



REVIEW ARTICLE

Biomixture-mediated pesticide biodegradation in biobeds: Insights into physicochemical and biological influencing factors

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Abstract

Agricultural farms are prone to pesticide contamination, with handling and disposal occurring before and after each spray. These activities, which result in point-source contamination, are the main cause of soil and water pollution (surface and groundwater). Farm contamination by pesticides is bioremediated through a system called a biobed. The study aims to evaluate the effects of physicochemical and biological factors on degradation efficiency and to optimise the system for improved performance. At least 50 % lignocellulosic substrate was required for effective degradation of the pesticide. Among coconut fibre, rice husk and coffee husk, coconut fibre exhibited greater pesticide degradation due to its higher carbon/nitrogen (C/N) ratio. Degradation of pesticides was increased with cotton fibre from 50 to 81.7 %. Novel biomixture's containing amendments such as ash, biochar and digestate increased the biomixture's removal efficiency. Even complete sorption of fipronil was achieved at the lowest level of wheat biochar (1 %). Temperature varied 13–20 °C increased the degradation rate and 99 % 2,4-D degraded within 21 days. Optimal pesticide degradation was achieved at 60 % of the water-holding capacity. The dissipation rate of pesticides was higher under a 15-day pre-incubation period than under a 7-day period. Bioaugmentation with microorganisms including *Anthracoophyllum discolor*, *Streptomyces* sp., *Phanerochaete chrysosporium*, *Lentinula edodes* and *Trametes versicolor* increased pesticide degradation. For example, *A. discolor* degraded atrazine 18 times faster than the control.

Keywords: biobed; biomixture; degradation; microbes; pesticides; sorption

Introduction

Pesticides are an essential part of contemporary farming, effectively controlling pests and diseases and thereby contributing to achieving self-sufficiency in food production. The agricultural sector remains the largest consumer of pesticides, accounting for about two-thirds of global pesticide usage (1). However, the excessive use of pesticides has induced adverse effects on the environment and health (2). Prolonged exposure to pesticides leads to reproductive disorders, endocrine disruption, respiratory problems, neurological changes, diabetes and cancer (3). It harms non-target species and beneficial organisms such as the living system of soil, arthropods, vegetation, birds, etc. that maintains the ecological balance (4). It has been reported that less than 1 % of applied pesticides actually reach their intended targets in agricultural fields, while the remainder is dispersed into soil, water and air, causing environmental pollution. Pesticides enter the environment through various pathways, including spray drift, runoff, leaching and subsurface drainage (5). Approximately 40–90 % of surface water contamination results from direct pesticide losses. These direct losses arise from spillages during equipment filling operations, pesticide mixing, leakages from the sprayer, leftover solutions and

rinsate disposal and contribute to point source pollution (6). These point sources are primarily associated with areas where filling and cleaning operations occur. Spillages or leakages of small quantities during the handling of pesticides impose a serious contamination risk. Residual pesticides in empty containers, spraying equipment and rinsates pose significant risks. However, many farmers discard leftover pesticide solutions and wash sprayers and containers in home yards, nearby rivers, canals, or farm water sources (7). Spilling just a few millilitres of the formulated product can release about 1 g of active ingredient. To dilute this quantity to a safe concentration of 0.1 µg L⁻¹, up to 10000 m³ of water would be required (7). Inappropriate handling and disposal of pesticide waste cause groundwater and surface water pollution, posing significant hazards not only to farmers but also to non-target organisms, especially when safety measures are not in place. Therefore, it is necessary to address the point source of contamination, particularly the improper handling and disposal of pesticides on farms. Biobed is a low-cost bioremediation system that has been successfully proposed to destroy/remove pesticides from wastewater. The biomixture component provides an adsorption site for pesticide retention, followed by microbial degradation over time. Biobeds are particularly effective in treating point-source

contamination from equipment washing effluents, improper disposal of pesticide residues, accidental spills and pesticide wastewater (8). This technology has been successfully accepted in several countries and implemented for pesticide decontamination. The present study introduced distinct designs of the biobed system for use under specific environmental conditions. The system could be modified easily according to the local conditions, making it more widely applicable. This review aimed to explore factors influencing biobed efficiency and to optimise them to improve performance.

Biobed system

A biobed is a type of biopurification system used on farms to absorb and break down pesticide waste, generated during spray tank filling, from leftover pesticide in containers and equipment and from rinsate produced during washing. Original biobed structure, developed in Sweden, consists of three components: (1) Grass layer, (2) Biomixture, (3) Clay layer. Each component of the biomixture plays a significant role in the biobed's performance.

Grass layer is the topmost cover of the biobed pit. It preserves moisture content and inhibits surface crusting through evapotranspiration. The rhizosphere of the grass layer helps retain pesticides, regulates their downward flow and provides a site for pesticide biodegradation (1).

Biomixture, core component of biobed system which is blended mixture of straw, peat and soil -2:1:1 (v/v/v) (2). (i) Straw (lignocellulosic biomass) is a primary substrate that induces the growth of white-rot fungi for microbial activity of the system. The inherent fungal population in biomixtures may contribute to pesticide biodegradation in the biobed. These fungi produce

peroxidases and laccases, which are associated with the oxidation of organic contaminants and degrade them. The fungi release oxidative enzymes, including laccases and peroxidases, to detoxify a variety of contaminants (9). Phenoloxidase activity and basal respiration are related to the quantity of straw components in a biomixture, which has been shown to be linked to the dissipation of certain pesticide groups. (ii) Peat (humic substances), a partially decomposed substrate containing high organic matter, increases porosity, enhances sorption and controls pH and moisture. Addition organic matters lower the pH of the mixture that regulates favourable condition for fungal growth (10). (iii) Soils with high cation exchange capacity offer sorption sites that retain pesticides for an extended period, reducing the risk of downward movement of contaminants. It supports the biodegradation of pesticides by bacteria and also provides food to microorganisms. Clay, an impermeable layer placed at the bottom of the system, with a thickness of 10 cm.

Types of biobeds

Biobeds are classified as unlined or lined based on the system's isolation from the surroundings. Various biobed designs have been described and depicted in Fig. 1.

Unlined biobed: Biobed materials are in direct contact with ground surface and no artificial impermeable layer is placed between them that isolates it from the ground. The biomixture fills the excavation and its boundary material is soil. In numerous instances, a clay layer is placed on the base of the biobed.

Lined biobed: It is similar in design to an unlined biobed, but is separated from the ground by a synthetic, impermeable barrier

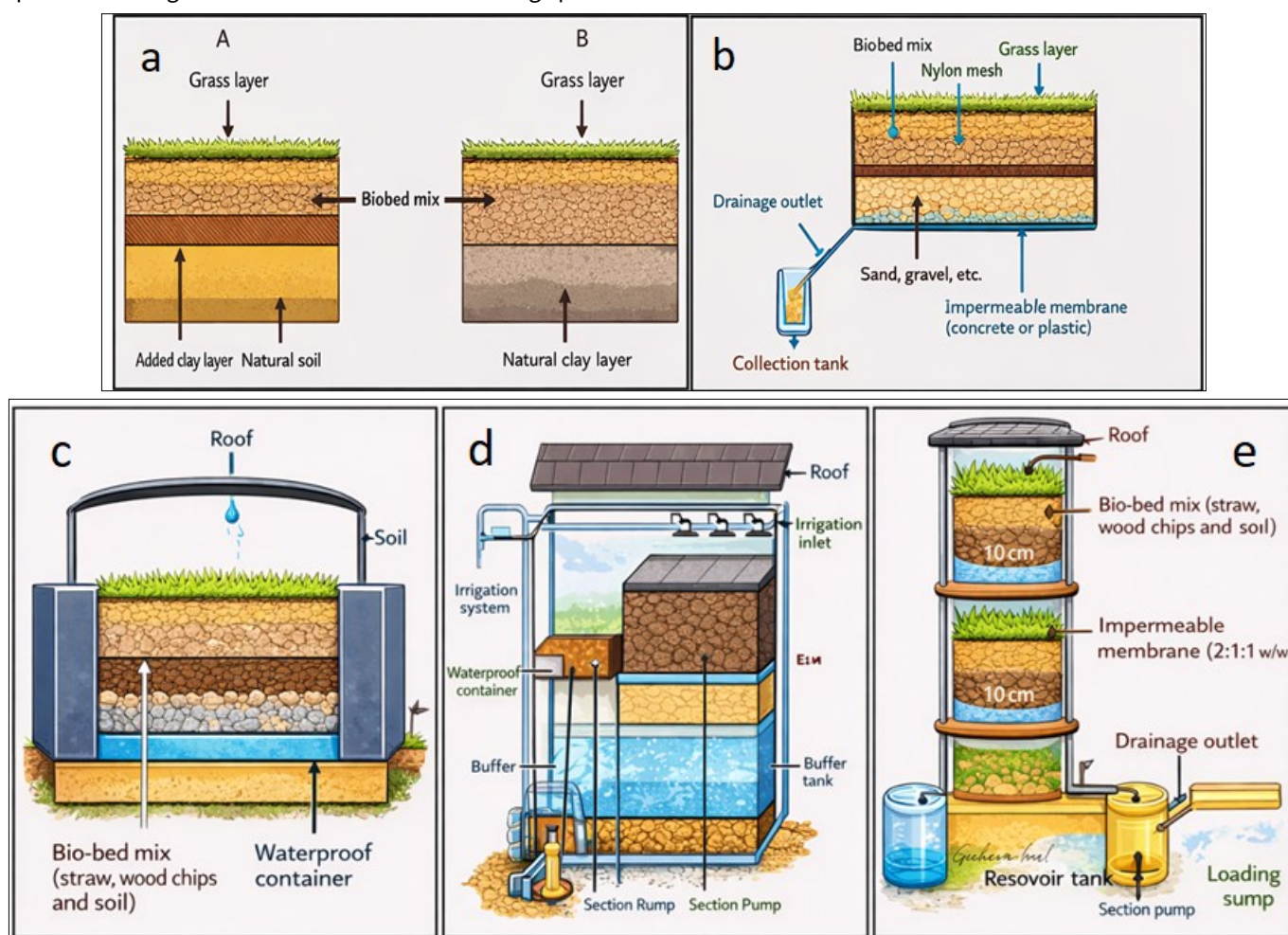


Fig. 1. Schematic representation of biobeds. (a) Unlined biobed; (b) Lined biobed; (c) Phytobac; (d) Biomassbed; (e) Biofilter.

(such as tarpaulin, concrete or plastic). This design makes it easier to pump leachate from the base of the biobed or to drain water into a nearby tank. This includes the original Swedish-designed biobed without a water collection system. This system provides favourable circumstances that allow pesticide degradation.

Engineered biobeds

The biobed design was easily adaptable to local conditions, which attracted global attention. It is known by various names in different countries, such as Phytobac® in France, Biobac in Belgium, Biofilters in Switzerland, Vertical Green Biobed in Guatemala, Biodep and Biomassbed in Italy and Biobed in the UK and Sweden. Adapting the biobed to different climates, ensuring that ingredients for the biobed mixture are available and reducing or eliminating the risk of pesticide leakage from the system are the goals of the changes to the basic biobed design. They are structurally different in their suitability for various climatic conditions, as shown in Fig. 1.

Phytobac®: Phytobac® developed in France by “Rhône-Poulenc Agro France”, now “Bayer CropScience”, resembles a lined biobed (11). It is constructed with a 60 cm water container, having concrete to allow complete retention of pesticide residue. It is anchored in the ground and the basin's sides are kept 30 cm above the ground to prevent flooding into the pit. A roof is installed to shield the whole system from precipitation (12). Even at high concentrations, the substrate in this system, composed of 70 % agricultural dirt and 30 % chopped straw, readily breaks down pesticides within a year. Since there isn't a grass layer on top, water evaporates from the system with ease. Controlling the system's moisture content is necessary to prevent waterlogging and excessive drying. Problems arise with the use of this system, including: (i) it requires large installations; (ii) bulk substrate is needed to prevent saturation or overflow; (iii) it creates problems during rainfall and (iv) a homogenous mixture is required for filling the pit. Upper layers tend to dry out quickly, increasing the potential for pesticide leaching.

Biobac: Biobac is developed by the National Institute of Agricultural Research in France. It is a tank filled with farm dirt and straw that is isolated from the subsoil. It was believed that the addition of C in the form of straw might maintain the natural detoxifying dynamics of the soil microflora and that agricultural soil would likely contain bacteria that could break down pesticides employed on the farm (12).

Biomassbed: The biomass bed was installed in Italy to create a useful system that could withstand the long dry seasons of the Mediterranean without compromising effectiveness. Although the biomass bed and lined biobed are structurally identical, the biomass bed has a tank to collect drained water. The bio-bed mix was made from locally available materials, including one part chopped vine branches, two parts green compost and two parts dirt. To collect leachate, it has a buffer tank. The first step in construction is to excavate a hole and insert a polyethylene tank into it. The plastic tank is divided into two sections by a metal grid that is positioned around one metre from the bottom. The biobed mix is kept in the upper half and leachate is collected in the lower part. Lining the metal grid with a layer of sand, a plastic net and a nylon filter improves drainage. In order to maintain moisture at an ideal level for biodegradation. An irrigation system is located at the top of the tank. The leached water is pumped back into the biobed mix. Typically, the system is covered with a roof to keep rainfall out of the biomass bed.

Biofilters: A modified version of the biobed, the biofilter, was developed in Belgium to handle large volumes of waste water. It consists of plastic tanks (1 m³) stacked vertically and connected by plastic pipes and valves. The number of tanks depends on the wastewater volume to be treated. A three-unit system is recommended for larger pesticide loads, while a two-unit system is appropriate for treating smaller volumes (less than 3000 L) and for pesticide loads between 1 and 100 g of active ingredient (12). Local soil, straw and peat/ compost are combined to create the biofilter substrate. Research indicates that the biofilter retained its adsorption and degradation capabilities when composted farmyard manure was utilised in place of straw and/or peat. According to reports, the breakdown of the applied pesticides was much enhanced when the substrate was recycled after a year by adding fresh organic material (straw and manure) (11). Channels may result from a biofilter issue, most likely due to the absence of a grass layer, which creates structural heterogeneities in the mixture.

Mechanisms of removal of pesticides

The efficiency of pesticide removal in a biobed is primarily based on two key mechanisms: sorption and degradation. When contaminated water is introduced into the biobed, pesticides are initially adsorbed onto the organic matter present in the biomixture. This adsorption process helps restrict the downward movement of contaminants, preventing their leaching into the environment. Over time, the sorbed pesticides become bioavailable for microbial degradation, where microorganisms break them down into less harmful compounds. This combined action of sorption and degradation enhances the effectiveness of biobed systems in removing pesticides from contaminated water. Pesticide removal via sorption and degradation in a biomixture is outlined in Fig. 2.

Sorption of pesticides on biomixture

The process of adsorption involves the concentration of constituents at the interface between two-phase systems, such as solids-liquids, gases-liquids, or gases-solids. Absorption, on the other hand, describes the process of transferring components between bulk states. Adsorption may be considered a beneficial mechanism that prevents pesticides from moving downward, thereby reducing the risk of groundwater and subsurface pollution. The characteristics of the pollutants and the substrate affect the sorption rate. The adsorption process mainly occurs on the lignocellulosic material in the biomixture, although it is also supported by soil and peat/ compost. They lock the pesticides within their matrix, preventing leaching into soil and groundwater. The sorbed pesticides should be reversible or bioavailable for further microbial degradation so that they can be decontaminated (13).

Degradation of pesticides in biomixture

Biobed is a type of bioremediation system in which microbes are the main agents of pesticides degradation. Mainly two microorganisms, fungi (especially white-rot fungi) and bacteria, biotransform or biodegrade the pesticides in the biomixture. Lignocellulosic material promotes the growth of white rot fungus (lignin-degrader) while the bacterial population is supported by the soil in biomixture (13).

Degradation mechanism of pesticides by white-rot fungi: The white-rot fungi contain lignin-degrading enzymes, polyphenoloxidases and peroxidases. Furthermore, peroxidases contain three important enzymes: lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and versatile peroxidase

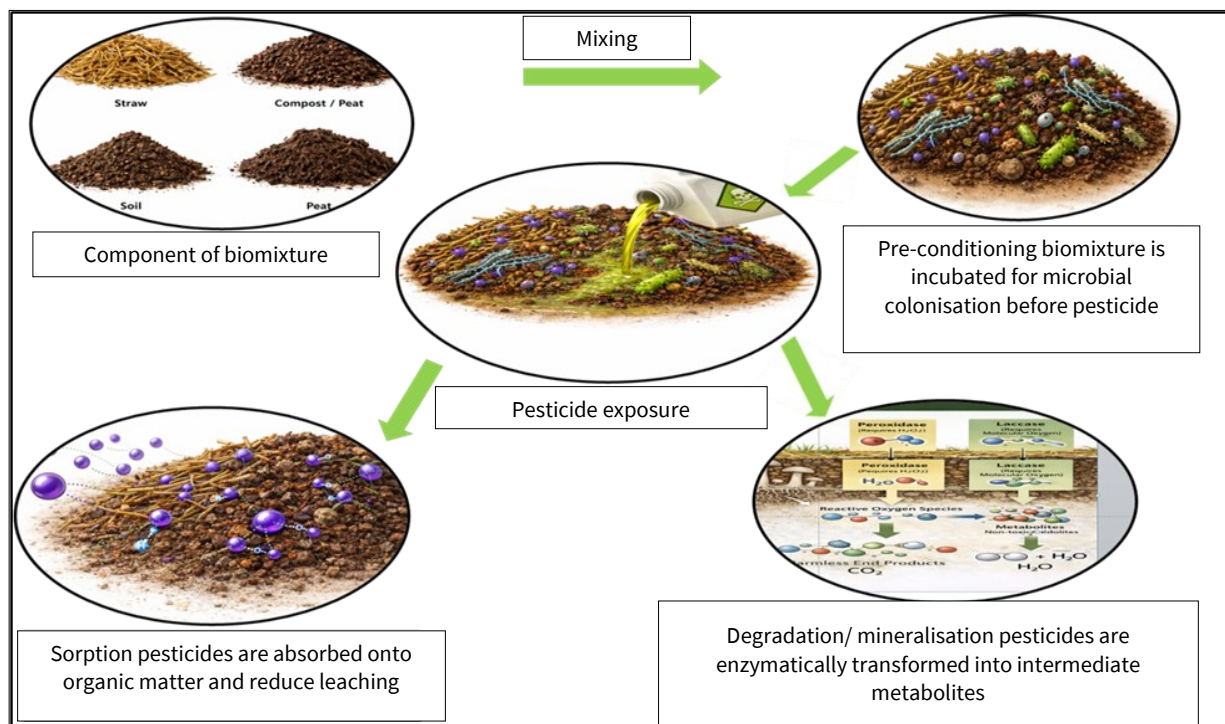


Fig. 2. Stepwise pesticide removal via sorption and degradation in biomixture.

(VP), which belong to the superfamily of nonanimal heme peroxidases. The enzymatic mechanism of peroxidase is illustrated in Fig. 3. Both laccases and peroxidases degrade organic substrates (pesticides) via oxidation.

Laccases use O_2 as an electron acceptor and oxidise a variety of organic molecules through a series of intermediary steps. Laccases have high redox potential; they oxidise aromatic compounds, especially phenols, polyphenols and methoxyphenols. Laccases only oxidise non-phenolic organic molecules when electron-transfer mediators are present. These mediators of laccases are produced during WRF metabolism (14). Peroxidases utilise hydrogen peroxide or organic hydroperoxides as electron acceptors in the oxidation process of organic substrates. Oxidation of organic compounds involves the formation of intermediates. During this process, hydrogen peroxide (H_2O_2) binds to the enzyme's heme group, facilitates the liberation of a water molecule and the enzyme returns to its inactive state through electron extraction from the reducing substrates (15).

Manganese peroxidase (MnP) catalyzes the oxidation of Mn^{2+} to Mn^{3+} in the presence of H_2O_2 . The resulting Mn^{3+} ions are stabilised through chelation with organic acids. These chelated Mn^{3+} complexes act as diffusible redox mediators, enhancing the oxidation of a wide range of organic substrates (16).

Lignin peroxidase, oxidises veratryl alcohol with catalytic action by H_2O_2 -mediated oxidation that acts as an oxidation-reduction mediator. The oxidised veratryl oxidises the non-phenolic aromatic residues in lignin, generating aryl cation radicals through a series of subsequent reactions. These radicals are ultimately mineralised (16).

Versatile peroxidase is regarded as a structural and functional hybrid of MnP and LiP, enabling broader oxidation of electron donors. The broad substrate oxidation ability of ligninolytic enzymes (LMEs) has been recognised as a key factor in the bio-transformation of various organic contaminants (16) (Fig. 3).

Degradation mechanism of pesticides by earthworm: Soil microorganisms are widely recognised as key agents in the

bioremediation of pesticide-contaminated environments. However, over the past decade, soil fauna has increasingly been incorporated into bioremediation efforts to restore polluted soils. Incorporating soil fauna into bioremediation strategies not only aids in the direct degradation of pollutants but also enhances soil structure and nutrient cycling. Recent advancements in bioremediation have highlighted the significant role of soil fauna, such as earthworms. As they interact with soil microorganisms, they contribute to a more robust, efficient breakdown of contaminants, leading to improved soil health and sustainability over time.

Earthworms contribute to pesticide degradation through two primary mechanisms: directly by releasing enzymes that detoxify pesticides in the gastrointestinal tract; and indirectly by stimulating and dispersing pesticide-detoxifying microbes along their crawling paths. Earthworms have the ability to break down pesticides in their digestive tract and in the soil where they secrete casts and make burrows. Another important factor in pesticide degradation is the earthworm species. The extent of degradation varies among species and is influenced by their ecological classification. Based on the habitat and feeding patterns, earthworms can be generally divided into three ecological groups: anecic (deep-burrowing), endogeic (topsoil-dwelling) and epigeic (surface-dwelling). Endogeic (topsoil-dwelling) and anecic (deep-burrowing) earthworms, which consume soil and engage in burrowing, exert a stronger influence on the breakdown of pesticides than epigeic earthworms. Pesticide breakdown is facilitated by earthworms in two major pathways: (i) the digestive canal (internal processes) and (ii) the burrow walls (external processes) along the microenvironment (17). Two mechanisms of pesticide degradation by earthworms are presented in Fig. 4.

Internal or direct detoxification process: The process induces pesticide degradation in earthworms' digestive tracts, in association with enzymes that detoxify pesticides. The enzymes generated by the gastrointestinal tract (glycolytic digestive enzymes) and symbiotic gut bacteria break down ingested organic materials. Enzymes called carboxylesterases, secreted by the gastrointestinal tract, can break down chemical pesticides. This

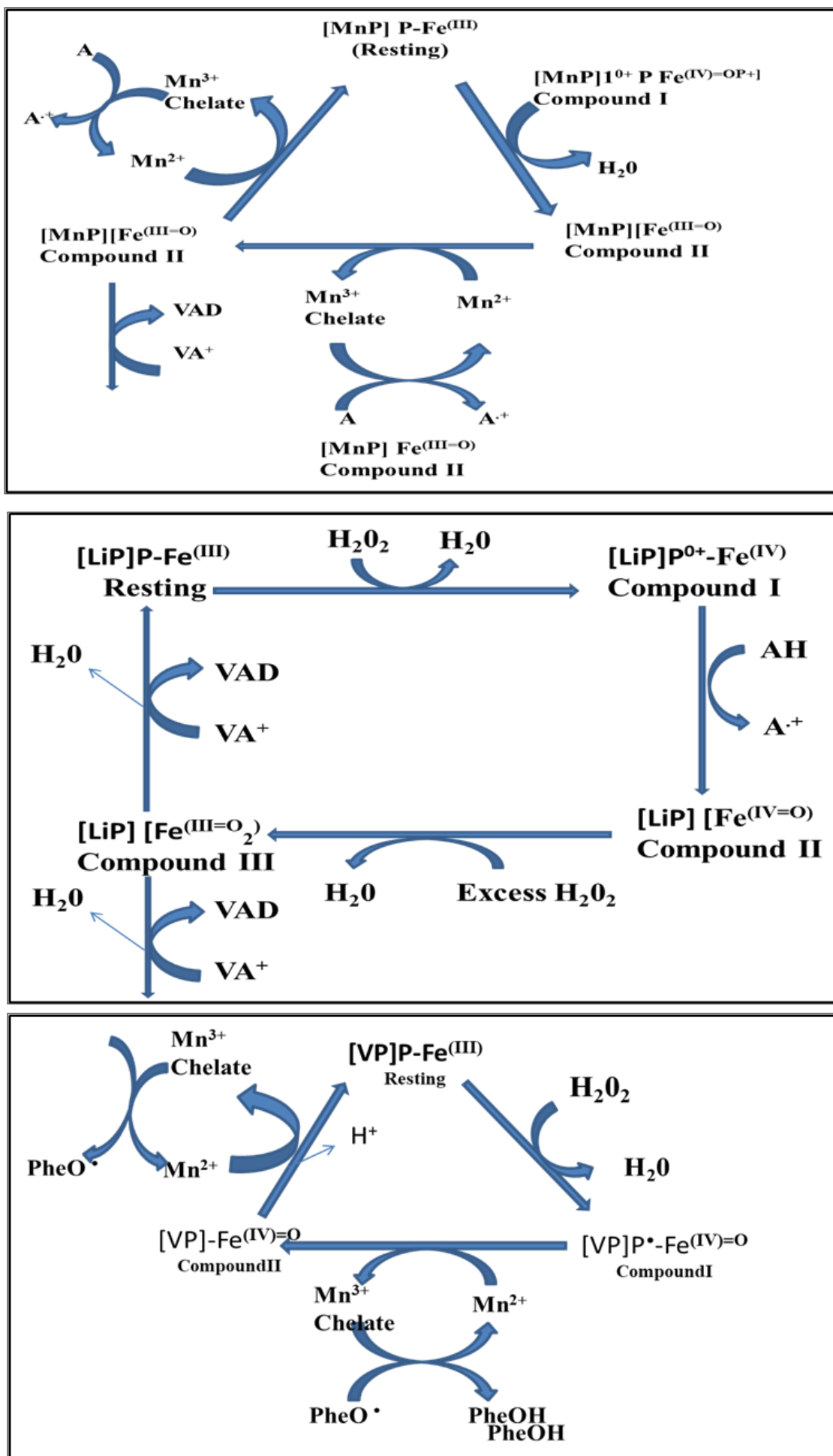


Fig. 3. (a) Catalytic cycle of manganese peroxidase (MnP); (b) Lignin peroxidase (LiP) mechanism; (c) Catalytic cycle of versatile peroxidase (VPLs).

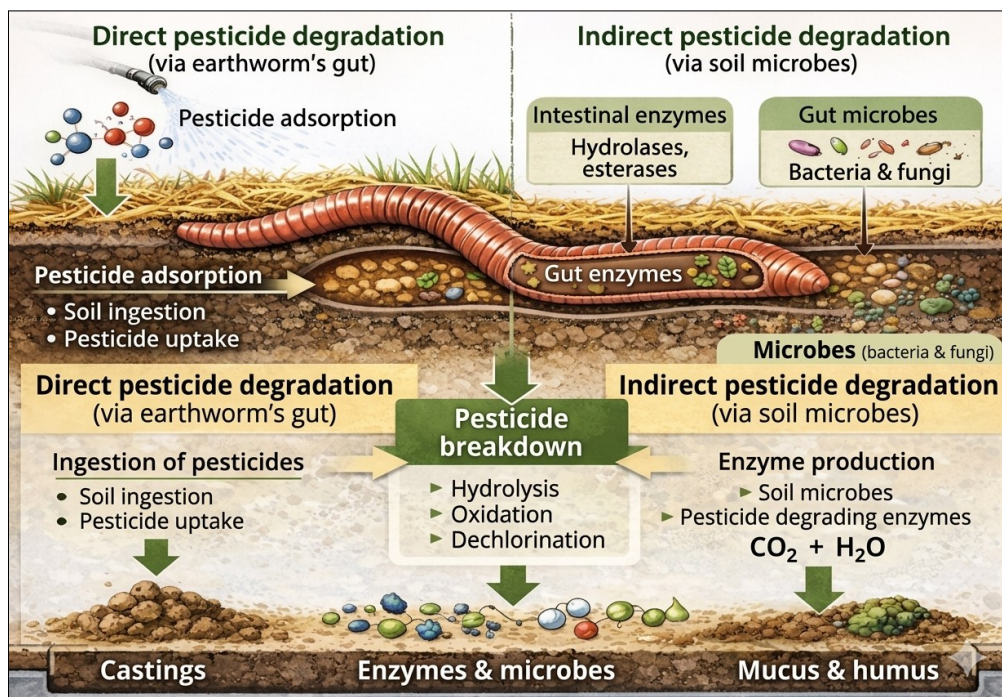


Fig. 4. Direct and indirect degradation mechanisms of pesticides mediated by earthworms.

enzyme catalyses the hydrolysis of synthetic pyrethroids, thereby detoxifying them into their acid and alcohol moieties. The irreversible attachment of carboxylesterases to the oxon molecule of toxic metabolite inactivates the organophosphorus insecticides. The same mechanism is employed for the carbamate group. However, chemical pesticides significantly affect the magnitude of inhibition (18).

External or indirect detoxification processes: Earthworm activity significantly enriches the surrounding soil with diverse microbes and enzymes, primarily through their casts and burrows. Earthworm casts contain high concentrations of extracellular enzymes, including phosphatase, protease, alkaline phosphatase, dehydrogenase and β -glucosidase. These enzymes stay active and grow more stable when they bind to the soil's organo-mineral moiety. Pesticide adsorption is enhanced by the drilosphere, which consists of organic detritus from earthworm activity along burrow walls. Earthworm burrows exhibit a distinct chemical composition that affects the translocation of pesticides via sorption onto organic ligands and biodegradation. It has been demonstrated that atrazine and its metabolites are less likely to move into the environment when *Lumbricus terrestris* is present in the soil (19). The carboxylesterase enzyme produced by earthworms acts as a molecular scavenger for organophosphorus pesticides in soil.

Factors affecting biobed performance

Biobeds create a favourable environment for microorganisms intended to remove high loads of pesticide wastes. Removal processes facilitate sorption and degradation. When wastewater containing pesticides is applied to a biomixture, it initially sorbs onto the organic substrate and is subsequently released in a bioavailable form for microbial decomposition. Pesticide adsorption in biomixtures must be prolonged sufficiently to minimise the threat of rapid downward movement that shields microflora from exposure to pesticides. The biomixture is a core component, made up of straw, peat or compost and soil, which mainly influences the sorption and dissipation of pesticides in biobed system. The composition of the biomixture significantly influences system performance; therefore, selecting appropriate materials and

optimising their ratios are key strategies for maintaining ideal conditions for pesticide removal. Extensive research from 1997 to 2025 has focused on developing biomixture formulations using locally sourced lignocellulosic and humified materials for pesticide treatment, as shown in Table 1. Recent studies developed novel biomixtures that include ash, biochar and digestate and vermicompost as supplementary or alternative constituents. Further, the performance of biobed performance system (BPS) can be influenced by other variables, such as temperature, water content (moisture), preconditioning, pesticide application, including its rate, co-application (more than one pesticide), frequent application of the same pesticide and mixtures.

Effect of nature and amount of lignocellulosic biomass

Lignocellulosic material encompasses a wide range of agricultural residues and is an indispensable component of the biomixture. The amount of lignocellulosic substrate is crucial in the biomixture, as sorption and degradation depend primarily on it. Straw was optimised to 50 % in a classic biomixture. A preliminary study suggested a high proportion of straw increased microbial activities and pesticide dissipation (20). Multiple studies followed the original composition of the biomixture, which degrades diverse classes of pesticides to varying extents, depending on their physicochemical properties listed in Table 1. Various agricultural residues of vegetable/fruit, fibre crop, oilseed crop and tree residues showed successful alternatives to cereal straw in treating high loads of pesticide contamination, as summarised in Table 2. Among the biomixtures of various agricultural residues, corn cobs (CC), spent mushroom substrate (SMS), orange peels (OP), sunflower crop residues (SFR) and olive leaves, grape stalks (GS), the most effective was the GS-mixture. Although the CC- and SFR-mixtures fared similarly to a STR-mixture. Furthermore, the pesticide mixture was effectively degraded by the SMS-biomixture, with degradation rates being associated with the quantities of SMS (21). Three lignocellulosic materials (50 %), coconut fibre, rice husk and coffee husk, coconut fibre efficiently degraded pesticides oxamyl, thiophanate-methyl, hexaconazole, fluzifop-p-butyl, carbendazim, epoxiconazole, propiconazole, triadimefon, chlorpyrifos,

Table 1. Composition of a traditional biomixture consisting of straw, peat and soil (2:1:1 ratio)

Lignocellulosic component	Humic component	Composition lignocellulose/humic/soil	Pesticide degradation
Wheat straw	Peat	2:1:1	Metamitron, chloridazon, methabenzthiazuron, terbuthylazine, metribuzin, isoproturon, linuron
			Azoxystrobin, bentazone, bromoxynil, fenpropimorph, ioxynil, dimethoate, diuron, fluazifop-p-butyl, prosulfocarb, glyphosate, propiconazole, kresoxim methyl, mecoprop-P, metha-benzthiazuron, MCPA, pirimicarb, propyzamide, metamitron, isoproturon, terbuthylazine, linuron, metalaxyl, chlorpyrifos, metribuzin and chloridazon
			Chlorpyrifos, iprodione and atrazine Iprodione Dithiocarbamates
	Compost	2:1:1	Azoxystrobin, bentazone, diuron, bromoxynil, ioxynil, dimethoate, fluazifop-p-butyl, glyphosate, kresoxim methyl, MCPA, mecoprop-P, fenpropimorph, pirimicarb, propiconazole, prosulfocarb, metamitron, isoproturon, metalaxyl, pendimethalin, propyzamide chlorothalonil, epoxiconazole
			Indoxacarb, buprofezin, metribuzin, terbuthylazine, azoxystrobin iprodione, dimethoate and metalaxyl
		2:1:1	Imazalil, thiabendazole, ortho-phenylphenol and diphenylamine
		2:1:1	2,4-D, bromoxynil, thifensulfuron-methyl, tribenuron-methyl, pyrasulfotole, thiencazabone-methyl and metsulfuron-methyl
		2:1:1	Chlorpyrifos
	River waste	1:0:1 2:1:1	Glyphosate

Table 2. Composition of biomixture with alternative lignocellulosic substrate

Lignocellulosic component	Humic component	Composition lignocellulose/humic/soil	Pesticide
Alfalfa straw (As), wheat stubble (Ws)	Peat	2:1:1	Atrazine, carbendazim, chlorpyrifos, isoproturon, iprodione and diazinon
Barley husks, pine sawdust and straw	Peat	2:1:1	Atrazine, chlorpyrifos and isoproturon
Barley husks /oat husk/saw dust/straw	Cow manure, garden waste compost, peat	2:1:1	Isoproturon, metalaxyl, bentazone and linuron
Straw, willow chopping	Compost	2:1:1	Metalaxyl, isoproturon, bentazon and chlorpyrifos,
Vine straw	Compost	2:2:1	Chlorpyrifos, metalaxyl and cymoxanil
	grape marc and olive leaves composts	2:1:1	Terbuthylazine, metribuzin, metalaxyl iprodione and chlorpyrifos
Corn cobs straw, grape stalks	Dried cow manure, peat mix	2:1:1	Metalaxyl, isoproturon, metamitron, linuron and bentazone
Coco chips	Seed residues compost	2:1:1	Chlorpyrifos and terbuthylazine
Cotton flower	Olive leaves compost, peat	2:1:1	Metribuzin, dimethoate, indoxacarb, metalaxyl buprofezin, terbuthylazine, azoxystrobin and iprodione
Straw, sunflower crop residues (SFR), olive leaves, grape stalks (GS), orange peels, corn cobs (CC) and spent mushroom substrate (SMS)	Olive leaves, compost, spent mushroom, grape stalk, substrate, orange peels,	2:1:1	Metribuzin, buprofezin, metalaxyl, azoxystrobin, iprodione, chlorpyrifos, dimethoate, indoxacarb and terbuthylazine
Biochar produced from pine woodchips	Vegetable compost	2:1:1/1:0:1	2,4-D, carbofuran, glyphosate, atrazine and diazinon
Sisal pulp, corn stover, seaweed	Olive cake and its vermicompost	2:1:1	Dimethoate, imidacloprid, oxyfluorfen, diclofenac, ibuprofen and triclosan Carbofuran
Coconut fibre	Peat and compost	2:1:1	Herbicides (triazines), fungicides (triazoles) and insecticides (organophosphates)
			Linuron, mixtures chlorpyrifos/metalaxyl and malathion/dimethomorph and antibiotics
Rice straw/ corn cob	Farm yard manures (FYM)	2:2:1	imidacloprid

imidacloprid, tebuconazole and triadimenol because of a higher carbon/nitrogen (C/N) ratio. Microbial and enzymatic activities are positively influenced by the substrate C/N ratio and because the C/N ratio of cotton fibre was highest, this ultimately enhanced degradation. In view of previous findings, coconut fibre was used in biomixtures and their compositional ratios were optimised to 29:7.3:63.7 and 11:7.3:81.7 of compost, soil and coconut fibre. Both compositions exhibited greater pesticide degradation than the traditional composition (50 % lignocellulosic material). The highest was observed at 11:7.3:81.7, as a high substrate amount facilitates a high C/N ratio, which supports microbial growth, activity and pesticide degradation (22). New formulations of various biomixtures are presented in Table 3. New formulated biomixtures were designed to decontaminate acetochlor, atrazine, pendimethalin and trifluralin, with straw content increasing from 12.5 to 62.5 % and their compositions were straw, soil and peat (12.5:62.5:25 and 25:50:25, respectively), or compost (50:25:25 and 62.5:12.5:25, respectively). The $t_{1/2}$ values were low in this composition 25:50:25 (straw < 50) for all pesticides except trifluralin (23). Some biomixtures, only utilised soil and lignocellulosic material at 50:50, show effective degradation of pesticides. Complete degradation of prometryn, glyphosate, carbendazim and atrazine after 180 days, except imidacloprid (60 %), was observed in soil and foxtail millet (1:1) mixture (24). Biomixtures containing 50 % soil, with the remaining proportion composed of compost and/or lignocellulosic materials and their compositions were 1+1 (M2 soil and compost/ M3 sisal/ M4 sea weed/ M5 corn stover), 2+1+1 (M6 soil, compost and sisal/M7 sea weed/ M8 corn stover), 2+1+1 (M9 soil, sisal and sea weed/ M10 corn stover), 2+1+1 (M11 soil, corn stover and seaweed), 4+1+1+1 (M12 soil-compost-seaweed-sisal-corn stover) treated five pesticides carbofuran, 2,4-D, atrazine, diazinon and glyphosate. Six biomixtures (M3, M4, M5, M9, M10, M11) contained 50 % lignocellulosic substrate, M6, M7, M8

contained 25 % and M12 with 37.50 lignocellulosic substrate. The results showed that 2,4-D dissipation (approx 99.98 %) was highest in M5 and M12 biomixtures. Biomixture M5 was also very effective in degrading carbofuran (99.72 %), diazinon (99.56 %) and atrazine (99.90 %). In the case of glyphosate, biomixture M7 was the most effective (99.61 %) (25). A biomixture of wheat straw and soil at a 1:1 ratio showed 99 % degradation of glyphosate, whereas alfalfa + soil indicated 85 % after 63 days. However, metabolite aminomethylphosphonic acid (AMPA) was found in both compositions (26). In a few studies, biomixtures were prepared with a 40 % lignin substrate and their component ratios were 2:2:1, with equal amounts of lignocellulosic and humic material (22, 27). Biomixtures were optimised for lignocellulosic materials, rice straw and corn cob at a 2:2:1 (Rice straw/corn cob : compost : soil) composition for imidacloprid and azoxystrobin degradation. Rice straw exhibited higher degradation efficiency than corn cob for both pesticides. Most findings showed that the biobed system effectively removes pesticides at high lignocellulosic material loads and it can also be inferred that at least 50 % lignin substrate is required in the biomixture for better performance. However, there are a few exceptions in which a 50 % lignin substrate is not used due to the substrate's physicochemical properties and the chemical characteristics of the pesticides. In today's scenario, there is a need to explore diverse lignocellulosic substrates for a biomixture to ensure its effective adaptation across various regions with local resources.

Effect of humic material (peat/compost/vermicompost)

Peat or compost is an integral component of the biomixture, which physically and chemically modifies the mixture, increasing sorption capacity, regulating moisture content and lowering pH, all of which create favourable conditions for the growth of fungus. Typical biomixture composed of straw, peat and soil (2:1:1) (28), where peat

Table 3. Compositions of newly developed biomixtures with varying component ratios

Lignocellulosic component	Humic component	Composition lignocellulose/humic/soil	Pesticide
Olive tree prunings	Compost	45:13:42	Carbofuran
		45:13:42	Chlorpyrifos, atrazine, ametryne and linuron
		63.7:29:7.3	Oxamyl, thiophanate-methyl, hexaconazole, fluazifop-p-butyl, carbendazim, epoxiconazole, propiconazole, triadimefon, chlorpyrifos, imidacloprid, tebuconazole and triadimenol
		81.7:11:7.3	
Coconut fibre	Peat and compost	12.5:25(peat): 62.5	
		25:25(peat):50	
		50:25(compost):25	Acetochlor, atrazine, pendimethalin and trifluralin
		62.5:25(compost):12.5	
Wheat straw	Prunings compost	1:4:0	Fludioxinil, penconazole, cyprodinil, iprovalicarb, metalaxyl and azoxystrobin
Wheat straw	Prunings compost	2:3:0	Fludioxinil, penconazole, cyprodinil, iprovalicarb, metalaxyl and azoxystrobin
	Compost/peat	2:2:1	Azoxystrobin and imidacloprid
Sunflower crop residues	Digestate	0:1:9	Bentazone, boscalid and pyrimethanil
Rice straw	Replacement of compost (5 and 10 %) with biochar/rice husk ash/sugarcane bagasse ash	2:1:1	Atrazine and fipronil
Rice straw	Leaf litter	2:1:1	Ethylenebisdithiocarbamate
Banana stem	-	1:0:1	Glyphosate, AMPA, atrazine, carbendazim, prometryn, imidacloprid
			Acetamiprid, thiacloprid, emamectin benzoate, quinalphos, tebuconazole, imidacloprid, carbendazim, thiophanate methyl, thiamethoxam, clothianidin, metalaxyl, carbofuran, chlorpyrifos, hexaconazole, profenophos, lambda cyhalothrin, fipronil, chlorothalonil, pyridalyl, deltamethrin
Rice straw	Vermicompost	1:2:8	

provides high organic matter, facilitating pesticide retention and minimising leaching losses, thereby supporting gradual degradation. In contrast, compost is characterised by a diverse and metabolically active microbial community along with higher nutrient availability, which significantly enhances enzymatic activity and accelerates pesticide biodegradation (29). When the three pesticides chlorpyrifos, pendimethalin and thiophanate-methyl were co-applied in compost biomixture, they enhanced the dissipation of chlorpyrifos and thiophanate-methyl but had no significant effect on pendimethalin degradation compared to its individual application (30). Moreover, the use of compost as a peat substitute has been validated by (31). Compost-based biomixtures not only maintain degradation efficiency but also enhance environmental sustainability and economic feasibility.

Latest studies have utilised vermicompost as a substitute for peat in the biomixtures. It has high microbial activity and nutrient richness. Vermicompost, produced by earthworms through the biodegradation of organic waste, contains a diverse microbial community and readily available nutrients, which markedly enhances enzymatic activities associated with pesticide degradation (32). As a result, vermicompost-amended biomixtures often exhibit accelerated degradation kinetics compared to conventional peat-based systems (33). However, there are certain limitations to the use of vermicompost. Vermicompost typically has an unstable structure and lower sorption capacity to peat and compost, which may reduce pesticide retention and increase the risk of leaching under high moisture levels (21). Although its high biological activity leads to rapid mineralisation, it potentially shortens the effective lifespan of the biomixture. In a study, emerging contaminants such as diclofenac, ibuprofen and triclosan present in wastewater effluents were treated in a mixture of soil, raw olive mill cake and olive residues and a mixture of soil, vermicompost and olive residues. The results showed that the degradation rates of these contaminants were similar in SVP and untreated soil, both of which were faster than in SCP. Additionally, 82 % of the applied pesticides were adsorbed onto the bioaccessible fraction (E), contributing to the higher decontamination efficiency observed in this biomixture (34). "Bioaccessible fraction is the portion of pesticides that is loosely bound and easily extractable, making it available for microbial degradation, transport, or uptake". Therefore, vermicompost offers an alternative to peat; its proportion must be carefully optimised in combination with other lignocellulosic materials to balance adsorption and biodegradation processes. Spent mushroom substrate (*Pleurotus eryngii*, *Lentinus edodes* and *Flammulina velutipes*) also acts as an alternative to peat as it showed a similar dissipation pattern when compared to peat biomixture (35).

Effect of amendment

Ashes and biochar are organic materials commonly used in pesticide decontamination. These materials improve sorption by high organic carbon and their chemical nature favour pesticide dissipation. Extensive studies have been carried out using ash to eliminate pesticides from wastewater (36–39). Research demonstrated that ashes improved the dissipation of pretilachlor and sulfosulfuron in soils due to the presence of alkaline minerals (40).

Similarly, biochar is recognised as the most effective adsorbing material for diverse classes of pesticides (41). Therefore, it is used for the decontamination process. Many physico-chemical characteristics of biochar, such as its surface area, organic carbon

and porous structure, determine its capacity to absorb pesticides (42). Moreover, carboxyl groups generated on the biochar surface by oxidation exhibited a significant cation-exchange capacity, thereby enhancing pesticide retention. A study confirmed that the application of biochar (wheat straw) accelerated the pesticide sorption (43). Biochar hinders the mobility, transport and bioavailability of pesticides for microbial uptake in agricultural soil.

A new concept has emerged in the decontamination of pesticides using biomixtures, in which ashes, biochars, or digestate are incorporated. A portion of compost was replaced with ashes (5, 10 %) of rice husk and sugarcane bagasse and wheat biochar (1, 5, 10 %). The sorption efficiency of ash/biochar-amended biomixtures for atrazine and fipronil was increased. The highest sorption was observed in wheat biochar biomixtures among all tested mixtures. This is attributed to their properties as ashes have unburnt carbon and a porous nature that facilitates the sorption of pesticides. Biochar with a high surface area, greater organic carbon (OC) content and porous structures has previously been reported to have high adsorption capacities for organic pollutants and pesticides (44). Compared with atrazine, fipronil had a higher octanol-water partition coefficient; therefore, it was more sorbed in all of the biomixtures. Total applied fipronil (100 %) was sorbed even at 1 % wheat biochar (45). Rice husk enhanced the degradation of atrazine at lower concentrations due to its alkaline nature. The sugarcane bagasse ash and wheat biochar biomixture showed no significant effect (46). Biochar partially replaced peat, decreasing the degradation of the pesticide's atrazine, carbendazim, chlorpyrifos, isoproturon and diazinon, except for iprodione, because of its high sorption capacity (47).

Digestate, a byproduct of anaerobic digestion, is high in organic matter and surface area, making it a good adsorbent for removing contaminants from soil and wastewater. Various dyes were removed from wastewater by adsorption using different digestates (48). Digestate was used as an adsorbent to remove oxyfluorfen and boscalid from. The Sorption result was explained by K_{ads} values. A higher K_{ads} value was found when the amount of digestate (soil + 5 % digestate) incorporated for oxyfluorfen treatment was compared to lower amounts (soil + 2 % digestate). But the trend was not similar for boscalid (49). Digestate was incorporated into the contaminated soil, enhancing the mineralisation of bentazone, boscalid and pyrimethanil, but decreasing it with biochar due to high sorption. However, the mineralisation rate was disproportionate to the digestate content. Therefore, a mixture of digestate and biochar (5 % + 5 %) was optimised for improved sorption and mineralisation of pesticides in BPS (50). Amended substrates have been optimised to improve the performance of the biobed system. These substances can alter the physicochemical characteristics of biomixtures, thereby affecting the mechanisms of pesticide sorption and degradation removal.

Effect of physical factor

Physical factors include moisture content and temperature (including seasonal fluctuations), both of which significantly influence pesticide biodegradation rates. Temperature, along with moisture, influences microbial community structure and function, ultimately impacting pesticide degradation (51). The half-life value of isoproturon was increased with lower moisture content, resulting in slower degradation. The optimal degradation was observed at 22 °C and 90 % moisture (52). Another study found that temperature and moisture had a substantial impact on the

dissipation of glyphosate and the synthesis and/or dissipation of its metabolite (AMPA) in soil (53). Pesticides, carbofuran and glyphosate dissipation increased with moisture at a constant temperature of 15 °C (54).

Temperature: A threshold temperature is crucial for microbial activity and pesticide biodegradation. As temperatures rise, the solubility of pesticides increases, making them bioavailable and it also enhances microbial activity, as evidenced by increased phenoloxidase content. Temperature increased phenoloxidase content 1.4 and 5.2-fold when the temperature rose from 2 to 10 °C and from 10 to 20 °C, respectively (9) and phenoloxidase content was associated with pesticide dissipation. The degradation rate was relatively higher at the high temperature (20 °C), achieving over 99 % degradation of 2,4-D and bromoxynil in 21 days, whereas at 13 °C, degradation took 28 days. Dissipation of pesticides decreased when the temperature was reduced from 20 to 5 °C and from 15 °C to 25 °C and half-life values ($t_{1/2}$) increased. Temperature of temperate region below 20 °C in the growing season, those regions require additional heat supplement to maintain the temperature in the biobed to enhance the pesticide (bromoxynil, tribenuron-methyl, pyrasulfotole, 2,4-D, thifensulfuron-methyl) degradation, but pesticides (metsulfuron-methyl and thien carbazon-methyl) were least sensitive to the temperature, did not show a significant effect on dissipation (55). Since microbial processes are temperature-dependent, an optimal threshold temperature can greatly enhance microbial activity, leading to higher degradation rates (56).

Moisture: Moisture content in the biobed system affects oxygen availability and microbial activity, ultimately influencing pesticide degradation. Water holding capacity (WHC) of biomixture at 60 % promotes faster pesticide dissipation than at 30 and 90 % WHC. Lower levels restricted microbial activity and pesticide availability, whereas higher levels created saturation and limited oxygen conditions that favoured aerobic degradation. Moreover, excess water created a risk of pesticide leaching from the biobed (10). Water flow strongly influenced the sorption behaviour of peat biomixtures and a water volume of 12.5 L m³ in the biomixture was effective for pesticide sorption in the biobed. The influence of water volume and its application frequency on the leaching behaviour of pesticides was determined in compost biomixture (BXs) and peat biomixture (OBX). Large water volumes with less frequent application (I), resulting in leaching ranging from 0.001 to 10.4 % of the applied pesticide. Frequent applications of smaller volumes of water (II) resulted in leaching losses of less than 1 %. The results showed that OBX excelled in (I), whereas the compost-biomixture (BX1) performed in (II). In contrast to BX1, OBX's dissipation efficacy was predominantly attributable to pesticide retention rather than degradation. They suggested that sorption capacity depends on both substrate and water management strategy (10).

Effect of preconditioning

Preconditioning is another crucial factor influencing the efficiency of biomixtures in the depurification process. Preconditioning is a preparatory step in which the biomixture substrate is incubated under controlled conditions-such as specific moisture, temperature and aeration levels-to stimulate microbial proliferation and enzymatic activity before pesticide exposure and treatment. Studies reported that chlorpyrifos degradation was higher under 15-day pre-incubation (29) and mostly at lower concentrations of 200 and 320 mg kg⁻¹ (57). Preconditioning significantly enhanced

the degradation of imidacloprid, reducing its half-life ($t_{1/2}$) by 5–9 times, whereas no significant effect was observed for azoxystrobin (22). Another report on preconditioning stated that the longer preconditioning period (15 days) can degrade pesticides more quickly than a shorter one (58). A longer time period favours sufficient microbial growth and its stabilisation for pesticide degradation. Understanding the role of preconditioning in pesticide dissipation is essential for optimising biobed performance and improving the effectiveness of biomixtures in mitigating pesticide contamination in agricultural environments.

Effect of pesticide/chemical factors

The depurification process in biobeds is significantly influenced by pesticide factors like pesticide concentration, repeated applications of the same pesticide, pesticide mixtures and their interactions. Several reports demonstrated that the degradation rate decreased as the concentrations of the pesticides isoproturon and chlorothalonil (59) and isoproturon and mecoprop (60) increased. Repeated pesticide applications have been found to reduce the degradation rate in biomixtures, as observed with chlorpyrifos (27), as well as with metribuzin, buprofezin, azoxystrobin, chlorpyrifos, dimethoate and terbuthylazine (61). Degradation retarded due to the toxic effect of high amounts of pesticides on the microbial population. Co-application of pesticides can influence degradation processes. Simultaneous application of metalaxyl and chlorpyrifos reduced metalaxyl's half-life due to a synergistic interaction between the pesticides; this effect was not observed for chlorpyrifos in the biomixture. A mixture of pesticides, carboxin (CBX), metalaxyl-M (MET-M), fluxapyroxad (FLX), fludioxonil (FLD), chlorothalonil (CHT) and thiabendazole (TBZ) slowed down the removal efficiency of the biomixture and the effect was more significant in soil compared to the biomixture. Moreover, interactions among pesticides in the mixture showed that CHT suppressed the degradation of TBZ and FLD more strongly. Sometimes, pesticide metabolites are more persistent and exert a greater inhibitory effect on microbial activity (62).

Effect of bioaugmentation

Natural microbiota facilitates the degradation of pesticides in the biobed system. However, bioaugmentation promotes the rapid and complete biodegradation of pesticides. It is a less expensive and environmentally friendly tactic than physicochemical methods (63). This process involves the addition of living organisms, especially bacterial and/or fungal strains, or their consortia.

Effect of microorganism

Microbial augmentation degraded persistent pesticides in the biobed system (64, 65). Microbial species exhibit enzymatic capabilities and preferences in the biodegradation of xenobiotics. While certain microorganisms are effective at decomposing saturated and unsaturated hydrocarbons, others show a preference for degrading mono- or polynuclear aromatics. Additionally, certain microbes can degrade both alkanes and aromatics. Pesticide degradation mainly achieved by bacteria (66) and fungi (67) and their consortia is presented in Tables 4–6. As a result, various bacteria and fungi have been augmented in a biomixture for pesticide biodegradation. A large number of microbial communities are listed in Table 4. For instance, fungal pellet (*Anthracyllum discolor* 10 %, w/w) enhanced the atrazine, chlorpyrifos, iprodione and isoproturon degradation in the augmented biomixture as compared to the uninoculated biomixture. After 30 days, atrazine was fully degraded

in the augmented biomixture, which was 18 times faster than in the uninoculated biomixture (68). Further study found that the half-life ($t_{1/2}$) of atrazine was reduced from 14 to 6 days in a biomixture when bioaugmented with *A. discolor* (69). The degradation of atrazine was enhanced by *Streptomyces* sp. M7 in the inoculated biomixture, reducing the half-life by up to 3-fold compared to the control. Microbial growth was not affected by the addition of atrazine. Although the microbial population in the contaminated biomixtures was lower after the experiment. *Streptomyces* sp. M7 inoculation raised acid phosphatase activity and may reverse the pesticide's impact on enzymatic activity (70). Another study investigated the effect of *Streptomyces* sp. M7 at 3 concentrations (2, 4, 8 kg g⁻¹) levels in 2 biomixtures (64). Biomixtures differed for organic matter (B1, sugarcane bagasse and B2, filter cake) composition. B1 showed higher microbial counts at 8 kg g⁻¹, whereas in B2, at 4 kg g⁻¹, higher. The differences in atrazine degradation were not significant in B1 and B2 and between the concentrations. Therefore, they optimised the cost-effective biobed at the lowest (2 kg g⁻¹) concentration (70). The fungal inoculum *Phanerochaete chrysosporium* degraded even the non-extractable portion of isoproturon in a biomixture (65). The *Lentinula edodes* accelerated the pesticide dissipation in the on-farm biopurification system. Maximum degradation of terbuthylazine (89.9), difenoconazole (75.0), diflufenican (65.0) and pendimethalin (99.4 %) in bioaugmented biomixture was recorded after 120 days (71). Bio-augmentation with *Trametes versicolor* in biomixture enhanced the removal of toxic chemical carbofuran (72). Another

report on inoculation with *T. versicolor* showed that the inoculum was effective in peat (GTS) biomixtures ($t_{1/2}$ 3.4 days, $k = 0.00248 \text{ day}^{-1}$) compared to compost (GCS) biomixtures (8.1 days, $k = 0.00188 \text{ day}^{-1}$) for carbofuran removal. Two metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran, were formed and degraded in the system. This was explained by the three parameters: lignin content, C/N ratio and pH. Higher lignin content, C/N ratio and lower pH of GTS created favourable environment for colonisation of *T. versicolor*, thereby enhancing enzymatic activity and pesticide degradation. Whereas in GCS, *T. versicolor* competed with indigenous microbes for growth (73). A study was conducted on the degradation of different types of fungicides in an inoculated (*T. versicolor*) biomixture. The removal of edifenphos, carbendazim and metalaxyl was achieved with high efficiency using a bioaugmented mixture. It was unsuccessful in eliminating all triazole fungicides (tebuconazole, triadimenol, epoxiconazole and fenbuconazole) from the biomixture. The removal of metalaxyl and carbendazim was significantly enhanced by repeated pesticide application. However, the effect of re-inoculation was not significantly observed on the overall performance of the bio-augmented biomixture. Acute tests on *Daphnia magna* showed that detoxification of the biomixture took 9 days after pesticide applications. The DGGE analysis revealed a modest establishment of *T. versicolor* in the biomixture and only a moderate temporal divergence in the bacterial and fungal populations. According to the results, fungicides other than triazoles can be effectively treated with the non-bioaugmented biomixture (74).

Table 4. Bioaugmentation of biomixture with fungal species

Fungus	Pesticide (s)	Biomixture composition	Degradation (%)
<i>Phanerochaete chrysosporium</i>	isoproturon	Straw: peat: soil (50:20:30, v/v/v)	78 % (28 days), 99 % (100 days)
<i>Stereum hirsutum</i> , <i>Hypholoma fasciculare</i> , <i>Coriolus versicolor</i>	metalaxyl, terbuthylazine, atrazine, diuron, iprodione, chlorpyrifos	Barley straw: Compost: soil (50:25:25, w/w/w)	75 % (42 days) <i>S. hirsutum</i> was excellent for all pesticides except chlorpyrifos
<i>Anthracophyllum discolor</i>	Iprodione, chlorpyrifos, isoproturon, atrazine	Straw: peat: soil (2:1:1, v/v/v)	100 % (30 days), 18% faster than control biomixture
	Atrazine	Wheat straw: peat: soil (2:1:1, v/v/v)	Half-life decreased from 14 to 6 days
	Carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran	Coconut fiber: compost: soil, (45:13:42, v/v/v)	~99 % (3 days)
	Carbofuran	Rice husk: compost: soil (50:25:25, v/v/v)	after 3 days, 22.9 % (50:25:25) 46.1% (50:33.3:16.7)
	Carbofuran	GCS (Rice husk: compost: soil)	30:43:27, best biomixture for degradation of carbofuran
	Carbofuran	GTS (Rice husk: peat: soil)	100 % (48 days)
<i>Trametes versicolor</i>	Aldicarb, methomyl, methiocarb, carbofuran	Rice husk: compost: soil (pre-exposed to Carbofuran) (30:43:27, v/v/v)	Not effective
	Tebuconazole, triadimenol, epoxiconazole, fenbuconazole, carbendazim, edifenphos, metalaxyl	Coconut fibre: compost: soil (45:13:42, v/v/v)	Failed to remove all the triazole fungicides
	Atrazine, terbuthylazine, terbutryn, chlorpyrifos	Coconut fibre/compost/soil (45:13:42, v/v/v)	Terbuthylazine - 15.1 % (20 days), Atrazine -45.4% (60 days); Chlorpyrifos- 65.3 %; No effect on terbutryn
	Atrazine, carbendazim, metalaxyl, carbofuran	Coconut fibre: compost: soil (45:13:42 volume basis)	No significant effect of bioaugmentation on $t_{1/2}$ of all four pesticides
<i>Lentinula edodes</i>	Pendimethalin, difenoconazole, diflufenican, terbuthylazine	Soil: granulated cork mixtures in different combinations	Higher degradation in bioaugmented biomixture pendimethalin (98.7, 99.4), difenoconazole (53.9, 75.0), diflufenican (44.7, 65.0), terbuthylazine (61.4, 89.9), respectively, after 120 days
<i>Abortiporus biennis</i> , Basidiomycete	Endosulfan, chlorpyrifos	Bran: peat: soil (50:25:25, v/v/v)	Endosulfan - 87 %, Chlorpyrifos- > 79 % in 27days

Table 5. Bioaugmentation of biomixture with bacterial species

Bacteria	Pesticide (s)	Biomixture composition	Degradation (%)
<i>Pseudomonas putida</i>	Fluopyram, myclobutanil, triticonazole	Sunflower residues:peat: soil (50:25:25); Sunflower residues:peat:zeolite:soil (50:12.5:12.5:25, v/v/v)	Half lives ($t_{1/2}$) values decreased for myclobutanil (9.5 days) fluopyram (7.7 days) and triticonazole (10.5 days) in bioaugmented biomixture than control biomixture 16.8, 10.5 and 15.7 days, respectively
<i>Sphingomonas haloaromaticamans</i> , <i>Pseudomonas putida</i>	Thiabendazole, imazalil, orthophenylphenol, diphenylamine	Straw: spent mushroom substrate: soil (50:25:25, v/v/v)	> 99.5 % for thiabendazole, imazalil, orthophenylphenol, diphenylamine
<i>Arthrobacter strain</i>	Iprodione	Wheat straw: peat: Soil (50:25:25, v/v/v)	Degradation increased
Mixed culture of <i>Streptomyces strains</i>	Chlorpyrifos	Wheat straw: peat:soil (2:1:1, v/v/v)	90 %
<i>Delftia acidovorans</i>	Chloroprotham	Straw: peat: soil (50:25:25, v/v/v)	Accelerated degradation
<i>Bordetella petrii</i> I GV 34, <i>B. petrii</i> II GV 36 and <i>Achromobacter xyloxiidans</i> GV 47	Cypermethrin	Clay:saw dust biochar (1, 5 and 10 %); Acacia concinna biochar (1, 5 and 10 %)	98 % (32 days)
Atrazine degrading enrichment culture and fipronil degrading <i>Bacillus megaterium</i>	Atrazine and fipronil	Rice straw: compost: soil (50:25:25, w/w) biomixtures mixed with rice husk ash (5 and 10 %), sugarcane bagasse ash (5 and 10 %) and wheat straw biochar (1, 5 %)	Increased degradation
Consortium of <i>Achromobacter sp.</i> and <i>Arthrobacter sp.</i>	Iprodione	Soil: wheat straw: peat (25:50:25 v/v/v)	Accelerated iprodione degradation. $t_{1/2}$ values control (9.1days), bio-augmented biobed (4.3days)

Table 6. Bioaugmentation of biomixture with mixed culture

Consortium	Pesticide (s)	Biomixture composition	Degradation (%)
Consortia of bacteria and fungus strains	Tebuconazole, metalaxyl, oxyfluorfen	Olive-pruning: vermicompost (olive mill by-product): soil, (1:2:1, v/v/v)	Accelerate removal of pesticides
Mixed culture of <i>Streptomyces sp.</i> and <i>Trametes versicolor</i>	Lindane	Sugarcane bagasse:peat: soil (50:25:25, v/v/v)	Increased the lindane dissipation 81–87 % of (66 th day)
Linuron primed soil	Pesticide-primed linuron primed soil	Straw: peat: soil (50:25:25, v/v/v)	Increased mineralisation capacity of biomixture
Pesticide primed soil	Isoproturon, metamitron, linuron, bentazon, metalaxyl	Straw: peat: coconut husk: chips: cowmanure (25:35:25:5:10, volume basis)	Primed soil in biomixture promoted degradation of linuron, metamitron and metalaxyl, but effect was not observed in isoproturon and bentazon degradation
Extract from matured biomixture	Diclofenac (dcf) and triclosan (tcs)	olive tree pruning: compost: soil (50:25:25, v/v/v)	90 % degradation observed for diclofenac and triclosan after 60 and 108 days Metabolite methyl-triclosan 12 times lower in inoculated biomixture than in controls

Some studies show that the inoculated biomixture did not enhance the efficiency of the biobed. In many cases reported, *T. versicolor* did not accelerate the degradation of aldicarb (ALD), methomyl (MTM) and methiocarb (MTC) (75), atrazine, terbutylazine, chlorpyrifos and terbutryn (76). Bioaugmentation with *T. versicolor* inhibited the native microbial population when inoculated at high concentrations in the solid phase, delaying pesticide removal during the first days of treatment compared to non-bioaugmented systems. Nonetheless, after 8 days, the removal was almost complete regardless of the process (77). Application of oxytetracycline and inoculation did not significantly affect pesticide elimination or the microbial community, as the half-life values ($t_{1/2}$) of the pesticides atrazine (9.9 days), carbendazim (3.0 days), carbofuran (2.8 days) and metalaxyl (2.7 days) were lower than those of the bioaugmented biomixture. Biomixture initially tolerated the antibiotic stress, which affected its performance. However, simultaneous removal of pesticides and oxytetracycline residues remains feasible (78).

A myriad of studies have demonstrated that mixed culture (consortium) is highly efficient at degrading a diverse range of pesticides. For example, A mixture of bacterial (*Brevundimonas sp.*, *Achromobacter sp.*, *Bacillus sp.*, *Staphylococcus sp.*, *Acremonium sp.*, *Ochrobactrum sp.*) and fungal (*Fusarium* and *Scopulariopsis sp.*) consortia was inoculated in biomixtures, which enhanced the degradation of tebuconazole (1.6 times), metalaxyl (3.8 times) and oxyfluorfen (7.7 times) (79). Another consortium of *T. versicolor* (SGNG1) and actinobacteria (*Streptomyces sp.* A2, A5, A11 and M7) accelerated the degradation of lindane. In test soils, consortia reduced lindane's half-life ($t_{1/2}$ = 24 days) by 6-fold. Even after repeated exposure, only the treated biomixture with inoculum enhanced lindane dissipation, as indicated by shorter half-life ($t_{1/2}$) values compared to the control biomixture (80). Removal efficiency for pesticide mixture (chlorpyrifos and diazinon) in sterile soil, biomixture, bioaugmented soil and inoculated biomixture was studied. Incubation was done with *Streptomyces* mixed culture in the targeted soil and biomixture. Pesticide degradation was not observed in sterile soil and biomixture. Dehydrogenase, acid

phosphatase and quantitative polymerase chain reaction (q-PCR) analysis demonstrated that bio-augmentation expanded the microbial population in treated soil and biomixture and colonised the substrates. However, inefficient chlorpyrifos degradation was observed in bioaugmented soil (14 %) and biomixture (6 %) after 4 weeks. Removal rate of diazinon was 0.024 day^{-1} and 0.060 day^{-1} in treated soil with inoculum ($t_{1/2} = 29$ days) and biomixture ($t_{1/2} = 11$ days), respectively, which was faster than chlorpyrifos degradation (81). According to the results of the aforementioned investigations, bioaugmentation may be a viable and sustainable method of minimising pesticide load in the environment.

Effect of soil fauna

Numerous studies have shown that earthworms facilitate pesticide degradation in soil. Building on this understanding, researchers are now investigating the bioremediation process using earthworms in a biobed. The effect of earthworms on glyphosate degradation was evaluated in a biobed system. The results showed that biomixtures with earthworms achieved 90 % glyphosate degradation within 90 days, whereas biomixtures without earthworms reached approximately 80 % degradation in the same period. Augmented biomixture also had excellent capacity to degrade the aminomethylphosphonic acid metabolite formed during degradation. Additionally, they noted that *E. fetida* can withstand high levels of glyphosate without experiencing any changes in its characteristics (82). The incorporation of earthworms into biomixtures presents a novel and promising approach for the degradation of a wide range of pesticides.

Conclusion

Decontamination of pesticides is largely affected by the biomixture components lignocellulosic substrate, compost, peat, or vermicompost. The amount of lignocellulosic substrate is crucial, as it determines sorption and degradation processes; at least 50 % is required for successful implementation. Many alternatives to wheat straw have been identified, including waste from vegetables, fruits, oilseeds, pulses and mushrooms, as well as coconut fibre, rice husk and coffee husk, thereby making the system more globally adaptable. The C/N ratio of the substrate positively correlated with microbial activities and pesticide dissipation. The compositional ratio of the mixture substrate, altered with various lignin substrates such as cotton fibre, increased the degradation rate, reaching 81.7 % with an increasing amount of cotton fibre, as a higher amount supports a higher C/N ratio. Pre-conditioning stabilises the diverse microbial population and enhances the degradation. Amendments like ash, biochar and digestate contain high levels of organic carbon, which facilitate greater sorption and microbial activity that effectively remove pesticides from the system. Microbial augmentation facilitates the complete and rapid degradation of pesticides, as widely reported, but in some cases, its effect is not observed. Hence, a specific biobed system needs to be developed in which the inoculum is compatible with indigenous microbes and synergistically removes pesticides from the system. However, most studies optimised the biomixture in the laboratory; therefore, there is a need to validate the system under field conditions.

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Authors' contributions

UK contributed substantially to the conceptualisation of the review and wrote the sections related to the biobed system and the physical and chemical effects on pesticide degradation. SK¹ wrote the bioaugmentation section and contributed to the interpretation of the relevant literature. KR conducted an extensive literature search and prepared the tables included in the manuscript. SK² and SP revised the manuscript following the reviewer's recommendations. SK³ prepared the tables and figures. PB critically reviewed and refined the manuscript and provided valuable suggestions to improve the overall quality of the article. All authors have read and approved the final manuscript [SK¹: Sandeep Kumar; SK²: Sonal Kumari; SK³: Swati Kumari].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflicts of interest to declare.

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