are insensitive to exogenous nitrogen sources. Wang et al. (2013) reported addition of both carbon and nitrogen sources promotes degradation rate of atrazine. Struthers et al. (1998) reported that addition of *Agrobacterium radiobacter* J14a cells in soil resulted in two to five times higher mineralization of atrazine than in the non inoculated soil. However, sucrose addition did not result in significantly faster mineralization rates or shorten degradation lag times. The mineralization of GP in soils is individually regulated and correlated by exchangeable H+, soil pH, oxalate extractable Al3+ and bacterial cell numbers. Recently, Al-Rajab & Hakami (2014) studied GP degradation in three agriculture soil and reported rapid degradation with a half-life of 14.5 d in the silt clay loam soil incubated at 20^{0C}. Johnson & Sims (2011) studied solvent toxicity in soil for bioavailability of 2,4-D toward microorganisms and suggested that solvent toxicity should be balanced with uniformity of substrate distribution when using organic carriers in soils.

Conclusion and future directions in biodegradation of herbicides

Due to excessive use of herbicides, ecosystems are under threat of its pollution. Microbes are the main vehicle for remediation of herbicides, and new discoveries, such as novel

biodegradation pathways, multispecies interactions and community- level responses to herbicides addition, are helping us to understand, predict and monitor the fate of herbicides. With the recent release of new metagenomic information from herbicides-associated environments determining the microbial players offers the promise of new discovery. Genomic, transcriptomic and mutant studies conducted with defined co cultures are yielding new information about how microbes interact and benefit energetically via different mechanisms of interspecies electron transfer. Despite this, there are many challenges, not least because of the heterogeneity of these ecosystems and the structure of herbicides. For example, there is growing awareness about the toxicity of the herbicides, which are difficult to degrade. Microbial metabolism is a process of energy conversion, and it is governed by enzymatic mechanisms, where reaction intermediates play a vital role. Screening of organisms that degrade herbicides or produce enzymes or enzyme systems that degrade herbicides may prove as environmentally profitable in the present time. A screening program for such organisms and enzymes is required but will require more universally uniform standards for assessment of their degradative ability. Current research provides an understanding of how the evolution of promiscuous enzymes and the recruitment of enzymes available from the metagenome allows for the assembly of biodegradation pathways. Nevertheless, physicochemical constraints including bioavailability, bioaccessibility and the structural variations of similar chemicals limit the evolution of biodegradation pathways. Further study is required in genetic modification of microorganisms. The use of gene probes for studying the distribution of set of genes in herbicides contaminated soils will be useful in identifying niches in which these kinds of genes prevail and the conditions under which the population of microbes bearing these genes increases. Phytoremediation in conjunction with rhizospheric microbes may provide a cheap, fast, eco-friendly and efficient rhizoremediation processes for the removal of explosive waste from the upper layers of the soil. The implementation of advanced technologies such as proteomics and bioinformatics should be investigated to provide more knowledge regarding the enzymatic mechanisms and intermediates involved in metabolic activities during biodegradation. In this regard, research may be also focused on examining the possibility of using the enzymes rather than the microorganisms in biological treatment. To sum it up, the synergistic performance of various microorganisms and various technologies is to be considered a research topic.

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