UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
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CAMPUS DE DRACENA
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Ferramentas biotecnológicas sustentáveis na remediação de solos agrícolas com tebuthiuron

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Dracena
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Tese apresentada junto ao Departamento de Produção Vegetal da Faculdade de Ciências Agrárias e Tecnológicas da Universidade Estadual Paulista “Júlio de Mesquita Filho” - Campus de Dracena, como parte das exigências do Concurso Público para Obtenção do Título de Livre-Docente em Biotecnologia Ambiental e Remediação de Solos.

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EPÍGRAFE

“Ensinar não é transferir conhecimento, mas criar as possibilidades para a sua própria produção ou a sua construção.”

Paulo Freire

(Pedagogia da autonomia, 1996)

“O Homem é parte da natureza e a sua guerra contra a natureza é, inevitavelmente, uma guerra contra si mesmo.(...) Temos pela frente um desafio como nunca a humanidade teve, de provar nossa maturidade e nosso domínio, não da natureza, mas de nós mesmos”

Rachel Carson

(Primavera Silenciosa, 1962)

“Fomos nos alienando desse organismo de que somos parte, a Terra,
e passamos a pensar que ele é uma coisa e nós, outra:
a Terra e a humanidade.”

Ailton Krenak

(Ideias para Adiar o Fim do Mundo, 2019)

DEDICATÓRIA

À minha esposa Marcela, companheira incondicional,
cuja dedicação como professora inspira não apenas os seus alunos,
mas também a mim, todos os dias.
Pelo amor, paciência e sensibilidade constantes,
que se fazem luz suave neste percurso.

Ao Raul, nosso filho e amor maior, que deu novo sentido à existência e
renovou em mim a esperança, a alegria e o compromisso com um futuro mais humano.
Espero que haja um mundo que respeite os ciclos da natureza e
acolha todas as formas de vida com equidade.
Que sua geração possa viver em uma sociedade que valorize a diversidade,
cuide do planeta e seja justa a todas as possibilidades de florescer.

Amo vocês!

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RESUMO

Esta tese buscou realizar uma reflexão criteriosa da produção científica e estabelecer uma conexão lógica e fundamentada entre alguns trabalhos desenvolvidos pelo grupo de pesquisa do autor, evidenciando as contribuições para o avanço da ciência, a formação de recursos humanos e o desenvolvimento de linhas de pesquisa consolidadas pelo GAIA (Grupo de Ação em Impactos Ambientais – FCAT/UNESP). Foram selecionados seis artigos científicos publicados em periódicos de reconhecida relevância editorial e alto impacto na comunidade científica, abordando a biorremediação de solos agrícolas com tebuthiuron. A partir dessas publicações, foi realizada uma análise crítica dos seus resultados, com especial atenção para a avaliação de diferentes ferramentas biotecnológicas nestes processos (fitorremediação, bioestimulação, bioaumentação, biossorção), cujo intuito foi reduzir os impactos negativos da concentração residual do herbicida e melhorar a qualidade agrícola e ambiental na promoção de sistemas de produção sustentáveis. Além disso, foram estabelecidas as relações de causa e efeito entre as técnicas empregadas pelo uso do tebuthiuron associado a outros compostos comumente utilizados na agricultura: pesticidas, resíduos agroindustriais e/ou bioinsumos. A organização baseou-se na cronologia das pesquisas com o propósito de demonstrar a evolução do autor enquanto cientista, considerando a progressão na qualidade dos experimentos, o aprofundamento nas metodologias, a sofisticação das abordagens analíticas, as colaborações estabelecidas e a formação de novos profissionais. Ao final, são apresentadas algumas reflexões sobre o estado da arte da área de pesquisa a partir das descobertas dos artigos selecionados, destacando as contribuições específicas na biorremediação de solos agrícolas com pesticidas, além da apresentação de desafios e perspectivas futuras para o avanço e a consolidação de práticas agrícolas com o mínimo de riscos ambientais. Portanto, as lacunas identificadas e os resultados apresentados pelos estudos relevaram o destacado potencial científico desta temática no estabelecimento de processos inovadores em aplicações práticas de manejo que estimulem a sustentabilidade dos sistemas agroindustriais.

Palavras-chave: biodegradação, biorremediação, fitorremediação, herbicida, modelagem de dados, toxicidade.

ABSTRACT

Sustainable biotechnological tools for the remediation of agricultural soils with tebuthiuron. This thesis aimed to conduct a critical analysis of scientific production and to establish a logical and well-founded connection among several studies developed by the author's research group, highlighting contributions to scientific advancement, human resource training, and the development of consolidated research lines within GAIA (Environmental Impact Action Group – FCAT/UNESP). Six scientific articles published in journals of recognized editorial relevance and high impact within the scientific community were selected, addressing the bioremediation of agricultural soils contaminated with tebuthiuron. Based on these publications, a critical evaluation of the results was performed, with special attention to the application of different biotechnological tools in these processes (phytoremediation, biostimulation, bioaugmentation, and biosorption), whose aim was to reduce the negative impacts of residual herbicide concentrations and to improve agricultural and environmental quality in the promotion of sustainable production systems. Furthermore, cause-and-effect relationships were established regarding the techniques employed using tebuthiuron associated with other compounds commonly used in agriculture: pesticides, agro-industrial residues, and/or bioinputs. The organization followed the chronological development of the research activities and publications to demonstrate the author's scientific evolution, considering the progression in the quality of the studies, the deepening of experimental methodologies, the sophistication of analytical approaches, the collaborations established, and the training of new professionals. Finally, reflections are presented on the state of the art in the research area based on the findings of the selected articles, emphasizing specific contributions to the field of pesticide-contaminated agricultural soil bioremediation, as well as highlighting future challenges and perspectives for advancing and consolidating agricultural practices with minimal environmental risk. Therefore, the identified gaps and the results presented by the studies highlighted the scientific potential of this topic for the establishment of innovative processes in practical management applications that foster the sustainability of agro-industrial systems.

Keywords: biodegradation, bioremediation, data modeling, herbicide, phytoremediation, toxicity.

SUMÁRIO

1. APRESENTAÇÃO

Em março de 2025, um estudo brasileiro publicado pelo periódico “Chemosphere” chamou a atenção por detectar a presença de pesticidas na chuva de cidades paulistas (DIAS et al., 2025). Os autores identificaram 14 moléculas e 5 produtos de degradação em amostras de água de chuva ao longo de dois anos em Brotas, Campinas e São Paulo. Seus resultados tornam ainda mais impactantes por estes municípios apresentarem grande heterogeneidade regional quanto ao uso do solo e à densidade populacional. A avaliação de risco ambiental indicou também que algumas moléculas e seus metabólitos secundários representam riscos à vida aquática. Os autores concluíram que a água de chuva é um compartimento ambiental relevante na dispersão de pesticidas e ressalta a necessidade de monitoramento e regulamentação mais rigorosa, especialmente considerando seu potencial uso como fonte alternativa para consumo humano.

Nota-se que as consequências ambientais pelo uso intensivo de pesticidas ainda não são completamente conhecidas e muitas situações prejudiciais são subestimadas pela complexidade dos agroecossistemas e pelos efeitos subletais pouco avaliados em organismos não-alvo. Em decorrência, é iminente que as próximas investigações promovam uma abordagem mais ampla dos impactos gerados pelas concentrações residuais destas moléculas e dos seus produtos de degradação por meio da multidisciplinaridade analítica.

Portanto, esta tese buscou avançar o conhecimento na área propondo alternativas ecologicamente adequadas, economicamente viáveis e agronomicamente benéficas a partir de processos biológicos na remediação de solos com um herbicida muito persistente e de elevada toxicidade: o tebuthiuron. Assim, estão apresentadas: uma breve introdução com síntese da bibliografia atualizada; a análise crítica de seis trabalhos publicados recentemente pelo grupo de pesquisa do autor (GAIA), disponibilizados como anexo; e reflexões finais sobre a evolução da biorremediação de solos agrícolas com pesticidas, destacando seu potencial científico para o desenvolvimento de práticas agrícolas inovadoras, sustentáveis e de baixo risco ambiental.

1.1. Referências

DIAS, M.A.; SANTOS, V.S.; VIZIOLI, B.C.; FERREIRA, B.S.; MONTAGNER, C.C. Pesticides in rainwater: A two-year occurrence study in an unexplored environmental compartment in regions with different land use in the State of São Paulo – Brazil. **Chemosphere,** v. 372, 144093, 2025.

2. INTRODUÇÃO

2.1. Cana-de-açúcar, tebuthiuron e reaproveitamento de resíduos na lavoura

A produção brasileira de cana-de-açúcar destaca-se mundialmente pela produtividade, mas para isso alguns fatores interferentes precisam ser mitigados. Apesar de sua via fotossintética eficiente (C4), seu desenvolvimento é altamente prejudicado pela competição por recursos com plantas daninhas (SANDANIEL et al., 2008), cujo prejuízo pode atingir até 85% na lavoura (SILVA et al., 2018). Assim, a primeira escolha dos produtores é o controle químico devido à sua facilidade de acesso, disponibilidade e baixos custos operacionais.

Dentre os herbicidas comercializados para cana-de-açúcar, destaca-se o tebuthiuron (N-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-N,N′-dimethylurea) que é utilizado para controlar plantas daninhas como *Urochloa decumbens*, *Commelina benghalensis*, *Digitaria horizontalis*, e *Panicum maximum* (BRASIL, 2023). Pertencente ao grupo das ureias substituídas (grupo C2), sua utilização em pré-emergência apresenta ação sistêmica e inibição do fotossistema II (AGROFIT, 2019). Quando aplicado em solo úmido, esta molécula atua imediatamente no controle das plantas infestantes que começarem a germinar. Já em solo seco, ela permanece na superfície do solo, aguardando a ocorrência de chuva para começar a atuar. Seu uso é recomendado após o plantio (em cana planta) ou depois do corte (em cana soca), sendo praticamente necessária uma única aplicação para manter a cana-de-açúcar livre destas espécies prejudiciais até o fechamento da cultura (BRASIL, 2023).

As principais características deste composto são: meia vida ($t_{1/2}$) entre 360 a 450 dias; solubilidade em água de 2.500 mg L^{-1} a 25 °C; coeficiente de partição octanol-água (log Kow) de 1,79, sendo considerado lipofílico; densidade 1,25 g mL^{-1}; pressão de vapor 0,27 mPA (25 °C); constante de dissociação (pKa) não ionizável, que favorece a sua mobilidade no perfil do solo; e coeficiente de sorção para o teor de carbono (Koc) igual a 80 mg L^{-1} (RODRIGUES; ALMEIDA, 2011; MANCUSO; NEGRISOLI; PERIM, 2011). Constata-se, assim, que se trata de uma molécula muito solúvel, de elevada persistência e baixa adsorção às partículas do solo. Somado a isso, aplicações sucessivas sem o manejo adequado podem tornar seu potencial de impacto ambiental ainda maior (FRANCO-BERNARDES et al., 2014).

Uma vez no solo, os pesticidas estão sujeitos à ação de diversos processos, os quais influenciam sua eficiência no controle de plantas daninhas, pragas e doenças

(SCORZA JÚNIOR; RIGITANO, 2009). Portanto, as condições de manejo da cultura afetam diretamente o comportamento ambiental dessas substâncias.

Quanto aos outros compostos aplicados no cultivo de cana-de-açúcar, o reaproveitamento de subprodutos agroindustriais representa uma importante ferramenta para a sustentabilidade do setor sucroenergético (CHRISTOFOLETTI et al., 2017). Entretanto, essa prática pode causar problemas a partir de estratégias sem controle que torna esta destinação alternativa inviável pelos efeitos negativos causados ao meio ambiente e à própria cultura (SOUZA et al., 2015; LIMA et al., 2016). Logo, o reaproveitamento desses resíduos é importante para a economia de insumos na lavoura, como também para não ocorrer impactos negativos pelo seu descarte incorreto no ambiente (CHRISTOFOLETTI et al., 2017).

Neste cenário, tem-se a fertirrigação com vinhaça como prática adotada por grande parte das indústrias sucroalcooleiras. Inúmeros estudos comprovam os resultados positivos obtidos na produtividade agrícola, associados à economia de adubos minerais (SOUZA et al., 2015; PRADO et al., 2017; SOTO; BASSO; KIANG, 2017; PINTO; ARAUJO, 2019). Contudo, há um limite de aplicação da vinhaça na cultura que varia em função da CTC (Capacidade de Troca Catiônica) e da concentração de potássio no solo e da concentração de potássio na vinhaça (CETESB, 2015).

Em decorrência disso, o prejuízo ocasionado pelo manejo incorreto da cultura canavieira na presença de diferentes substâncias no solo pode potencializar o impacto ambiental, uma vez que suas características físico-químicas e toxicológicas promovem a alteração do seu comportamento quando associados em agroecossistemas.

2.2. Comportamento ambiental do tebuthiuron em agroecossistemas

Em 2013, Bicalho e Langenbach (2013) já alertavam sobre a alta persistência do tebuthiuron no solo e a dificuldade de biodegradação da molécula. Baseada na preservação sustentável do meio ambiente, os autores indicavam a possibilidade de sua substituição e sugeriam o seu banimento para uso no país, bem como ocorreu na União Europeia em 2002. No entanto, o tebuthiuron é permitido para uso no Brasil e liberado para 20 culturas (HYPENESS, 2021), dentre elas a cana-de-açúcar. Outro país com destaque na produção canavieira que possibilita o uso deste herbicida é a China, fato que justifica a maioria das estudos sobre o tema serem de origem brasileira e chinesa (FUNDAÇÃO HEINRICH BÖLL, 2023).

Neste contexto, o “Atlas dos Agrotóxicos”, publicado em dezembro de 2023, evidenciou que o Brasil é um dos principais importadores e consumidores destas substâncias, permitindo a presença de concentrações mais elevadas dos seus resíduos em comparação aos países europeus (FUNDAÇÃO HEINRICH BÖLL, 2023). A publicação alertou ainda que ensaios laboratoriais isolados utilizados na aprovação de pesticidas não refletem a complexidade do ambiente natural, pois a análise está sujeita a incertezas e não contempla as relações intra e interespecíficas. Portanto, existem informações de apenas uma parte dos potenciais riscos e impactos dos pesticidas em função das condições simplificadas, pois os protocolos experimentais geralmente demonstram os efeitos individuais, sem estudar a mistura de ingredientes ativos que geralmente acontece nos agroecossistemas.

Sabendo que o destino de um pesticida é o solo, esta molécula pode ser sorvida, estar biodisponível ou ser degradada por microrganismos (MENDES et al., 2021a). No entanto, compostos solúveis e com alta mobilidade, como o tebuthiuron, apresentam potencial para serem lixiviados (PEREIRA et al., 2015), o que torna fundamental conhecer suas interações com os atributos físico e químicos do solo e os efeitos das condições ambientais (GUIMARÃES et al., 2018). Consequentemente, a variação da sua dinâmica em função destas propriedades pode aumentar os riscos de contaminação ambiental.

Em 2002, o governo brasileiro alterou as diretrizes para o cultivo de cana-de-açúcar com a proibição de queimadas. Esta decisão ocasionou uma maior aplicação de herbicidas em virtude da presença da camada de palha cobrindo o solo (TONIÊTO et al., 2016). Giori et al. (2014a) e Bonfleur et al. (2015) ressaltaram o impacto direto desta alteração no comportamento ambiental de herbicidas no solo. Pela manutenção da palhada no sistema de plantio direto (SPD), foi observada maior permanência destas moléculas no solo, principalmente pelo acúmulo de matéria orgânica. O reaproveitamento de resíduos nas lavouras também reduziu a contaminação de águas superficiais e subterrâneas em função de uma maior sorção dos pesticidas no solo (GIORI et al., 2014b).

Considerando esse panorama, a análise focada unicamente no coeficiente de partição linear (Kd) pode superestimar a lixiviação dos pesticidas. A sua disponibilidade na solução do solo é o fator determinante em relação ao transporte, à degradação e à eficácia, mas é inversamente relacionada ao potencial de sorção. Sendo este parâmetro influenciado pelas práticas de cultivo (GIORI et al., 2014a,b; BONFLEUR et al., 2015;

TONIÊTO et al., 2016), nota-se a influência direta das condições de manejo, tipos e propriedades do solos, e associação com ingredientes ativos e resíduos.

Desta forma, foi demonstrada uma menor mobilidade e maior taxa de sorção (Kd) do tebuthiuron quando presentes características distintas no solo em relação: ao teor de matéria orgânica (GIORI et al., 2014a; 2014b; BONFLEUR et al., 2015; TONIETO et al., 2016; FARIA et al., 2018; MENDES et al., 2021b; PIERRI et al., 2022); à granulometria e à classificação (TONIETO et al., 2016; FARIA et al., 2018; SILVA JUNIOR et al., 2018; MENDES et al., 2021b; GUIMARÃES et al., 2022); às interações organominerais (BONFLEUR et al., 2015; MENDES et al., 2021b); à pluviosidade (TONIETO et al., 2016; SILVA JUNIOR et al., 2018); ao tempo de permanência (TONIETO et al., 2016); e ao pH (FARIA et al., 2018).

Mendes et al. (2021b) observaram que o percentual de sorção de um herbicida é uma estimativa prática, enquanto o parâmetro Kd trata-se apenas de um coeficiente da equação linear que descreve a relação entre as concentrações sorvida e em equilíbrio na solução do solo. Salienta-se que o comportamento do tebuthiuron já foi vastamente estudado em condições isoladas, porém ainda há pouca exploração sobre os efeitos em conjunto com outros pesticidas e bioinsumos. Além disso, os seus efeitos colaterais em organismos não-alvo permanecem pouco explorados (QIAN et al., 2017).

A preocupação com os possíveis prejuízos ambientais ocasionados pelo tebuthiuron foi reforçada por Reinhardt et al. (2022), que confirmaram a existência de resíduos no solo mesmo após mais de uma década da última aplicação do herbicida (1996-2004). Obviamente que as condições ambientais e do solo favoreceram esta longa persistência, mas um importante fator que impulsionou este resultado foi a recorrência de aplicação, do mesmo modo como acontece nas lavouras canavieiras do Brasil.

A presença de resíduos de tebuthiuron em altas concentrações já foi documentada em áreas circunvizinhas a canaviais brasileiros (BICHALHO et al., 2010) e chineses (QIAN et al., 2017). Estes autores alertaram que os potenciais riscos não devem ser negligenciados e sugeriram avaliações quanto à comunidade microbiana do solo, uma vez que já foram relatados prejuízos com outros pesticidas (SAGARKAR et al., 2013; WAN et al., 2014; ZHANG et al., 2014).

Por fim, algumas publicações recentes identificaram organismos bioindicadores para realização de análises laboratoriais que verificam indiretamente as consequências da presença deste herbicida em solos brasileiros (FARIA et al., 2018; SILVA JUNIOR et al., 2018; FRIAS et al., 2023). Como resultado, uma avaliação detalhada nestas condições auxiliará em tomadas de decisão mais sustentáveis para melhorar a eficiência no controle de plantas daninhas, aumentar a produtividade agrícola e diminuir o risco de contaminação ambiental.

2.3. Processos biológicos de tratamento de solos com pesticidas

O processo de detoxificação de áreas agrícolas com pesticidas não é simples, mas felizmente muitas soluções foram aprimoradas nas últimas décadas seguindo quatro requisitos, conforme proposto por Ferro, Sims e Bugbee (1994): (i) alta eficiência de descontaminação, (ii) execução simples, (iii) protocolos rápidos e confiáveis, e (iv) custo-benefício. Partindo dessas premissas, a biorremediação aparece como uma estratégia ecologicamente viável para o tratamento de áreas impactadas.

De acordo com Kumar et al. (2011), a biorremediação é a transformação de poluentes nocivos em compostos de menor toxicidade por agentes biológicos. A partir do metabolismo de microrganismos e/ou vegetais, os contaminantes são imobilizados ou degradados, possibilitando a restauração das boas condições do ambiente. Esta estratégia apresenta vantagens como: baixo custo, elevada eficiência, alta aceitação pública e, principalmente, menor impacto no ecossistema (LIU et al., 2018).

Diversos processos possibilitam o aperfeiçoamento da atenuação natural por microrganismos como a bioestimulação e a bioaumentação, que podem reduzir o tempo de tratamento biológico, respectivamente, pela suplementação de nutrientes e a inoculação de microrganismos específicos. Em paralelo, o apelo agronômico pelo cultivo de plantas de interesse na fitorremediação garante a recuperação sustentável de solos contaminados. Neste sentido, estudos até a última década baseavam-se principalmente em espécies vegetais de clima temperado, sendo limitada a aplicação no Brasil. Contudo, há uma crescente exploração de plantas tropicais aplicadas na fitorremediação para reduzir a concentração de herbicidas no solo, tais como: sulfentrazone (MADALÃO et al., 2013; ALVES et al., 2019; MIELKE et al., 2020), fomesafen (ALVES et al., 2019), quinclorac (MENDES et al., 2021c) e tebuthiuron (PIRES et al., 2005; 2008; MENDES et al., 2021c; CONCIANI et al., 2023; FRIAS et al., 2023). Barroso et al. (2023) ainda revelaram que a fitorremediação pode reduzir em pelo menos 50% a presença de resíduos dos herbicidas, com a diminuição de até 92% da sua concentração no solo.

Especificamente relacionado ao tebuthiuron, o longo efeito residual deve ser considerado na escolha da espécie em sucessão à cana-de-açúcar a fim de evitar perdas econômicas, maiores riscos de contaminação, e prejuízos ao sistema de rotação de cultura. Deste modo, torna-se interessante o uso de adubos verdes na fitorremediação, pois, além de atuar como agente descontaminante, pode proporcionar ganhos agronômicos na fertilidade do solo e no intervalo entre a aplicação do herbicida e o próximo plantio (MONQUERO et al. 2013; GUIMARÃES et al., 2022). Dentre essas espécies, pode-se destacar: *Canavalia ensiformis* (MADALÃO et al., 2013; MENDES et al., 2021c), *Mucuna pruriens* (PIRES et al., 2005; 2008; FRIAS et al., 2023), *Pennisetum glaucum* (PIRES et al., 2008), *Arachis hypogea, Sorghum bicolor* (CONCIANI et al., 2023), *Crotalaria spectabilis, Lupinus albus, Stizolobium aterrimum,* e *Ceropia hololeuca* (MENDES et al., 2021c).

Entretanto, estas investigações comumente não analisam a ecotoxicidade do solo antes e após o desenvolvimento vegetal. A avaliação do potencial ecotoxicológico é crucial para comprovar a eficácia da biorremediação, pois a degradação de moléculas orgânicas pode originar compostos intermediários ainda mais tóxicos (ROCHA et al., 2018). Consequentemente, é imperativo avaliar a ecotoxicidade em um enfoque mais abrangente e dependente do tempo para demonstrar o sucesso das estratégias de remediação (BANKS; SCHULTZ, 2005). Mesmo em 2023, pesquisadores continuam evidenciando a quantidade limitada de estudos sobre fitorremediação de solos agrícolas, principalmente por meio de uma abordagem mais ampla deste processo (BARROSO et al., 2023; CONICANI et al., 2023). Devido ao frequente uso do tebuthiuron na cultura da cana-de-açúcar e o do seu elevado potencial de toxidez ambiental, torna-se fundamental a introdução de metodologias multidisciplinares.

Neste contexto, o Brasil pode se tornar um grande expoente na área em virtude de suas condições climáticas, de sua biodiversidade e de seu destaque em pesquisas agronômicas. Em síntese, a integração de fatores comuns em práticas agrícolas pode resultar em importantes planos de manejo sustentáveis e legislações específicas com políticas públicas voltadas para a preservação da qualidade ambiental, melhor aproveitamento de regiões agrícolas e promoção de benefícios socioeconômicos.

2.4. Validação ecotoxicológica por bioensaios padronizados

Finalizado o tratamento da área contaminada, é imprescindível que sejam realizadas análises que comprovem a redução do potencial tóxico do meio. O monitoramento das condições ambientais é indispensável para definir as melhores estratégias de tratamento, cujos aspectos são explorados pela Ecotoxicologia (VASSEUR; MASFARAUD; BLAISE, 2021).

Esta ciência identifica os efeitos tóxicos de substâncias poluentes a partir de organismos-teste em ensaios laboratoriais padronizados para a determinação da toxicidade de amostras ambientais. Seus protocolos baseiam-se em expor o organismo escolhido em diferentes condições para analisar e quantificar os efeitos deletérios e prejudiciais às espécies sentinelas (MAGALHÃES; FERRÃO FILHO, 2008).

Reconhece-se que vegetais superiores se caracterizam como modelos biológicos de destaque na avaliação da ecotoxicidade de amostras ambientais ou substâncias isoladas (IQBAL et al., 2019). A espécie *Lactuca sativa* (alface) é muito utilizada neste monitoramento pela simplicidade, boas características fisiológicas e alta sensibilidade à contaminação do meio (GONÇALVES; COELHO; CAMILI, 2016), sendo adequada para avaliar efeitos de pesticidas (ARAGÃO et al., 2019). Nesta perspectiva, os bioensaios permitem a investigação da fitotoxicidade pela determinação da taxa de germinação de sementes e do crescimento das plântulas, gerando dados que suportam o cálculo da taxa de inibição do desenvolvimento vegetal (SOBRERO; RONCO, 2004).

Em relação à avaliação do potencial ecotoxicológico de amostras de solo com pesticidas, alguns estudos reforçam a importância desses bioindicadores em protocolos experimentais consolidados, tendo em vista a complexidade de variáveis em diferentes culturas e condições de manejo (CHELINHO et al., 2014; LEITÃO et al., 2014; BERNARDINO, 2019; NEVES et al., 2021; FRIAS et al., 2023).

Portanto, pesquisas na temática da biorremediação de solos agrícolas com pesticidas com uma abordagem ampla e multidisciplinar contribuem significativamente para a compreensão do potencial dos processos biológicos como solução ecologicamente viável na restauração de agroecossistemas. Neste sentido, suas propostas contribuem diretamente para os Objetivos de Desenvolvimento Sustentável (ODS) da ONU, com destaque ao: 2 - Fome zero e agricultura sustentável, 12 - Consumo e produção responsáveis, 14 - Vida na água, e 15 - Vida terrestre.

2.5. Referências

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3. ANÁLISE DA PRODUÇÃO CIENTÍFICA

Sabendo dos potenciais impactos a espécies não-alvo ou culturas de sucessão pelo uso de tebuthiuron em áreas de cultivo de cana-de-açúcar, o GAIA (Grupo de Ação em Impacto Ambientais - FCAT/UNESP) iniciou as suas atividades para elucidar estas questões e desenvolver estratégias de mitigação destes danos. Suas linhas de pesquisa visam buscar soluções sustentáveis para os agroecossistemas, destacando a biorremediação para a restauração da qualidade de solos agrícolas com pesticidas associados ou não a diferentes compostos utilizados na lavoura.

Logo, esta tese apresenta seis trabalhos já publicados com resultados de pesquisas realizadas por membros do GAIA. A seleção destes manuscritos está justificada com uma breve apresentação das suas principais ideias, além de identificar potencialidades e novas estratégias para uma produção agrícola sustentável e perspectivas para a redução de possíveis prejuízos a partir do uso do tebuthiuron junto a outros pesticidas, resíduos agroindustriais do setor sucroenergético e/ou bioinsumos.

3.1. Anexo I – Ferreira et al. (2021)

FERREIRA L.C.; MOREIRA, B.R.A.; MONTAGNOLLI, R.N.; PRADO, E.P.; VIANA, R.S.; TOMAZ, R.S.; CRUZ, J.M.; BIDOIA, E.D.; FRIAS, Y.A.; LOPES, P.R.M. Green manure species for phytoremediation of soil with tebuthiuron and vinasse. Frontiers in Bioengineering and Biotechnology (ISSN 2296-4185; JCR 2023: 4.3), v. 8, 613642, 2021. DOI: http://dx.doi.org/10.3389/fbioe.2020.613642

Este trabalho surgiu de uma dissertação de mestrado no Programa de Pós-Graduação em Agronomia – Sistemas de Produção (PPGA) da FEIS/UNESP, câmpus de Ilha Solteira.

Sendo o primeiro relato com o uso de plantas na remediação de solo com tebuthiuron, foram utilizadas espécies de interesse agronômico que já apresentavam boa tolerância e desenvolvimento para outros herbicidas. Diante de todo embasamento teórico, esta pesquisa observou o efeito do tebuthiuron em espécies não-alvo, especialmente em culturas de sucessão que podem ser utilizadas na reforma de canaviais como adubos verdes. Portanto, foram investigados o desenvolvimento destas plantas e o seu potencial fitorremediador na redução dos prejuízos ambientais pela concentração residual do herbicida no solo.

Nesta perspectiva, detectou-se que a combinação do tebuthiuron com outras substâncias ainda não havia sido explorada na literatura, além de raramente incluírem a análise da ecotoxicidade nas amostras de solo. O sucesso da estratégia de tratamento para a redução dos danos não é simplesmente identificado pela quantificação da concentração residual do pesticida, visto que a degradação de compostos orgânicos pode gerar moléculas intermediárias ainda mais tóxicas do que a original.

Dentro desse quadro, o delineamento experimental baseou-se em quatro plantas de interesse agronômico, utilizadas como adubo verde na rotação da cultura canavieira, e que não são alvo do tebuthiuron (feijão-de-porco ou *Canavalia ensiformis;* feijão-gandu ou *Cajanus cajan*, mucuna-cinza ou *Mucuna pruriens;* e milheto ou *Pennisetum glaucum*), além de analisar a combinação do herbicida junto à vinhaça no solo. A abordagem ecotoxicológica com organismo-teste padronizado (*Lactuca sativa*) complementou esta avaliação junto ao monitoramento do desenvolvimento da espécie sentinela *Crotalaria juncea* (crotalária).

Inicialmente, seus resultados foram interessantes por identificar a morte de alguns indivíduos e o baixo desenvolvimento de *C. ensiformis* e *C. cajan*, cujas espécies são muito exploradas pela sua eficiência na fitorremediação de solos com poluentes orgânicos e inorgânicos. Ou seja, não se tratava de plantas tolerantes ao tebuthiuron, por mais que não fossem espécies-alvo pela bula do produto comercial. Por outro lado, as outras duas plantas (*M. pruriens* e *P. glaucum*) demonstram um bom desenvolvimento no solo com o herbicida e, principalmente, permitiram o crescimento de *C. juncea* quando estiveram previamente cultivadas. Em paralelo, os bioensaios de ecotoxicidade endossaram este resultado.

Além disso, outro fator que se destacou positivamente no solo com tebuthiuron foi a presença conjunta da vinhaça. Em virtude da sua composição físico-química, houve um efeito negativo nos primeiros dias que foi superado pelos significativos benefícios gerados no desenvolvimento da mucuna-cinza e do milheto e, posteriormente, da planta bioindicadora (crotalária). Destaca-se ainda o seu impacto na redução da ecotoxicidade do solo em comparação com as amostras contendo apenas o tebuthiuron isoladamente.

Este efeito positivo da associação da vinhaça com o tebuthiuron no solo foi comprovado pela abordagem estatística de Gompertz. O ajuste dos dados de variância temporal possibilitou realizar previsões com probabilidade suficiente para determinar a taxa de desenvolvimento vegetal em função do tempo. Seus parâmetros cinéticos indicaram as potenciais espécies fitorremediadoras e as melhores estratégias na mitigação dos efeitos colaterais da concentração residual do tebuthiuron no solo. Assim, foi possível comparar o período limitante no crescimento e desenvolvimento das plantas pela presença ou não do herbicida. Nota-se que este foi o primeiro relato desta abordagem estatística na biorremediação de solos agrícolas.

Finalmente, *M. pruriens* e *P. glaucum* foram indicadas como potenciais plantas fitorremediadoras de solo com tebuthiuron por apresentarem não só o interesse agronômico na recuperação da qualidade do solo na rotação da cultura da cana-de-açúcar, mas também pelo apelo ambiental na mitigação dos impactos negativos.

Entretanto, há uma comunidade de organismos no solo muito importante para a ciclagem de nutrientes e, consequentemente, para a produtividade vegetal e a degradação de compostos poluentes, que é geralmente negligenciada em estudos agrícolas: a microbiota. Conhecendo o efeito da vinhaça junto ao tebuthiuron para as plantas, desejava-se saber como os microrganismos nativos do solo reagiriam a esta associação. Além disso, o comportamento ambiental do herbicida também pode ser influenciado pela sua aplicação conjunta com outro pesticida. Portanto, estes questionamentos nortearam um segundo trabalho apresentado no Anexo II.

3.2. Anexo II – Nantes et al. (2023)

NANTES, L.S.; ARAGÃO, M.B.; MOREIRA, B.R.A.; FRIAS, Y.A.; VALÉRIO, T.S.; LIMA, E.W.; VIANA, R.S.; <u>LOPES, P.R.M.</u> Synergism and antagonism in environmental behavior of tebuthiuron and thiamethoxam in soil with vinasse by natural attenuation. International Journal of Environmental Science and Technology (ISSN 1735-1472; JCR 2023: 3.0), vol. 20, p. 4883-4892, 2023. DOI: http://dx.doi.org/10.1007/s13762-022-04276-8

Esta publicação foi originada de uma iniciação científica financiada pela FAPESP que culminou em um trabalho de conclusão de curso (TCC) em Engenharia Agronômica da FCAT/UNESP.

Seu delineamento experimental propôs avaliar a atenuação natural do solo na presença do tebuthiuron em associação com a vinhaça e/ou o inseticida thiamethoxam. Pelo método respirométrico, o metabolismo da microbiota nativa foi mensurado de acordo com diferentes combinações destas substâncias em esquema fatorial triplo, além de observar o potencial ecotoxicológico ao longo do tempo por meio organismo-teste padronizado. A abordagem estatística mais uma vez utilizou modelos matemáticos para auxiliar na interpretação dos dados e seus resultados foram promissores.

O modelo de Gompertz revelou-se como uma ferramenta útil para processos estocásticos do metabolismo microbiano por ser capaz de descrever a respiração basal do solo, sendo mais adequado do que funções de primeira ordem. Além disso, as simulações da produção de CO_2 pelo modelo de Monte-Carlo permitiram elucidar o comportamento ambiental das associações tebuthiuron-thiamethoxam-vinhaça a longo prazo.

Neste sentido, foi observado que as amostras contendo apenas tebuthiuron ou thiamethoxam apresentaram taxas de produção de CO_2 semelhantes ao solo controle. Igualmente ao Anexo I, a adição de vinhaça teve um impacto significativo na respiração do solo e na potencialização desse bioprocesso, uma vez que se trata de uma fonte de energia de fácil assimilação pelos microrganismos.

Porém, a validação da atenuação natural pelos testes de ecotoxicidade revelaram resultados opostos. A associação da vinhaça com o tebuthiuron propiciou um efeito positivo com uma maior taxa de produção de CO_2 e menor toxicidade após o período. Em contrapartida, sua presença com o thiamethoxam promoveu um antagonismo pelo aumento do potencial ecotoxicológico nas amostras de solo. Quando presente os três compostos, o tebuthiuron compensou esta relação antagônica do thiamethoxam com a vinhaça.

Esta consequência adversa endossa a necessidade de novas pesquisas sobre o uso de pesticidas de forma integral. A literatura científica geralmente descreve o efeito isolado destas substâncias, principalmente com relação aos fatores físicos e químicos. Sem considerar as associações entre compostos no solo e o impacto em organismos não-alvo, a real implicação dos pesticidas nos agroecossistemas muitas vezes é subdimensionada ou até desconhecida. Diante disso, a comparação com resultados anteriores quanto à atenuação natural do thiamethoxam foi dificultada. Esta lacuna ressalta a preocupação pela sua ação limitante na atividade microbiana e alta ecotoxicidade, mesmo na presença da vinhaça. Portanto, uma abordagem ampla e multidisciplinar que considere os diferentes aspectos das práticas agrícolas é essencial para futuras investigações sobre manejo sustentável de pesticidas na agricultura.

Embora a presença de vinhaça promova alguns prejuízos ao organismo-teste nos períodos iniciais, não foi observado nenhuma fitotoxicidade nestas amostras de solo ao final do período. O desempenho superior da associação tebuthiuron-vinhaça confirmou este impacto positivo. Contudo, é importante esclarecer que o processo de atenuação natural foi claramente dependente do tempo. Pela simulação de Monte-Carlo, a ação sinérgica da vinhaça com o tebuthiuron em períodos mais longos diminuirá continuamente o impacto negativo, inclusive com o thiamethoxam. Entretanto, a atenuação natural eficaz de ambos os pesticidas em solo sem vinhaça não é provável a longo prazo, pois seus resultados tiveram desempenho inferior à amostra controle.

Estas duas conclusões limitantes do processo incentivaram a realização da pesquisa disposta no Anexo III: (I) a atenuação natural não foi eficaz na remediação do solo com o tebuthiuron isoladamente, e (II) o metabolismo microbiano demonstrou ser altamente dependente do tempo. Com o intuito de promover um tratamento eficiente e mais rápido, o novo estudo assumiu estes desafios a partir do isolamento de microrganismos de interesse biotecnológico.

3.3. Anexo III – Lima et al. (2022)

LIMA, E.W.; BRUNALDI, B.P.; FRIAS, Y.A.; MOREIRA, B.R.A.; ALVES, L.S.; <u>LOPES, P.R.M.</u> A synergistic bacterial pool decomposes tebuthiuron in soil. Scientific Reports (ISSN 2045-2322; JCR 2023: 3.8), v. 12, 9225, 2022. DOI: http://dx.doi.org/10.1038/s41598-022-13147-8

O terceiro artigo baseou-se em uma dissertação de mestrado defendida no PPGA (FEIS/UNESP) junto a uma iniciação científica financiada pela FAPESP, que originou um TCC em Engenharia Agronômica (FCAT/UNESP).

Seus objetivos foram isolar microrganismos nativos de solos agrícolas com capacidade de metabolizar o tebuthiuron e propor uma tecnologia ecologicamente compatível e economicamente adequada para reduzir a contaminação de agroecossistemas. Até aquele momento, não havia nenhuma pesquisa aprofundada sobre o potencial microbiano na degradação desta molécula como alternativa de recuperação ambiental.

Dessa maneira, os microrganismos potencialmente degradadores foram isolados de áreas tradicionais de produção de cana-de-açúcar em dois sistemas distintos: plantio direto em cana-soca (SPD) e convencional em cana-planta (SC). A escolha destas duas áreas com histórico da aplicação do ingrediente-ativo foi baseada na hipótese de que manejos conservacionistas poderiam promover maior diversidade microbiana no solo.

A avaliação da eficiência metabólica das linhagens bioprospectadas e o monitoramento dos impactos antes e após a inoculação destes isolados foram realizados, respectivamente, por meio do monitoramento respirométrico e ecotoxicológico. Foram utilizados modelos matemáticos consolidados pelo grupo de pesquisa (GAIA) na apresentação e interpretação dos dados. Desta forma, testou-se três condições pela dose recomendada de tebuthiuron (zero, 0,5x e 1,0x) e outras quatro de acordo com a inoculação dos microrganismos bioprospectados (zero, SPD, SC e consórcio SPD+SC).

Inicialmente, a metodologia por cultivo sequencial em meio salino contendo apenas tebuthiuron como fonte de carbono foi capaz de isolar microrganismos em todas as amostras de solo. A caracterização morfológica das linhagens obtidas identificou todas como colônias bacterianas, independente do sistema de cultivo.

A co-inoculação das linhagens isoladas nos dois sistemas em um pool microbiano (SPD+SC) aumentou a eficiência de degradação do herbicida. Este consórcio apresentou um efeito sinérgico e levou cerca de 24 dias para se aproximar do valor máximo para a biotransformação do tebuthiuron na dose mais alta em CO_2. A presença dos microrganismos isolados de forma separada (SPD ou SC) apresentou taxa metabólica maior do que o ensaio sem inóculo (controle), mas necessitaram de períodos mais longos do que a sua ação conjunta pelo modelo de Gompertz.

Os resultados dos bioensaios ecotoxicológicos corroboraram com a respirometria, uma vez que a inoculação simultânea dos isolados (SPD+SC) também promoveu menor toxicidade. Nestas condições, o organismo-teste apresentou um desenvolvimento maior e mais rápido em comparação às amostras de solo com tebuthiuron na presença separada de cada grupo microbiano (SPD ou SC) ou ainda na sua ausência (controle). A taxa de crescimento relativo pelo modelo de Gompertz também foi maior na presença dos isolados juntos, reforçando ainda mais sua distinta capacidade de desintoxicar o meio. Em suma, os resultados dos testes de ecotoxicidade ajustados ao modelo matemático possibilitaram validar a eficácia deste pool bacteriano na mineralização da molécula-alvo.

Do mesmo modo, a análise de componentes principais (PCA) foi capaz de explicar cerca de 70% da variabilidade dos resultados interdependentes da respirometria e da ecotoxicidade. A correlação positiva da biodegradação microbiana e do desenvolvimento da espécie bioindicadora ressaltou a eficácia das linhagens em conjunto (SPD+SC) na remediação do solo com tebuthiuron.

Então, concluiu-se que microrganismos nativos de solo cultivado com cana-de-açúcar podem ser úteis para a biorremediação de agroecossistemas com tebuthiuron (bioaumentação). Em relação a isso, foi observada uma maior eficiência na sua mineralização quando presente 50% da dose recomendada, cujo valor é muito próximo da concentração residual do herbicida após um ciclo da cultura de 5 anos/cortes. Ou seja, a inoculação dos microrganismos degradadores pode ser realizada junto à reforma do canavial como estratégia eficiente de recuperação da qualidade do solo e de redução dos impactos a organismos não-alvo ou a até à cultura de sucessão.

Ressalta-se que estava prevista a identificação molecular dos isolados de melhor resultado na biodegradação do tebuthiuron. No entanto, a pandemia de Covid-19 não permitiu esta análise e infelizmente as linhagens microbianas foram perdidas em virtude do cenário ocasionado pela crise sanitária mundial. Apesar disso, este estudo preliminar foi oportuno e relevante para o fomentar novas ferramentas biotecnológicas na recuperação de solos agrícolas com pesticidas. Seus resultados certamente possibilitaram a abertura de soluções sustentáveis para a descontaminação de agroecossistemas com a manutenção da produtividade agrícola.

Desta forma, foi observada a existência de inúmeras publicações sobre a ação microbiana na biorremediação de solos com pesticidas. Porém, cada uma abordava questões muito particulares e as revisões até então publicadas resumiam evidências de artigos independentes para responder essas perguntas específicas. Uma visão geral foi ilustrada pela Figura 1 (Anexo III), com as primeiras ocorrências de palavras-chave relacionadas à “biorremediação de pesticidas” nas principais bases de dados científicas consultadas em 2021. Em decorrência, surgiu a ideia do próximo trabalho desta tese (Anexo IV) que reuniu diferentes cientistas da área para o desafio de uma meta-análise crítica a respeito da temática sobre biossistemas microbianos na degradação de pesticidas em solos agrícolas.

3.4. Anexo IV – Lopes et al. (2022)

<u>LOPES, P.R.M.</u>; CRUZ, V.H.; MENEZES, A.B.; GADANHOTO, B.P.; MOREIRA, B.R.A.; MENDES, C.R.; MAZZEO, D.E.C.; DILARRI, G.; MONTAGNOLLI, R.N. Microbial bioremediation of pesticides in agricultural soils: an integrative review on natural attenuation, bioaugmentation and biostimulation. Reviews in Environmental Science and Bio/Technology, v. 21 (ISSN 1569-1705; JCR 2023: 8.6), p. 851-876, 2022. DOI: http://dx.doi.org/10.1007/s11157-022-09637-w

Este artigo surgiu após uma discussão entre pesquisadores do Brasil e do exterior que observaram a necessidade de explorar, compilar e sistematizar as estratégias microbiológicas no tratamento de solos agrícolas com pesticidas, uma vez que as publicações disponíveis geralmente apresentam evidências resumidas e independentes. Deste modo, as revisões existentes não propunham uma aplicação estatística nos metadados para sintetizar tendências e fornecer insights analíticos sobre a temática. Por outro lado, esta meta-análise propôs garantir informações mais críticas e precisas sobre a biorremediação microbiana de pesticidas de solos agrícolas em contraste a uma simples coletânea descritiva em uma revisão sistemática.

A metodologia foi baseada na busca nas bases de dados Scopus e Web of Science (2018-2021) usando combinações dos termos “microbial bioremediation” e “pesticide” e focadas em quatro eixos principais: “natural attenuation, bioaugmentation and biostimulation, microbial community, and metabolism”. Foram selecionados 55 artigos originais que foram submetidos a um checklist de 14 critérios para elaboração da planilha de dados. A exclusão dos manuscritos baseou-se no escopo fora da temática agrícola, na omissão de algumas variáveis-chave e em revisões sem dados primários. Por último, os dados finais foram sintetizados e disponibilizados como material suplementar.

De forma geral, o enfoque integrativo permitiu mapear lacunas de qualidade em alguns destes estudos recentes, mas a ausência de dados experimentais completos em muitos artigos limitou análises meta-estatísticas robustas. Foi observado que a produção científica na temática era dominada por análises sobre a comunidade microbiana (47%) e seu metabolismo (40%), com somente 6% tratando de atenuação natural e 8% abordando as técnicas de aperfeiçoamento como bioaumentação e bioestimulação. Ou seja, geralmente os trabalhos possuíam avaliações isoladas sem um direcionamento prático às estratégias de biorremediação. Neste sentido, as publicações apresentaram-se com procedimentos analíticos heterogêneos sem a padronização de protocolos que limitaram a comparação e o avanço nas alternativas microbianas para o tratamento de solos agrícolas com pesticidas.

Em relação às estratégias de remediação, a atenuação natural foi identificada com eficiência regular, principalmente para o herbicida lindane, sendo pouco utilizada pela necessidade de um tempo maior e da sua forte correlação com as características do solo (pH, textura e matéria orgânica). Como técnica aceleradora pela introdução dirigida de microrganismos (autóctones, alóctones ou OGM), a bioaumentação possibilitou maior remoção de pesticidas persistentes no solo. Um exemplo foi o aumento de mais de duas vezes na degradação da atrazine pela inoculação de *Pigmentiphaga* spp. (Alcaligenaceae). Todavia, avaliações sobre as consequências posteriores são necessárias para evitar desequilíbrio ecológico nas comunidades microbianas indígenas por meio de relações desarmônicas com as espécies introduzidas. Da mesma forma, a bioestimulação também promove o aperfeiçoamento do tratamento e impulsiona o crescimento microbiano autóctone pela suplementação de nutrientes ou ajustes nas condições físico-químicas do ambiente. Porém, considera-se que a adição de fertilizantes químicos pode ocasionar efeitos negativos, como a eutrofização e o favorecimento de outros grupos de organismos. Como opção sustentável e mais cuidadosa de suplemento orgânico, o esterco animal foi indicado com benefícios à fertilidade do solo e à bioestimulação na diminuição da concentração de pesticidas, como o chlorpyrifos.

Percebeu-se a dominância das bactérias como grupo microbiano utilizado na biorremediação de solos com pesticidas. Curiosamente, mais da metade dos artigos (55%) baseava-se em monoculturas ao invés de explorar a complexidade metabólica de consórcios microbianos. E, apesar de cepas fúngicas já demonstrarem capacidade elevada na remoção de pesticidas em solo, pesquisas com estes organismos ainda são escassas, o que indica um grande potencial inovador e biotecnológico a ser explorado.

Outra lacuna identificada foi em relação às rotas metabólicas da biodegradação microbiana frente aos pesticidas. Apenas 2 dos 21 trabalhos encontrados sobre este assunto reportavam integralmente a via de degradação com compostos precursores e metabólitos, além de enzimas e genes envolvidos. Logo, a existência de produtos secundários às vezes mais tóxicos que a molécula original pode não ser rastreada e, consequentemente, gerar impactos desconhecidos após a ação microbiana.

Como conclusão, esta revisão integrativa propõe alguns aspectos interessantes para o avanço científico na área, baseando-se em: (a) tecnológicas ômicas para mapear rotas completas de degradação dos pesticidas e prever formação de subprodutos; (b) consórcios microbianos e OGMs para aumentar a eficiência do tratamento com a preocupação constante com a biossegurança e a bioética; (c) fitorremediação associada a bioinsumos agrícolas para desenvolver sistemas integrados na produção agrícola sustentável e com menos dependência de fertilizantes minerais; (d) monitoramento ecológico constante para consolidar uma abordagem multidisciplinar com avaliações da diversidade microbiana e da ecotoxicidade do ambiente; e (e) economia circular para fomentar o reaproveitamento sustentável de resíduos agroindustriais como compostos alternativos para a manutenção da produtividade com redução de custos e mitigação de possíveis impactos ambientais.

A partir da experiência do grupo de pesquisa e da análise crítica desta meta-análise, foi proposto um trabalho com o uso de plantas leguminosas associado à bioaumentação com inoculantes microbianos para acelerar a degradação do herbicida tebuthiuron (Anexo V).

3.5. Anexo V – Cruz et al. (2023)

CRUZ, V.H.; MOREIRA, B.R.A.; VALÉRIO, T.S.; FRIAS, Y.A.; SILVA, V.L.; MORAIS, E.B.; VASCONCELOS, L.G.; TROPALDI, L.; PRADO, E.P.; MONTAGNOLLI, R.N.; LOPES, P.R.M. Leguminous plants and microbial inoculation: an approach for biocatalytic phytoremediation of tebuthiuron in agricultural soil. Agronomy (ISSN 2073-4395; JCR 2023: 3.3), v. 14, n. 12, 2805, 2024. DOI: http://dx.doi.org/10.3390/agronomy14122805

O quinto manuscrito apresenta os resultados de uma dissertação de mestrado defendida no PPGA e financiada pela FAPESP.

Sua hipótese foi utilizar processos fisiológicos de plantas e microrganismos de interesse agronômico em um sistema biocatalítico propondo uma situação de reforma da lavoura canavieira para melhorar a qualidade do solo concomitante à redução do impacto toxicológico do tebuthiuron. Assim, foi realizado um arranjo fatorial 2x3x3 pelos seguintes fatores: tebuthiuron (ausência e presença), inoculantes microbianos comerciais (ausência, bacteriano e fúngico), e espécies vegetais utilizadas como adubo verde e com potencial fitorremediador (ausência, *Canavalia ensiformis* ou feijão-de-porco, e *Mucuna pruriens* ou mucuna-cinza). Este delineamento foi aplicado no monitoramento do desenvolvimento vegetal e da ecotoxicidade das amostras de solo em cinco tempos até 70 dias após a semeadura, totalizando 630 vasos como unidades experimentais. Transcorrido este período, foi observado o crescimento de *Crotalaria juncea* como planta indicadora para avaliar a eficácia do processo de biorremediação. Por fim, a modelagem matemática foi aplicada nas análises cinéticas dos dados.

Primeiramente, foi comprovada a maior tolerância e desenvolvimento da *Mucuna pruriens* em solo com tebuthiuron em comparação a *Canavalia ensiformis*. Este resultado corrobora com o Anexo I. Porém, a relação destas plantas com cada inoculante foi bem diferente. O bioinsumo com predominância de bactérias favoreceu o crescimento de *M. pruriens* sob estresse da presença do herbicida, enquanto o inoculante com maior concentração fúngica foi mais eficaz com *C. ensiformis*. Este resultado sugere uma interessante especificidade planta-microrganismos que pode ser ainda mais explorada futuramente, assim como destacado no Anexo IV.

Após 70 dias de cultivo prévio destas plantas, o desenvolvimento de *Crotalaria juncea* nos vasos e de *Lactuca sativa* nos bioensaios consolidou o protocolo experimental desenvolvido pelo grupo de pesquisa. Seus resultados demonstraram alta sensibilidade destas espécies pela presença do tebuthiuron e indicaram a detoxificação do solo e consequente sucesso das estratégias biológicas na redução do seu impacto, mesmo em concentrações residuais. Neste experimento, o cultivo anterior de *M. pruriens* possibilitou maior taxa específica de crescimento e biomassa seca para a crotalária e maior germinação e desenvolvimento para o organismo-teste (alface), reforçando o seu potencial fitorremediador em solos com tebuthiuron.

Portanto, combinar leguminosas com inoculantes microbianos pode representar uma alternativa ecologicamente viável, pois a bioaumentação dirigida beneficiou diretamente a fitorremediação, além de propiciar ganhos agronômicos na recuperação da qualidade do solo para o próximo ciclo da cultura. Embora as plantas tenham sofrido inibição inicial pelo herbicida, os bioinsumos atenuaram significativamente a fitotoxicidade, sugerindo vias metabólicas capazes de transformar esta molécula em derivados menos tóxicos. Este desempenho positivo da fitorremediação junto à bioaumentação foi também validado com as espécies bioindicadoras (*C. juncea* e *L. sativa*). Um componente que pode servir de modelo para a avaliação de outros pesticidas persistentes em solos é abordagem estatística multiparamétrica e com modelos matemáticos.

Ao final, este artigo ainda reconhece algumas limitações e indica diretrizes para melhorar o processo, além discorrer sobre perspectivas futuras. O fato deste experimento ser realizado dentro de estufa agrícola permite evitar variáveis indesejadas do campo, mas não reflete toda a complexidade das práticas agrícolas. Em cenários reais, torna-se interessante a avaliação de longa duração comparando condições de solo distintas que modulam a eficiência da biorremediação (textura, composição físico-química, matéria orgânica, diversidade microbiana, etc.). A mistura do tebuthiuron com outros pesticidas, bioinsumos e resíduos agroindustriais no solo também promovem quadros desconhecidos que interferem diretamente no comportamento ambiental da molécula.

Em relação às novas abordagens para o avanço da sustentabilidade agrícola, é sugerida a integração de: (a) pesquisas avançadas sobre interações planta-microrganismos; (b) tecnologias ômicas e engenharia genética; (c) estratégias combinadas com outros processos físicos, químicos e biológicos de tratamento;

(d) aplicações do processo em ambientes aquáticos e urbanos; e (e) políticas públicas e conscientização da sociedade. À medida que as preocupações ambientais aumentam globalmente, a evolução da fitorremediação em larga escala torna-se cada vez mais significativa, principalmente pelos ganhos agronômicos em paralelo. À visto disso, esclarecer as suas limitações e projetar perspectivas pode torná-la uma ferramenta mais sólida e versátil para mitigar a contaminação de agroecossistemas e apoiar práticas agrícolas sustentáveis.

O próximo trabalho (Anexo VI) buscou um novo caminho para o enfrentamento dos prejuízos ocasionados pelo tebuthiuron a partir do reuso de resíduos orgânicos na agricultura. Por consequência, buscou-se uma solução sustentável para atuar em conjunto com as estratégias de biorremediação por meio de uma alternativa inovadora baseada em um material adsorvente de baixo custo e origem renovável: o hydrochar.

3.6. Anexo VI – Moreira et al. (2023)

MOREIRA, B.R.A.; CRUZ, V.H.; BARBOSA JÚNIOR, M.R.; VASCONCELOS, L.G.; SILVA, R.P.; LOPES, P.R.M. Adsorption of tebuthiuron on hydrochar: structural, kinetic, isothermal, and mechanistic modeling, and ecotoxicological validation of remediative treatment of aqueous system. Biomass Conversion and Biorefinery (ISSN 2190-6823 JCR 2023: 3.5), v. 13, p. 21741–21755, 2024. DOI: http://dx.doi.org/10.1007/s13399-023-04365-9

O sexto artigo foi realizado em paralelo com os projetos de pesquisa conduzidos pelos membros do GAIA avaliando uma nova técnica para o aperfeiçoamento no tratamento biológico de ambientes com tebuthiuron: a biossorção.

A ideia surgiu da constatação de que o tebuthiuron, embora eficaz no controle de plantas daninhas, representa um risco significativo ao meio ambiente devido à sua alta solubilidade em água, persistência química e capacidade de lixiviação, que o torna um potencial contaminante em corpos hídricos e ecossistemas agrícolas. Com esta crescente preocupação somada às limitações da fitorremediação e da biodegradação microbiana, foi proposta a fabricação de um hydrochar a partir de resíduos alimentares para a diminuição da concentração do herbicida por processos biossortivos.

A pesquisa também foi motivada por uma lacuna de estudos que interliguem caracterização físico-química, modelagem cinética/isotérmica e validação ecotoxicológica de materiais bioadsorventes na remediação de pesticidas. Seus objetivos foram: (I) sintetizar um material poroso e funcional a partir de resíduos alimentares; (II) avaliar sua capacidade de remover tebuthiuron de soluções aquosas; e (III) monitorar o uso deste hydrochar em múltiplos ciclos com baixo risco residual ecotoxicológico.

A metodologia utilizou restos de alimentos de origem vegetal coletados em restaurantes em um processo simultâneo de carbonização hidrotérmica (HTC) e ativação química com KOH. O hydrochar preparado foi caracterizado físico-química e morfologicamente por diferentes procedimentos e testado quanto à sua capacidade adsortiva em relação ao tebuthiuron em solução aquosa, com posterior análise de modelagem cinética (pseudo-primeira ordem e pseudo-segunda ordem) e isotérmica (Freundlich, Langmuir e Temkin). Avaliou-se também a regeneração deste bioadsorvente por até 10 ciclos, com monitoramento da ecotoxicidade por bioensaios com *Lactuca sativa*. Este delineamento permitiu a execução de um experimento articulando desempenho, sustentabilidade e viabilidade de sua aplicação ambiental.

O hydrochar preparado demonstrou excelente desempenho na adsorção do tebuthiuron, atingindo taxas de remoção superiores a 98% em concentrações de até 1,5 $mg \cdot L^{-1}$. Essa elevada eficiência foi atribuída à sua área superficial e mesoporosidade, colocando-o no mesmo patamar de adsorventes comerciais. No entanto, vale ressaltar que toda a análise foi realizada em condições laboratoriais controladas.

Quanto aos mecanismos de adsorção, foi observada a atuação conjunta de processos físicos e químicos com destaque à capacidade de regeneração e reuso do material por até sete ciclos, mantendo mais de 80% da eficiência inicial. Apesar destes resultados indicarem a sua clara viabilidade de aplicação prática, os bioensaios indicaram uma redução parcial da ecotoxicidade residual do tebuthiuron. Logo, esta descoberta abre novas possibilidades de uso conjunto do hydrochar com outras estratégias de biorremediação que possivelmente diminuíram ainda mais este impacto negativo e proporcionaria um ambiente mais saudável.

O comportamento adsortivo do tebuthiuron pelo hydrochar ajustou-se melhor à cinética de pseudo-segunda ordem e à isoterma de Freundlich, que indicam um processo de quimiossorção em multicamadas e superfície heterogênea. Essa combinação sugeriu tanto uma vantagem em termos de capacidade de adsorção, quanto um desafio para controle e padronização industrial.

Portanto, este hydrochar representa um passo promissor na direção de soluções sustentáveis para a remediação de ambientes com tebuthiuron e outros pesticidas. A combinação de seu alto desempenho adsortivo, baixo custo e caráter regenerável posiciona este material como uma alternativa tecnicamente viável e ambientalmente alinhada com os princípios da economia circular. Entretanto, para consolidar essa tecnologia em situações reais de contaminação, é imprescindível avançar com testes em ambientes complexos, padronizar matérias-primas, integrar análises químicas e ecotoxicológicas, e incorporar critérios econômicos e normativos.

4. CONSIDERAÇÕES FINAIS

Esta tese consolida as descobertas de seis artigos recentes realizados pelo "GAIA – Grupo de Ação em Impactos Ambientais" da FCAT/UNESP sobre a remediação ambiental de tebuthiuron, um herbicida persistente e com elevada toxicidade amplamente utilizado no cultivo da cana-de-açúcar. Esta coletânea sequencial explora diferentes abordagens biológicas como: fitorremediação, atenuação natural (biodegradação microbiana), bioaumentação, bioestimulação e biossorção. A análise crítica dos seus resultados indica lacunas de conhecimento na área e possíveis diretrizes para futuras investigações, destacando a necessidade de integração de estratégias e da avaliação de impactos ecotoxicológicos a longo prazo para fundamentar sistemas agrícolas sustentáveis.

Neste contexto, a multidisciplinaridade é essencial para discutir alternativas práticas de manejo com pesticidas para manter a produtividade agrícola, recuperar a qualidade do solo, mitigar o potencial de contaminação destes compostos e evitar prejuízos aos agroecossistemas. A avaliação isolada de fatores muitas vezes subdimensiona as consequências reais e não reconhece possíveis efeitos negativos a organismos não-alvo e a outras matrizes ambientais.

Os trabalhos selecionados asseguram a implantação de estratégias de tratamento de solos com tebuthiuron em paralelo com o desenvolvimento sustentável da produção agrícola. Inicialmente, tem-se resultados notáveis sobre espécies vegetais de interesse agronômico para a rotação da cultura canavieira (*Mucuna pruriens* e *Pennisetum glaucum*) que podem auxiliar na detoxificação de solos com este herbicida, promovendo a sustentação de culturas sentinelas como *Crotalaria juncea* e o desenvolvimento de organismo-teste sensível como *Lactuca sativa* (Anexo I). A vinhaça, utilizada na fertirrigação das áreas de cultivo, potencializou esta capacidade fitorremediadora das plantas e incentivou a biodegradação microbiana do tebuthiuron pelo processo de bioestimulação (Anexo II), revelando mais um benefício da sua aplicação além dos ganhos agronômicos e econômicos. Outra técnica de aperfeiçoamento da biorremediação que apresentou alta eficiência foi a bioaumentação a partir de linhagens microbianas isoladas de solo de lavouras canavieiras, principalmente quando associadas em um pool bacteriano sinergético (Anexo III). Apesar dos desafios e da necessidade de novas pesquisas, a meta-análise ressaltou a aptidão da biorremediação por microrganismos para reduzir efeitos ecológicos adversos e propiciar uma produção agrícola mais saudável pelo uso de pesticidas (Anexo IV). Assim, a inoculação microbiana junto às plantas foi capaz de aumentar a tolerância vegetal pelo estresse ocasionado pelo tebuthiuron, potencializar a degradação desta molécula, e reduzir a ecotoxicidade residual do solo, reforçando a tendência da integração dos processos biotecnológicos (Anexo V). Finalmente, foi apresentado um bioadsorvente sustentável (hydrochar) com alta regenerabilidade e elevada capacidade de adsorção de tebuthiuron em soluções aquosas, que fomenta ainda mais a sustentabilidade agrícola por favorecer a economia circular no reaproveitamento de resíduos.

Sabendo que o enfrentamento da contaminação ambiental de solos agrícolas com pesticidas, como o tebuthiuron, requer uma abordagem verdadeiramente multidisciplinar, integrando conhecimentos de diferentes áreas do conhecimento (agronomia, microbiologia, química ambiental, engenharia de materiais, ciência de dados, etc.), esta cooperação é essencial para desenvolver soluções poderosas, sustentáveis e adaptáveis às condições de campo. À vista disso, os estudos apresentados nesta tese acrescentam interessantes descobertas na área que podem ser exploradas de forma mais aprofundada em pesquisas futuras, destacando: o emprego de análises estatísticas com modelos matemáticos, que permitiram uma previsibilidade e compreensão mais precisa da dinâmica de degradação do tebuthiuron no solo; e a validação das estratégias por protocolos de ecotoxicidade com bioindicadores.

A partir das lacunas identificadas e dos resultados apresentados, esta linha de pesquisa concentra um enorme potencial científico e prático para a criação de processos inovadores para otimizar suas aplicações com o mínimo de riscos ambientais e maior estímulo à sustentabilidade dos sistemas agroindustriais. Logo, possíveis caminhos para expansão do conhecimento científico na temática fundamentam-se em: (1) uso de outras espécies vegetais de interesse agronômico; (2) busca por novas espécies e consórcios microbianos com potencial sinérgico; (3) associação com outros resíduos agroindustriais e bioinsumos; (4) aprofundamento do uso de bioadsorventes; (5) implementação de estratégias e ferramentas biotecnológicas combinadas; (6) desenvolvimento de protocolos amplos de monitoramento ecotoxicológico a longo prazo com diferentes organismos-teste; (7) avaliação do efeito combinado com outros pesticidas; (8) determinação da concentração residual do pesticida e de seus metabólitos secundários; (9) identificação de subprodutos intermediários e estabelecimento da rota de degradação da molécula contaminante; (10) viabilidade econômica do processo; e (11) validação dos processos a campo em condições práticas.

5. ANEXOS

5.1. Anexo I

Luziane Cristina FERREIRA; Bruno Rafael de Almeida MOREIRA; Renato Nallin MONTAGNOLLI; Evandro Pereira PRADO; Ronaldo da Silva VIANA; Rafael Simões TOMAZ; Jaqueline Matos CRUZ; Ederio Dino BIDOIA; Yanca Araujo FRIAS; Paulo Renato Matos LOPES.

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Green Manure Species for Phytoremediation of Soil With Tebuthiuron and Vinasse

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Tebuthiuron is often used to control weed growth in sugarcane cultures. This herbicide is highly toxic and can persist in soil for up to 2 years according to its degradation half-life. Hence, its residual effect is highly hazardous for the environment and local habitants via leaching, surface runoff. Screening out of species of green manure as potential phytoremediators for tebuthiuron in soil, with and with no vinasse, accordingly is the scientific point of this study. Green manure species selected for the trial in greenhouse were jack bean [*Canavalia ensiformis* (L.) DC.], pigeon pea [*Cajanus cajan* (L. Millsp.)], velvet bean [*Mucuna pruriens* (L.) DC.)], and millet [*Pennisetum glaucum* (L.) R.Br.], and *Crotalaria juncea* L. as bioindicator of this herbicide. The determination/quantification of height, stem diameter, and number of leaves in all plants were monitored, as well as other morphological traits for drafting any inference on biomass production. Moreover, ecotoxicity bioassays were performed from soil samples at the beginning and at the end of the experiment. Results showed preliminary evidence of effective phytoremediation capacity by *M. pruriens* and *P. glaucum* in soils with tebuthiuron, as the growth of *C. juncea* was sustained. Both Gompertz approach and principal component analysis predicted that these green manure species could grow healthier and for longer periods in soils containing tebuthiuron and vinasse and, thus, reduce physiological anomalies due to ecotoxicity. The implications of this study may aid in the implementation of cost-effective strategies targeting decontamination of tebuthiuron in sugarcane crops with vinasse application in fertigation.

Keywords: bioremediation, ecotoxicity, fertigation, herbicide, sugarcane

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INTRODUCTION

Sugarcane is affected by weeds, despite its highly efficient photosynthetic pathway (C_4) that promotes adequate development, especially in its early stages. Weeds compete for available soil resources and therefore undermine agricultural yields (Victoria Filho and Christoffoleti, 2004; Sandaniel et al., 2008).

The planting of sugarcane takes place in wide open areas so that high productivity is achieved, aided by technological tools for the proper weed management as herbicides (Kuva et al., 2008; Oliveira and Brighenti, 2011). Such chemical method is the first choice of agricultural producers due to its ease of access, availability, and low operational costs, compared to other control techniques (Kuva et al., 2008).

Among the herbicides commercialized for sugarcane, tebuthiuron is the most used, whose selective pre-emergent action controls main weeds in the crop (Moraes et al., 2016). This molecule [1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea] has a systemic action and acts in the inhibition of photosystem II (Breitenbach et al., 2001). However, tebuthiuron can cause environmental damage since it is considered dangerous to the environment due to its high persistence and long half-life in the environment, moderate to extreme toxicity (Rodrigues and Almeida, 2011), low sorting capacity in soil (Koskinen et al., 1996), and high solubility in water (Franco-Bernardes et al., 2014). Hence, residual concentration is an extremely important factor, as it results in a greater potential for contamination. Therefore, successive applications without proper management can make its potential for impact even greater on soil and groundwater (Christofoletti et al., 2017).

Vinasse can also be applied to farmlands as growth-inducing agents, as opposed to herbicides. Vinasse is one of the many by-products of sugar production also found in alcohol distilleries in enormous quantities. It is highly applicable as fertilizer during crop production (Andrade, 2007). It is considered a residue from the alcohol production, generated at a 10–14:1 ratio (Assad, 2017). The outstandingly large amount of vinasse generated everyday highlights its expressive polluting potential and, therefore, demands the development of proper disposal protocols. However, vinasse can also be used to enrich soils due to its nutritional value. Still, its environmental effects combined with herbicides are yet to be determined.

The cleaning up process of areas with previous pesticides release is not simple, but fortunately, many solutions have been improved in the past decades. Feasible solutions should follow four requirements, as proposed by Ferro et al. (1994): (i) high decontamination efficiency, (ii) straightforward execution, (iii) fast and reliable protocols, and (iv) cost effectiveness. Bioremediation is as an ecologically viable strategy that meets such requirements during the treatment of impacted areas by organic pollutants. The acceleration of natural biological processes that reduce the concentration and toxic effects of polluting agents is the core of all bioremediation strategies (Fasanella and Cardoso, 2016).

Phytoremediation further expands this definition by using plants to reduce the toxicity of contaminants in the environment (Ali et al., 2013). Research related to this technique seeks to understand the plant–contaminant interactions that may lead to full pollutant removal (Vasconcellos et al., 2012). Therefore, the plants must be capable of absorbing toxic elements in the soil to promote decontamination (Souza et al., 2011).

Pires et al. (2008) reported that millet (*Pennisetum typhoides*), velvet bean (*Stizolobium aterrimum*), Jack bean (*Canavalia ensiformis*), and pigeon pea (*Cajanus cajan*) were highly effective toward tebuthiuron phytoremediation. They used sunn hemp (*Crotalarea juncea*) as the bioindicator plant. Several studies reinforce this approach, as many authors have observed a decrease in pesticides concentration in soils by using phytoremediation (Pires et al., 2003, 2005, 2006; Pires et al., 2008; Madalão et al., 2013; Melo et al., 2017).

However, there is a major drawback in all those studies: the toxicity of these samples has not been quantified before and after the treatments. The degradation of organic compounds could potentially generate intermediate compounds that are often more toxic than the original formulation (Rocha et al., 2018). We argue that it is imperative to evaluate the ecotoxicological potential in a broader time-dependent approach to demonstrate the success of bioremediation strategies (Banks and Schultz, 2005).

In this context, we evaluated the potential of four plant species to remediate soil samples contaminated with tebuthiuron and the effects of vinasse in the process.

MATERIALS AND METHODS

Experiments were set up in a greenhouse located at the College of Agricultural and Technological Sciences, Sao Paulo State University (Unesp), Dracena, São Paulo, Brazil, with geographical coordinates of 21°28′57′′ S 51°31′58′′ W and 400 m elevation.

According to the Köppen (1948) classification, the regional climate type is Aw (tropical humid). The average local temperature and precipitation are 22.1°C and 1,200 mm, respectively. The meteorological data were provided by our own station (Dracena EMA/FCAT).

The observations occurred between May and July 2019. The average temperature and relative humidity were 22.6°C and 62.9%, respectively, also obtained from the Dracena EMA/FCAT weather station.

Soil, Vinasse, and Tebuthiuron Sampling

The regional soil is a dystrophic red-yellow argisol type according to the classification proposed by Santos et al. (2018). The physical analysis revealed that it is composed of 89.9% sand, 7.1% clay, and 3.0% silt.

The soil has the following chemical characteristics: phosphorus, 5 mg/dm^3; organic matter, 3 mg/dm^3; pH 5.2; potassium, 1.7 mmolc/dm^3; calcium, 15 mmolc/dm^3; magnesium, 4 mmolc/dm^3; H + Al, 13 mmolc/dm^3; CTC, 34 mmolc/dm^3; sum of bases, 21 mmolc/dm^3; and base CTC saturation (V%), 61%. This characterization served as a basis to the optimal fertilizing conditions in our pots for all the proposed species.

Fertilizer dosages per pot were set individually to meet each species needs. We applied 80 g of urea diluted in 1.5 L of water, divided into three applications, in *Pennisetum glaucum* (L.) R.Br. We applied 8 g of urea diluted in 4.5 L of water, applied only once at sowing, in legumes [*C. ensiformis* (L.) DC., *C. cajan* (L. Millsp.), *Mucuna pruriens* (L.) DC.]. For all other pots, we added 125 g of KCl diluted in 6 L of water, divided into three applications, and 445 g of simple super phosphate to 320 L of soil, necessary

TABLE 1 | Morphological aspects of green manure species in soil with tebuthiuron and vinasse.

Species	Test			
	T-V-	**T-V+**	**T+V-**	**T+V+**
		Height, cm		
C. cajan	64.40 Aa	49.20 Bb	ND*	ND*
C. ensiformis	75.00 Aa	64.80 Bb	ND*	ND*
M. pruriens	125.80 Aa	134.60 Aa	115.50 Aab	44.00 Bb
P. glaucum	71.00 Aa	53.60 Ba	60.75 Ba	56.65 Aa
		Diameter, mm		
C. cajan	5.40 Ba	4.69 Bb	ND*	ND*
C. ensiformis	5.65 Ba	5.60 Bb	ND*	ND*
M. pruriens	5.05 Ba	5.05 Ba	4.45 Bab	3.50 Bb
P. glaucum	52.70 Aa	42.30 Aa	35.90 Aa	23.15 Ab
		Leaves		
C. cajan	16.80 Aba	13.00 Bb	ND*	ND*
C. ensiformis	4.80 Ca	4.20 Cb	ND*	ND*
M. pruriens	14.40 Ba	11.00 Ba	11.50 Ba	2.00 Ab
P. glaucum	22.50 Aa	20.80 Aa	20.50 Aa	16.30 Ba

Same letters, whether uppercase in the column and lowercase in the row, describe statistically similar means by post hoc Tukey's HSD test at $p < 0.05$;.
**As the plant dead, the trait became undetected (ND).*

for filling the pot total volume. The volume of each vessel was 4 dm^3.

The vinasse was collected from a sugar-energy plant in the Dracena-SP region using sterile glass bottles. The vinasse was subsequently stored in a refrigerator at 4°C until its use in the experimental units preparation (stored for 3 days).

The herbicide tebuthiuron was provided by Combine® 500SC—Dow AgroSciences Industrial Ltd.

Plant Species

The plant species were chosen according to their capability to remediate pesticide-contaminated soils. Their agricultural potential to improve overall soil quality was another criterion. We narrowed our plants selection to species that are often found as green manure and/or forage in the rotation of the sugarcane cultures. Thus, we used the following species: pigeon pea (*C. cajan*), jack bean (*C. ensiformis*) (Madalão et al., 2013, 2016), velvet bean (*M. pruriens*) (Pires et al., 2005, 2008), and millet (*P. glaucum*) (Pires et al., 2008).

Experimental Setup

We designed the experiments according to a randomized blocks approach at a 2 × 2 × 4 factorial scheme with five repetitions. The parameters were tebuthiuron concentration, vinasse volume, and the four plant species.

The treatments are further referred to in this paper as indicated in the brackets: [T–], absence of tebuthiuron; [T+], presence of tebuthiuron; [V–], absence of vinasse; and [V+] presence of vinasse.

Preparation of Experimental Units

Experimental units (pots 4 dm^3 and their contents) were filled with soil and received the four potentially phytoremediating species. Treatments containing vinasse had to undergo manual compound application to endure homogeneity. The vinasse was added first, at 150 m^3 ha^{-1} (150 ml dm^{-3}), following the CETESB Technical Standard P4.231/2005 (2005) on the procedures to apply vinasse to agricultural soils.

The Combine® 500 SC was sprayed at the following day, at 2 L ha^{-1} as the recommended rate of this herbicide in sugarcane crops, using a CO_2 pressurized sprayer (Herbicat®) equipped with six XR 8002 flat jet nozzles at a pressure of 2 bar (0.65 L min^{-1} flowrate) from a minimum distance of 0.5 m. The application was carried out 0.75 m above the pots at a constant speed (5 km h^{-1}) until 250 L ha^{-1} had been applied. Environmental conditions such as temperature and relative humidity were monitored at the time of spraying using a portable digital thermo-hygro-anemometer-luximeter (Instrutherm® model THAL-300). The spraying occurred inside our greenhouse to avoid wind interference during the application. An equivalent volume of deionized water was added to treatments without vinasse and/or tebuthiuron.

Finally, 10 seeds per pot were sown the day after the tebuthiuron application. Thinning was performed on the eight DAS to keep only one plant per pot. These were irrigated daily by microsprinklers for 60 min (30 min at 6:00 a.m. and 30 min at 6:00 p.m.) to ensure adequate conditions for plant growth.

The cultivation of species with phytoremediation potential was performed for 50 days. Therefore, the final phytoremediation evaluation time for these plants is t_{50}.

Ten days after harvesting the plants of these species, we sowed 10 seeds of the bioindicator species sunn hemp (*C. juncea* L).

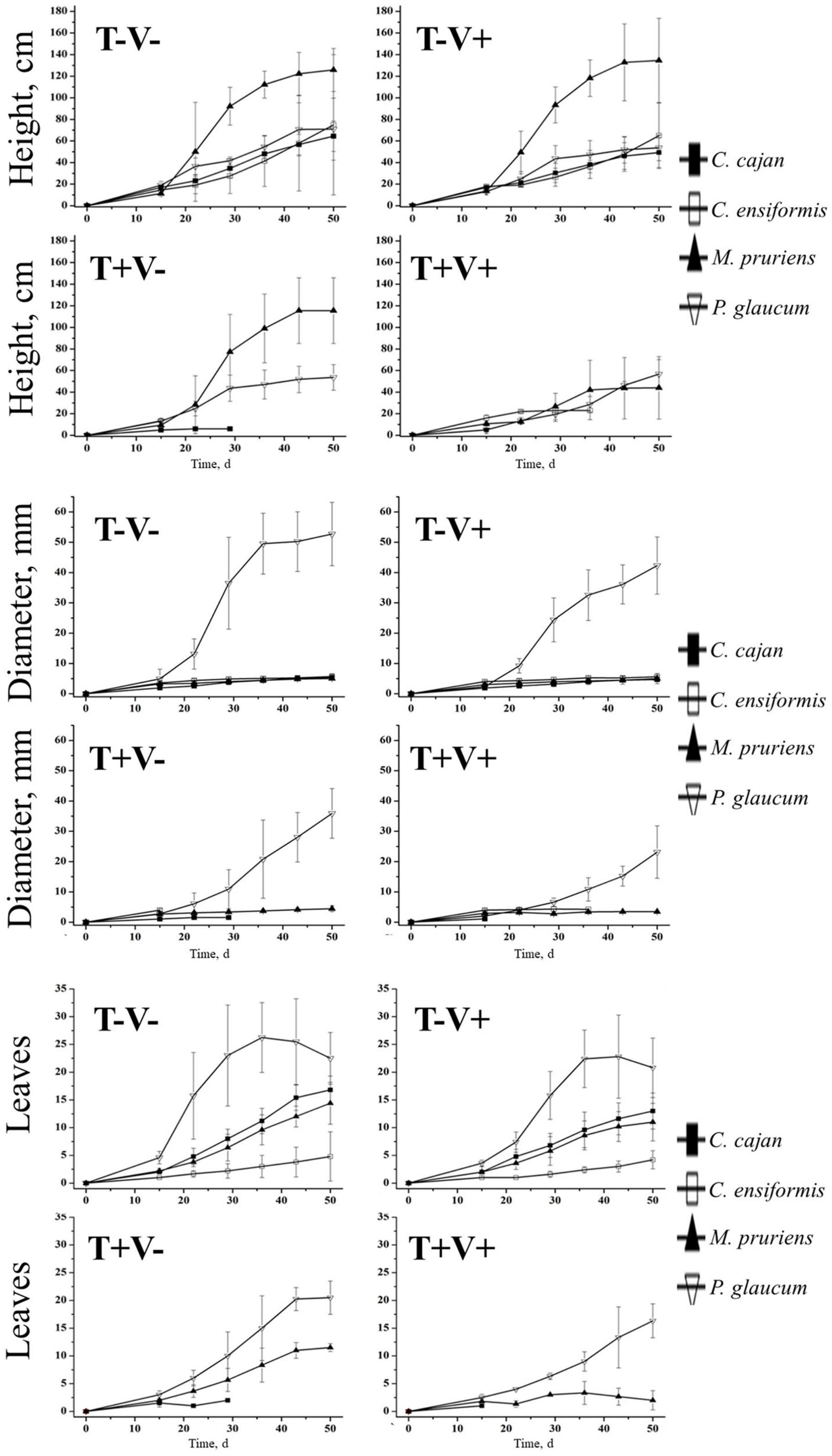

FIGURE 1 | Production of biomass by green manure species in soil with tebuthiuron and vinasse.

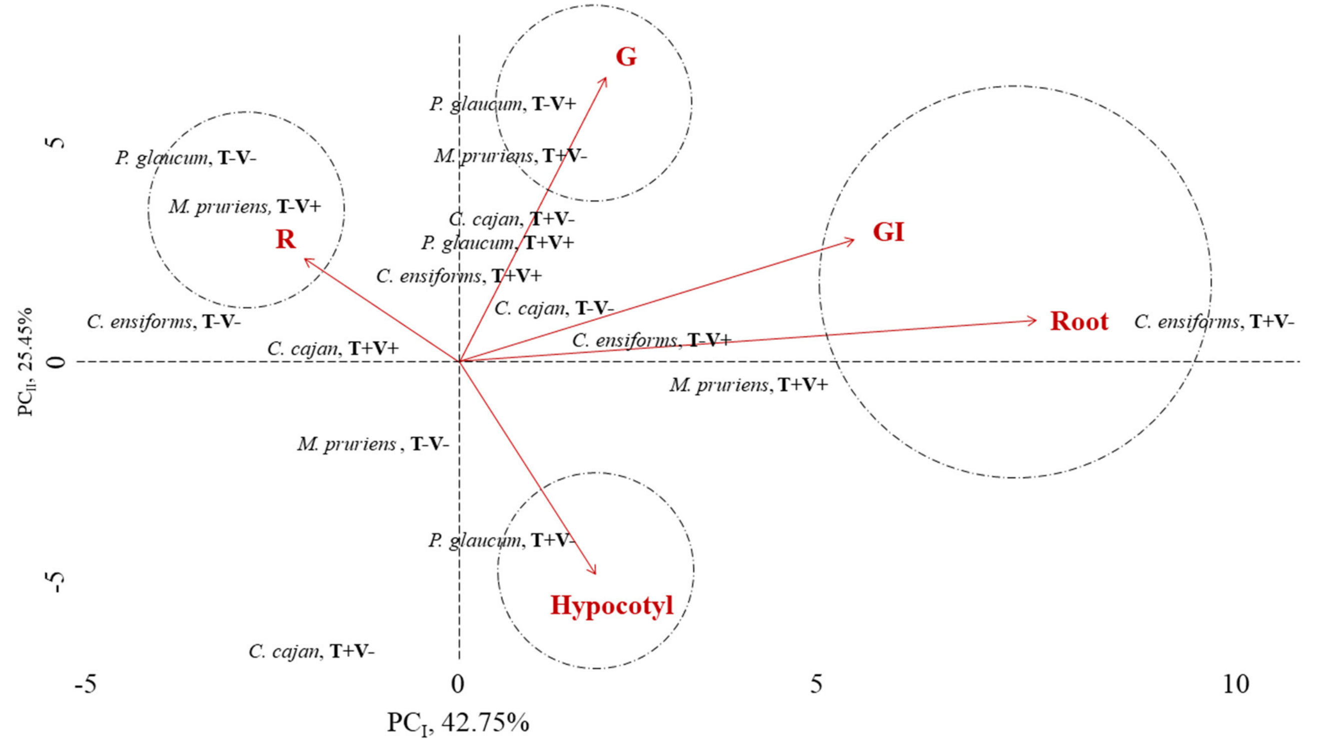

FIGURE 2 | Kinetics of growth and development of green manure species in soil with tebuthiuron and vinasse.

Thinning was performed on the 10th DAS. These were also irrigated daily by microsprinkling at 6 mm/h for 60 min (30 min at 6:00 A.M. and 30 min at 6:00 P.M.) to sustain proper plant development conditions.

Evaluation of Plant Growth

Plant growth was quantified weekly. The monitored parameters were (i) stem diameter in millimeter, (ii) height of shoot in centimeter, and (iii) number of leaves.

For *C. cajan*, *C. ensiformis*, *M. pruriens*, and *P. glaucum*, the periodic monitoring running from the 15th to the 50th DAS yielded six time-point datasets until the end (t_{50}) of cultivation. *C. juncea* was planted in all experimental units, and its morphological parameters were monitored from 17th to the 45th DAS, thus yielding five time-point datasets until the end of experiment (t_{95}).

After cultivation, plants were separated for quantification of biomass: the fresh and dry matter of shoots and roots. The separation of the shoots and roots occurred by cutting the stalks close to the soil between the stem and the root. The roots were thoroughly washed so that all the soil was removed. After separation, each fraction was weighed separately and then packed in a paper bag to dryness in oven at 65°C, over 72 h. The resulting samples were weighted again to obtain the dry mass.

Ecotoxicity Bioassays

Bioassays monitored the ecotoxicological potential of each treatment over the proposed time frame. The phytotoxicity of the soil samples was determined at the initial (t_0) and final (t_{50}) times when it was cultivated the phytoremediation species (*C. cajan*, *C. ensiformis, M. pruriens*, and *P. glaucum*), just before the *C. juncea* introduction.

Lettuce seeds (*Lactuca sativa*) were the test organism, according to Sobrero and Ronco (2004). Phytotoxic effect determination of each treatment was performed in six replicates from the solubilized soil extract, according to the NBR 10.006 (ABNT , 2004).

Ecotoxicity tests were prepared in Petri dishes with filter paper supported with 2.0 ml of the solubilized extract and 10 lettuce seeds. Petri dishes were then wrapped with polyvinyl chloride (PVC) film and incubated at 20 ± 2°C for 120 h in the dark.

Positive control was prepared using 0.05 M zinc sulfate to inhibit seed germination and negative control using deionized water to test the base germination and growth values of the seeds (Sobrero and Ronco, 2004).

The following parameters were determined: seed germination, root and hypocotyl elongation ($\geq$0.1 mm), and the Germination Index (GI) that combines seed germination (% G) and root elongation (% R) at the CN. The GI was used to assess the toxicity

TABLE 2 | Kinetic parameters for the growth and development of green manure species in soil with tebuthiuron and vinasse.

Species	**Test**	**Parameter**				$\mathbf{R^2_{adj}}$
		α	β	κ	$\alpha\kappa e^{-1}$	
		Height				
C. cajan	T-V-	78.50	4.40	0.40	11.55	0.9895**
	T-V+	61.65	4.50	0.45	10.20	0.9585*
	T+V-	5.10	560.65	4.80	9.00	0.0350
	T+V+	5.05	1.05	1.45	2.70	0.9995**
C. ensiformis	T-V-	217.90	4.70	0.20	16.05	0.9895**
	T-V+	193.05	4.20	0.20	14.20	0.9710*
	T+V-	4.50	$3.20e^{-7}$	−5.20	−8.60	0.2285
	T+V+	16.85	730.90	4.80	29.75	0.9995**
M. pruriens	T-V-	127.95	18.25	1.00	47.05	0.8755*
	T-V+	140.40	14.40	0.90	46.50	0.8495*
	T+V-	121.80	24.95	0.95	42.55	0.7815*
	T+V+	50.85	7.45	0.60	11.20	0.7405*
P. glaucum	T-V-	80.30	4.55	0.50	14.75	0.8190*
	T-V+	54.85	8.40	0.80	16.15	0.8765*
	T+V-	133.10	5.20	0.25	12.25	0.6075
	T+V+	134.55	5.45	0.25	12.35	0.6290
		Diameter, mm				
C. cajan	T-V-	4.85	2.25	0.60	2.90	0.9655*
	T-V+	4.85	2.25	0.60	2.90	0.9655*
	T+V-	1.05	1.25	−6.50	−6.80	0.5235
	T+V+	0.05	$4.80e^{-10}$	−1.10	−0.05	0.3690
C. ensiformis	T-V-	5.20	3.10	1.90	9.90	0.9735*
	T-V+	5.05	3.65	2.70	13.65	0.9570*
	T+V-	1.95	$5.50e^{-10}$	−1.15	−2.25	0.4040
	T+V+	3.35	$1.10e^{-12}$	−5.70	−19.10	0.5220
M. pruriens	T-V-	4.50	2.60	1.70	7.65	0.9280*
	T-V+	4.40	2.65	1.65	7.25	0.9390*
	T+V-	3.90	2.60	1.65	6.40	0.9245*
	T+V+	3.30	4.25	3.15	10.40	0.9780*
P. glaucum	T-V-	53.05	17.55	1.30	68.95	0.9940**
	T-V+	43.20	7.25	0.80	34.55	0.9940**
	T+V-	62.60	4.90	0.35	21.90	0.9970**
	T+V+	243.05	5.55	0.15	36.45	0.9960**

*Significant code: **p < 0.01; *p < 0.05.*

of soil samples in the test organism, according to Equation 1 (Labouriau and Agudo, 1987):

$$G_I = \frac{G \times R}{100}. \tag{1}$$

Data Analysis

Procedures of Shapiro–Wilk and Bartlett checked the normalcy and homoscedasticity of the dataset, respectively, and the analysis of variance tested the significance of effect of factors on the soil phytoremediation. The tests were separated by *post hoc* Tukey's honest significant difference (HSD) test. The data of temporal variability were fitted for Gompertz (Equation 2). This stochastic model has probability density function enough to predict how long would it take the green manure in soil with tebuthiuron and stillage to reach the maximum of growth and develop and stop. The kinetic parameters, α, β, and κ, will assist in drafting inferences about the potential phytoremediators and figuring out the best chance to deal with how to solve the side effects of these contaminants as much suitably as possible.

$$f = \alpha e^{-\beta e^{-kx}} \tag{2}$$

where:

$f(x)$: height or stem diameter;

x: time of sampling;

α: upper asymptote or the maximum of height or stem diameter;

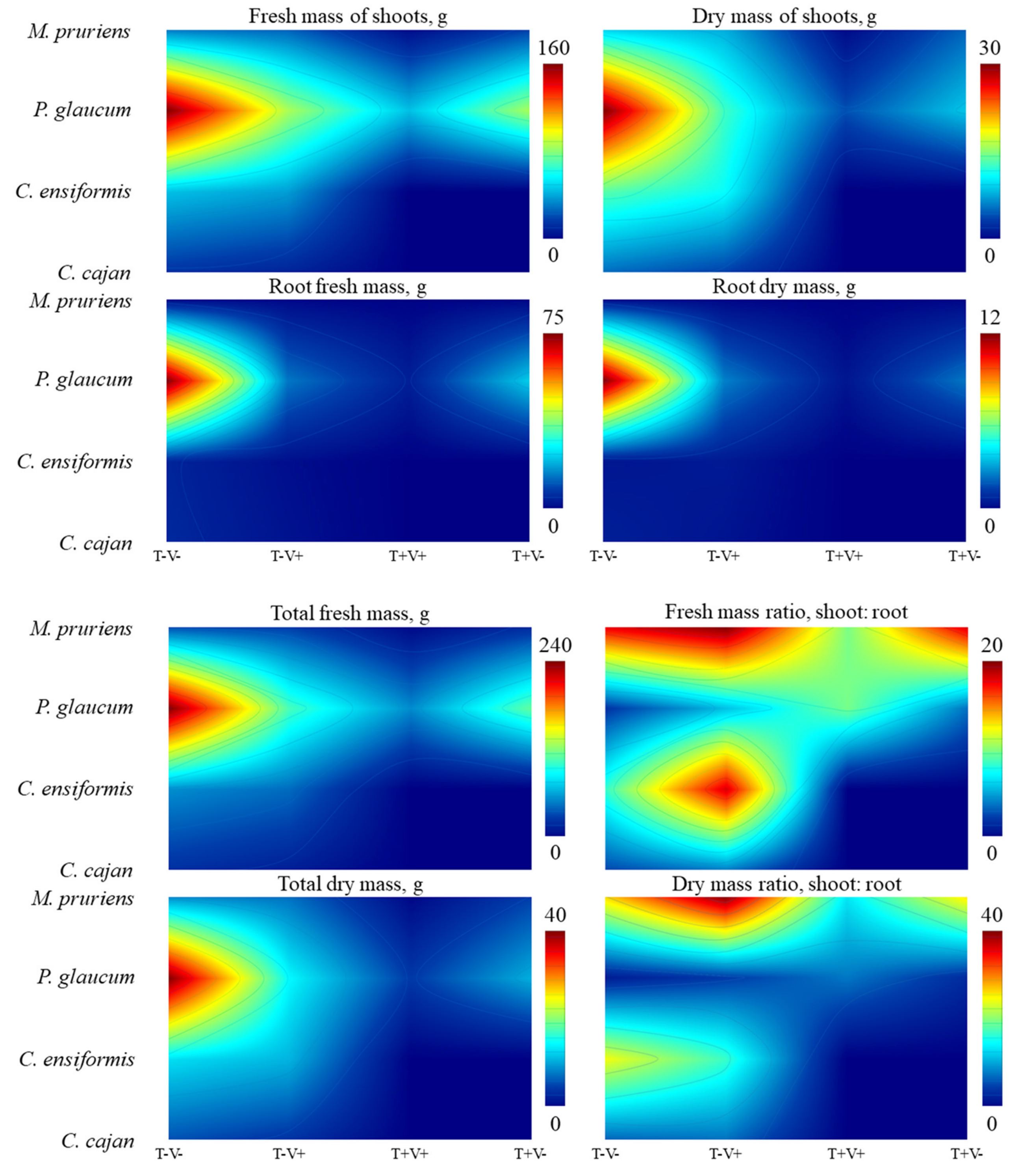

FIGURE 3 | Growth and development of *C. juncea* as bioindicator of phytoremediation of green manure species in soil with tebuthiuron and vinasse.

β: inflection point;
κ: specific-growth rate;
$\alpha\kappa e^{-1}$: absolute-growth rate;
e: Euler number.

An unbiased soft computing technique of contour plotting was performed to chart the spatial production of phytomass by the models for green manure. To an optimization of visualization of non-Boolean patterns in chromatic wireframe by contour

TABLE 3 | Performance of *Crotalaria juncea* after green manure species cultivation in soil with tebuthiuron and vinasse.

Species	**Test**			
	T-V-	**T-V+**	**T+V-**	**T+V+**
		Height, cm		
C. cajan	74.60 Aa	60.00 Aa	ND*	ND*
C. ensiformis	73.00 Aa	72.20 Aa	ND*	ND*
M. pruriens	78.40 Aa	85.80 Aa	58.50 Aab	18.75 Ab
P. glaucum	72.80 Aa	66.80 Aa	57.00 Aab	11.50 Ab
		Diameter, mm		
C. cajan	4.60 Aa	4.15 Aa	ND*	ND*
C. ensiformis	4.45 Aa	4.25 Aa	ND*	ND*
M. pruriens	4.85 Aa	4.90 Aa	3.40 Aab	1.80 Ab
P. glaucum	4.40 Aa	3.60 Aa	2.80 Aab	1.60 Ab
		Leaves		
C. cajan	32.40 Aa	22.80 Aab	ND*	ND*
C. ensiformis	31.40 Aa	32.80 Aa	ND*	ND*
M. pruriens	32.20 Aa	34.00 Aa	24.50 Aab	10.00 Ab
P. glaucum	34.80 Aa	25.60 Aa	22.00 Aab	7.50 Ab
		Shoot fresh mass, g		
C. cajan	25.45 Aa	21.20 Aa	ND*	ND*
C. ensiformis	23.35 Aa	30.80 Aa	ND*	ND*
M. pruriens	29.75 Aa	34.55 Aa	12.00 Ab	2.35 Ab
P. glaucum	28.95 Aa	21.25 Bab	7.80 Ab	0.25 Ab
		Shoot dry mass, g		
C. cajan	22.80 Aa	13.80 Ab	ND*	ND*
C. ensiformis	23.95 Aa	15.65 Aa	ND*	ND*
M. pruriens	7.90 Aa	6.90 Aa	2.20 Aab	0.35 Ab
P. glaucum	6.05 Aa	3.70 Bab	1.25 Ab	0.10 Ab
		Root fresh mass, g		
C. cajan	5.20 Aa	4.30 Aa	ND*	ND*
C. ensiformis	7.20 Aa	5.65 Aa	ND*	ND*
M. pruriens	24.05 Aa	19.20 Aa	9.55 Aa	2.80 Ab
P. glaucum	19.10 Aab	24.50 Aa	6.70 Aab	0.10 Ab
		Root dry mass, g		
C. cajan	2.15 Aa	1.30 Aa	ND*	ND*
C. ensiformis	3.40 Aa	1.75 Aab	ND*	ND*
M. pruriens	3.00 Aa	2.55 Aa	0.90 Aab	0.15 Ab
P. glaucum	1.95 Aab	2.75 Aa	0.45 Abc	0.05 Ac

Same letters, whether uppercase in the column and lowercase in the row, describe statistically similar means by post hoc Tukey's HSD test at $p < 0.05$;.
**As the plant dead, the trait became undetected (ND).*

plotting approach, fuzzy logic to turn any ambiguity off from the data was implemented. Another method of applying non-traditional mathematics to establish an eventual effect of green manure on the decontamination of the soil included principal component analysis (PCA). The Kaiser–Meyer–Olkin test was applied to determine how many components should be necessary to reduce the high-dimensionality data, while preserving as much attributable variability as possible into orthogonal subsets without collinearities. The software was R-project. This multiparadigm programming open-coding language provides a user-friendly environment for statistical computing and graphs.

RESULTS AND DISCUSSION

Performance of Species of Green Manure

Morphological Traits

The effects of green manure species and the microenvironment toward the phytoremediation potential was determined. Phytoremediators response occurred regardless of their morphological traits. The variations found in each assay allowed us to determine their sources (**Table 1**).

Tebuthiuron and vinasse underwent reactions in the soil, thus collectively influenced the phytotoxicity of the microclimate.

TABLE 4 | Principal components into ecotoxicity bioassays in soil samples with green manure species, tebuthiuron and vinasse.

Index/variable	Bartlett's test of sphericity	
Chi-squared	104.95	
Degree of freedom	10	
p-value	<0.01**	
	Kaiser-Meyer-Olkin test	
	Component	
	PC_{I}	**PC_{II}**
Eigenvalue	2.05*	1.20*
Percentage of variance	42.75	25.45
Cumulative percentage of variance	42.75	68.20
	Loading	
Hypocotyl	0.45	−0.55*
Root	0.95**	−0.05
%G	0.05	0.90**
%R	−0.35	0.25
GI	0.95**	0.30
	Contribution, %	
Hypocotyl	9.05	24.40*
Root	43.45**	0.05
%G	0.15	63.95**
%R	5.50	4.35
GI	41.85**	7.25
	Physiological vigor	Physiological anomaly

Significant code: $^{**}p < 0.01$; $^{*}p < 0.05$.

The herbicide alone was more toxic to *C. cajan* and *C. ensiforms*. In contrast, *M. pruriens* and *P. glaucum* resisted longer to chemically stressed microenvironment. Vinasse addition significantly reduced the toxicity. Hence, green manure species produced larger amounts of mass of roots and shoots in these soil samples (**Figure 1**).

Advantages of vinasse on the ecotoxicity were more prominent in *M. pruriens* and *P. glaucum*. These were the most effective strategies of manuring for phytoremediation potential. The primary assumption for vinasse attenuation by on phytotoxicity of soil may be its availability of soluble carbon. Thus, it is likely to power up the microbial metabolism and enhance the subsequent degradation of pesticides (Prata et al., 2000, 2001; Villaverde et al., 2008). Tebuthiuron is highly available and can move smoothly through the structure of agricultural soils with lows levels of organic matter and clay (Chang and Stritzke, 1977). These authors reported 40.00 and 1.00% herbicide adsorbed in particles at 4.8 and 0.30% organic matter, respectively. Bioavailability is one of the keys to an effective and consistent biodegradation. If the pollutant or contaminant is not available from the environment, (micro)organism cannot successfully perform longer. The nature and physicochemical properties of the pesticide (e.g., chemical stability, spatial structure, feedback effect, and intermediate metabolites) and its multiplicity of interactions with the rhizosphere are factors influencing greatly its bioavailability and, of course, kinetics of biodegradation.

Kinetics of Growth and Development

The Gompertz approach predicted accurately how long would it take for the chemical contamination of soil by tebuthiuron to become limiting for the growth and development of green manure species (**Figure 2**). Estimates for the absolute primary growth rate for the *C. cajan* in soil with tebuthiuron and vinasse combined was the lowest (**Table 2**). As long as the target molecule is rather persistent than readily degradable, the more probable the strategy of manuring is to inefficiently decontaminate an area. The highest estimations for both the maximum of size and absolute growth rate for this potential phytoremediator in soil with no tebuthiuron supported the high phytotoxicity of this herbicide to the primary growth.

In contrast, the association of tebuthiuron and vinasse allowed *C. ensiformis* to achieve its highest primary growth rate, whether specific or absolute. The negative estimation for absolute growth rate for this specie in soil with tebuthiuron alone was proof that vinasse was an effective source of nutrients to speed up the plant's growth and development, thus assisting in phytoremediation. The use of this agroindustrial residue also enhanced the height of *M. pruriens* and *P. glaucum* in soil samples with tebuthiuron. The herbicide severely limited the development of *C. cajan* and *C. ensiformis*, according to the lowest estimations for stem diameter. Thus, *M. pruriens* and *P. glaucum* are recommended over *C. cajan* and *C. ensiforms* for the phytoremediation of tebuthiuron in fields of sugarcane, even without the application of vinasse. The growth and development of *C. juncea*, the bioindicator chosen for this contaminant, became healthier and longer when sowing either *M. pruriens* and *P. glaucum*.

Performance of *C. juncea* as Bioindicator of Phytoremediation

The combination of tebuthiuron and vinasse considerably dropped the height, stem diameter, and number of leaves in *C. juncea* over time, compared to the control (**Figure 3**). In contrast, the herbicide alone had no significant effect, whether negative or positive, on the growth and development by the bioindicator species sowed after growing green manure species, consistent with the outcomes of explanatory analysis.

The explanation for the extensive decrease in leaves production (**Table 3**) may be either phytotoxicity by the compounds at high concentrations in soil or natural senescence, as plants become metabolically and physiologically ineffective over time. In contrast, the control soil sample (without tebuthiuron and vinasse) peaked in height, stem diameter, and number of leaves. Therefore, *C. juncea* was highly susceptible to tebuthiuron. Practically, this molecule more severely disabled both *C. cajan* and *C. ensiformis* to grow and develop as healthily as possible prior to sowing *C. juncea* for monitoring the potential phytoremediation of soil with green manure.

The behavior of bioindicator species *C. juncea* supported how persistent should be tebuthiuron in a microenvironment,

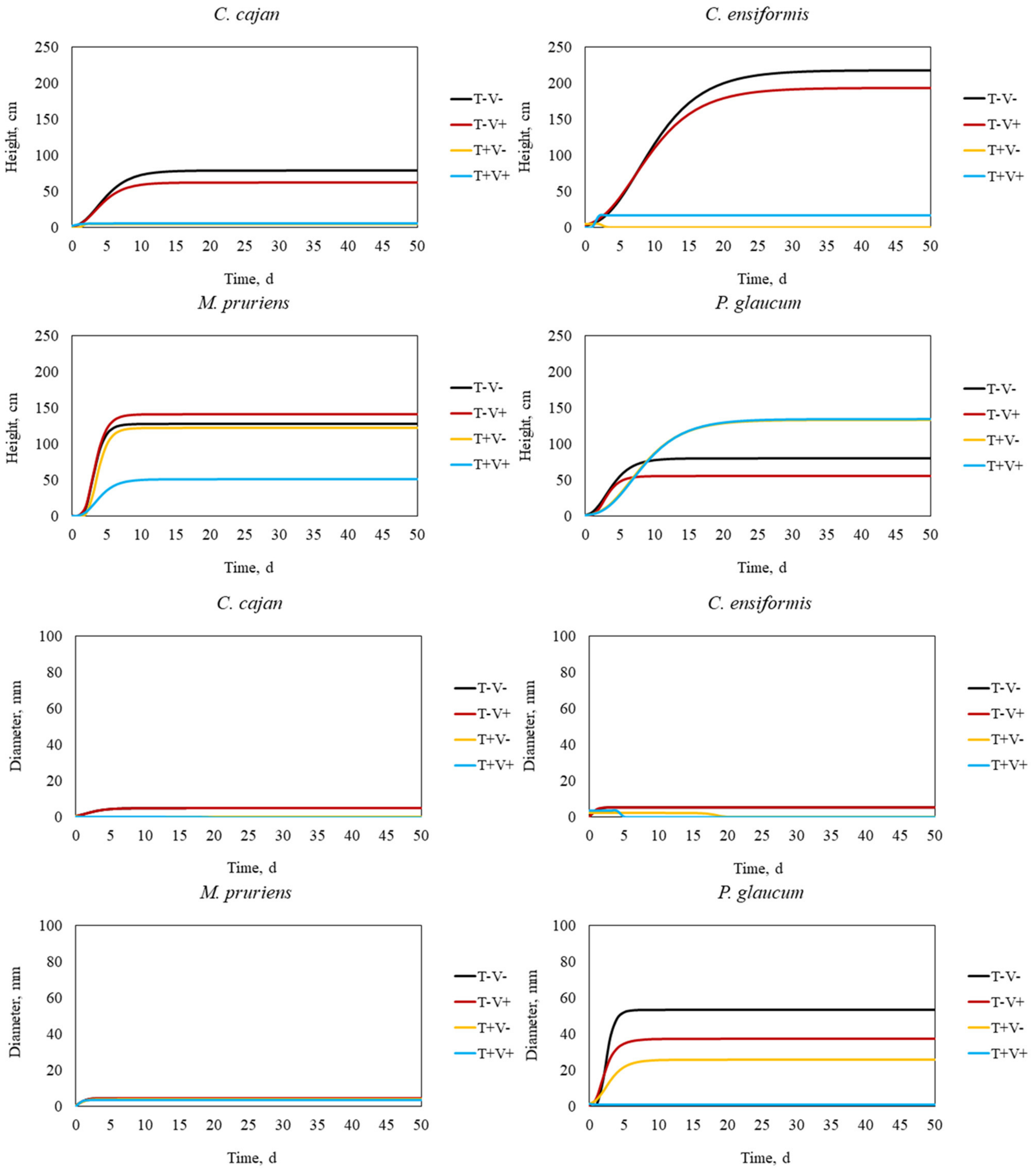

FIGURE 4 | Principal components into the ecotoxicity bioassays after 50 days of cultivation of green manure species in soil with tebuthiuron and vinasse.

regardless of vinasse application as source of nutrients to speed up the growth and development and, hence, assist green manure species in extensively remediating the herbicide. *C. cajan* and *C. ensiformis* ended up much more effectively remediating tebuthiuron and, hence, ensured the soil more suitable for the bioindicator species' growth and development

(Pires et al., 2008), inconsistent with the trends in this study. Some plants are capable of highly remediating contaminants (Ferraço et al., 2017). Cultivation of *C. juncea* slightly reduced sulfentrazone concentration in soil. Bioindicators of this herbicide then grew more consistently over time, regardless of sowing density, but not as consistent as control assay (Ferraço et al., 2019).

Franco et al. (2014) reported phytoremediation benefits by *Phaseolus vulgaris* on the growth and development of *Urochloa brizantha*. Plant heigh, leaf production, and area all increased in *C. juncea* with decreasing concentration of contaminant. Thus, the longer the postcultivation is, the more probable the phytoremediation is to becoming effective.

Beans in soil with picloram at 32.00 g ha^{-1} produced low amount of dry mass due to plants' death at high pesticide concentration. However, this morphological trait increased with phytoremediation by the *Urochloa* sp. (Franco et al., 2015). Belo et al. (2016) reported similar trend for the dry mass of *P. glaucum* after phytoremediation by *C. juncea* and *C. ensiformis*. Data on phytoremediation potential of herbicides, like sulfentrazone, by *P. glaucum* are neither conclusive nor conducive to commercial application yet, and this requires further investigations (Madalão et al., 2012a,b). These references supported the major findings in this study on the negative effect of tebuthiuron on biomass accumulation in roots and shoots of green manures species. Other reliable and executable bioindicators of soil decontamination are finger millet (*Eleusine cocracana)*, for chlorimuron-ethyl and sulfumeturon-methyl (Assis et al., 2010), and cucumber for picloram (Galon et al., 2017).

Ecotoxicity Bioassays

The principal component analysis robustly reduced the dimensionality of dataset and preserved as much interpretable variability as possible into the components, PC_I and PC_{II}. These components, collectively, explained ∼70.00% variance in ecotoxicity of soil samples on germination, growth, and development of the test-organism *L. sativa* (**Table 4**).

The first component, attributable to seed physiological vigor, had positive correlations with hypocotyl length and germination index (GI). Cartesian coordinates for soil samples with phytoremediation by either *M. pruriens* and *P. glaucum* structurally were positive in the upper right quadrant in the factorial map (**Figure 4**). Therefore, the more effective the tebuthiuron biodegradation is, the less probable the contaminant is to become toxic during the germination and primary growth of *L. sativa* seeds. The second component, attributable to physiological anomaly by phytotoxicity, had positive and negative loadings with germination and hypocotyl length, respectively. Soil samples with *P. glaucum* and hypocotyl were closer together in the lower right quadrant. Thus, this species should be of greater relevance to ensure plant growing and development without any severe physiological anomaly by tebuthiuron in site without vinasse.

CONCLUSION

Green manure species and vinasse can remediate soils with tebuthiuron. Preliminary evidence of *M. pruriens* and *P. glaucum* show their increased capabilities of phytoremediating sites where the target herbicide exists. These species, in association with vinasse as source of soluble carbon, can decontaminate the system more effectively than *C. cajan* and *C. ensiformis*, thus enabling the bioindicator *C. juncea* to grow and develop healthier in the presence of residual tebuthiuron. As long as the manuring by fertilizing agents is effective in remediating the soil, the less probable tebuthiuron persists at high concentrations in soil and, thus, becoming harmful to non-target organisms as shown in ecotoxicity bioassays. Undergerminated seeds and severe physiological anomalies due to phytotoxicity in roots and hypocotyl of *L. sativa* are likely to decrease quickly with phytoremediation by *M. pruriens* and *P. glaucum*, which is the best chance to do this. Findings of this study are timely and should be of great importance to development and implementation of cost-effective strategies to assist in mitigating contamination of soil by tebuthiuron in sugarcane crops with vinasse application as biofertilizer. The fate of this herbicide and its potential metabolites in soil and into tissues of green manure species, especially *M. pruriens* and *P. glaucum*, is prone to scaled up designs toward an effective and safe industrial usage and could be the focus of further investigations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article could be made available by the authors.

AUTHOR CONTRIBUTIONS

LF, EP, RT, EB, and PL: conceptualization. LF, RM, YF, and PL: methodology. LF, BM, RM, RV, and PL: validation. LF, BM, and PL: formal analysis, data curation, and writing—original draft. LF and YF: investigation. PL: resources, supervision, project administration, and funding acquisition. RM, EP, RV, RT, JC, EB, YF, and PL: writing—review & editing. BM, JC, and PL: visualization. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.2. Anexo II

Laura Silva NANTES; Munick Beato ARAGÃO; Bruno Rafael de Almeida MOREIRA; Yanca Araujo FRIAS; Thalia Silva VALÉRIO; Edivaldo Wilson de LIMA; Ronaldo da Silva VIANA; Paulo Renato Matos LOPES.

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ORIGINAL PAPER

Synergism and antagonism in environmental behavior of tebuthiuron and thiamethoxam in soil with vinasse by natural attenuation

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Abstract

Full-scale farming systems rely on tebuthiuron and thiamethoxam to manage sugarcane for controlling weeds and insects, respectively. Both are effective pesticides, but they can harm the surroundings. Moreover, an additional level of routine management, such as spreading vinasse onto field for fertigation, can impact the environmental pollution/contamination positively or negatively. Therefore, we hypothesized the environmental behavior in and analyzed if it could be possible for natural attenuation to effectively dissipate them from the soil. The respirometric bioassay to quantify the microbial metabolism upon the pesticides in association with vinasse or not lasted for 130 days. Vinasse acted similar to an organic source of metabolizable energy. Hence, it enhanced the microbial transformation of target-pesticides into CO_2. Soil samples respirometers, where vinasse and pesticides co-existed, became less toxic over a sensitive organism. Therefore, ecotoxicological bioassay cross-validated the synergism of vinasse towards the natural attenuation of tebuthiuron with the opposite true for thiamethoxam. The sigmoidal Gompertz function was more adequate to describe the microbial mineralization of tebuthiuron-thiamethoxam-vinasse associations in soil. This model presented more adequate to describe microbiological degradation than first-order functions. Plainly, analytical insights into microbiological-ecotoxicological ramifications of our exploratory study are timely and provided forward knowledge in naturally remediating agroecosystems.

Keywords Agrochemicals · Bioremediation · Ecotoxicity · Microbial respiration · *Saccharum* spp

Introduction

Sugarcane (*Saccharum* spp.) is a specialty of grassy crop. It can massively produce sucrose and lignocellulosic biomass, whether for making commercial sugar and bioethanol and cogeneration of biopower (Daniels and Roach 1987; Silva 2016). Therefore, sugarcane proves an appealing source of renewable and sustainable energy both economically and environmentally to replace fossil fuel in global energy grid (Huang et al. 2020). However, its management-intensive can generate vinasse as a high-BOD organic waste from bioethanol distillation. Large-scale facilities usually spread it onto field as a biofertilizer via fertigation (Rossetto and Santiago 2006). Nevertheless, an overreliance application can pollute/contaminate the soil rather than amend it by salinization and acidification (Carralero and Garlobo 1999). Another potential disadvantage refers to further leaching or carryover of water-soluble residual pesticides existing in the agroecosystem (Siqueira et al. 1994; IBAMA 2019).

Pesticides application to control pests in areas producing sugarcane is intensive. The most usual are herbicides and insecticides by representing 60% and 15% of the total, respectively (SINDIVEG 2018). Well over 200 countries produce sugarcane on an industrial scale worldwide (Huang et al. 2020), they rely on tebuthiuron and thiamethoxam as the most reliable herbicide and insecticide to control weeds (i.e., grassy and broad-leaf) and prevent insects from outbreaking, respectively. Both are highly capable of controlling pests, but present a potential harmful to the environment and society (Siqueira et al. 1994; IBAMA 2019). These compounds are highly soluble in water (Mercurio et al. 2016) and thereby vinasse can potentially make them easily

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transportable from the system (Gloria and Orlando Filho 1983; Rossetto and Santiago 2006).

In order to recover these areas with potential contamination, bioremediation proves useful to dissipate pollutants from the soil, even if concentration of the target-molecule is significant (Gaylarde et al. 2005; Procópio et al. 2009). Microorganisms and plants can effectively stabilize and/or degrade complex contaminant molecules into simpler and harmless compounds, such as carbon monoxide (CO_2) via mineralization. For example, roots can stabilize or remove an inorganic/organic substances out of the system by vegetable metabolism in association with other processes (Gaylarde et al. 2005; Procópio et al. 2009). Hence, ecotoxicological bioassay can be of any assistance in assessing the soil toxicity before and after bioremediation for cross-validation (Azevedo and Chasin 2004; Knie and Lopes, 2004; Banks and Schultz, 2005; Rocha et al. 2018; Ferreira et al. 2021).

Therefore, in light of research and innovation in bioremediation, our study aimed to analyze if it could be possible for natural attenuation to dissipate either tebuthiuron or thiamethoxam in soil with or without vinasse.

Material and methods

Soil, pesticides and vinasse

Ultisol was collected in an experimental area near 21°28' S and 51°31' W (Brazil) predominantly sandy soil with 89.9% sand, 7.1% clay and 3% silt. Its physicochemical composition was: pH (6.00); organic matter (6.00 g dm^{-3}); potassium (6.60 $mmol_c$ dm^{-3}); calcium (27.00 $mmol_c$ dm^{-3}); magnesium (27.00 $mmol_c$ dm^{-3}); potential acidity (14.00 $mmol_c$ dm^{-3}); phosphorus (2.00 mg dm^{-3}); boron (0.15 mg dm^{-3}); copper (0.90 mg dm^{-3}); iron (2.00 mg dm^{-3}); manganese (0.30 mg dm^{-3}); zinc (0.10 mg dm^{-3}); sum of bases (61.00 $mmol_c$ dm^{-3}); and cation exchange capacity (75.00 $mmol_c$ dm^{-3}). Pesticides used were herbicide tebuthiuron (Combine® 500, Dow AgroSciences) and insecticide thiamethoxam (Actara® 250, Syngenta). The vinasse was acquired from an industrial sugar mill plant in Brazil. Materials were stored individually in airtight recipients in laboratory at 4 ℃ to preserve properties until experimental setting-up.

Experimentation

Experimentation was performed according to a 2 × 2 × 2 factorial design, corresponded respectively to presence and absence of tebuthiuron, thiamethoxam and vinasse. In the control sample (baseline) no product was added to the soil. Hence, a special combination of both pesticides and vinasse was elaborate to study synergistic/antagonistic between molecules and if organic supplement could change the relationship both positively both negatively. All tests consisted of three replications to control systematic errors.

To set-up experimentation in a greenhouse, soil was screened on a sieve to particles finer than 0.15 mm. Afterwards, soil and pesticides were thoroughly mixed together, manually, until obtaining a homogeneous material. The material was then filled into 4.0 L pots and vinasse was dropped onto experimental units after one week. Experimental pots were organized in the facility in vinasse vs. no vinasse groups to streamline the workflow. The experimentation lasted for 130 days to quantify the performance of a long-term natural attenuation. Pesticides were applied according to specifications by the manufacturers and vinasse volume was defined as per technical standard P4.231 (CETESB 2015) to resemble conditions on an industrial scale.

Respirometric bioassay

Respirometric bioassays was performed in triplicate to monitor microbial activity upon transforming pesticides into CO_2 (Bartha and Pramer 1965). Representative soil samples were extracted from pots then transferred to respirometer flasks. The bioassay lasted for 130 days in a dark room at 28 °° to avoid photodegradation with renewing the atmosphere inside flasks every carbon dioxide quantification. Evolution of microbial production of CO_2 was quantified indirectly (Eq. 1; $r^2 \sim 0.9999$) from electroconductivity data (weekly resolution) on carbonate (Faria et al. 2013):

$$Y = 1554.8 - 95.726 \times \mathrm{C} \tag{1}$$

where

Y: CO_2 production, mg;

C: electroconductivity of the analyzed sample, mS; and.

1554.8 and 95.726 stand as the factors for transforming the concentration of an analyte, from mS to mg CO_2.

Ecotoxicological bioassay

To validate natural attenuation, an ecotoxicological bioassay was carried out on soil samples at the 30th, 60th, 90th, and 120.th days of natural attenuation. Soil aqueous extract was prepared according to the NBR 10.006 (ABNT 2004). Thus, *Lactuca sativa* L. seeds were used as sensitive test-organism to xenobiotics as per Sobrero and Ronco (2004). For baseline, plates were prepared with distilled water (negative control) and 0.05 M zinc sulphate as germination inhibitor (positive control). Results were assessed to germination index (Eq. 2) as an indicator of phytotoxicity of an environmental sample (Labouriau and Agudo 1987)

$$GI = \frac{G \times R}{100} \tag{2}$$

where

GI: germination index;

G: sample germination rate in relation to negative control, %;

R: sample root elongation in relation to negative control, %.

Data analytics

Shapiro–Wilk's and Bartlett's tests of normality and homoscedasticity were performed to check distribution and variance, respectively. A sigmoid Gompertz function (Eq. 3, Tjørve and Tjørve 2017) was fitted for respirometric data to model kinematic natural attenuation and a Monte-Carlo simulation (Dai 2019) was accomplished to predict what we might expect on periods longer than 250, 500 and 1000 days. Surface-response method and fuzzy contour plotting were carried out to "visualize" variability in both bioassays and solve the puzzle of whether pesticides were synergistic or antagonistic. The criteria to analyze the adequacy of the stochastic model included Akaike information criterion (AIC), Bayesian information criterion (BIC), and adjusted coefficient of determination (r_{adj}^2). All analyzes were carried out in the R-project's application programming interface for statistical computing and graphics (R Core Team 2016).

$$f_x = \alpha e^{-\beta e^{-kx}} \tag{3}$$

where

f_x: statistic CO_2 production, mg or %;

x: time, d;

α: upper asymptote or the maximal CO_2 production, mg;

β: inflection point;

κ: exponential decay of specific-growth rate, mg CO_2 d^{-1};

e: Euler constant.

Results and discussion

Microbial activity: biodegradation

The sigmoid Gompertz approach adequately described the kinematic natural attenuation of pesticides over 130 days respirometry (Fig. 1). Microbial respiration was positive towards both tebuthiuron and thiamethoxam and also the vinasse notably boosted the bioprocess. Plainly, vinasse acted similar to an organic source of metabolizable energy to microorganisms.

Hence, it enabled the biosystem to transform both target-pesticides into CO_2 faster and effectively when present associated. A steeper breakthrough curve for soil containing only vinasse, relative to other experimental units, validated its synergistic environmental behavior as an organic energy-dense supplement (Prata 1998) towards natural attenuation.

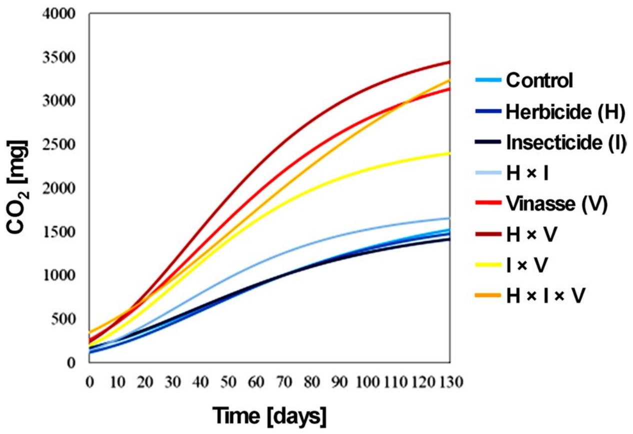


Fig. 1 Sigmoidal production of CO_2 from microbial metabolism in soil with tebuthiuron-thiamethoxam-vinasse associations

Furthermore, soil containing only either tebuthiuron or thiamethoxam produced CO_2 similar as the control soil sample. By contrast, pesticides together generated more CO_2 than both alone. Therefore, tebuthiuron and thiamethoxam proved synergistic one towards another, which turned easier for the biosystem to dissipate them in soil.

Results showed that thiamethoxam was antagonistic towards vinasse. It skewed downwards the breakthrough curve on respirometry for the supplement alone. By contrast, tebuthiuron and vinasse proved synergistic one towards another. Thereby, they produced the steepest breakthrough curve possible. It is highlighting that tebuthiuron compensated for the antagonistic thiamethoxam-vinasse relationship. Thus, microbial activity was increased for the complex herbicide-insecticide-vinasse.

However, special association between tebuthiuron, thiamethoxam and vinasse is likely to produce the largest amount of CO_2 possible on Monte-Carlo simulation (Fig. 2). Therefore, synergistic action by tebuthiuron is likely to become stronger, further decreasing the negative impact of an antagonistic thiamethoxam on long-term natural attenuation. Vinasse is likely to upregulate the bioprocess by providing attenuators an additional source of energy to metabolize pesticides both together and alone. An effective natural attenuation of both pesticides in soil without vinasse is not likely at long term and results demonstrated that these soil samples underperform the control bioassay.

Furthermore, Gompertz model for the long-term natural attenuation of these pesticides in soil allowed to observe similar conclusion about their biodegradation in soil. Estimate of α was the highest for herbicide-insecticide-vinasse association, but the opposite was found for the insecticide alone (Table 1). Certainly, vinasse impacted positively in soil natural attenuation and tebuthiuron minimized the antagonism by thiamethoxam over microbial activity. Hence, herbicide-supplement nexus maximized the amount of

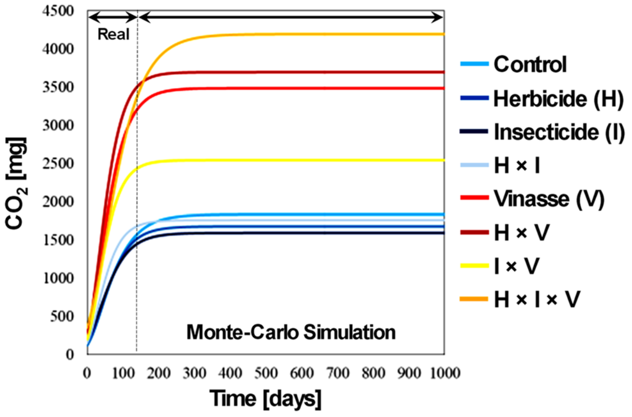


Fig. 2 Monte-Carlo simulation in a sigmoidal long-term production of CO_2 from microbial metabolism in soil with tebuthiuron-thiamethoxam-vinasse associations

biotransformation, although the insecticide component considerably decreased the specific-growth rate to 17.40 mg g^{-1}.

The estimate of k for the control bioassay was 19.65 mg g^{-1}, which turn it kinematically comparable to the most productive test due to the higher the k the faster the natural attenuation. Furthermore, $\beta > 1$ is an indicator of relative decreasing growth accelerates with time. By contrast, $\beta < 1$ decelerates the relative decrease while the $\beta = 1$ keeps it constant. Estimates of β were greater than the unit for all tests. Yet, slightly lower value for the insecticide, relative to herbicide and vinasse, further supported the capability for thiamethoxam to limit the microbial activity. On the other hand, the highest estimate of β for herbicide-vinasse association further supported the antagonistic environmental behavior one towards another, making the fastest kinematic natural attenuation possible. Thereby, microorganisms in soil containing tebuthiuron and vinasse are likely to demand 130 days to convert most of compounds into CO_2. Introducing thiamethoxam into the system is required a longer period (~ 240 days) for the native microbiota to approach the plateau on the breakthrough curve. A greater magnitude of T for tebuthiuron-thiamethoxam-vinasse system supported both complexity and larger amount of substrate.

Table S1 (Supplementary material) demonstrated that both pesticides proved remediable, yet tebuthiuron (14.40 mg d^{-1}) outstripped the thiamethoxam (13.30 mg d^{-1}) in CO_2 absolute production. An herbicide-insecticide framework synergistically allowed a further microbial biotransformation (18.55 mg d^{-1}) throughout the respirometric bioassay. A tangible assumption would be an increasing bioavailability and another a less toxic substrate (Laabs et al. 2000; Oliveira 2007; Gupta et al. 2008).

A fuzzy contour plotting (Fig. 3) could adequately describe the importance of both bioavailability and ecotoxicity of a substrate to microbial performance and how vinasse can balance the system. Any association other than herbicide-insecticide and herbicide-vinasse can negatively impact the microbial activity. For instance, insecticide-vinasse and herbicide-insecticide-vinasse were antagonistic, so did not perform as we might expect on a synergistic environmental behavior. Thereby, they associated to relative values of CO_2 absolute production below 1.

Camargo et al. (2009) reported the importance of the organic matter as an enabler for microbial metabolization on vinasse biodegradation in soil. Neves et al. (2021) and Faria et al. (2019a) also performed respirometric bioassays to analyze time-dependent mineralization of tebuthiuron in soil with and without vinasse, and measured an increasing CO_2 production over time regardless of the microcosm. However, they stressed the role of supplementary compound in accelerating the bioprocess, which supported trends from this study for the natural attenuation in soill containing herbicide and vinasse. Vinasse facilitated diuron and ametrine degradation by microorganisms and reduced their persistence in soil (Prata 1998).

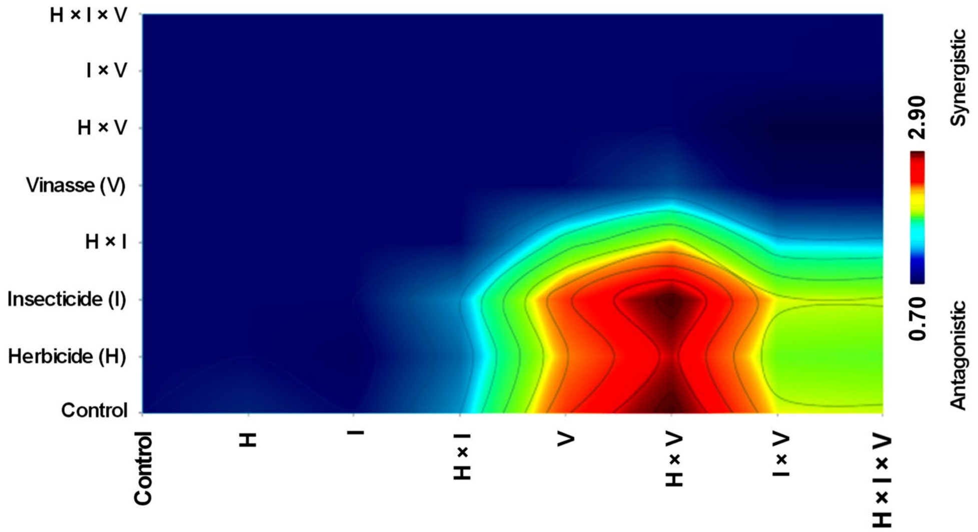
Therefore, outperformance of tebuthiuron-vinasse in this study can further support the synergism of the by-product

Table 1 Parametrization and adequacy of sigmoid Gompertz model for the long-term microbial metabolism in soil with tebuthiuron-thiamethoxam-vinasse associations

Test	Parameter				Metric		
	α, mg	β	κ, mg d^{-1}	T, d	AIC	BIC	r_{adj}^2
Control	1834.75	2.40	19.65	93.35	209.90	213.90	0.99305**
Herbicide (H)	1678.45	2.65	23.35	71.90	216.85	220.80	0.99085**
Insecticide (I)	1593.30	2.25	22.70	70.30	228.20	232.20	0.98010*
H×I	1759.35	2.50	28.65	61.40	226.20	230.20	0.98825*
Vinasse (V)	3486.35	2.60	24.60	141.70	246.20	250.20	0.99110**
H×V	3696.90	2.70	28.10	131.55	242.45	246.45	0.99420**
I×V	2543.75	2.55	29.05	87.55	244.45	248.40	0.98640*
H×I×V	4196.55	2.50	17.40	241.20	262.05	266.05	0.97990*

T time to reach the plateau; Significant code: ** $p < 0.010$; * $p < 0.05$

Fig. 3 Relative rate of CO_2 absolute production (mg d^{-1}) from microbial metabolism in soil with tebuthiuron-thiamethoxam-vinasse associations. On the fuzzy contour plotting, the redder the frame, more synergistic process, and the bluer the frame, the more antagonism process



towards herbicides. By contrast, natural attenuation of thiamethoxam is often not available from regular literature, making it hard for contrasting the trends from this exploratory study to earlier investigations. While the Gompertz model accurately predicted the microbial CO_2 production from thiamethoxam in Table 1, Scorza Junior and Rigitano (2009, 2012) could not find a sigmoid kinetics for the degradation of this insecticide. They more adequately modeled the phenomenon on a first-order function, as the transformation did not change upon the time. Kinetic function fitting is instrumental to study mineralization and first-order models are capable of predicting mineralization (Johnsen et al. 2013). However, they have the limitation of either overfitting or underfitting data on molecules with half-life independent upon time and concentration (Johnsen et al. 2013).

If density of degrading microorganisms or pesticide concentration do not change with time, then fitting of first-order functions becomes rather complex and inaccurate. As pesticides often sustain the degraders growth, mineralization breakthrough curves for microbial populations are sigmoidal rather than as simple as linear (Johnsen et al. 2013). Therefore, we screened out sigmoid Gompertz out of similar microbial growth models (Mahdinia et al. 2020) as an option to first-order functions for fitting of mineralization of tebuthiuron and thiamethoxam into CO_2.

Certainly, sigmoidal Gompertz function can adequately describe the microbial mineralization of both pesticides in soil with and without vinasse. However, it cannot fit non-growth regions and numerous samples could make it challenging for interpreting its parameters. Overall, sigmoid Gompertz function and its variants (Gompertz 1825) can prove useful to stochastic microbiological studies by predicting degradation (Fan et al. 2004) and mineralization (Rousseaux et al. 2003; Zablotowicz et al. 2007; LeFevre et al. 2012) of pollutants/contaminants more accurately than first-order functions. Therefore, it offers an excellent option for regulatory agencies, researchers and policymakers to replace first-order kinetics in evaluating and elaborating approval procedures of pesticides.

Ecotoxicology

L. sativa seeds germinated on plates throughout the ecotoxicological bioassays irrespective of soil samples with tebuthiuron- thiamethoxam-vinasse associations (Fig. 4). Results showed an increase in GI when pesticides and vinasse coexisted in soil. Therefore, ecotoxicity analysis cross-validated the role of vinasse in naturally dissipating tebuthiuron and thiamethoxam as reducing the sample potential toxicity to test-organism.

Although vinasse allowed a further natural attenuation of both pesticides, it could make it harder for an *L. sativa* germination and development at 40-60th day soil samples. An appreciable amount of compounds in the system could be toxic rather than beneficial to seeds (Faria et al. 2019b; Ferreira et al. 2021), which present higher results in soil after 100 days of natural attenuation. At 120th day, no phytotoxic residual existed in soil with vinasse. Plainly, biodegradation by microorganisms changed the composition of potential toxic compounds to a less harmful scenario in soil natural attenuation.

The dangerousness of the insecticide was also evidenced as thiamethoxam limited the germination and development of the test-organism. Certainly, it demonstrated a worse effect compared to the herbicide over *L. sativa*'s seeds. However, either tebuthiuron or vinasse compensated for

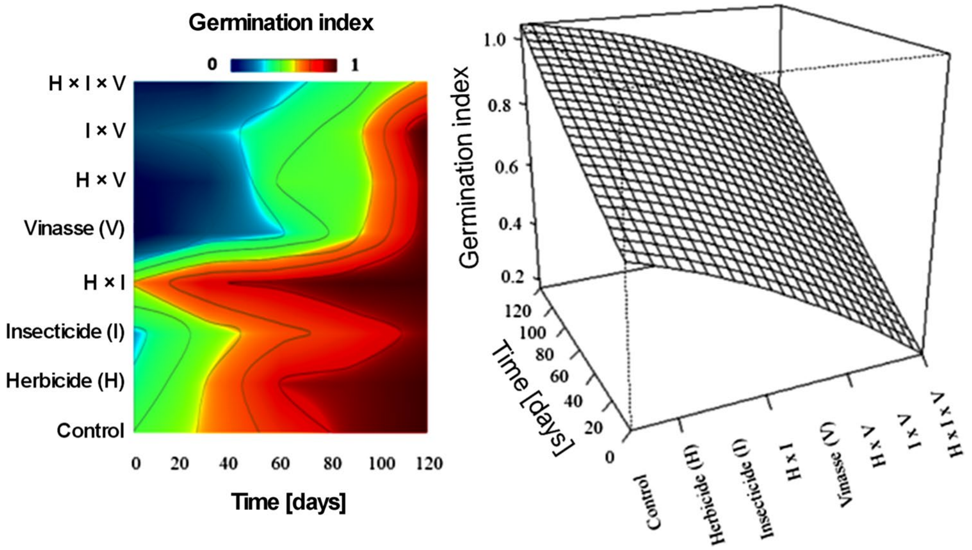


Fig. 4 Germination index (GI) of *L. sativa* ‘’ ecotoxicity bioassays in soil with tebuthiuron-thiamethoxam-vinasse associations. On the fuzzy contour plotting, the redder the frame, the higher the GI, and the bluer the frame, the lower the GI

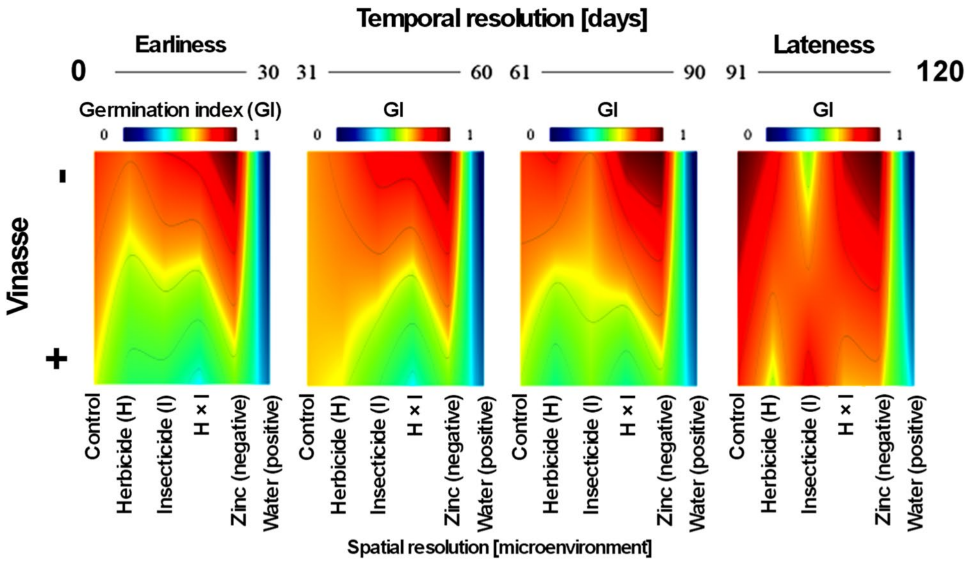


Fig. 5 Spatial–temporal germination index of *L. sativa* by ecotoxicity bioassays in soil with tebuthiuron-thiamethoxam-vinasse associations

its harmfulness, hence seeds germinated and seedlings developed more and faster than possible both temporally and spatially (Fig. 5). Therefore, ecotoxicological bioassay also cross-validated the synergistic behavior of tebuthiuron towards the thiamethoxam, yet its presence in herbicide-insecticide-vinasse composition can significantly reduced the germination index.

Negative estimate for test supported the limitation for test-organism in soil containing compounds as toxic as pesticides and vinasse (Table 2). Most notably, it further supported how natural attenuation could gradually dissipate a harmful scenario in soil, which become suitable rather than toxic to support growth and development of *L. sativa*.

Table 2 Parametrization and adequacy of surface-response model for the germination index of *L. sativa* by ecotoxicity bioassays in soil with tebuthiuron-thiamethoxam-vinasse associations

Parameter	Estimate	Standard Error	p < I t I
Intercept, β_o	0.65	0.09500	0.00010**
Test, β_x	− 0.05	0.04000	0.44600
Time, β_y	0.0030	0.00200	0.01019*
$\beta_x \times \beta_y$	0.0002	0.00020	0.00980**
B_x^2	− 0.004	0.00410	0.33860
B_y^2	–	–	0.87780

Significant code **$p < 0.010$; *$p < 0.05$

Plainly, natural attenuation depended upon time, regardless of the pesticide tested.

Overall, natural attenuation proved effective to dissipate tebuthiuron and thiamethoxam in soil. Hence, samples became potentially less toxic over time, which enabled a pesticide-sensitive organism to germinate and to develop in ecotoxicological bioassays. Even if pesticides are antagonistic one towards another and nutrient-dense vinasse presents in the system, germination index after natural attenuation was similar as we might expect on an environment without a critical pollution/contamination. However, our study is preliminary and further in-depth investigations are necessary for clarity and validation of protocol.

Conclusion

Natural attenuation proved useful to dissipate tebuthiuron and thiamethoxam out of the soil. Both pesticides were able to be microbiologically mineralizable into CO_2, but vinasse can catalyze this biotransformation by providing an extra energy to microbiota. In relation to mathematical modeling, it was demonstrated that sigmoidal Gompertz function was more adequate to describe the microbial mineralization of tebuthiuron-thiamethoxam-vinasse associations in soil. This model presents as a useful tool to stochastic microbiological degradation studies and demonstrated more accurately than first-order functions.

Therefore, insights into microbiological-ecotoxicological ramifications of our explanatory study were timely. They provided valuable knowledge of particular relevance to progress in the field's prominence of remediating geolocations, where an intensive phytosanitary and nutritional management of sugarcane can harm the surroundings via leaching and run-off. Our approach can drive thriving and responsive farming systems towards an emerging yet exciting sustainable global sugar-energy ecosystem. Stakeholders will be able to explore natural attenuation to address an increasing pressure to feed/power the world with greater social and environmental responsiveness, leveling-up the cost-effectiveness of the sector.

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Author contributions All authors contributed to this research. Material preparation, data collection and analysis performed by LSN, MBA, BRAM, YAF, TSV, EWL, RSV and PRML. The first draft was written by LSN and BRAM and review and editing by PRML. All authors commented on previous versions of the manuscript. Finally, authors have read and approved to the published version of the manuscript.

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Data availability The original data can be available upon request unto authors.

Declarations

Conflict of interest The authors declare no potential conflict of interest.

Ethical approval Authors confirm that the manuscript has not been submitted to journal for simultaneous consideration and has not been previously published. Results collection, selection, and processing performed personally. Authors' institution informed about this submission.

Consent for publication All authors approved the manuscript before submission and consent to the submission to International Journal of Environmental Science and Technology.

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5.3. Anexo III

Edivaldo Wilson de LIMA; Bruno Pinheiro BRUNALDI; Yanca Araujo FRIAS; Bruno Rafael de Almeida MOREIRA; Lucas da Silva ALVES; Paulo Renato Matos LOPES.

A synergistic bacterial pool decomposes tebuthiuron in soil. Scientific Reports, volume 12, article 9225, 2022.

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A synergistic bacterial pool decomposes tebuthiuron in soil

Edivaldo Wilson de Lima, Bruno Pinheiro Brunaldi, Yanca Araujo Frias, Bruno Rafael de Almeida Moreira, Lucas da Silva Alves & Paulo Renato Matos Lopes

This study aimed to propose an eco-compatible strategy to mitigate the possible environmental contamination caused by tebuthiuron. Therefore, we screened potential tebuthiuron-degrading microorganisms from conventional (CS) and no-till (NTS) systems producing sugarcane. Then, they were bioprospected for their ability of decomposing the target-molecule at 2.48 mmol g^{-1} and 4.96 mmol g^{-1} into CO_2 via respirometry. Integrating microbiota from CS and NTS into an advantageously synergistic bacterial pool produced the highest specific-growth rate of CO_2 of 89.60 mg day^{-1}, so outstripped the other inoculum. The bacterial CN-NTS framework notably stabilized the sigmoidal Gompertz curve on microbial degradation earliest and enabled the seeds of *Lactuca sativa* to germinate healthiest throughout ecotoxicological bioassay for cross-validation. Our study is preliminary, but timely to provide knowledge of particular relevance to progress in the field's prominence in remediating terrestrial ecosystems where residual tebuthiuron can persist and contaminate. The analytical insights will act as an opening of solutions to develop high-throughput biotechnological strategies for environmental decontamination.

By searching for the academic specific topic of "pesticide bioremediation", we can screen-out numerous harmful molecules. Chlorpyrifos, malathion, atrazine, lindane and imidacloprid have greater intellectual interest by researchers and science policymakers than any other active compound (Fig. 1). All of them are sources of neurotoxins to pollinizers and invertebrates[1,2]. However, no single in-depth study exists for the microbiological detoxification or mineralization of tebuthiuron in soil.

Tebuthiuron is the dominant member of phenylurea[3]. It is useful to control grassy and broadleaf weeds in areas producing sugarcane. However, it is highly water-soluble (2.50 g L^{-1}) and can escape easily into ecosystems, where non-target organisms do not resist xenobiotics[4,5]. Carryover of biotoxins by residual tebuthiuron leaching can promote contamination and loss of biodiversity by food chain bioaccumulation or environmental exposure[6,7].

Although tebuthiuron is not the focus of literature on pesticide depollution, we can find few contemporary examples of its successful bioremediation. Mendes et al.[8] when studying the phytoremediation of pesticides by green manure, reported the ability for *Crotalaria spectabilis*, *Canavalia ensiformis*, *Stizolobium aterrimum*, and *Lupinus albus* to effectively remove C-tebuthiuron at 266.40 g ha^{-1}. *Mucuna pruriens* and *Pennisetum glaucum* were also able to dissipate C-tebuthiuron at 500 g ha^{-1} in soil with stillage as an organic matter to boost performance[9]. The authors[9] cross-validated the potential phytoremediators by checking the normal germination of an organism sensible to the target-molecule throughout ecotoxicological bioassay.

Thus, phytoremediation proves useful to remediate tebuthiuron. However, it often requires a special management and makes it challenging for agricultural systems to produce food, energy and natural fiber in off-season[8,9]. Furthermore, it is not easy to simulate conditions on an industrial scale. Therefore, an option to compensate the complexities of phytoremediation would be microbial degradation.

Microbial degradation is the major route of dissipation for photosystem II herbicides in aquatic ecosystems[2]. Bacteria can effectively degrade diuron, atrazine, hexazinone and tebuthiuron in seawater, with straightforward evidence on hydrolysis as the predominant pathway[10]. However, the authors[2,10] highlighted the importance of reproducing systematic studies for clarity and, most notably, analyzing environments other than coastal waters to progress in the field's prominence in microbiologically dissipating pesticides.

To the best of our knowledge, no in-depth investigation exists on the potential of microorganisms to remediate tebuthiuron in soil. Therefore, in light of research and innovation in pesticide-remediating eco-solutions, the novelty of our paper refers to the elaboration of a synergistic bacterial pool to dissipate tebuthiuron out of agricultural soil. Our exploratory study is still at an early stage of development. However, preliminary analytical insights into respirometric-ecotoxicological ramifications of microbial biotransformation of the target-pollutant into a simpler and harmless compound are timely. Our remedial approach is effective and will be likely useful for

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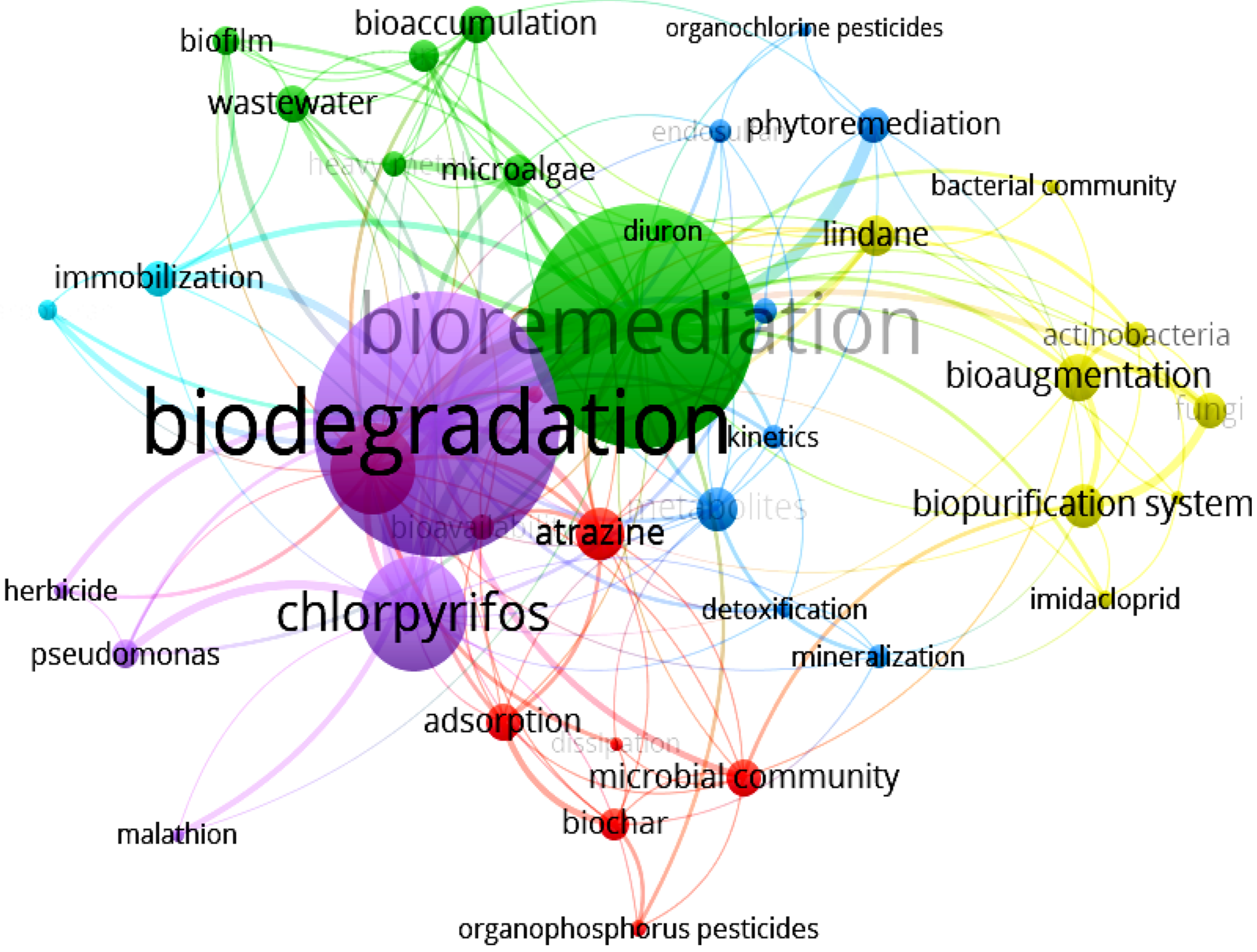

Figure 1. Co-occurrence of keywords on the specific topic of "pesticide bioremediation" (*Scopus, Web of Science*, and *Wiley Online Library* on 12th April, 2021).

the purpose of environmental recovery in agroecosystems, such as intensive sugarcane producing areas, where tebuthiuron acts as a highly toxic and recalcitrant pollutant and disrupts the agronomic functionalities besides the ecological sustenance.

Material and methods

Target-pesticide and microbial isolates. The pesticide used was tebuthiuron (Combine 500, Down AgroSciences Industrial). We isolated the potential tebuthiuron-degrading native microorganisms from conventional (longer historic of tebuthiuron) and no-till (shorter historic of tebuthiuron) systems producing sugarcane. Both sites were treated with the broad-spectrum herbicide at 800.00 g kg^{-1}. The in-situ microbial bioprospection consisted of randomly sampling five points at 0.00–0.15 m depth around the radicles. Then, the soil was transferred to air-tight bags. The material was stored in freezer at −5.00 °C to cryopreservation until further laboratorial procedures of spread plating and inoculation (Fig. 2).

Inoculum preparation. The nutrient-broth, nutrient-agar and mineral minimum broth were autoclaved (121 °C × 0.25 h) to formulate selective culture media. Particularly the mineral minimum broth consisted of 0.70 g KCl, 0.20 g KH_2PO_4, 3.00 g Na_2HPO_4, and 1.00 g NH_4NO_3 per liter, with an additional 1.00 mL L^{-1} solution of micronutrients: 4.00 mg $MgSO_4$, 0.20 mg $FeSO_4$, 0.20 mg $MnCl_2$, and 0.20 mg $CaCl_2$[11]. Mimetic microcosms were elaborated by introducing 25.00 g L^{-1} of tebuthiuron into all media at pH 7.20 in order to adapt the isolates to the pesticide until pre-selection. Aliquots of 90.00 mL were then thoroughly mixed with 50.00 g of soil (2 mm granulometry) in Erlenmeyer flasks and were mechanically stirred at 120.00 rpm, 30.00 °C for 72.00 h for homogenization. The material was incubated at room temperature for 12 days. After incubation, 0.50 mL of all microbial suspensions was diluted in sterile saline solutions (0.90% NaCl) to prevent cross-contamination. Aliquots of 1.00 mL were evenly streaked out over nutrient-agar plates at 10^{-4}, 10^{-5} and 10^{-6} and stored at 37 °C for 48.00 h. Finally, we automatically counted the colony-forming units on the surface of plates to check cellular viability. Thereafter, we performed spectrophotometric measurements at 600.00 ηm[12] to standardize inoculum at 0.80 absorbance unit for the respirometric bioassay.

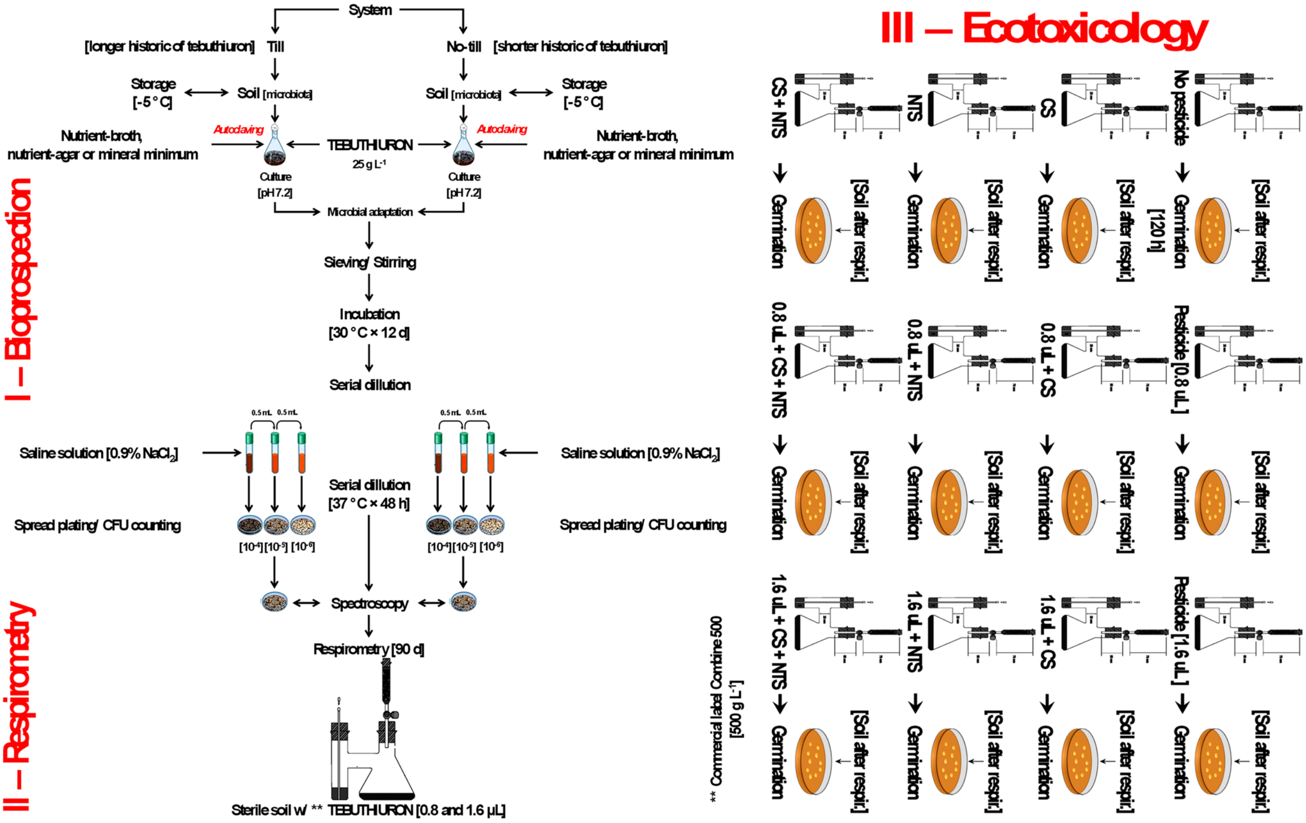

Figure 2. Scheme of isolating native microorganisms to mineralize tebuthiuron into CO_2. In the diagram, the doses of 0.8 µL and 1.6 µL are equivalent to the concentrations of 2.48 mmol g^{-1} and 4.96 mmol g^{-1} in the soil (half-dose and full-dose of tebuthiuron, respectively.

Respirometric bioassay. A respirometric bioassay was performed to quantitatively analyze the ability of the isolates to mineralize tebuthiuron into CO_2, according to methodology described by Bartha and Pramer[13]. Samples of soil (Table S1, Supplementary material) were collected at the layer of 0.00–0.30 m in the experimental field of the Plant Production Division of the College of Agricultural and Technological Sciences, São Paulo State University (Unesp). The material was oven dried at 65 °C until constant mass, sieved to 2.00 mm granulometry and autoclaved at 121.00 °C for 0.25 h for sterilization before preparing media to the respirometry. Aliquots of 0.05 kg of sterile soil and tebuthiuron at 0.8–1.6 µL were introduced together into respirometers and then the isolates were inoculated. After inoculation, 10.00 mL of KOH at 1 M were transferred to flasks to capture the production of CO_2 upon the target-pesticide at the concentrations of 2.48 mmol g^{-1} and 4.96 mmol g^{-1} as the half-dose and full-dose, respectively. We quantified the product of microbial respiration by electroconductivity of CO_3^{-2} (Eq. 1) every day after titrating the KOH with 10.00 mL of $BaCl_2$ at 1 M. After quantification, 10.00 mL of KOH was added to respirometers for the next samples[13]. The bioassay lasted for 90 days. Flasks were incubated at 25.00 ± 2.50 °C and $60.00 \pm 5.00\%$ of relative humidity of the air, in dark room to prevent photodecomposition[10]. The set was aerated for renewing atmosphere every day after quantifying the production of CO_2 from the experimental units (Table 1) in triplicate to reduce systematic errors.

$$G = 1554.80 - 95.7OC \quad (1)$$

where: G is the production of CO_2, mg; C is the electroconductivity, mS cm^{-1}; and the constants stand as factors of transformation of concentration of the analyte from mmol to mg[13].

Ecotoxicological bioassay. To cross-validate our approach, ecotoxicity of tebuthiuron after an eventual microbial bioremediation was determined in triplicate in seeds of a utilitarian organism (*Lactuca sativa*) based on experimental protocols. Samples of soil (0.0025 kg) were collected from flasks at the beginning (t_0) and the end (t_{90}) of the respirometric bioassay. Twenty-five seeds were randomly selected, then evenly distributed over acrylic plates to have contact with the xenobiotic. The plates were incubated in biochemical oxygen chamber at 25.00 ± 2.50 °C, $50.00 \pm 5.00\%$ relative humidity, and 16:8 h of photoperiod. The ecotoxicological bioassay lasted for 120.00 h, and the tests were sampled every 24.00 h to quantify the germination and radicle-to-hypocotyl ratio as indicators of vigor and phenotypical morphophysiology, respectively[14]. Media with water and zinc sulphate as an inhibitor of germination were prepared as positive and negative controls, respectively.

Data analysis. We fitted the data on respirometry and ecotoxicology for sigmoidal Gompertz function (Eq. 2), starting the parametrization with $\alpha = 1000$, $\beta = 10$ and $\kappa = 0.50$[15]. The criteria to analyze the adequacy of

Test	Tebuthiuron, mmol g^{-1}	Source of native microbiota	
		Conventional system	No-till system
I	No pesticide	−	−
II	2.48, half-dose	−	−
III	4.96, full dose	−	−
IV	No pesticide	+	−
V	2.48, half-dose	+	−
VI	4.96, full dose	+	−
VII	No pesticide	−	+
VIII	2.48, half-dose	−	+
IX	4.96, full dose	−	+
X	No pesticide	+	+
XI	2.48, half-dose	+	+
XII	4.96, full dose	+	+

Table 1. Set of tests for the respirometric bioassay of potential microbial biodegradation of tebuthiuron.

the stochastic model included Akaike information criterion (AIC), Bayesian information criterion (BIC), and adjusted coefficient of determination (r_{adj}^2).

$$f_x = \alpha e^{-\beta e^{-kx}} \tag{2}$$

where: f_x is the production of CO_2 or germination, mg or %; x is the time, days; α is the upper asymptote or the maximum of production of CO_2 or germination, mg or %; β is the inflection point; κ is the exponential decay of specific-growth rate of production of CO_2 and germination, mg CO_2 day^{-1} and % day^{-1}; and e is the Euler number.

A box-plot diagram was elaborated to describe the radicle-to-hypocotyl ratio for samples from ecotoxicological bioassay, separating them using the *post-hoc* Tukey's test. Principal component analysis[16] was conducted to extract functional relationships between respirometry and ecotoxicology. All analyses were performed in the environment of the software R-project for statistical computing and graphics[17].

Ethics approval. Authors confirm that the manuscript has not been submitted to journal for simultaneous consideration and has not been previously published. Results collection, selection, and processing performed personally. Authors' institution informed about this submission.

Consent for publication. All authors approved the manuscript before submission and consent to the submission to *Scientific Reports*.

Results

Morphological characterization of isolates.

The isolates, irrespective of origination, consisted of bacterial colonies. Mineral broth medium produced more colony-forming units than nutrient-broth and nutrient-agar. Therefore, it proved the most reliable option to culture potential tebuthiuron-degrading bacteria.

Kinetic mineralization.

Integrating CS and NTS into a bacterial pool enhanced the sigmoidal mineralization of tebuthiuron into CO_2 (Fig. 3D). Plainly, composite inoculum proved synergistic effect and, hence, outstripped both CS and NTS in stabilizing the Gompertz breakthrough curve for the target-pesticide at 4.96 mmol g^{-1} (Fig. 3D). The bacterial CS-NTS framework required about 24 days to approach the maximum value for biotransformation of tebuthiuron at the highest dose into CO_2 ($\alpha \sim 2141.00$ mg) (Table 2), and our stochastic analysis on Gompertz adequately predicted its ability for accelerating the process into an inflection point of 1.95 and relative growth-rate of 89.60 mg CO_2 day^{-1}. Comparatively, values of β and k for CS and NTS were 1.80 and 39.50 mg CO_2 day^{-1} (Fig. 3B) and 1.90 and 38.50 mg CO_2 day^{-1} (Fig. 3C), respectively, for 4.96 mmol g^{-1}. For the lowest dose, we could parametrize 1.80 and 49.55 mg CO_2 day^{-1} and 1.95 and 34.60 mg CO_2 day^{-1}, respectively. Therefore, both CS and NTS required longer periods of time to reach the maximum relative value to CS-NTS. As CS ($\beta = 1.95$; $k = 80.5625$ mg day^{-1}) (Fig. 3B) generated CO_2 more intensively than NTS ($\beta = 1.95$; $k = 40.5785$ mg day^{-1}) (Fig. 3C), it likely contributed more to the ability of the composite inoculum to accelerate the sigmoidal mineralization throughout 90 days respirometry. If CS-NTS proved synergistic effect towards skewing upwards the breakthrough curve for the mineralization of tebuthiuron at 2.48 mmol g^{-1} (Fig. 3D), a flatter and longer stationary phase on Gompertz function for its performance at 4.96 mmol g^{-1} followed the dependence of growing bacterial population on concentration. Most notably, curves for CS-NTS at 2.48–4.96 mmol g^{-1} steeper than its contrasting curve without the pesticide (Fig. 3D) supported the role of tebuthiuron in providing the bacterial growth by acting as a source of mineralizable carbon, besides the adequacy of Gompertz to describe growth sigmoidal rather than linear. Furthermore, tebuthiuron was not necessarily the only source of energy to microorganisms, as it released CO_2 (Fig. 3A) without introducing isolates, irrespective of origination, into the soil for respirometry.

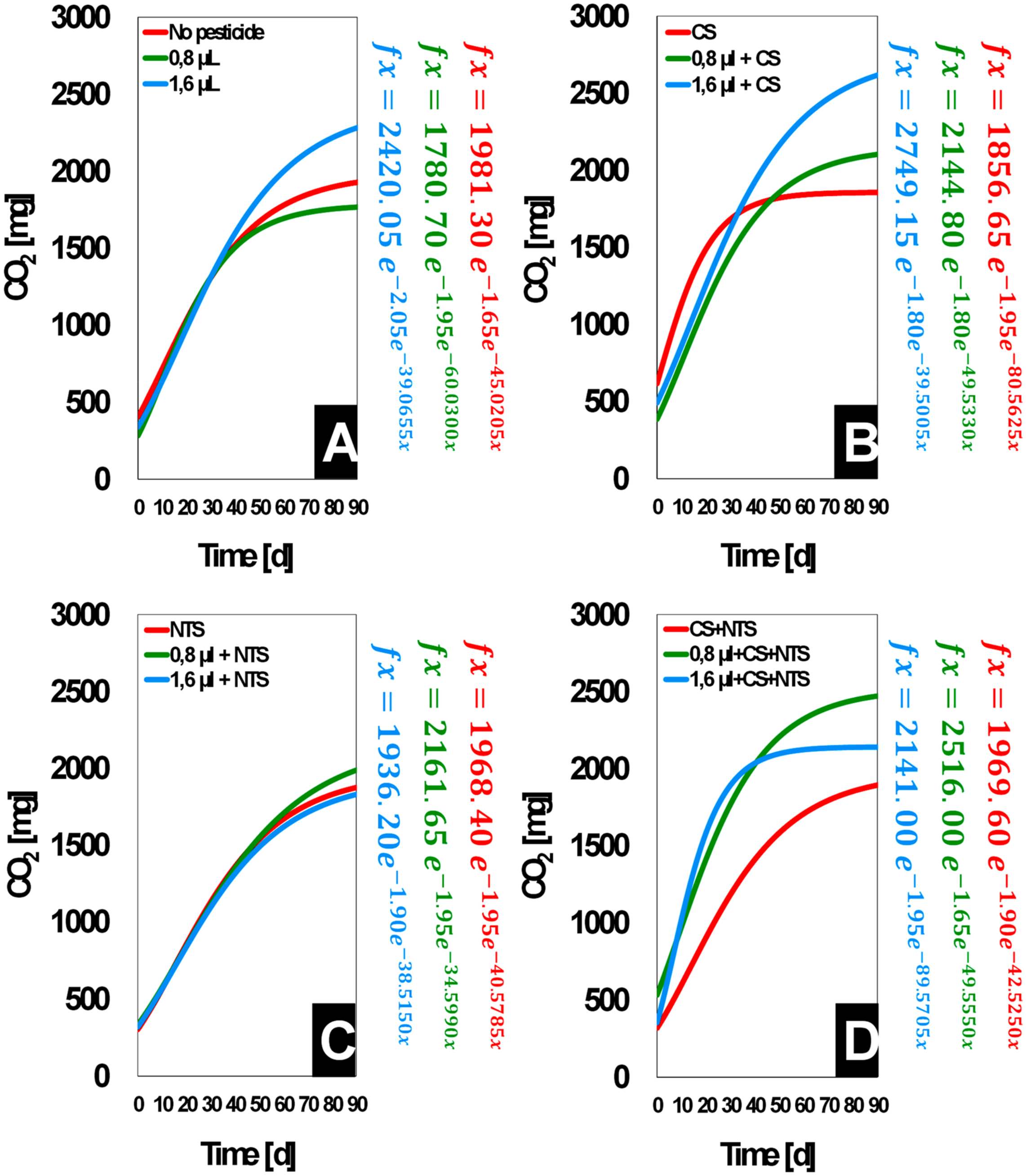

Figure 3. Sigmoidal mineralization of tebuthiuron in media containing only soil (**A**) and soil with isolates from conventional system (**B**) and no-till system (**C**) and both (**D**). In the diagram, the doses of 0.8 µL and 1.6 µL are equivalent to the concentrations of 2.48 mmol g^{-1} and 4.96 mmol g^{-1} in the soil (half-dose and full-dose of tebuthiuron, respectively); Conventional (CS) and no-till (NTS) systems; $\beta = 1$ keeps the relative decrease with time constant; $\beta > 1$ accelerates the relative decrease with time; $\beta < 1$ decelerates the relative decrease with time[15].

Ecotoxicology. Since bacterial CS-NTS framework most effectively mineralized the herbicide at 2.48 mmol g^{-1} into CO_2, it enabled the seeds to have the highest percentage germination over time (Fig. 4, D0 and D90). In addition, seedlings developed fastest and healthiest with radicle-to-hypocotyl ratio closest to 1 (Fig. 5, D0 and D90). The best result of the CS-NTS microbial consortium can be observed when comparing with the results of the *Lactuca sativa*'s germination index in relation to the presence of isolated inoculums CS (Fig. 4, B0 and B90) and NTS (Fig. 4, C0 and C90). Consequently, the same relationship can be performed with the radicle-to-hypocotyl ratio of this organism sensitive to tebuthiuron for isolates CS (Fig. 5, B0 and B90) and NTS (Fig. 5, C0 and C90).

However, ecotoxicological units containing soil from respirometers without microbiota, irrespective of composition, delayed the germination (Fig. 4, A0 and A90) and induced an atypical radicle-to-hypocotyl ratio of ≥ 1.45 (Fig. 5, A0 and A90). Sensitive organism also proved to be able to germinate on plates containing only tebuthiuron (Fig. 4, A0 and A90). Certainly, the addition of 2.48 mmol g^{-1} and 4.96 mmol g^{-1} tebuthiuron in

	Parameterization			Adequacy		
Test	α, mg	β, days	κ, mg day^{-1}	AIC	BIC	r_{adj}^2
No pesticide	1981.30	1.65	45.0205	364.2270	369.8315	0.9540*
2.48 mmol g^{-1}	1780.70	1.95	60.0300	335.6625	341.2670	0.9465*
4.96 mmol g^{-1}	2420.05	2.05	39.0655	360.8330	366.4380	0.9450*
CS	1856.65	1.95	80.5625	334.5820	340.1865	0.9810*
2.48 mmol g^{-1} + CS	2144.80	1.80	49.5330	323.4520	329.0570	0.9905**
4.96 mmol g^{-1} + CS	2749.15	1.80	39.5005	354.0185	359.6230	0.9825*
NTS	1968.40	1.95	40.5785	290.5405	296.5410	0.9960**
2.48 mmol g^{-1} + NTS	2161.65	1.95	34.5990	317.3815	322.9865	0.9910**
4.96 mmol g^{-1} + NTS	1936.20	1.90	38.5150	298.4065	304.0115	0.9945**
CS + NTS	1969.60	1.90	42.5250	313.4130	319.0175	0.9920**
2.48 mmol g^{-1} + CS + NTS	2516.00	1.65	49.5550	369.1560	374.7610	0.9655*
4.96 mmol g^{-1} + CS + NTS	2141.00	1.95	89.5705	365.3595	370.9640	0.9725*

Table 2. Parametrization and adequacy of Gompertz model for the kinematic microbial biodegradation of tebuthiuron. 2.48 and 4.96 mmol g^{-1} are half-dose and full-dose of tebuthiuron, respectively. *CS* conventional system, *NTS* no-till system, *AIC* Akaike information criterion, *BIC* Bayesian information criterion. Significant code: $^{**}p < 0.01$; $^{*}p < 0.05$.

soil were not sufficient to prevent seed germination. However, these concentrations induced longer and hairier radicle and shorter hypocotyl for *L. sativa* (Fig. 5, A0 and A90). These results indicated developmental abnormalities or adaptations to the challenging microenvironment for the seedlings. By fitting the sigmoidal Gompertz function to the germination data, we could estimate $\beta > 1$ for all tests except negative control containing water (Table 3). A $\beta \geq 1$ is an indicator that the growth relative decreasing accelerates with time. On the other hand, $\beta < 1$ decelerate the growth relative decrease, while the $\beta = 1$ keeps it constant. Inflection point was greatest for bacterial CS-NTS framework ($\beta = 956.95$), so seeds germinated and developed fastest into seedlings on plates containing soil from respirometric flasks where concentration of tebuthiuron initially was 4.96 mmol g^{-1} (Fig. 4, D90). Relative growth-rate also was the largest for the CS-NTS ($k = 0.23835$), further supporting its distinct ability for detoxifying the medium. Overall, applying Gompertz to ecotoxicological bioassay, it was possible to validate the effectiveness of the synergistic bacterial pool to mineralize the target-molecule into CO_2 throughout 90 days respirometry, which potentially make the substrate less harmful. Hence, we could verify that the organism germinated and developed throughout 5 days without any critical abnormality, despite its sensitivity to tebuthiuron.

Mineralization-ecotoxicology nexus. The PCA robustly divided the high-dimensionality dataset and exported only the useful statistics into the latent orthogonal hits, namely PC_I and PC_{II} (Fig. 6). The two PC together explained about 70% of variability in the interdependent respirometric and ecotoxicological bioassays (Table 4). The PC_I explained the mineralization. It correlated positively with the specific-growth rate of both biodegradation and germination. However, it correlated negatively with the radicle-to-hypocotyl ratio. On the other hand, the PC_{II} explained the ecotoxicity. It correlated positively with both inflection of biodegradation and radicle-to-hypocotyl ratio. The bacterial CS-NTS framework moved towards the left lower quadrant in the bi-plot map, further supporting its effectiveness to mineralize tebuthiuron and make it less toxic over seeds. Therefore, the multivariate analysis of data validated the effectiveness of the synergistic bacterial pool to remediate the target-molecule.

Discussion

Native microorganisms from sugarcane's rhizosphere can be useful to bioremediate the tebuthiuron in soil. By integrating microbiota from conventional and no-till systems into an advantageously synergistic bacterial pool, we can optimize the biotransformation of the target-pesticide into CO_2. The higher the dose the more effective the mineralization, as the xenobiotic likely becomes more bioavailable from the substrate. Plainly, bioavailability is primordial to bioremediation. If contaminant is available, microorganisms are able to mineralize the carbon[18]. If not, they cannot access the target-molecule, so an effective bioremediation is not likely. The bacterial CS-NTS framework can mineralize the tebuthiuron at half-dose more effectively than CS and NTS, making it an option to compensate the insufficient either electronic donation/acception or systematic stimulation, which are limiting factors to pesticide-metabolizing enzymes[19].

Isolates from conventional system can adapt to the mimetic microcosm more effectively than isolates from no-till system, supporting our hypothesis of the impact of environmental pressure on the microbial behavior. A longer historic and more frequent applications of tebuthiuron can upregulate the ability for autochthone microorganisms to resist mimetic microcosm containing the pesticide as an elemental component. Therefore, they can accelerate the lag phase for the composite inoculum. The shorter the lag phase the more efficient the microbiota in mineralizing tebuthiuron in the log or exponential phase. Production of CO_2 is the largest in the log phase and gradually decreases as the biotransformation levels off. Thus, we could find no significant activity for the stationary phase.

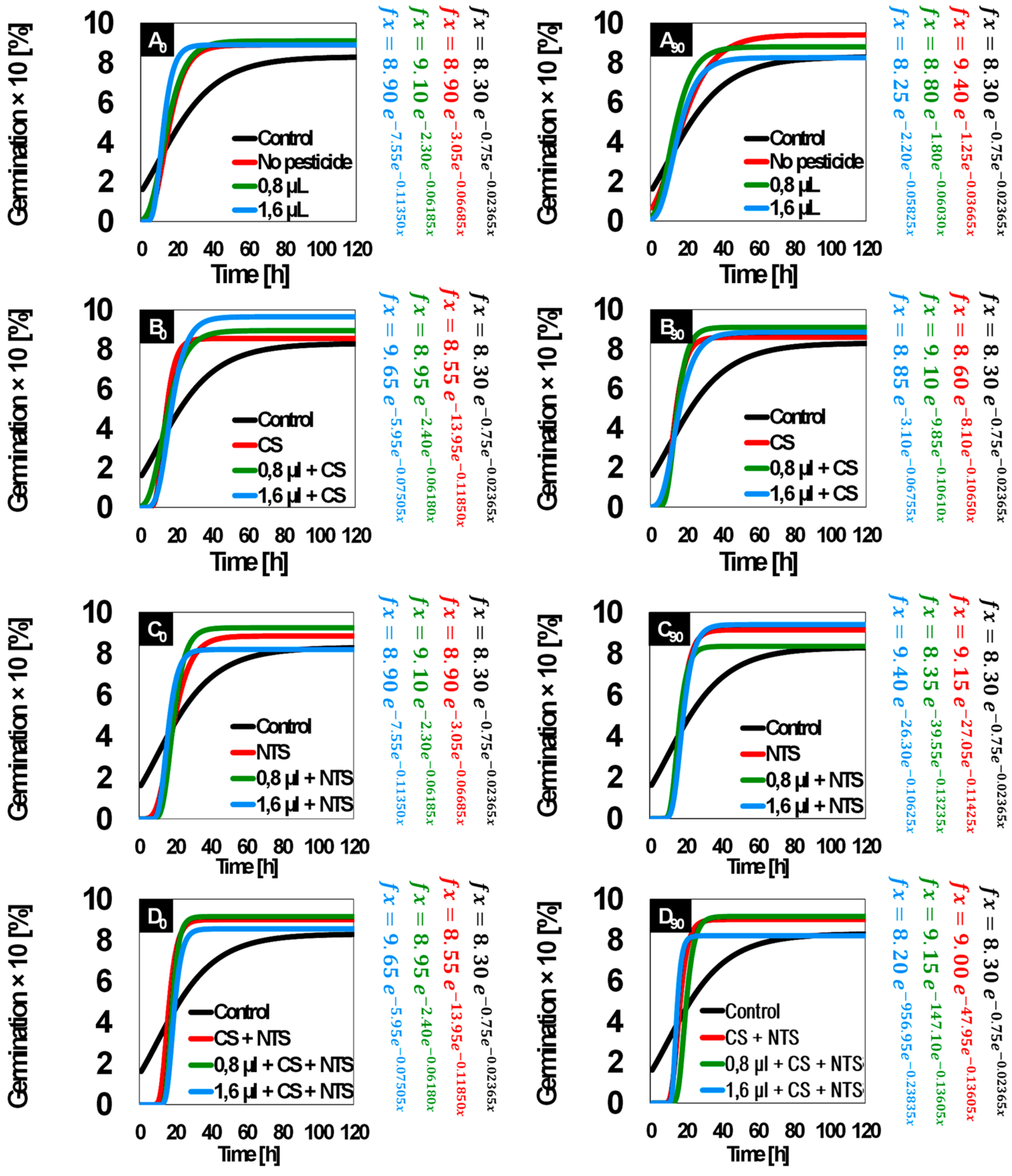

Figure 4. Sigmoidal germination of an organism sensitive to tebuthiuron on plates containing respirometric samples of only soil (**A**) and soil with isolates from conventional system (**B**) and no-till system (**C**) and both (**D**). In the diagram, the doses of 0.8 µL and 1.6 µL are equivalent to the concentrations of 2.48 mmol g^{-1} and 4.96 mmol g^{-1} in the soil (half-dose and full-dose of tebuthiuron, respectively); Conventional (CS) and no-till (NTS) systems; Left-panel (t_0—initial time) and right-panel (t_{50}—after 90 days of biodegradation); $\beta = 1$ keeps the relative decrease with time constant; $\beta > 1$ accelerates the relative decrease with time; $\beta < 1$ decelerates the relative decrease with time[15].

Studies on microbial degradation of tebuthiuron focus on aquatic ecosystems[2,10], making it challenging for contrasting our trends with the existing literature. Although our study can demonstrate the potential microbial degradation of the target-pesticide in soil, it is still preliminary on phylogenic profile of bacteria and metabolism are missing out. Thus, further in-depth investigations are necessary to clarify the possible pathways. A possible future broad time-dependent analytical approach would be to reveal the soil-specific microbial world around degradation of tebuthiuron. Another one would be to investigate if it could be possible for N-demethylation[20] to be a metabolic pathway for native microorganisms to mineralize the target-pesticide into CO_2. We expect N-demethylation supports the role of tebuthiuron as an electron donor to bacteria as acceptors. Electron donation is an important attribute for the pesticide to be degradable and become harmless or less toxic over an organism,

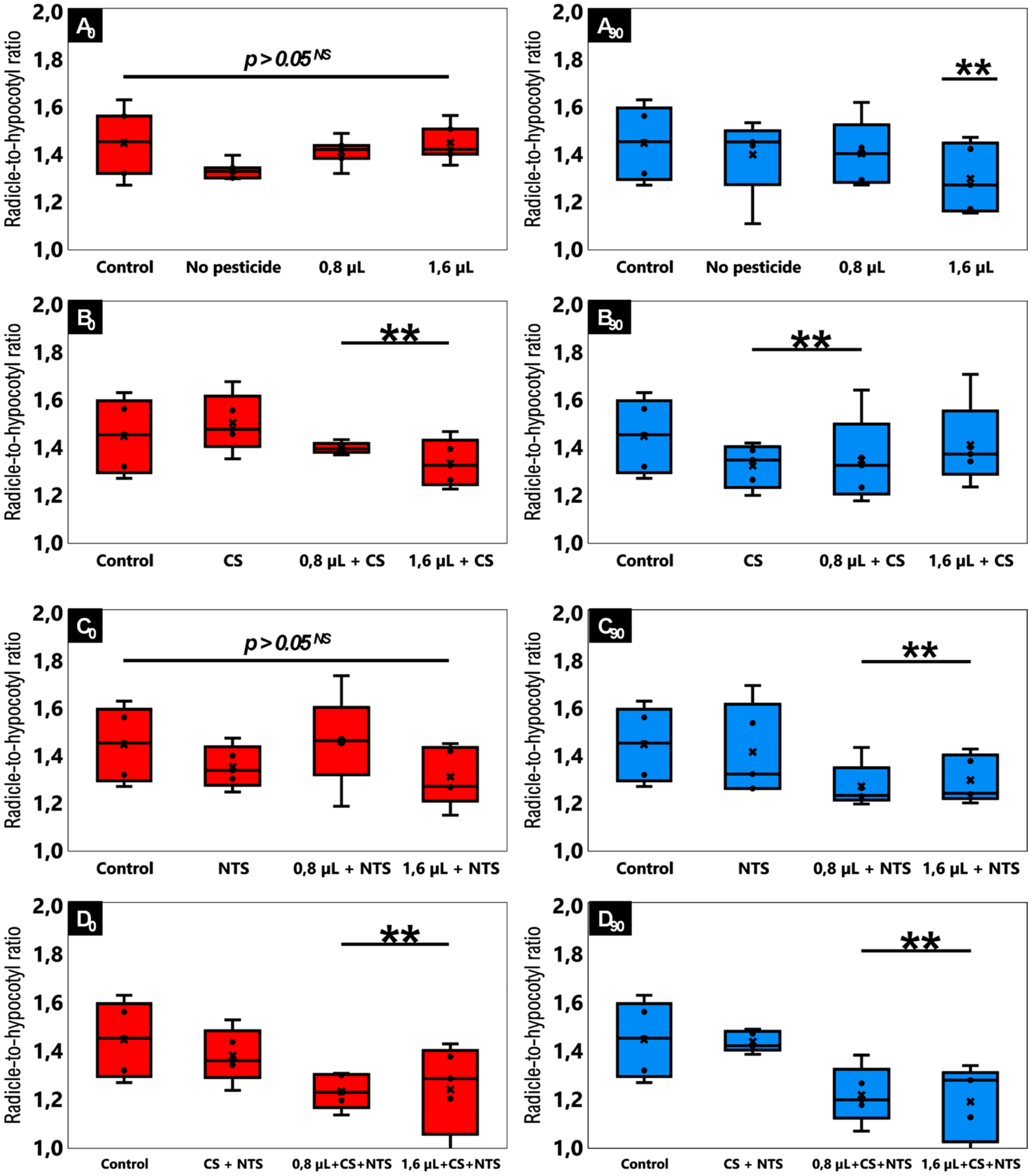

Figure 5. Radicle-to-hypocotyl ratio of an organism sensitive to tebuthiuron on plates containing respirometric samples of only soil (**A**) and soil with isolates from conventional system (**B**) and no-till system (**C**) and both (**D**). In the diagram, the doses of 0.8 μL and 1.6 μL are equivalent to the concentrations of 2.48 mmol g^{-1} and 4.96 mmol g^{-1} in the soil (half-dose and full-dose of tebuthiuron, respectively); *Significant and *NS* non-significant by post hoc Tukey's test at $p < 0.05$; conventional (CS) and no-till (NTS) systems; left-panel (t_0—initial time) and right-panel (t_{50}—after 90 days of biodegradation).

as our ecotoxicological bioassay can describe towards the impact of tebuthiuron on germination and seedling of *L. sativa*.

After 90 days respirometry, samples of soil from flasks, where tebuthiuron and bacterial CN-NTS consortium co-exist, could be toxic enough to limit germination or even promote developmental abnormalities, such as stubby radicle or twisting hypocotyl[21]. Hence, seeds can develop into seedlings with radicle-to-hypocotyl ratio closer to 1, which is an indicator of healthy plant[21]. Although ecotoxicological bioassay can cross-validate the potential of rhizospheric native microorganisms to detoxify soil with tebuthiuron, the pesticide could not necessarily be the only source of energy to the microcosm. Flasks containing the target-molecule without isolates, irrespective of composition, can also produce CO_2. Underlying mechanisms of auto-mineralization of tebuthiuron is unclear, and thus further in-depth investigations are necessary to clarify the fate of the pesticide either directly or through the formation of metabolites to confirm degradation. Interestingly, the sensitive organism can

	Parameterization			Adequacy		
Test	$\alpha \times 10$, %	β	$\kappa \times 10$, % h^{-1}	AIC	BIC	r_{adj}^2
Initial time (t_0)						
Control	8.30	0.75	0.02365	−5.90	−7.50	0.9955 **
No pesticide	8.90	3.05	0.06685	3.85	2.30	0.9895 *
2.48 mmol g^{-1}	9.10	2.30	0.06185	−2.55	−4.10	0.9960 **
4.96 mmol g^{-1}	8.90	7.55	0.11350	−4.45	−6.00	0.9970 **
CS	8.55	13.95	0.11850	5.40	3.80	0.9900 **
2.48 mmol g^{-1} + CS	8.95	2.40	0.06180	−3.70	−5.25	0.9970 **
4.96 mmol g^{-1} + CS	9.65	5.95	0.07505	0.65	-0.92	0.9975 **
NTS	8.85	5.10	0.06500	2.90	1.30	0.9955 **
2.48 mmol g^{-1} + NTS	9.25	20.55	0.09630	−3.70	−5.25	0.9995 **
4.96 mmol g^{-1} + NTS	8.20	24.45	0.11885	0.55	−1.00	0.9975 **
CS + NTS	9.00	47.95	0.13535	0.90	−0.65	0.9985 **
2.48 mmol g^{-1} + CS + NTS	9.15	352.35	0.16850	−4.80	−6.35	0.9995 **
4.96 mmol g^{-1} + CS + NTS	8.55	188.40	0.14570	−6.20	−7.75	0.9995 **
Final time (t_{90})						
No pesticide	9.40	1.25	0.03665	1.40	−0.15	0.9915 **
2.48 mmol g^{-1}	8.80	1.80	0.06030	−19.40	−20.95	0.9990 *
4.96 mmol g^{-1}	8.25	2.20	0.05825	−17.00	−18.55	0.9995 **
CS	8.60	8.10	0.10650	−22.55	−24.10	0.9995 **
2.48 mmol g^{-1} + CS	9.10	9.85	0.10610	1.95	0.40	0.9950 **
4.96 mmol g^{-1} + CS	8.85	3.10	0.06755	−3.85	−5.40	0.9975 **
NTS	9.15	27.05	0.11425	−7.75	−9.30	0.9995 **
2.48 mmol g^{-1} + NTS	8.35	39.55	0.13235	−5.20	−6.75	0.9995 **
4.96 mmol g^{-1} + NTS	9.40	26.30	0.10625	−11.95	−13.55	0.9995 **
CS + NTS	9.00	47.95	0.13535	0.90	−0.65	0.9985 **
2.48 mmol g^{-1} + CS + NTS	9.15	147.10	0.13605	−8.00	−9.60	0.9995 **
4.96 mmol g^{-1} + CS + NTS	8.20	956.95	0.23835	−46.80	−48.35	0.9995 **

Table 3. Parametrization and adequacy of Gompertz model for kinetic germination of an organism sensible to tebuthiuron on plate containing soil from respirometric bioassay. 2.48 and 4.96 mmol g^{-1} are half-dose and full-dose of tebuthiuron, respectively. *CS* conventional system, *NTS* no-till system, *AIC* Akaike information criterion, *BIC* Bayesian information criterion. Significant code: **$p < 0.01$; *$p < 0.05$.

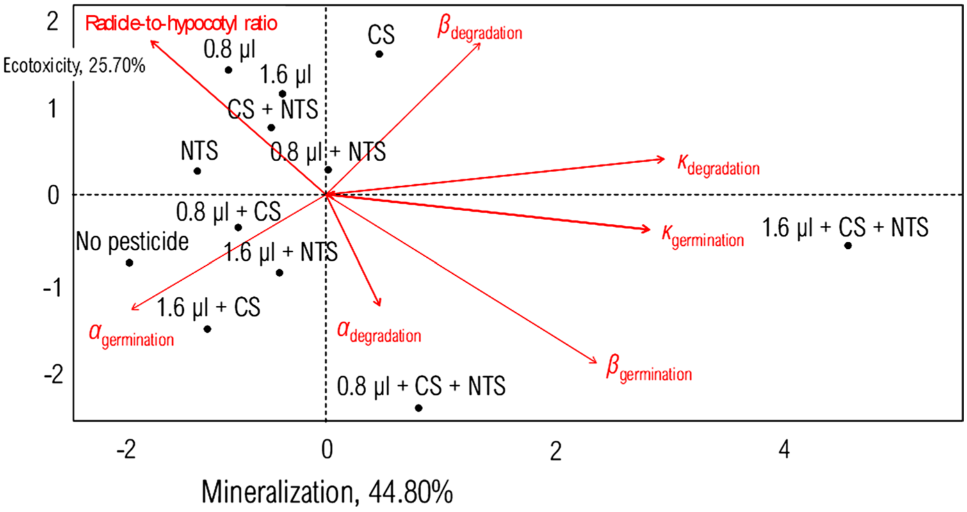

Figure 6. Bi-plot map for the mineralization-ecotoxicology nexus. In the diagram, the doses of 0.8 μL and 1.6 μL are equivalent to the concentrations of 2.48 mmol g^{-1} and 4.96 mmol g^{-1} in the soil (half-dose and full-dose of tebuthiuron, respectively); (CS) conventional and no-till (NTS) systems.

	Latent orthogonal hit	
Eigenvector	**PC_I, mineralization**	**PC_{II}, ecotoxicology**
Eigenvalue	2.85*	1.65*
Percentage of variance	44.75	25.70
Cumulative percentage of variance	44.75	70.45
	Correlation	
Maximum degradation	0.05	−0.55
Inflection of degradation	0.25	0.85*
Specific-growth rate of degradation	0.75*	0.20
Maximum germination	−0.60*	−0.50
Inflection of germination	0.95**	−0.15
Specific-growth rate of germination	0.90 **	−0.10
Root-to-hypocotyl ratio	-0.65 *	0.65*

Table 4. Principal components into respirometric and ecotoxicological bioassays. *CS* conventional system, *NTS* no-till system. Significant code: **$p < 0.01$; *$p < 0.05$.

germinate on plates containing soil of 90 days from respirometers, where concentration of tebuthiuron initially range from 2.48 to 4.96 mmol g^{-1} and microbiota does not exist. However, its radicle could be longer/hairier and hypocotyl shorter than normal, which are adaptative response of plant to a stressing microenvironment[22].

Fitting of kinetic functions is primordial to study mineralization[23]. First-order models are able to predict mineralization. However, they have the limitation of either overfitting or underfitting data on molecules with half-life independent upon time and concentration[23]. If density of degrading microorganisms does not change with time or concentration of pesticide, fitting of first-order functions becomes rather complex and inaccurate. However, as pesticides often sustain growth of degraders, mineralization curves for growing microbial populations are sigmoidal rather than linear[23]. Thereby, we screen Gompertz out of similar microbial growth models[24] as an option to first-order functions for fitting of mineralization of tebuthiuron into CO_2. Certainly, sigmoidal Gompertz function can adequately describe the microbial mineralization of tebuthiuron throughout respirometric bioassay, although it cannot fit nongrowth regions and numerous samples could make it challenging for interpreting its parameters. Overall, Gompertz function and its variation[25] can prove useful to stochastic microbiological studies by predicting degradation[26], mineralization[27–29] and ecotoxicology of pollutants/contaminants with accuracy. Therefore, it offers an excellent option for regulatory agencies, researchers and policymakers to replace first-order kinetics in evaluating and elaborating approval procedures for pesticides.

Definitely, the innovative strategy of isolating native rhizospheric microorganisms from areas producing sugarcane with history of exposure to tebuthiuron can prove useful to develop a functional pesticide-degrading framework. Our timely exploratory study introduces the microbial biodegradation of tebuthiuron in agricultural soil. However, it is still preliminary. Thus, further in-depth research is needed to explore the composition and function of the tebuthiuron-degrading bacterial pool and to clarify how the soil changes as the microbiota synergistically and dynamically reduces the target-pollutant into simpler compounds over time. Furthermore, future studies should focus on the microbial metabolism to elucidate the possible genes, enzymes, and pathways controlling the catabolism of tebuthiuron to cross-validate and add information to our preliminary analytical insights into the respirometric-ecotoxicological ramifications of biodegradation. Most importantly, researchers should analyze whether an eventual metabolite could be more toxic and recalcitrant than the parent molecule in order to develop an environmentally safe and responsive microbial bioremediation, which should include an holistical ecotoxicological approach.

Conclusion

Our preliminary study clearly demonstrates the microbial degradation of tebuthiuron in soil. Native microorganisms from sugarcane's rhizosphere prove useful to elaborate a synergistic bacterial pool able to produce 89.60 mg CO_2 day^{-1} upon the target-pesticide at 4.96 mmol g^{-1}. The composite inoculum is likely to require 25 days to stabilize the sigmoidal biotransformation on Gompertz function. Our insights provide knowledge of particular relevance to progress in the field's prominence in microbiologically remediating terrestrial ecosystems, where residual tebuthiuron can persist and contaminate.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request. Moreover, they are preserved in the cloud in the Repository Institutional by Unesp, whose periodic backup system promotes greater security.

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Competing interests

The authors declare no competing interests.

Additional information

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5.4. Anexo IV

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REVIEW PAPER

Microbial bioremediation of pesticides in agricultural soils: an integrative review on natural attenuation, bioaugmentation and biostimulation

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Abstract Pesticides can impact the agriculture and environmental sector both positively and negatively. An over-reliance on their application to crops to control pests can disturb ecosystems. Therefore, the scientific community and policymakers must be aware of the commitment and active stance they need to take up to effectively elaborate on solutions toward mitigating environmental contamination over the coming few years. We, therefore, reviewed the academic literature on bioremediation from 2018 to 2021 (the latest year of complete publication) to provide a meta-analysis of microbial systems capable of dissipating pesticides from agricultural soils. Natural attenuation can control lindane; however, it is time-consuming and unconvincing to scale. By introducing a suite of microorganisms into the system for substrate-specific biodegradation, we can boost the bioprocess and ultimately level up its cost-effectiveness. Options of microorganisms for bioaugmentation include the fungus *Trametes versicolor* and the bacteria *Pigmentiphaga* spp. and *Paenanthrobacter* spp. Bioaugmentation and biostimulation are enablers of environmental reclamation in agroecosystems. However, those biocatalytic strategies can be costly while manifesting as degraders to ecological sustainability. For instance, allochthonous and recombinant microorganisms can reduce genetic diversity by promoting antagonistic relationships. In addition, some stimulant minerals can be more toxic and harmful to beneficial non-target organisms than the target pesticide. Prudence and safety are significant aspects of ensuring environmentally safer applications for

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pesticide-degrading approaches. Therefore, our analytical insights can provide knowledge to progress the field's prominence in developing high-throughput microbiological removal of hazardous active compounds from agricultural soils.

Keywords Agroecosystems · Environmental depollution · Harmful compounds · Metabolic pathways

Abbreviations

CFU	Colony-forming unit
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
FAO	Food and agriculture organization
OM	Organic matter
PLFA	Phospholipid fatty acid analysis
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
T-RLFP	Terminal restriction fragment length polymorphism

1 Introduction

FAO 's food security and nutrition experts estimate the world population at 10 billion by 2050 (FAO 2021). Therefore, agriculture will likely require an increasingly higher level of resource inputs to produce food and feed people sustainably over the coming years (Sharma et al. 2019). However, intensifying agricultural systems will likely make it harder for stakeholders (e.g., governments, regulatory agencies, and producers) to address an increasing pressure to farm responsibly and reduce the negative environmental impacts such as pollution and contamination by fertilizers and pesticides (Gyawali 2018; Sharma et al. 2019; Gomes et al. 2020). For instance, contamination, particularly by more than one active pesticide ingredient over global arable land, is approximately 24.5 million km^2 (Tang et al. 2021). An over-reliance on the application of pesticides onto crops to control pests can be hazardous and effectively harm the biota by exposing it both directly and indirectly to genotoxicity (Ferreira et al. 2021; Kapeleka et al. 2021; Cuenca et al. 2019), cancer-causing malignant tumor (Matich et al. 2021; Ventura et al. 2019), neurotoxicity (Mostafalou and Abdollahi 2017), and reproductive and metabolic disruption (Richardson et al. 2019).

Unsuitable and unsustainable application of pesticides in high-throughput agroecosystems can threaten society and the environment (Gonçalves and Delabona 2022; Gomes et al. 2020). Therefore, elaborating on strategic, catalytic, resilience-building actions toward mitigating persistent residues can be significant for environmental protection and sustainable production (Mir et al. 2020). An option would be microbial bioremediation, including strategies such as natural attenuation, bioaugmentation, biostimulation, and synergistic plant–microbe associations (Fig. 1). In natural attenuation, a decrease in the concentration of contaminants occurs due to a combination of natural events, such as volatilization, dilution, run-off, adsorption, microbial degradation, and others, without human interference. Bioaugmentation is related to the incorporation of microorganisms (autochthonous, allochthonous, and/or OGM) presenting an enzymatic complex capable of biodegrading the target contaminant. Biostimulation accelerates autochthonous microbial growth by nutrient addition or adjustments to the physicochemical conditions in the polluted site. In contrast, in plant–microbe associations plants and microorganisms act in cooperation, in which plants supply appropriate environmental conditions to enhance microbial degradation of contaminants (Dehnavi, Ebrahimipour 2022; Laczi et al. 2020; Mazzeo et al. 2014).

As microorganisms can act as biodegraders or biotransformers of pollutants/contaminants (Bhatt et al. 2021a, 2021b), we review the recent academic literature on bioremediation to provide a meta-analysis of microbial pesticide-degrading biosystems. Researchers often collect and summarize evidence from eligible independent studies to answer specific questions, such as the detoxification of chlorpyrifos or 2,4-dichlorophenol from terrestrial ecosystems and industrial wastewater streams, implementation of biosafety for GMOs, and legislation and regulation of environmental restoration and reclamation worldwide (Table 1). However, they do not emphasize applying statistics to metadata to synthesize trends and provide analytical insights into microbial bioremediation of pesticides from agricultural soils, as we focus on in our review. Our meta-analysis generates in-depth knowledge while ensuring more critical and accurate information on a variety of technical-scientific topics

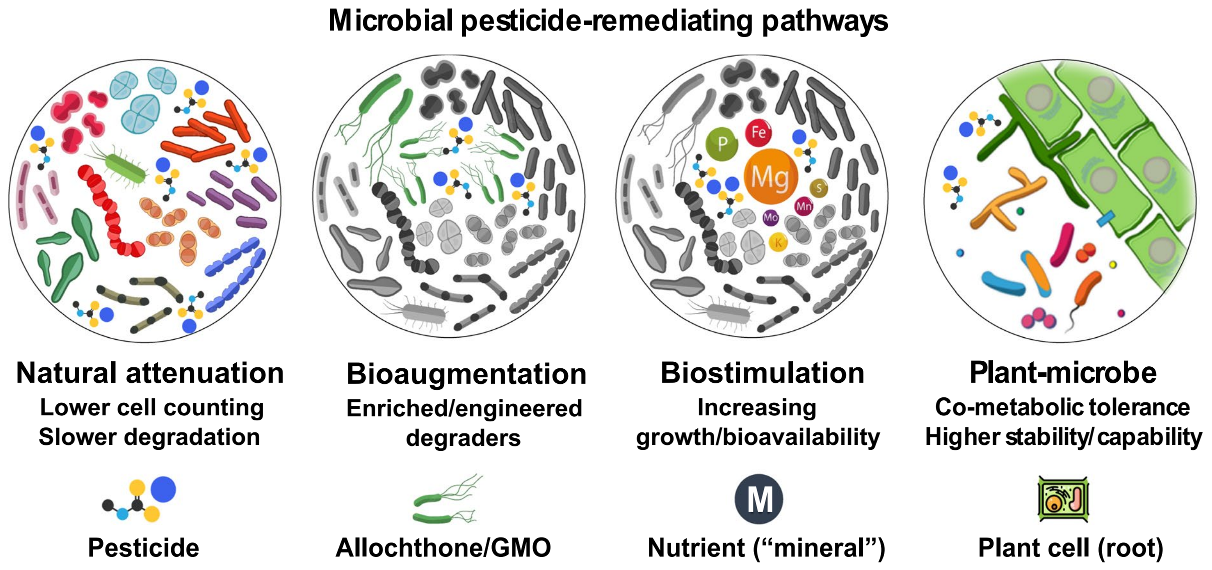

Fig. 1 Natural attenuation, bioaugmentation, biostimulation, and symbiotic plant–microbe interaction as approaches for microbial bioremediation of pesticides from agricultural soils

(such as those listed below) for the community-spanning readership compared to a compilation of investigations or trials within a systematic review, which generally sounds descriptive. We additionally shed light on the quality of research to identify methodological inconsistencies and improve the experimental soundness and addressability in conducting cutting-edge science.

2 General quality of research and biotechnological development in microbial bioremediation

The recent academic literature on microbial systems for bioremediation is heterogeneous. It unfolds into the topic clusters, namely natural attenuation, bioaugmentation and biostimulation, microbial community, and metabolism according to our search on Elsevier's Scopus and Clarivate's Web of Science (Figure S1, Supplementary data). Comparatively, microbial community (47.20%) and metabolism (39.60%) have a greater intellectual interest of the scientific community than bioaugmentation and biostimulation (7.55%), and natural attenuation (5.65%) (Figure S2, Supplementary data). Clearly, research and biotechnological development in microbial bioremediation progress at the molecular level. However, by analyzing the period of our integrative review, we cannot hit up a single perfect study for soundness. The authors do not provide sufficient information approximately any topic cluster, nor describe methods and analyzes clearly. Some studies are not be methodologically rigorous and reproducible by another independent researcher. Instrumental data on pesticide, microorganism, substrate, and molecular analysis are missing across full-text articles. Thus, they can on average provide only 72.55% of the information we might expect from an intricate scientific study for progressing the field of agricultural and environmental research.

Natural attenuation is the most consistent topic cluster. It can meet 86.75% of the total field-level criteria (Table S1, Supplementary material). The opposite is true for the microbial community. It can only meet 66.25% of the total field-level criteria. Bioaugmentation and biostimulation, and metabolism (molecular analysis) are intermediate. They can on average meet 76.25% of the total arbitrary keys to the overall quality of the research and biotechnological development. Precisely, 2 (~66.65%) out of 3 eligible full-text articles on natural attenuation can provide 90.00% of the total information approximately the soil-pesticide-microorganism nexus. Therefore,

Table 1 Pesticide bioremediation review articles in the period of publication of 2018–2021 on Scopus and Web of Science

Subject	Reference
Microbial biodegradation of chlorpyrifos by autochthonous (natural attenuation) and allochthonous microorganisms for bioaugmentation	Varghese et al. (2021)
Microbial biodegradation of OPs and OCs by surfactant-synthesizing microbes in the context of India	Raj et al. (2021)
Microbial biodegradation of insecticides (neonicotinoids) from agroecosystems	Ahmad et al. (2021)
Microbial biodegradation of chlorpyrifos from wastewater streams with an auxiliary photocatalysis	Bose et al. (2021)
Microbial biodegradation of CECs by synthetically and metabolically engineered microbial scavengers to minimize the negative impacts of GMOs on ecosystems (biosafety)	Tran et al. (2021)
Mycoremediation of OCs, such as HCH, endosulfan, DDT, and pentachlorophenol, by ligninolytic fungi and enzymes (e.g., dehalogenases)	Bokade et al. (2021)
Microbial biodegradation and biochemistry of carbamates, such as aldicarb, oxamyl, carbofuran, carbaryl, and methomyl, by high-performance strains	Mishra et al. (2021)
Microbial biodegradation of OPs, OCs, anilinopyrimidines, carbamates, pyrethroids, neonicotinoids, and triazines, by bacteria, fungi, algae, and biocatalytic (enzymatic) systems from agroecosystems	Sarker et al. (2021)
Bioremediation of pesticides from agricultural soils: classification and toxicity of prevalent active compounds to humans, animals, and plants, and elements of the legislation	Raffa and Chiampo (2021)
Biodiversity of bacteria and fungi capable of degrading carbamates, OCs, OPs, and pyrethroids, and their impacts on agriculture and human health	Kumar et al. (2021)
Microbial biodegradation of neonicotinoids, such as acetamiprid, nitenpyram, thiamethoxam, and clothianidin, by bacteria fungi, and yeasts in soils, biobeds, and biomixtures	Anjos et al. (2021)
Microbial biodegradation of dichlorvos (organophosphorus insecticide/acaricide) by bacteria and fungi	Zhang et al. (2021)
Bacterial removal of aromatic compounds, heavy, PAHs, and pesticides (i.e., HCH) from terrestrial and aquatic niches, such as agricultural soil, by *Sphingopyxis* sp.	Sharma et al. (2021)
Microbial removal of pesticides from the environment by consortia	Bhatt et al. (2021a)
Degradation of OPs, such as chlorpyrifos and malathion, by chemical (e.g., oxidation, catalytic hydrolysis, ionizing radiation) and biological (e.g., microbes and substrate-specific enzymes) processes	Kaushal et al. (2021)
Bacterial biodegradation of herbicides: bioaugmentation, consortia, plasmid-containing pesticide-degrading genes, and omics approaches for screening, monitoring, and environmental control	Pileggi et al. (2020)
Phytoremediation and microbial bioremediation of pesticides from soils	Tarla et al. (2020)
Environmental dissipation of OCs by phytoremediation and microbial bioremediation with auxiliary substrate-specific semiconductors or photocatalysts	Ajiboye et al. (2020)
Microbial bioremediation of phenolic compounds, such as 2,4-D and its metabolite 2,4-DCP, from agroecosystems	Magnoli et al. (2020)
Phytoremediation, microbial bioremediation, and integrative plant–microbe remediation of co-contaminants, such as heavy metals (e.g., Cr, Cu, Pb, Zn, As, and Cd) and pesticides (e.g., HCH and carbendazim), from soils	Zhang et al. (2020a, b)
Occurrence, bioremediation, and management of pesticides, such as OPs, OCs, and inorganics, in the context of India	Rajmohan et al. (2020)
Microbial biodegradation and biochemistry of neonicotinoids, such as imidacloprid, nitenpyram, acetamiprid, thiacloprid, thiamethoxam, clothianidin, and dinotefuran	Pang et al. (2020)
Microbial biodegradation of pesticides from natural ecosystems and omics approaches (e.g., genomics, transcriptomics, proteomics, and metabolomics) to study communities qualitatively and quantitatively	Rodríguez et al. (2020)
Catalytic (bio)degradation of OPs, such as chlorpyrifos and malathion, by highly specific and stable hydrolyzing enzymes chemical methods	Thakur et al. (2019)
Bacterial (enzymatic) biodegradation of chlorpyrifos from agricultural and industrial sites and natural aquatic ecosystems	Dar et al. (2019)
Biological removal of endosulfan from ecosystems through natural attenuation, bacterial degradation, co-mycoremediation, and phytoremediation with bioaugmentation or biostimulation	Mudhoo et al. (2019)
Microbial biodegradation of POPs, such as pesticides, PCBs, PAHs, and PPCPs, from wastewater: Bioaugmentation, biostimulation, and GMOs for high-throughput bioremediation	Gaur et al. (2018)

Table 1 (continued)

Subject	Reference
Decontamination of pesticides, such as OPs, OCs, nitrogen-benzenes, phenols, and metallo-organics, in agricultural soils: phytoremediation, microbial biodegradation, and biosorption with bioaugmentation or biostimulation	Sun et al. (2018)
Biological (bacteria and fungi), physical (e.g., clays and zeolites), and chemical (e.g., UV-H_2O_2, UV-ozone, zero-valent iron, and photocatalysis) remediation of pesticides from terrestrial and aquatic systems	Marican et al. (2018)

Contaminants of emerging concern, CECs: aromatic compounds, organic halogens, heavy metals, greenhouse gases, microplastics, and pesticides;

GMOs Genetically modified organisms, *HCH* Hexachlorocyclohexane; *DDT* dichlorodiphenyltrichloroethane; *PHAs* polycyclic aromatic hydrocarbons; *DCP* dichlorophenol; *OPs* organophosphates; *OCs* organochlorines; *POPs* persistent organic compounds; *PCBs* polychlorinated biphenyls; *PPCPs* pharmaceuticals and personal care products

they are missing 10.00% of data on the purity/analytical grade of the active ingredient, and quantification/qualification of the metabolic outcome. The purity/analytical degree does score 0.60, while the metabolic outcome does not score, making it the most limiting key for soundness of research and biotechnological development in natural attenuation.

Half of the contemporary publications particularly in bioaugmentation and biostimulation can describe the experimentation at an appreciable rate of 95.00%, while another half can detail it at up to 70.00%. Therefore, "down-scoring" studies could not be consistent by missing data on purity/analytical grade of pesticide, colony-forming unit, soil's physicochemical properties (i.e., pH, OM, and texture), and quantification/qualification of the metabolic outcome. Purity/analytical degree, CFU, pH, and OM score equally at 0.75, while the score for the texture is 0.50. In contrast, quantification/qualification of metabolic outcome does not score, making it again the most limiting variable for soundness of research and biotechnological development in bioaugmentation and biostimulation.

A single study on the microbial community can address all relevant filters for soundness of research and biotechnological development, according to our methodological rigor. Of the eligible 26 full-text articles, 12 and 9 can meet 80.00% and 60.00% of the total field-level criteria, respectively, while the rate for the other 4 can range from 20.00% to 40.00%. A significant piece of information on the nature of the active ingredient, characterization of autochthonous or allochthonous microorganisms, and specification of soil is missing. Thus, the series of soil does score only 0.20, making it the most limiting variable. The opposite is true for molecular analysis. All studies can completely describe the analysis of the composition and function of potential pesticide-degrading microorganisms through microbiome sequencing, metagenomics, PLFA, or DGGE.

Out of 21 studies on metabolism, only 2 fulfill the checklist. They completely provide information on precursor and metabolite at the level of pesticide level, specification and the origin of biodegraders/biotransformers at the organismal level, pathway, enzyme and gene at the metabolic level, and database and instrumentation (e.g., chromatographer, spectrometer, and thermocycler) at the analytical level. Of the other 19 studies not scoring perfectly, 12 can describe the experimentation at an acceptable rate of 70.00–90.00%, and 7 can provide only 44.45–66.65% of the total information. They are missing most of the data at the metabolic level, so the pathway, enzyme, and gene do score 0.80, 0.50, and 0.30, respectively. Other intermediate limiting variables refer to metabolite/instrumentation and database by scoring 0.75 and 0.50, respectively. Therefore, quantification/qualification of metabolic outcome is missing once again. Clearly, it is the most limiting scholar trade-off in making cutting-edge science in microbial bioremediation. Therefore, the scientific community must correct this if it is to effectively advance the field's prominence in natural attenuation and bioaugmentation, and biostimulation over the coming few years.

3 The state-of-the-art dissipating pesticides out of agricultural soils

3.1 Pesticides

The scientific community is extremely likely to elaborate natural attenuation, bioaugmentation, and biostimulation into actionable solutions to remediate an extensive list of pesticides in soils in croplands and grasslands. Thus, the scholarly set of chemicals is heterogeneous. Conceptually, it consists of fungicides, herbicides, insecticides/acaricides, molluscicides, and nematicides. Researchers focus more on insecticides/acaricides and herbicides (Figure S3, Supplementary data). Thus, they represent 40.00% and 37.75% of the total readable thirty-one records across fifty-five full-text articles, respectively. In contrast, molluscicides and nematicides are less frequent. Together they represent 6.70% of the total. Fungicides comprise the intermediate group representing 15.55% of the total.

Pyrethroids (35.00%), organophosphates (20.00%), neonicotinoids (20.00%), organochlorines (15.00%), carbamates (5.00%), and benzothiadiazoles (5.00%) are the most influential classes of insecticide. Triazinones (45.00%), phenylureas (25.00%), dinitroanilines (20.00%), and imidazolinones (10.00%) are the most influential classes of herbicide, while the group of insecticides distributes equally (25.00%) into the classes, namely benzimidazoles, imidazoles/sulphonamides, phenylamides, and triazoles. Out of 70 observations particularly for insecticides, approximately 15.70% and 10.50% refer to lindane (organochlorine) and nitenpyram (neonicotinoid), respectively. Out of 60 observations particularly for herbicides, atrazine (triazinone) and diuron (phenylurea) are the most frequent active ingredients. They represent approximately 31.25% and 12.50% of the total set, respectively. No predominant molecule exists for fungicides/acaricides, molluscicides, and nematicides.

3.2 Microorganisms

Bacteria are the foundation of microbial remediation of pesticides. Approximately 83.00% of the articles reviewed are extremely likely to rely on powerful bacterial metabolism to elaborate pure and composite microbial systems highly capable of biodegradation or biotransformation. In contrast, research in fungi is still at an early stage of development, and they represent only 17.00% of the total set. The set of bacteria is comprehensive. However, we can unfold it into bacterial phyla, namely Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Gammaproteobacteria, Alphaproteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Kaiserbacteria, Planctomycetes, Sphingobacteria, and Verrumicrobia. Meanwhile, the most active phyla of fungi in contemporary literature are Ascomycota, Basidiomycota, Chytridiomycota, Mucoromycota, Oomycota, and Zygomycota.

Precisely, Proteobacteria represent 52.00% of the total subset, ranking it first out of 13 bacterial phyla attributable to our database. Actinobacteria, Firmicutes, Bacteroidetes, and Gammaproteobacteria rank next in the bibliographic collection's top 5 bacterial phyla by representing 14.00%, 13.00%, 8.00%, and 4.00% of the total, respectively. Complementarily, Ascomycota, and Basidiomycota dominate over the other phyla of fungi. They represent 53.50% and 26.50% of the total set, respectively. Chytridiomycota, Mucoromycota, Oomycota, and Zygomycota are likely to contribute equally (5.00%). Thus, together they represent 20.00% of other phyla of fungi.

Overall, by cross-analyzing the 90 records, particularly for phyla of bacteria (68.45%) and fungi (31.55%), we can identify the most frequent ones, namely Proteobacteria (42.60%), Actinobacteria (11.50%), Firmicutes (10.60%), Ascomycota (8.95%), and Bacteroidetes (6.20%). Basidiomycota, Gammaproteobacteria, and Alphaproteobacteria are intermediate by representing approximately 4.40%, 3.55%, and 1.75% of the total, respectively. In contrast, Acidobacteria, Chlorobacteria, Gemmatimonadetes, Kaiserbacteria, Planctomycetes, Sphingobacteria, Verrumicrobia, Chytridiomycota, Mucoromycota, Oomycota, and Zygomycota are likely to have a lower intellectual interest of the scientific community. They contribute equally (0.95%) and, together, represent only 10.45% of the total subset.

3.3 Insights into microbial remedial approaches for environmental reclamation in agroecosystems

3.3.1 Natural attenuation

Pesticides can negatively impact the society and environment. For instance, lindane is hazardous and can harm beneficial non-target living things, such as pollinators, arthropods, and mammals, by polluting/contaminating the agricultural landscapes and surroundings or via bioaccumulation of the food web (Feng et al. 2020a, 2020b). Thus, official regulatory authorities and the scientific community do advise against spreading lindane or any hazardous organochlorine onto farms to control insects for occupational safety and human health, and welfare (Nwankwegu et al. 2022). However, by reviewing the contemporary literature on micro-scale pesticide-degrading biosystems, we can screen experimental data on residual lindane in agricultural soil from the study by Feng et al. (2020a). It is persistent and can thrive through the ecosystem for a longer time than we might expect on an adequate administration and functional agroecosystem. However, it can be biodegraded (Feng et al. 2020b, 2020c). A community of wild microorganisms capable of enduring a harsher environment can dissipate 72.10% lindane out of an oxidic substrate naturally. In contrast, it can attenuate only 22.15% sulfentrazone, although sulfentrazone concentration in soil was much higher than lindane and may therefore have impacted attenuation to a greater extent (Santos et al. 2019) (Fig. 2). Natural attenuation over lindane or any similar alpha/beta/gamma isomer of HCH will likely succeed more cost-effectively in farming systems, where either maize, rice, soybean, or any crop can preserve functional microorganisms

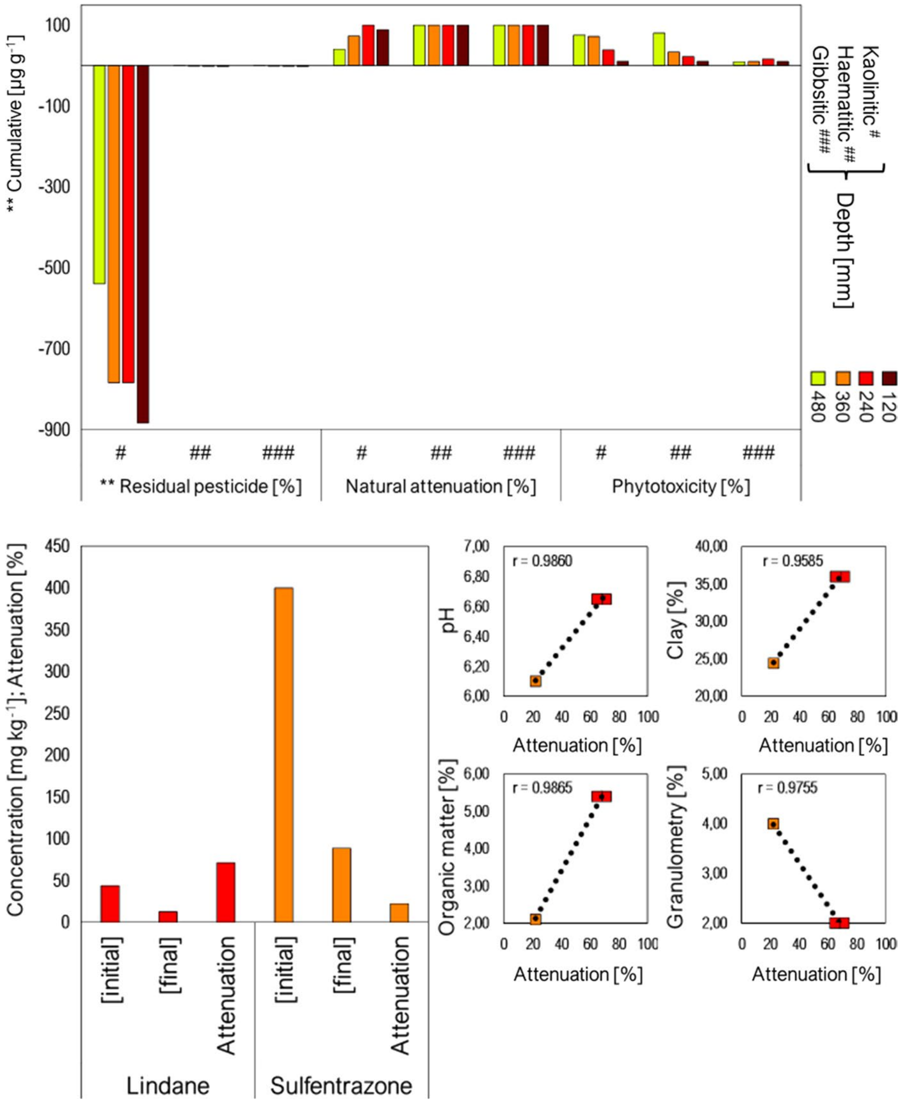

Fig. 2 Microorganisms can dissipate lindane and sulfentrazone from agricultural soils naturally. The bioprocess depends on pH, organic matter, texture, and granulometry. Thus, the more alkaline and texturally heavier the medium the greater the systematic capability of natural attenuation, as it makes it harder for the water-soluble pesticide to leach out. In contrast, the more acidic and texturally lighter the medium, the harder it is for bacteria to thrive, and thus the effectiveness of natural attenuation decreases. It becomes more critical in gibbsitic soil because of a more intensive isoelectric weathering (meta-analysis of 3 studies in the period of 2018–2021)

in the rhizoplane/rhizosphere (Almario et al. 2017; Santos-Medellín et al. 2017; Zhang et al. 2018). How organisms interact to synergistically thrive through a challenging condition is instrumental to natural attenuation to successfully dissipate xenobiotics. The colonization of a plant by a group of microorganisms is species-specific and dynamic. While the plant provides the microbial population a wealth of nutritive exudates (e.g., organic acids) from roots and even protection against stressing abiotic and biotic agents (Marasco et al. 2018; Zhang et al. 2018), the role of bacteria and fungi in biodegrading or biotransforming a toxic compound, both naturally and artificially, can enable the vegetable component to grow healthily and productively (Santos-Medellín et al. 2017). Even if a pollutant is highly toxic and recalcitrant, such as lindane, a stable yet environmentally responsive microbiota can metabolize it into a simpler and less harmful outcome (e.g., H_2O and CO_2) than the precursor for the plant-soil system. However, biodegradation/biotransformation could inadvertently make an outcome of microbial metabolism further toxic, potentially disrupting ecological sustenance (Salam et al. 2017).

Solubility and volatility are determinants of the fate and bioavailability of a pollutant (Table S2, Supplementary material). Water-soluble pesticides can easily leach out of the system to anaerobic sites, where oxygen insufficient can limit the natural attenuation by constraining the supply of energy to the aerobic microbial community (Rissato et al. 2015). Furthermore, run-off can transport a pesticide to bodies of water existing on the surface or underground, making it unavailable rather than accessible to autochthonous/allochthonous biodegraders and biotransformers (Zhu et al. 2019). Our meta-analysis can timely show the benefit of texturally heavier substrate for the bioremediation of highly leachable active ingredients into the significant positive spatial correlation ($r=0.9585$) between the content of clay in kaolinitic/haematitic/gibbsitic soil and attenuation of lindane and sulfentrazone. Texture influences the natural attenuation both positively and negatively. For instance, sand makes it easier for sulfentrazone or any hydrophilic pesticide to flow through the profile than clay (Reis et al. 2017). A “smoother motion” of the molecule, from the top to deeper layers, where oxygen often is deficient, can limit the decomposition in most instances. Most importantly, it can promote further leaching, making it harder for microbiota to access water-soluble molecules (Santos et al. 2019). Therefore, natural attenuation of mobile active ingredients in texturally lighter and more acidic soils is inefficient (Braga 2016; Mansour 2018), it becomes rather obsolete, driving the need to develop and implement high-throughput bioremediation (Randika et al. 2022).

By managing organic matter (significant positive correlation with attenuation; $r=0.9865$) to amend the soil at a pH lower than the pKa of sulfentrazone (~ 6.55), we can effectively control both the ionization and the mobility of the residual herbicide. Particularly in tropical systems, where isoelectric weathering is more intensive, natural attenuation can support remediation and build-up environmental resilience (Santos et al. 2019). Furthermore, increasing pH to near neutrality can enhance the natural attenuation of sulfentrazone by *Pseudomonas* spp. (Mueller et al. 2014). *Bacillus* spp., *Streptomyces* spp., *Sphingomonas* spp., *Clostridium* spp., and *Desulfovibrio* spp. can effectively mineralize organochlorines, even in drylands (Xu et al. 2018a). The role of *Bacillus* spp., *Sphingomonas* spp., and *Streptomyces* spp. in biologically dissipating lindane is more prominent in the rhizosphere of maize, relative to soybean and rice (Feng et al. 2020b). Maize’s exudates (e.g., organic acids and sugars) are more beneficial to aerobic rhizospheric biodegraders (Xu et al. 2018b). Additionally, they can more effectively promote microbial decomposers of endosulfan and atrazine (Ishag et al. 2017). However, they do not necessarily benefit the alpha diversity as is the case with the exudates from rice (Feng et al. 2020a).

The anoxic rhizoplane of rice can improve the activity of *Geobacter* spp., *Clostridium* spp., and *Flavobacterium* spp. as electron-donating agents in the process of oxidation–reduction. Hence, it makes them capable of decomposing lindane under flooding even faster than we might expect in an aerobic condition (Xu et al. 2018a). By analyzing the evidence from the exploratory study by Xu et al. (2018a), we could hypothesize whether a gradual release of radial oxygen by rice is capable of compensating for the deficient energy supply, enabling bacteria to carry out effective attenuation. Another reasonable assumption would be facultative anaerobic metabolism (Feng et al. 2020a), although anaerobiosis rarely outperforms aerobiosis in conditioning bacteria and fungi to the removal of pesticides. Microbes under an anoxic condition are likely to underperform in dechlorination and reduction of either Fe^{3+} or SO_4^{2-} (Zhu et al. 2019). Dechlorinating/reducing bacteria are significant to an effective and resilience-building natural attenuation of organochlorines. However, dechlorination is time-consuming and requires the soil to be fertile. If not, the substrate could be nutritionally insufficient to support microbial cometabolism. Thereby, biostimulation of S or sulfite salt or even bioaugmentation of highly chlorine-degrading strains can be an enabler for boosting the biological process and making the application of natural attenuation in the environmental depollution of lindane feasible. Overall, research and biotechnological development on natural attenuation is at an embryonic stage, yet provides forward knowledge of relevance to progress in the field's prominence in remediating lindane and sulfentrazone.

3.3.2 *Bioaugmentation and biostimulation*

Bioaugmentation is a specialty of bioremediation (Xu et al. 2019; Randika et al. 2022). It is an eco-compatible approach and, conceptually, consists of introducing into the system a group of enhancer microorganisms, whether for removing a pollutant/contaminant relevant to a context (e.g., agriculture) (Pimmata et al. 2013; Das and Dash 2014; Cycoń et al. 2017; Raimondo et al. 2020a, 2020b, Lima et al. 2022).

Thus, bioaugmentation is flexible and can run in situ and ex-situ. Most notably, it can even degrade a highly toxic and recalcitrant compound in an ecosystem where we might not expect to effectively remediate it on natural attenuation (Iwamoto and Nasu 2001). Bioaugmentation and biostimulation can overcome xenobiotics by dissipating them out of the target site with greater cost-effectiveness than possible with mainstream bioremediation (Pimmata et al. 2013; Raimondo et al. 2020a, 2020b; Zhang et al. 2020a, b).

Bioaugmentation provides us with the opportunity to leverage autochthonous, allochthonous, and, most excitingly, genetically modified microorganisms with recombinant DNA technology (Nwankwegu et al. 2022) to develop bioremediation strategies for harsher environments (Varghese et al. 2021). In such circumstances, it can be more difficult to intervene properly in depollution or decontamination due to a more complex plant-soil-molecule nexus (Varghese et al. 2021). Autochthonous bioremediation consists of reintroducing into the original site a group of native microorganisms from another mimetic microcosm containing the target-pesticide for adaptation (Varghese et al. 2021). Allochthonous bioremediation refers to the seamless action of isolating highly pesticide-degrading microorganisms. Introducing them into the target geolocation where we might expect to expedite a remedial approach (Mehta et al. 2021; Varghese et al. 2021). The objective of genetically modified bioaugmentation is to explore the power and expression of functional proteins as catalyzers for more thriving and responsive bioremediation (Varghese et al. 2021).

Bioaugmentation is dependent upon the environment and microbial system, besides the nature, concentration, bioavailability, and recalcitrance of the pollutant. For instance, abiotic and biotic agents can impact bioaugmentation both positively and negatively. And immobilization or any other carrier or matrix to support an additional level of biodegraders or biotransformers can enable remediating an environment, where temperature, pH, fertility, or even antagonistic relationships (e.g., succession and competition) make it harder for breaking down a pollutant/contaminant (Varghese et al. 2021).

Biostimulation is the enrichment of the indigenous microbial population by fundamentally optimizing environmental conditions, such as aeration, nutrient content, pH, and temperature (Andreolli et al. 2015; Aldas-Vargas et al. 2021). Hence, it acts as an opening of enablers for building-up cutting-edge biosystems to remediate sites where the high concentration of a compound hinders the application of mainstream bioremediation or even special bioaugmentation (Aldas-Vargas et al. 2021).

For instance, strategically adding into the ecosystem an amount of limiting nutrients (e.g., P, N, and C) for the microbial growth, can compensate for the deficiency of either electron donors or acceptors, or stimulate metabolic pathways to an effective rather than inhibitory or lagging remedial intervention (Andreolli et al. 2015; Aldas-Vargas et al. 2021). An option of organic enhancer for biostimulation would be livestock manure. It is highly effective to improve soil fertility and, by scaling up, it could support the abatement of persistent pesticides such as chlorpyrifos in the real world (Vischetti et al. 2007; Romyen et al. 2007).

Other potential organic sources include compost and municipal solid waste, while the industrial NPK fertilizers comprise inorganic solutions (Vischetti et al. 2007; Romyen et al. 2007). Synthetic formulations are capable of effective biostimulation by releasing metabolizable inorganics into the microbiota. However, they could be costly to deploy or even toxic to microorganisms. Most importantly, they could limit the environmental footprint of biotechnology by emitting greenhouse gases and promoting eutrophication or any other disruption into agroecosystems and surroundings (Kadian et al. 2012).

Bacteria are the foundation of microbial bioremediation. In contrast, research and biotechnological development in fungi are at an embryonic stage (Fang et al. 2008; Chen et al. 2012). However, by analyzing the evidence from our integrative review, we can tentatively identify fungal strains highly capable of boosting the removal of pesticides from agricultural soils as competently as bacteria. Studies on bioaugmentation and biostimulation focus on pyrethroids, namely cypermethrin, cyhalothrin, and cyfluthrin (Fig. 3).

Secondary yet scholarly relevant active ingredients include atrazine (triazinone), carbofuran (carbamate), carbendazim (benzimidazole), metalaxyl (phenylamide), and acetamiprid (neonicotinoid). For instance, introducing *Trametes versicolor*, *Pigmentiphaga* spp. and *Paenarthrobacter* spp. in the system can enhance the bioremediation of cypermethrin, cyhalothrin, and cyfluthrin. Yet, the natural dissipation of pyrethroids can phenomenally range from 90.00 to 94.50%. Hence, fungal bioaugmentation of an isolate from geolocation with a history of cypermethrin/cyhalothrin/cyfluthrin can on average enhance it slightly by 3.45% (Kaur and Balomajumder 2020). The highly pyrethroid-degrading strain is more responsive to cypermethrin and cyfluthrin by promoting further bioremediation of 6.60% and 4.75%, respectively. However, if the target-molecule is cyhalothrin, fungal bioaugmentation was not expressive, increasing it only by up to 0.55% (Kaur and Balomajumder 2020). An assumption for the molecule-specific ability of the fungal strain to decompose more cyhalothrin than cypermethrin and cyfluthrin is the more structurally

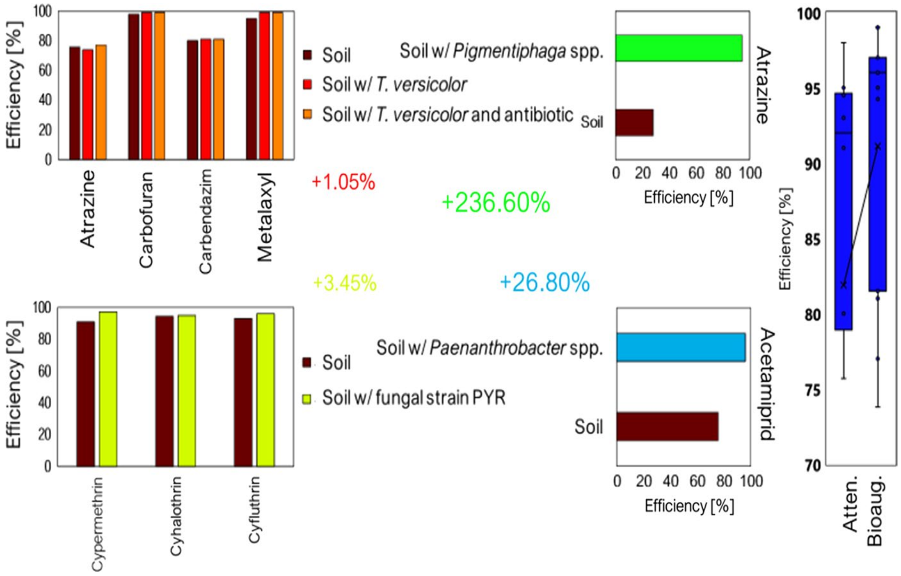

Fig. 3 Bioaugmentation and biostimulation can prove useful to enhance the removal of pesticides from agroecosystems. For instance, *Pigmentiphaga* spp. or *Paenanthrobacter* spp. for bacterial bioaugmentation can notably enhance the bioremediation of atrazine and acetamiprid. *T. versicolor* can decompose carbofuran, carbendazim, and, metalaxyl, yet it is insufficient to extensively improve bioremediation. Even with the addition of an antibiotic to the soil to select for the lignocellulolytic fungus, it does not perform effective bioaugmentation. Thus, further in-depth research and biotechnological development in fungal bioaugmentation are imperative to advance the field (meta-analysis of 4 papers from the period of publication of 2018–2021)

complex functional group of cyano or nitrile existing in the molecule.

The application of *T. versicolor* can increase the dissipation of metalaxyl, carbendazim, and carbofuran by 4.20, 1.25, and 1.05%, respectively. However, effective fungal bioaugmentation cannot be true for atrazine, as it potentially decreases the potential removal by 2.50% (Castro-Gutiérrez et al. 2019). Thus, selective antibiotics could be an option to make the introduction of *T. versicolor* into the process of attenuating atrazine feasible. For instance, oxytetracycline and gentamicin can eliminate the competing autochthonous microorganisms, enabling the *T. versicolor* to thrive in a stressed rhizospheric condition and more effectively decompose the herbicidal active ingredient by decreasing it by 1.70%, relative to the baseline of 75.70% for the natural attenuation. If natural attenuation of any pesticide is sufficient, it rather hinders bioaugmentation. Hence, *T. versicolor* cannot act similar to an effective biological booster, even if administering it with a selective antimicrobial solution to inhibit any antagonistic effect (Castro-Gutiérrez et al. 2019).

While the *T. versicolor* cannot expressively improve the dissipation of atrazine, members of the bacterial genus *Pigmentiphaga* (order Burkholderiales, family Alcaligenaceae) can considerably enhance the remedial bioprocess by 236.60%, relative to the natural attenuation of 28.00% (Chen et al. 2021a). Thereby, we could hypothesize whether *Pigmentiphaga* spp. is capable of more effectively producing atrazine-degrading genes, such as *atzA*, *atzB*, *atzC*, *atzD*, *atzE* and *atzF* (Souza et al. 1995). Another relevant gene family refers to trz. The bacterial degradation of atrazine involves, firstly, the transformation into hydroxyatrazine by *atzA* and *trzN*; secondly, the reduction of primary metabolite into cyanuric acid by *atzB* and *atzC* via the s-triazinic cycle, while the *trzD* or *atzD*, *atzE,* and *atzF* promote further breakdown to complete the metabolic process. Another assumption would be the inherent ability of the bacterial genus to benefit from available nutrients in the system to perform cometabolism (Yang et al. 2021). Ammoniacal nitrogen and CO_2 are sources of energy to existing microbiota and could support the distinct potential of bioaugmentation/biodegradation by *Pigmentiphaga* spp. The bacterial genus can outperform the ligninolytic fungus *T. versicolor* in the potential for atrazine-specific bioaugmentation (Chen et al. 2021a).

Outside of our period of meta-analysis, *Arthrobacter* spp. (Zhao et al. 2017), *Shewanella* spp. (Ye et al. 2000), *Rhodococcus* spp. (Fazlurrahman et al. 2009), *Pseudomonas* spp. (Monard et al. 2008), *Agrobacterium* spp. (Devers et al. 2007), *Chelatobacter* spp., *Aminobacter* spp., and *Stenotrophomonas* spp. (Rousseaux et al. 2001), and *Nocardioides* spp. (Topp et al. 2000) are also capable of expressing the genes atzABCDEF and trzDN to mineralize atrazine into NH_4^+ and CO_2 as the simplest metabolite possible from the dechlorination-degradation pathway (Souza et al. 1995). Overall, the research and biotechnological development in bacterial bioaugmentation focus on *Arthrobacter* spp., *Pseudomonas* spp., *Rhodococcus* spp., and *Stenotrophomonas* spp. However, by analyzing evidence from our integrative review, we could hypothesize whether both *Pigmentiphaga* spp. and *Paenarthrobacter* spp. are capable of replacing the in-situ ozonolysis or electrochemical cell (Cruz-Alcalde et al. 2017; Chen et al. 2018) in the process of dissipating acetamiprid or any similar insecticide. If our perspective is right, we will be able to elaborate on eco-compatible solutions toward leveling up the cost-effectiveness of environmental reclamation (Chen et al. 2021b).

A review by Nwankwegu et al. (2022) elaborates on the benefits and trade-offs of bioremediation and brings valuable information on the complexity of achieving a complete remedial outcome from cometabolism. Bioremediation requires multiple arrays of microbial enzymes and metabolic mechanisms to function properly. Thus, deploying an assemblage of high-density cellular frameworks is instrumental to the approach to succeed over xenobiotics even dealing with recalcitrant pollutants/contaminants. For instance, *Mycobacterium vaccae* can oxidize cyclohexane into cyclohexanone, however, without gaining any energy or carbon from the metabolic transformation. The outcome of microbial metabolism could either be innocuous or deleterious to the functioning of the primary metabolic producer, however, it could act as a vital source of energy and carbon for other microorganisms in the complex ecosystem (Mrozik and Piotrowska-Seget 2010). If microbial relationships are synergistic then bioremediation promotes dissipation continuously, without any critical delay or inhibition (Zafra et al. 2017).

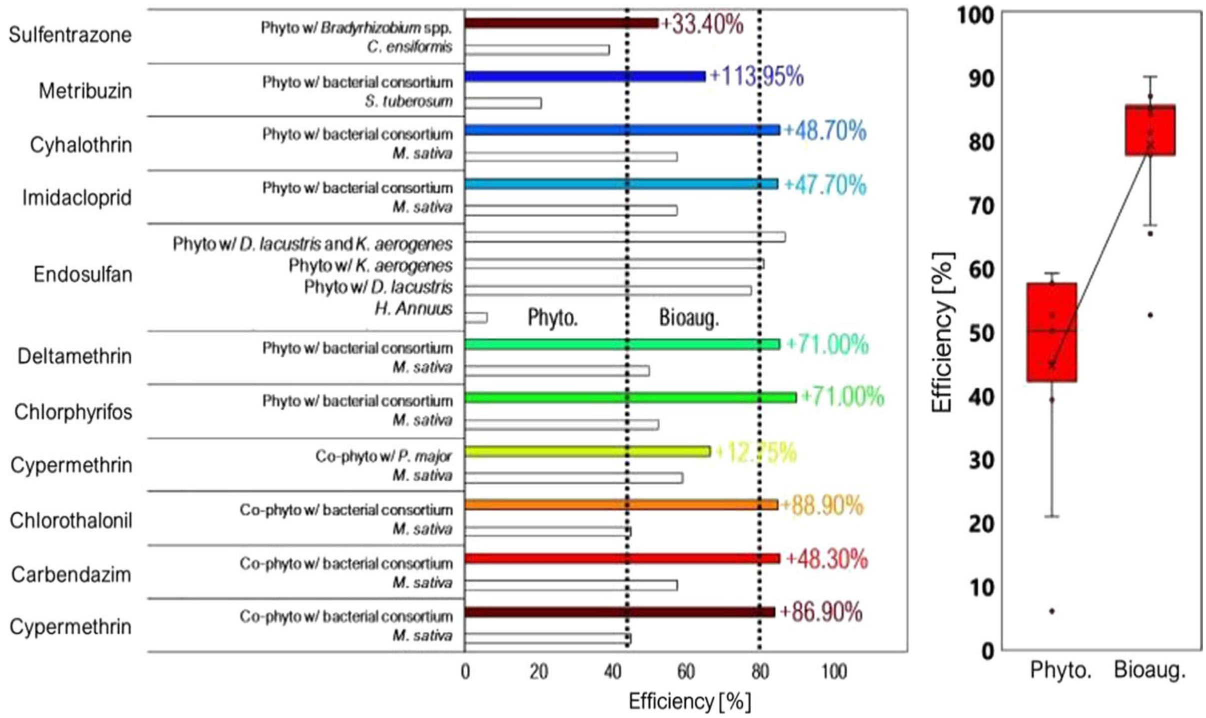

Fig. 4 Bacterial bioaugmentation can enhance the phytoextraction of a wide range of pesticides. For instance, *D. lacustris* and *K. aerogenes* are synergistic with each other, making up an exceptional endosymbiotic/ectosymbiotic consortium to *H. annus* to remediate endosulfan. However, the performance is likely unrealistic and requires further in-depth research for cross-validation of the potential phytomicrobioremediation (meta-analysis of 4 papers from the period of publication of 2018–2021)

Furthermore, our meta-review can timely capture the ability of bioaugmentation to make the rhizospheric microbial population denser than possible with natural attenuation. It can significantly improve the ability of crops to either stabilize or extract pesticides out of the system (Fig. 4).

Successful examples of introducing highly pesticide-degrading microbes into phytoremediation systems are available in contemporary literature. A bacterial pool can on average increase the phytoremediation of pesticides by 78.10%. *Bradyrhizobium* spp. and *Canavalia ensiformis* form an excellent group for further removing 33.40% sulfentrazone (Mielke et al. 2020). *Delftia lacustris* and *Klebsiella aerogenes* and *Helianthus annuus* make up other effective solutions of integrative bioremediation to endosulfan by removing it at 77.65–86.85%. The phytoremediation can remove only 5.00%, supporting the exceptional potential of both bacteria to boost the bioprocess (Rani et al. 2019). The most effective strategy possible to use metabolically active cells of *D. lacustris* and *K. aerogenes* in the phytoremediation of endosulfan by *H. annus* is by mixing them into an endosymbiotic/ectosymbiotic consortium. Furthermore, numerous functional bacterial consortia exist for the "phytomicrobioremediation" of metribuzin by *Solanum tuberosum* and imidacloprid, deltamethrin, cyhalothrin, chlorpyrifos, chlorothalonil, carbendazim and cypermethrin by *Medicago sativa* (Li et al. 2020). They are capable of doubling the effectiveness of the integrative biological process.

Exploratory studies on "phytomicrobioremediation" often are not available in contemporary literature. However, we can identify consistent trials elucidating the beneficial traits of bacterial degraders to the phytoextraction of pesticides. Microbes can benefit from the monocarboxylic/dicarboxylic/tricarboxylic acids and phenolic compounds from the plant to grow and develop as healthy as possible. However, not all exudates are chemoattractants. For instance,

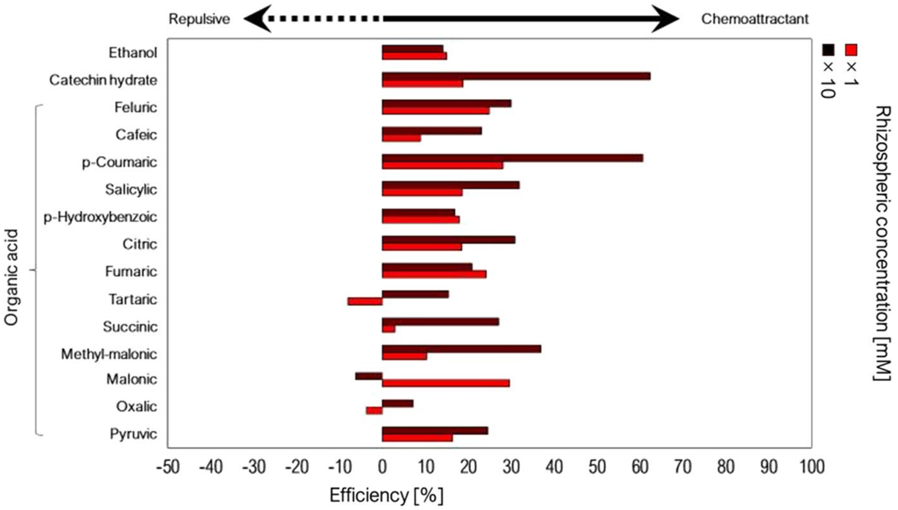

Fig. 5 Synergistic and antagonistic exudates to microorganisms in the process of phytoextraction with bioaugmentation. While the microbial population degrades a highly toxic pesticide into a simpler and less harmful metabolic outcome for the plant, the plant component provides it with a wealth of beneficial substances for microbial growth and development. Phytogenic carboxylic and phenolic compounds could be potential solutions to replace synthetic additives (e.g., fertilizers and surfactants) in biostimulation, potentially making it environmentally safer and less disruptive to ecological health and agronomic functions (meta-analysis of 4 papers from the period of publication of 2018–2021)

malonic, tartaric, and citric acids can downregulate cell transport, so an effective rhizostabilization is not likely. In contrast, pyruvic, succinic, fumaric, citric, hydroxybenzoic, salicylic, caffeic, and ferulic acids as well as catechin hydrate are beneficial biocompounds. They can upregulate the composition and function of bacteria in the rhizosphere, potentially offering the possibility of organic salts for biostimulation (Fig. 5).

Therefore, the scientific community must take every effort to amplify our knowledge on how to introduce plants capable of secreting synergistic carboxylic and phenolic groups into the system to promote pesticide-decomposing microorganisms, or culture them for biosynthetization, extraction, and formulation of phytogenic organic salts to biostimulation. Organic salts could replace sulfite/sulfur in the process of biostimulation. It will likely level-up the cost-effectiveness of purposely modifying the environment, where existing microorganisms are capable of bioremediation.

Overall, bioaugmentation and biostimulation can prove useful to enhance dissipation of insecticides, herbicides, and fungicides out of agricultural soils. However, by analyzing evidence from our integrative review and other contemporary comprehensive studies on the benefits and trade-offs of bioremediation, we can recognize that their deployment is expensive while another "burning issue" refers to ecological disturbance. Introducing microorganisms into an environment can optimize detoxification. However, autochthonous, allochthonous, and genetically modified bioaugmentation as well as the outcome of the remedial intervention could inadvertently deteriorate the natural ecosystem by decreasing genetic diversity via antagonistic relationships (e.g., competition, predation, and succession) and ecotoxicity (Hassan et al. 2016; Randika et al. 2022). Chemical formulations for biostimulation often consist of N, P, K, and, eventually, S. Such inorganic stimulants can promote a complete cometabolic biodegradation or biotransformation. However, they could be more harmful than the primary pollutant or contaminant and, hence, do not necessarily sustain the ecosystem in self-recovering from disturbance for potential agronomic functions. The poor soil condition will likely become less tractable rather than reversible where an artificial additive (e.g., biosurfactant) (Fanaei et al. 2020) added to expedite an actionable remedial approach is capable of either severely unbalancing soil's physicochemical properties (e.g., pH, cation exchange, conductivity, etc.) or constraining microbial consortia (Nwankwegu et al. 2022). Furthermore, nitrogenous and phosphorous agents could easily escape from the land via leaching and run-off directly into aquatic ecosystems, promoting a dramatic exacerbation of eutrophication and triggering an episodic "harmful algal bloom". Thus, it could make the application of biostimulation in environmental reclamation for agroecosystems rather unsuitable and unsustainable.

Therefore, the scientific community and governance must be aware of the commitment, cooperation, and coordination that is needed if they are to effectively oversee and regulate the development and implementation of bioaugmentation and biostimulation with efficiency, safety, prudence, and transparency for every stakeholder in the agriculture and environmental sector. However, by analyzing the evidence from our systematic analysis, we cannot access sufficient information on pesticide (e.g., analytical purity, log Kow and secondary metabolite), soil (e.g., pH, OM, and temperature), microbiota (e.g., structuration, diversification, and function), and microbial metabolism (e.g., pathway, enzyme, and pesticide-degrading gene), nor descriptions of methods and analyzes. And because a piece of instrumental data is missing from the recent academic collection on microbial bioremediation, authors need to improve methodological rigor to progress in research and biotechnological development in natural attenuation, bioaugmentation, and biostimulation over the coming few years. If not, our community-spanning knowledge will be limited to elaborate protocols and standards and provide an intricate reference for properly exploring the metabolism of microorganisms, whether for dissipating pesticides out of agroecosystems, while conserving/preserving the natural and aesthetic beauty, and functionality of the environment. Particularly for genetically modified bioremediation, further in-depth research must focus on developing and disseminating ethical open-source recombinant DNA technology to improve the delivery of microbial engineering and public acceptance, while leveling-up the ecological and financial cost-effectiveness. Molecular bioengineering and an extensive range of pesticide-degrading microbes will likely reshape our critical thinking and enable safer sustainable management of agroecosystems to address an increasing pressure to promote and perpetuate an environmentally healthier planet.

3.4 Microbial community

3.4.1 Molecular analytical methods

Approximately 40.75% of 25 scientific studies reporting on the impact of pesticides on microbial community focus on herbicides, ranking them first out of 4 chemical groups. Insecticides (33.35%) and fungicides (22.20%) rank next, while molluscicides (3.70%) are the less frequent (Figure S4, Supplementary material). The active ingredients, namely atrazine (29.60%) and metolachlor (29.60%), have the greatest intellectual interest of the scientific community. Together they represent well over half of the total field-level citation. The microbiome is a key to biodegradation and biotransformation of any pollutant/contaminant in the environment. As it is a driver of bioremediation, authors are extremely likely (72.00%) to benefit from high-throughput sequencing of 16S rRNA to describe microbial communities and understand how pesticides could impact them. Secondary yet bibliometrically expressive approaches include shot-gun metagenomics (12.00%) and PLFA (12.00%). In contrast, DGGE is less frequent by representing only 4.00% of the total field-level citation.

3.4.2 The impact of pesticides on beta-diversity

Pesticides can impact the microbial community, both negatively and positively, or not affect them. For instance, no significant effect exists for either metaldehyde (Castro-Gutiérrez et al. 2019) or prosulfocarb (Barba et al. 2019) on native microbiota based on rRNA-sequencing and PLFA to profile the beta-diversity. Even when lignocellulose is available as metabolizable energy in the ecosystem, the behavior and ecotoxicity of metaldehyde and microbial cometabolism are unchanged (Castro-Gutiérrez et al. 2019). Furthermore, no scientific evidence exists for the impact of carbofuran, carbendazim, and metalaxyl on native microbiota based on DGGE, and T-RFLP data from the study by Castro-Gutiérrez et al. (2019). In contrast, atrazine (Aguiar et al. 2020; Bhardwaj et al. 2020; Zhai et al. 2020), deltamethrin (Bragança et al. 2019), iprodione (Katsoula et al. 2020), metolachlor (Sun et al. 2020), oxamyl (Gallego et al. 2019), and sulfometuron-methyl (Obregón-Álvarez et al. 2021) are likely to significantly influence the diversity and functioning of microbial communities (Figure S5, Supplementary material).

A substantial fraction of studies does not provide trustworthy information on the impact of pesticides on microorganisms. However, we can tentatively identify in a few trials, a functional relationship (e.g., depletion) between the degree of degradation and shifts in community structure of communities co-existing with diuron (Liu et al. 2019), metalaxyl (You et al. 2021), metolachlor (Li et al. 2019), metribuzin (Wahla et al. 2020), organochlorines (Ali et al. 2019), or sulfamethoxazole (Liang et al. 2021). Proteobacteria is likely the most responsive phylum to an extensive range of active ingredients, namely atrazine, carbendazim, chlorothalonil, diuron, imazethapyr, imidacloprid, iprodione, metalaxyl, metaldehyde, metolachlor, organochlorines, oxamyl, sulfamethoxazole, and sulfometuron-methyl. Actinobacteria rank next and are associated with biodegradation or application of atrazine, azoxystrobin, diuron, iprodione, metaldehyde, metolachlor, organochlorines, and oxamyl. Other relevant phyla are Acidobacteria and Firmicutes. Both are responsive to atrazine, diuron, metaldehyde, metolachlor, organochlorines, oxamyl, sulfamethoxazole, and sulfometuron-methyl, similar to Proteobacteria.

Proteobacteria consist of a wide range of microorganisms. They are metabolically complex and, hence, highly capable of biodegrading/biotransforming an environmental pollutant/contaminant (Xiong et al. 2021). At a finer taxonomic level, Burkholderiales (Betaproteobacteria), and Hyphomicrobiaceae (Alphaproteobacteria) are likely to degrade atrazine, diuron, imazethapyr, iprodione, metolachlor, sulfometuron-methyl more effectively than Nitrosomonadales (Betaproteobacteria), Pseudomonadales (Gammaproteobacteria), and Xanthomonadales (Gammaproteobacteria). Complementarily, notable genera include *Arthrobacter* spp. for atrazine, azoxystrobin, diuron, and *Pseudomonas* spp. for carbendazim, imidacloprid, sulfamethoxazole, sulfometuron-methyl. In contrast, the impact of pesticides on fungal communities often is not available from the recent bibliographic collection on microbial bioremediation. However, we can capture how the fungicides, namely carbendazim (systemic) and chlorothalonil (nonsystemic), could negatively influence the orders of Ascomycota, namely Eurotiales and Hypocreales. Other active ingredients capable of modulating Ascomycota

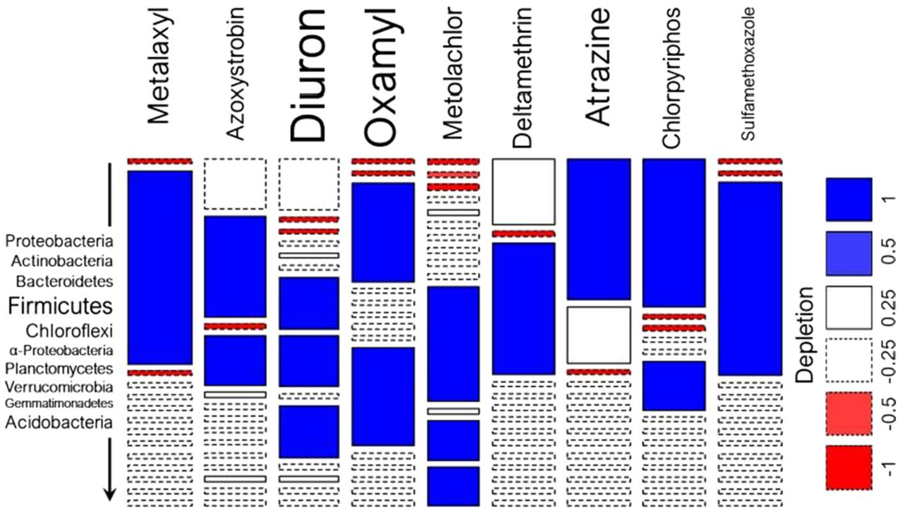

Fig. 6 Microbial sensitivity to pesticides. Bacteria and fungi prove useful to dissipate herbicides, fungicides, insecticides, nematicides, and molluscicides out of agricultural soils. However, if the target molecule is more toxic than biodegradable, it can disturb the microbiota, and thus both diversity (alpha and beta) and potential bioremediation decrease. The microbial response is pesticide-dependent. For instance, Proteobacteria respond negatively to metalaxyl, oxamyl, metolachlor, and sulfamethoxazole, so the key in the diagram is blue. In contrast, the phylum can resist environmental pressure by atrazine and chlorpyrifos, so the key in the diagram is red. Meanwhile, no significant effect exists for the azoxystrobin, diuron, and deltamethrin, so the key in the diagram is white (meta-analysis on 25 papers from the period of publication of 2018–2021)

include iprodione and metolachlor, while chlorothalonil can impact the Basidiomycota. Additionally, Chytridiomycota, Mucoromycota, and Zygomycota can perform functional biodegradation of metolachlor, allowing their growth in an ecotoxicological microcosm.

Microbial response to pesticides is dynamic (Fig. 6). While a portion of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria can resist a wide range of active ingredients, another portion is not capable of surviving any stress caused by the original pollutant/contaminant or the metabolic outcome of biodegradation/biotransformation. For instance, Sphingomonadales (Alphaproteobacteria) could not resist azoxystrobin (Han et al. 2020), metolachlor (Sun et al. 2020), and metribuzin (Wahla et al. 2020). Likewise, Bacillales (Firmicutes) can prove sensitive to atrazine (Bhardwaj et al. 2020), azoxystrobin (Han et al. 2020), and diuron (Liu et al. 2019). Azoxystrobin, metolachlor, and diuron can also be harmful to Xanthomonadales (Liu et al. 2019; Han et al. 2020; Wahla et al. 2020), Burkholderiales (Betaproteobacteria) (Liu et al. 2019; Xu et al. 2019), Pseudomonadales (Gammaproteobacteria) (Liu et al. 2019; Bhardwaj et al. 2020), and Hyphomicrobiales (Alphaproteobacteria) (Liu et al. 2019; Xu et al. 2019; Bhardwaj et al. 2020). Particularly the genus *Altererythrobacter* spp. can respond negatively to azoxystrobin (Han et al. 2020) and positively to iprodione (Katsoula et al. 2020). *Bacillus* spp. is also capable of surviving atrazine (Xu et al. 2019; Bhardwaj et al. 2020) and metolachlor (Sun et al. 2020). However, it is highly sensitive to azoxystrobin (Han et al. 2020) and diuron (Liu et al. 2019), supporting the ability of bacteria to adapt the stress, and pesticide-dependent metabolism. Furthermore, studies by Aguiar et al. (2020), Liu et al. (2019), Xu et al. (2019), Bhardwaj et al. (2020), Zhai et al. (2020), and Chen et al. (2021a) provide substantial references of the distinct ability of *Bradyrhizobium* spp., *Halomonas* spp., *Lysobacter* spp., and *Pseudomonas* spp. to positively respond to atrazine, carbendazim, imidacloprid, sulfamethoxazole, and sulfometuron-methyl during natural attenuation and bioaugmentation. Introducing citrate into the ecosystem can stimulate the cometabolism of atrazine by *Pseudomonas* spp., with the opposite true for mustard seed meal and molasses. Both can inhibit the bacterial genus in enhancing the dissipation of atrazine by decreasing abundance (Bhardwaj et al. 2020). Therefore, biostimulation does not necessarily benefit bioremediation. An additive could be more toxic than the target-pesticide to the microbial community and, therefore, capable of reducing ecological sustenance (Randika et al. 2022; Nwankwegu et al. 2022).

3.4.3 *The impact of pesticides on alpha-diversity*

The impact of pesticides on beta-diversity is significant. However, they do not change or rarely change the alpha-diversity or rather the structure of an ecological community relative to the number of taxonomic groups in a site at a local scale. For instance, metribuzin (Wahla et al. 2020), oxamyl (Gallego et al. 2019), and iprodione (Katsoula et al. 2020) are not likely to influence the Shannon–Wiener and Simpson's metrics. They do not impact the active (RNA) and total (DNA) bacterial communities, nor condition the determination of p-nitrophenol via T-RFLP. In contrast, metolachlor can increase the alpha-diversity in a circuit, where external influence on the microbial community, such as competition of exoeletrogenic bacteria for organic carbon, does not exist. Therefore, the herbicide could be a source of vital energy for microbial metabolism (Li et al. 2020). The opposite is true for both sulfometuron-methyl (Obregón-Álvarez et al. 2021) and atrazine (Liu et al. 2019). It can selectively decrease the alpha-diversity of bacterial consortia incapable of resisting xenobiotics. Furthermore, Zhao et al. (2020) provide a reference of the synergistic iprodione towards fungal communities in the phyllosphere of a pepper plant. The herbicide can increase the Simpson's diversity and, most notably, the archaeal Pielou's evenness. However, information on the impact of pesticides on fungal alpha-diversity is neither consistent nor convincing to deploy a remedial strategy in the real world. Particularly for mycorrhizae and saprotrophic taxa, we could not identify a single reference. Thus, further in-depth research must focus on developing such functional groups to strengthen fungal bioremediation.

3.4.4 *Synergism and antagonism between pesticide-degrading microorganisms*

A pesticide can impact a wide range of microorganisms. Likewise, numerous microorganisms can simultaneously degrade a pesticide. Thereby delving

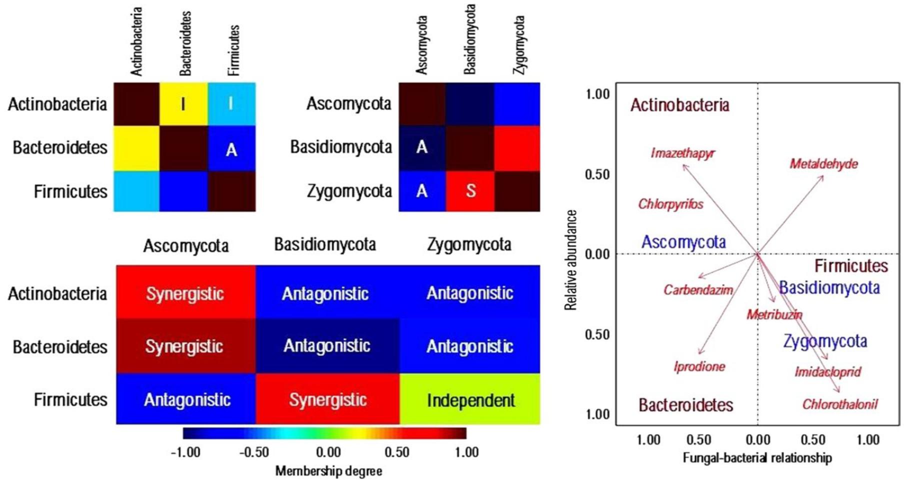

Fig. 7 The canonical structure of synergistic and antagonistic between pesticide-degrading microorganisms. Microbiota consists of a wide range of bacteria, fungi, and other microscopic living organisms. The microcosm is dynamic. An agent could be synergistic or antagonistic towards another agent and a pollutant/contaminant or even an active stimulant co-existing in the ecosystem could impact the microbial relationship both positively and negatively. For instance, Actinobacteria are synergistic towards Ascomycota in the co-biodegradation of imazethapyr and chlorpyrifos, making them a potential merger for elaborating a remedial consortium. In contrast, they are antagonists towards Basidiomycota and Zygomycota in the co-biodegradation of metribuzin, imidacloprid, and chlorothalonil. Therefore, the outcome of fungal metabolism of either chlorothalonil or imidacloprid could be a toxic rather than a vital source of energy to Actinobacteria. In some cases, no relationship exists between microorganisms, so they are independent such as Firmicutes and Zygomycota, making them not an option for the consortium (meta-analysis of 25 papers from the period of publication of 2018–2021)

deeper into the studies by Nantes et al. (2022), Castro-Gutiérrez et al. (2019), Katsoula et al. (2020), Li et al. (2020), and Wahla et al. (2020), we can decide whether microbe-microbe, pesticide-pesticide, and microbe-pesticide relationships are synergistic or antagonistic. Most notably, we can predict them on a canonical diagram (Fig. 7) and explore the outcome of multivariate analysis to bring consortia into implementation for bioremediation in an environment where more than one active ingredient does co-exist.

For instance, Actinobacteria, Bacteroidetes, and Ascomycota could be potentially added together for dissipating imazethapyr, chlorpyrifos, carbendazim, and iprodione cost-effectively. A microbial biosystem consisting of Firmicutes and Basidiomycota would make-up another eco-compatible solution to remediate metribuzin, imidacloprid, and chlorothalonil. A massive portion of members of pesticide-degrading consortia is of similar bacterial lineages. For instance, *Mycobacterium* spp. (Micrococcales) can dominate over other taxa composing an acetamiprid-degrading consortium (Li et al. 2019). It reduces the pesticide into a simpler and less harmful metabolite, proving synergistic towards increasing the relative abundance of *Arthrobacter* spp. (Xu et al. 2019; Han et al. 2020; Zhai et al. 2020), *Pseudoarthorbacter* spp. (Liu et al. 2019; Chen et al. 2021a, b), *Clavibacter* spp. (Aguiar et al. 2020), *Agromyces* spp. (Chen et al. 2021a, b) and *Pseudogymnoascus* spp. (Li et al. 2019). The following most abundant taxa in the acetamiprid-degrading consortium are *Afipia* spp. (Hyphomicrobiales) (Liu et al. 2019; Aguiar et al. 2020) and *Acinetobacter* spp. (Pseudomonadales) (Liu et al. 2019; You et al. 2021; Liang et al. 2020; Obregón Alvarez et al. 2021; Zhai et al. 2020). *Arthrobacter* spp. can dominate over *Methylophilus* spp. (Nitrosomonadales) and *Hyphomicrobium* spp. in atrazine-degrading consortia (Liu et al. 2019; Cao et al. 2021). A hierarchy also exists for other consortia, where *Pseudomonas* spp., *Bacillus* spp., and *Halobacillus* spp. are likely prevalent relative to other taxa capable of dissipating imidacloprid, carbendazim, lambda-cyhalothrin, beta-cypermethrin, chlorpyrifos, chlorothalonil (Xu et al. 2019; Wahla et al. 2020; Esikova et al. 2021).

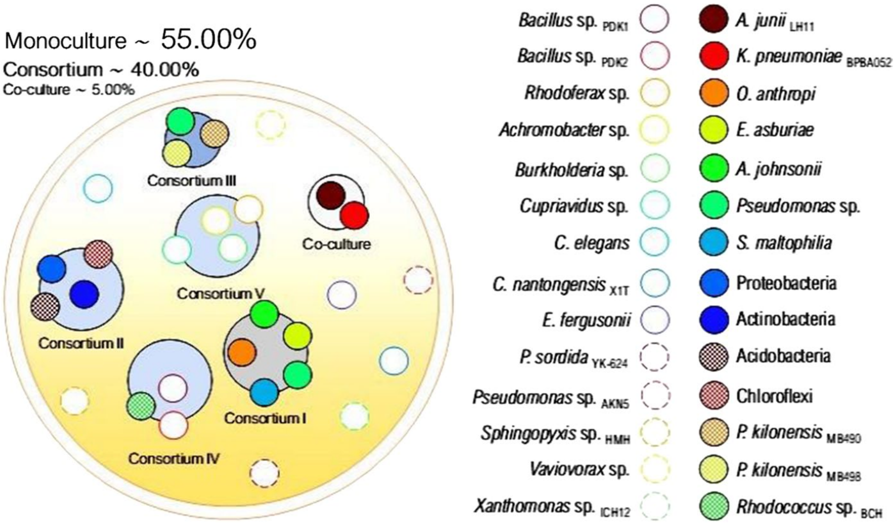

Fig. 8 Microbial biosystems capable of bioremediation of pesticides are complex and, fundamentally, unfold into modalities, namely monoculture, consortium, and co-culture (meta-analysis on 21 papers from the period of publication of 2018–2021)

3.5 Metabolism of pesticide-degrading microorganisms: pathways, enzymes, and genes

Studies on microbial metabolism of pesticides focus more on herbicides (52.40%) and insecticides (38.10%). In contrast, fungicides (4.75%) and nematicides (4.75%) have less interest by the scientific community (Figure S6, Supplementary material). While atrazine (25.00%) and diuron (16.50%) are the most scholarly relevant herbicides, nitenpyram (25.00%) is the fungicide having the greatest intellectual interest in the scientific community.

Approximately 30 microbial agents are capable of degrading pesticides, irrespective of the chemical group. Thus, researchers are extremely likely to bring them into functional monocultures (55.00%) and consortia (40.00%). In contrast, co-cultures (e.g., A. *junii* LH11 and *K. pneumoniae* BPBA052) are less frequent, representing only 5.00% of the total subset (Fig. 8). *A. johnsonii*, *E. asburiae*, *Pseudomonas* spp., *S. maltophilia*, and *O. anthropi* make-up the quantitatively most complex pesticide-degrading consortium. The consortium of *Rhodoferax* sp., *Achromobacter* sp., and *Burkholderia* sp., ranks next. In contrast, *P. kilonensis* (strains MB490 and MB498) and *Pseudomonas* sp., *Bacillus* sp. (strains PDK1 and PDK2), and *Rhodococcus* sp. are the quantitatively fewer complex consortia. Co-occurrence of *Cupriavidus* spp. and members of Proteobacteria, Chloroflexi, Actinobacteria, and Acidobacteria can support the complexity and how bacteria can interact to degrade a pesticide and thrive through a stressful ecosystem.

Microorganisms can trigger a wide range of enzymes as catalyzers of biodegradation/biotransformation of pesticides, and the most frequent in the literature are monooxygenases. They represent 18.50% of the total subset. Flavin-reductases, hydrolases, amidohydrolases, and dioxygenases rank next. They equally contribute (14.80%) to the scholarly set of enzymatic precursors of pesticide-degrading metabolic pathways. In contrast, ferredoxins, laccases, dehalogenases, and dehydrogenases are the less frequent functional proteins. Together they represent only 14.80% of the total subset (Figure S7, Supplementary material). A study by Zhao et al. (2020) provides a reference of the enzymatic powerfulness of *Cunninghamella elegans* (strain ATCC36112) towards biodegradation of diazinon (O, O-diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate). The fungus can degrade the organophosphate pesticide at appreciable effectiveness of approximately 90.00%, and probable secondary metabolites include diethyl (2-isopropyl-6-methylpyrimidin-4-yl) phosphate (diazoxon) and 2-isopropyl-6-methyl-4-pyrimidinol (pyrimidinol). Pyrimidinol is likely less toxic than the precursor, with the opposite true for diazoxon (Shemer and Linden 2006; Malakootian et al. 2020). Therefore, the incomplete metabolism of diazinon is hazardous and could negatively impact the ecological sustenance and make it harder for the agroecosystem to recover from any functional disorder than possible with the original pesticide. By analyzing the metabolic profile of *C. elegans* (strain ATCC36112), we can hypothesize whether microsomal enzymes are capable of modulating the biodegradation of diazinon. The pathway may involve, firstly, the oxidation of the pesticide via desulfurization; secondly, the hydrolysis of the intermediate compound via hydroxylation (Zhu et al. 2017a). Monooxygenases or heme-containing enzymes of the cytochrome P450 complex could regulate the catabolism (Liu et al. 2006; Zhu et al. 2017b). *Sphingopyxis* sp. (strain HMH) can trigger flavin-nitroreductases to catalyze the oxidative hydroxylation of butralin (N-sec-butyl-4-tert-butyl-2,6-dinitroaniline). The nitroreduction of the pesticide generates N-(sec-butyl)-4-(tert-butyl)-6-nitrobenzene-1,2-diamine and a further formation of 5-(tert-butyl) -3-nitrobenzene-1,2-diamine and butanone are completely possible via N-dealkylation of the amine group to N-(sec-butyl) (Ghatge et al. 2021).

A pure bacterial culture of *Cupriavidus nantongensis* (strain X1T) can degrade chlorpyrifos (O, O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) more effectively than a native microbial community (Fang et al. 2019). It can halve the concentration (100 mg L^{-1}) of the insecticide every 6 h, generating 3,5,6-trichloro-2-pyridinol (TCP) and 3,6-dihydroxypyridine-2,5-dione (DHP) as the major metabolic outcomes (Fig. 9). Both are highly toxic and water-soluble. Hence, they could escape from the agroecosystem directly into the environment, potentially polluting more severely than we might expect on the leaching of the parent molecule. However, the strain X1T can express the genes, namely tcpA and fre, making it capable of completely degrading the TPC at 20 mg L^{-1}, with a half-life of 8 h. Monooxygenases and flavin-reductases are likely the precursors of the bioprocess. The biodegradation of chlorpyrifos by *C. nantongensis* (strain X1T) involves, firstly, the formation of ethanol and diethyl-chlorpyrifos; secondly; the hydrolyzation of either ethanol or diethyl-chlorpyrifos into TCP or diethyl thiophosphate (DETP) by a catabolic organophosphorus hydrolase; thirdly, the desulfurization of TCP and further dealkylation/dechlorination until the oxidation levels off and yields CO_2 and H_2O as the simplest metabolites. Another highly chlorpyrifos-degrading microorganism is *Paracoccus* sp. (Fang et al. 2019).

Although bacteria predominate the literature reporting on the catabolism of pesticides, we can review in the study by Wang et al. (2019) the potential biodegradation of nitenpyram ((E)-N-(6-chloro-3-pyridylmethyl)-N-ethyl-N'-methyl-2-nitro vinylidene diamine) by *Phanerochaete sordida* (strain YK-624). The fungus can produce powerful ligninolytic enzymes, making it highly capable of converting the neonicotinoid molecule into (E)-N-((6-chloropyridin-3-yl) methyl)-N-ethyl-N′-hydroxy-acetamide (CPMHA). Heme-containing enzymes of the P450 complex modulate the reduction of the nitro group and the further deamination. No scientific evidence exists for genetic mutations

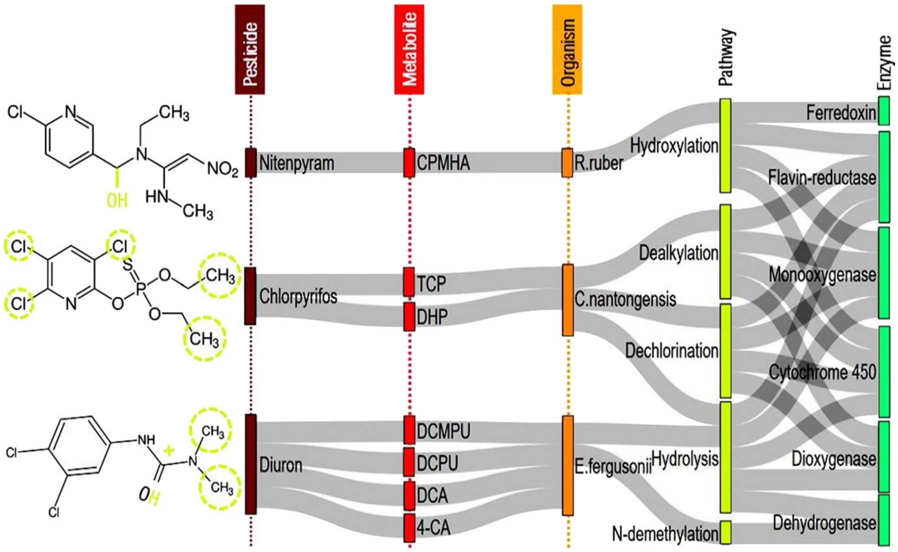

Fig. 9 Prevalent metabolic pathways of pesticide-degrading microorganisms in the academic literature on microbial bioremediation (meta-analysis of 21 papers from the period of publication of 2018–2021)

in the metabolism. However, exposure to the pesticide can increase the production of monooxygenases in association with the genes, namely CYP12a5 and CYP6ER1, in *Drosophila melanogaster* and *Nilaparvata lugens* (Zhang et al. 2017; Harrop et al. 2018), respectively. A higher enzymatic activity may be an indicator of pesticide-resistant metabolism. Another nitenpyram-degrading agent is *Rhodococcus ruber* (strain CGMCC17550). Of the 5198 genes it can express during biodegradation of the pesticide, 18 do associate with the cytochrome P450 complex. We can recognize heme-containing enzymes of the cytochrome P450 complex from microorganisms capable of degradation/biotransformation of highly toxic and recalcitrant compounds (Dai et al. 2021). Glucose, fructose, and pyruvate can improve the biodegradation of nitenpyram by providing an additional level of energy to cometabolism by another strain of *R. ruber*, namely DSM44319 (Dai et al. 2021). *Streptomyces canus* (strain CGMCC13662) also degrades neonicotinoids (Guo et al. 2019), whereas *Escherichia fergusonii* (strain ATCC 35,469) proves useful as an aerobic degrader of diuron [1-(3,4-dichlorophenyl)-3,3 dimethyl urea] via N-demethylation and hydrolysis (Silva Moretto et al. 2019; Tandon et al. 2019). Dioxygenases, by replacing the chlorine atom with hydroxyl in the aromatic ring, catalyze the reduction of the herbicide to a wide range of metabolites such as 3-(3,4-dichlorophenyl)-3-methylurea (DCPMU), 3,4-dichlorophenyl urea (DCPU), 3,4-dichloroaniline (DCA) and 4-chloroaniline (4-CA) (Silva Moretto et al. 2019; Tandon et al. 2019). A further metabolization of DCA can be catechol, a highly toxic compound to living things in nature (Egea et al. 2018).

Overall, numerous microorganisms capable of degrading pesticides are available from the recent academic literature on bioremediation. However, by analyzing the evidence from our meta-analysis, we can identify fewer trials focusing on mixtures of pesticide and microbial consortia. Consortia can remove a pollutant more effectively than monoculture, as they consist of multiple arrays of detoxifying mechanisms (Kumar and Philip 2006). However, eventually, they can produce antagonistic relationships (e.g., competition and succession), so they cannot perform an effective biodegradation/biotransformation as we might expect on a synergistic cometabolism. By isolating a group of microorganisms from an extreme environment containing the compound we want to treat and then enriching it with another group of synergistic microorganisms, we can optimize bioremediation.

Even if the target-molecule is highly toxic and recalcitrant, a composite microbial system becomes capable of effective biodegradation (Krishna and Philip 2008). For instance, studies focusing on the bioremediation of neonicotinoids can demonstrate the effectiveness of bacteria to catalyze the bioprocess through an extensive range of enzymes (Hussain et al. 2015; Huang et al. 2019). A nutritional supplement in the system can enable the microcosm to perform cometabolic biodegradation, as the pesticide becomes not the single source of energy, such as carbon or nitrogen. The outcome of cometabolism depends upon the chemical structure of the pesticide, catabolic activity, and work-up condition (e.g., medium, pH, temperature, etc.). However, it often can become more toxic and persistent than the original molecule, and thus analyzing the secondary metabolite becomes imperative for environmental reclamation in agriculture or any other relevant context with prudence and safety (Hussain et al. 2015).

4 Conclusion

Our integrative review provides insights into microbial bioremediation of pesticides from agricultural soils with higher accuracy and reliability than is achievable through a systematic review, which sounds descriptive rather than analytical. Approximately 83% of the studies rely on bacteria to elaborate monocultures (55%), consortia (40%), and co-cultures (5%) for biocatalytic remediation. In contrast, research in fungi is at an early stage of biotechnological development; however, it can provide information about genetically engineered strains capable of degrading pyrethroids. Natural attenuation is time-consuming and not convincing to scale up. By introducing allochthonous or genetically modified microorganisms into native communities for bioaugmentation, we can improve the removal of active compounds from croplands and grasslands. Another eco-compatible solution to boost pesticide-remediating biosystems refers to biostimulation. However, the scientific community and policymakers need to improve the overseeing and regulation of the addition of microorganisms and compounds to the target site to avoid hazardous impacts on ecological sustenance and agronomic functions. Researchers focus on microbial communities. However, they do not emphasize analyzing the bioremediation for secondary metabolites, pathways, enzymes, and genes, driving the need to conduct further in-depth omics studies and trials. Additionally, bringing a disruptive recombinant DNA technology into biocatalytic frameworks could offer opportunities to implement environmentally cleaner and safer bioremediation while ensuring ethical standards for stakeholders. Therefore, scientists must tap the research and biotechnological development in pesticide-degrading microorganisms into rigorous scientific methods to provide intricate knowledge for advancing the field's prominence in developing high-throughput remediating approaches.

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Data availability The datasets used and/or analyzed are available from the corresponding author on reasonable request. They are also preserved in the Repository Institutional by Unesp, whose periodic backup system promotes greater security.

Declarations

Conflict of interest The authors declare no potential conflict of interest.

Ethical approval Authors confirm that the manuscript has not been submitted to journal for simultaneous consideration and has not been previously published.

Consent for publication All authors approved the manuscript before submission and consent.

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5.5. Anexo V

Victor Hugo CRUZ; Bruno Rafael de Almeida MOREIRA; Thalia Silva VALÉRIO; Yanca Araujo FRIAS; Vinicius Luiz da SILVA; Eduardo Beraldo de MORAIS; Leonardo Gomes de VASCONCELOS; Leandro TROPALDI; Evandro Pereira PRADO; Renato Nallin MONTAGNOLLI; Paulo Renato Matos LOPES.

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Article

Leguminous Plants and Microbial Inoculation: An Approach for Biocatalytic Phytoremediation of Tebuthiuron in Agricultural Soil

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Abstract: Herbicides are important for weed control but can severely impact ecosystems, causing soil and water contamination, biodiversity loss, and harm to non-target organisms. Tebuthiuron, widely used in sugarcane cultivation, is highly soluble and persistent, posing significant environmental risks. Microbial inoculation has emerged as a sustainable strategy to mitigate such damage. This study investigated the phytoremediation potential of *Mucuna pruriens* and *Canavalia ensiformis* in tebuthiuron-contaminated soils, enhanced by fungal and bacterial inoculants. *Crotalaria juncea* served as a bioindicator plant, and *Lactuca sativa* was used in ecotoxicological bioassays. During a 140-day greenhouse experiment from September 2021 to March 2022, *M. pruriens* showed faster growth than *C. ensiformis* in uncontaminated soils but was more affected by tebuthiuron. Bacterial inoculants improved *M. pruriens* growth under stress, while fungal inoculants mitigated tebuthiuron's effects on *C. ensiformis*. *C. juncea* exhibited high sensitivity to tebuthiuron but grew beyond 100 cm with bacterial inoculants. Ecotoxicological assays showed that bacterial bioaugmentation significantly reduced soil toxicity. Natural attenuation further decreased tebuthiuron toxicity, and prior cultivation of *M. pruriens* enhanced soil detoxification. This integrated approach combining phytoremediation and bioaugmentation offers a sustainable method to degrade tebuthiuron, foster safer agriculture, and reduce environmental and health risks.

Keywords: bioaugmentation; bioremediation; *Canavalia ensiformis*; *Crotalaria juncea*; ecotoxicity; herbicide degradation; *Lactuca sativa*; *Mucuna pruriens*; sustainable agriculture

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1. Introduction

The herbicide tebuthiuron raises significant environmental concerns due to its high water solubility (2.5 g L^{-1} at 25 °C) [1], prolonged soil persistence (log K_ow = 1.8) [2], and potential to contaminate terrestrial and aquatic ecosystems [3]. Given the increasing evidence of tebuthiuron's detrimental environmental impacts, there is an urgent need for innovative and sustainable remediation strategies. Phytoremediation has emerged as a promising approach, utilizing plants to detoxify environments contaminated by various pollutants, including heavy metals, hydrocarbons, dyes, and pesticides [4].

Our study investigates the tolerance and phytoremediation potential of two leguminous species, *Mucuna pruriens* (L.) DC. var. *pruriens* and *Canavalia ensiformis* L., in combination with microbial inoculants. These annual plants not only have the potential to degrade tebuthiuron but also fix atmospheric nitrogen, enriching the soil and improving its physical, chemical, and biological properties [5]. Previous studies have indicated that these species exhibit resilience to tebuthiuron, making them promising candidates for remediating contaminated environments.

Several studies have evaluated the potential of leguminous species for phytoremediation of tebuthiuron-contaminated soils. For instance, Mendes et al. [6] assessed the use of *Crotalaria spectabilis*, *C. ensiformis*, *Stizolobium aterrimum*, and *Lupinus albus* in soils treated with quinclorac and tebuthiuron. They found that all four species absorbed more tebuthiuron than quinclorac, with *C. ensiformis* identified as the most efficient species for remediating tebuthiuron-contaminated soils. Ferreira et al. [7] further advanced the field by identifying suitable phytoremediator organisms for tebuthiuron in agricultural soils. Their experiments involved *Cajanus cajan*, *C. ensiformis*, *M. pruriens*, and *Pennisetum glaucum*, which successfully removed tebuthiuron applied at 2 L·ha^{-1}, enabling subsequent growth of *Crotalaria juncea* and *Lactuca sativa* in the presence of residual herbicide. They also highlighted the potential of repurposing vinasse as a source of organic carbon to enhance plant development and improve the ecological viability of phytoremediation.

Contrastingly, Frias et al. [8] investigated the efficacy of *M. pruriens* as a phytoremediator in soil supplemented with vinasse and found it ineffective at removing tebuthiuron. *M. pruriens* was exposed to tebuthiuron at 0.5, 1, 1.5, and 2 L·ha^{-1} and vinasse at 75, 150, and 300 m^3·ha^{-1}. The herbicide caused phytotoxicity, severely inhibiting germination and growth. The addition of vinasse exacerbated damage to both photosynthetic and non-photosynthetic structures, reducing biomass production. Consequently, neither *C. juncea* nor *L. sativa* could grow in the presence of residual pesticide.

Despite its promise, phytoremediation faces challenges, as its success depends on factors such as soil characteristics, climate, and co-contaminants [9]. To optimize phytoremediation effectiveness in addressing tebuthiuron contamination, a comprehensive understanding of the interactions between selected plant species and the herbicide is essential [7,8]. To enhance remediation efficiency, we incorporate bioaugmentation as a pivotal strategy. Bioaugmentation involves introducing microorganisms into a contaminated environment to accelerate pollutant degradation [10]. By introducing selected microbial strains with high pesticide degradation capabilities, resilience, and adaptability [11], we establish a symbiotic alliance that expedites pesticide degradation in soil. The integration of phytoremediation and bioaugmentation offers a powerful synergy [9], significantly improving tebuthiuron degradation efficiency and overall remediation outcomes.

Our approach emphasizes safety and efficacy by evaluating the environmental toxicity levels of tebuthiuron during the remediation process. We designed tests to realistically predict the behavior of substances in the environment. Specifically, we employed *C. juncea* as a bioindicator species sensitive to tebuthiuron after prior cultivation of *M. pruriens* and *C. ensiformis*. Additionally, *L. sativa* was used in ecotoxicological assays to assess residual toxicity. These complementary experiments aimed to verify the presence of tebuthiuron in the soil [7–16].

Therefore, this research aims to investigate the tolerance and phytoremediation potential of *M. pruriens* and *C. ensiformis*, in conjunction with microbial inoculants, for the remediation of tebuthiuron-contaminated agricultural soil. Our findings will advance our understanding of the viability and effectiveness of these techniques in addressing tebuthiuron contamination and the associated ecological concerns. Innovative and sustainable approaches are crucial for the successful remediation of tebuthiuron-contaminated soils.

2. Materials and Methods

2.1. Soil, Tebuthiuron, and Microbial Inoculant

The soil used in this study was classified as a Dystrophic Red-Yellow Oxisol. This soil was sourced from an agricultural facility in the Dracena region with no recent history of phytosanitary treatments, ensuring minimal prior contamination. Upon acquisition, the soil was transported to a greenhouse, air-dried, sieved through a 2.0 mm mesh, and stored in hermetically sealed plastic containers for chemical characterization. Soil samples were collected both before and after the experiment to determine chemical composition (Table 1). For each analysis period, data from all treatments were averaged to present the overall soil properties. Additional soil samples were collected at the end of the experimental period for further chemical analysis.

Table 1. Soil chemical analysis at 0 and 70 DAS of *C. ensiformis* and *M. pruriens*.

Attributes	Unit	0 DAS	70 DAS	Indication
pH (H_2O)	-	4.0	7.5	Increased
Organic matter	g dm^{-3}	4.0	10	Increased
Potassium	mmol dm^{-3}	0.3	1.6	Increased
Calcium	mmol dm^{-3}	6	51	Increased
Magnesium	mmol dm^{-3}	2	23	Increased
Hydrogen + Aluminum	mmol dm^{-3}	33	8	Decreased
Aluminum^{3+}	mmol dm^{-3}	13	0	Decreased
Phosphor	mg dm^{-3}	1	6	Increased
Sulfur	mg dm^{-3}	7	-	Not detected
Boron	mg dm^{-3}	0.10	0.02	Decreased
Copper	mg dm^{-3}	0.1	0.2	Increased
Iron	mg dm^{-3}	4	2	Decreased
Manganese	mg dm^{-3}	1.8	1.2	Increased
Zinc	mg dm^{-3}	0.1	0.3	Increased
Sum of bases	mg dm^{-3}	8	75.6	Increased
Cation exchange capacity	mg dm^{-3}	41	83.6	Increased
Base saturation	%	20	90	Increased
Aluminum saturation	%	61	0	Decreased

Increased—the value in the soil was higher in 70 DAS (day after the sowing) than the initial time (0 DAS); Decreased—the value in the soil was lower in 70 DAS than the initial time (0 DAS); Not detected—there was no comparison between 0 and 70 DAS due to the minimum concentration of the parameter not detected by the analysis.

The herbicide used was Combine® 500SC (Batch: 041-14-2000) from Dow AgroSciences Industrial Ltda. (São Paulo, Brazil), a commercially available formulation of tebuthiuron (TBT).

To augment the soil microbial community and enhance phytoremediation, microbial inoculants were obtained from Microgreen® Ltda. (http://microgreen.agr.br/, Piracicaba, Brazil), a company specializing in soil microbial reclamation. Two types of inoculants were utilized: a bacterial inoculant (BACT) rich in actinomycetes, *Bacillus* spp., and lactic acid bacteria and a fungal inoculant (FUNG) containing *Trichoderma* spp., *Purpureocillium* spp., and *Beauveria* spp., whose application is for the restoration of microbiota in agricultural soils.

2.2. Plant Species: Phytoremediator Species, Indicator Plant, and Test Organism

Mucuna pruriens (MP) and *Canavalia ensiformis* (CE) were selected for their well-documented phytoremediation capabilities, especially in soils contaminated with tebuthiuron [6–8]. These leguminous species form symbiotic relationships with nitrogen-fixing bacteria, enhancing nutrient availability and improving soil fertility.

Sunn hemp (*Crotalaria juncea*) was chosen as a bioindicator plant due to its known sensitivity to tebuthiuron [15]. Acting as a sentinel species, *C. juncea* aids in assessing soil contamination levels and the efficacy of phytoremediation efforts. Seeds of *C. juncea*, *C.*

ensiformis, and *M. pruriens* were obtained from BR SEEDS® (Araçatuba, Brazil), ensuring uniformity and reliability of the experimental material.

For ecotoxicological bioassays, commercially available seeds of *Lactuca sativa* L. (variety Butterhead) were procured from Feltrin Sementes® (Caxias do Sul, Brazil).

2.3. Experimental Setup

The experiment was conducted using a completely randomized design with a 2 × 3 × 3 factorial scheme, comprising seven replicates and five analysis times, totaling 630 pots. The factors studied were tebuthiuron concentration (presence or absence), microbial inoculant type (bacterial, fungal, or none), and plant species (*C. ensiformis*, *M. pruriens*, or none). This comprehensive design allowed for a thorough investigation of the independent and combined effects of these variables on the study parameters (Figure 1). Randomization minimized bias and ensured equal representation of treatment groups across experimental units.

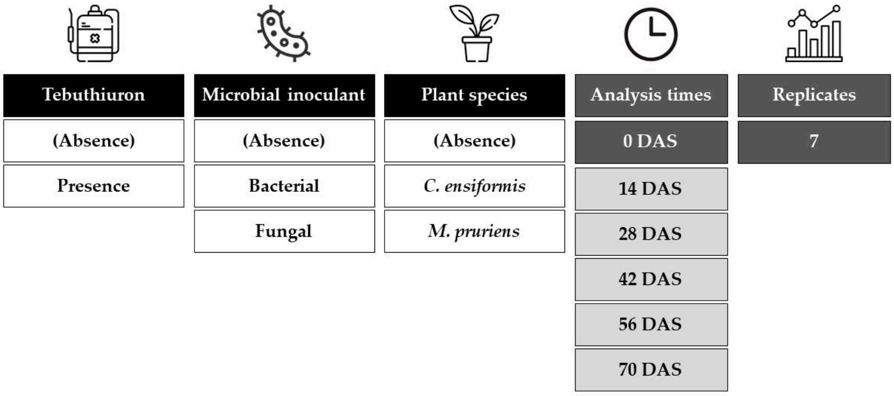

Figure 1. Summary of the experimental design with the three factors (tebuthiuron, microbial inoculant, and plant species), the five analysis times (0, 14, 28, 42, 56, and 70 DAS—days after sowing), and the number of replicates.

Prior to the experiment, the soil underwent a preparatory phase to adjust its acidity and fertility, following the procedures of Ferreira et al. [7] and Frias et al. [8]. For every 504 kg of soil, amendments were meticulously applied as follows: 454 g (1.8 t ha^{-1}) of limestone to regulate pH, 10 g (40 kg ha^{-1}) of urea as a nitrogen source, 56 g (222 kg ha^{-1}) of single superphosphate for phosphorus supplementation, and 13 g (52 kg ha^{-1}) of potassium chloride to ensure adequate potassium levels for optimal plant growth. After thorough mixing through uniform distribution, the soil was used to fill pots with a capacity of approximately 4.0 L each (19 cm × 15 cm × 19 cm).

The microbial inoculants were incorporated into the soil according to their respective treatment groups. The fungal inoculant (FUNG) was applied at a rate of 0.36 g (180 t ha^{-1}) per pot, while the bacterial inoculant (BACT) was added at a volume of 50 mL (25 m^3 ha^{-1}) per pot. Three days after inoculant incorporation, the herbicide Combine® was applied at the recommended dosage for sandy soils of 2.0 L ha^{-1} (1000 g active ingredient ha^{-1}). The application was performed using a laboratory sprayer equipped with four XR 11002 flat-fan nozzles (Jacto®, Pompéia, Brazil), operating at a pressure of 2 bar and a flow rate of 0.65 L$\cdot$ min^{-1}. Following the manufacturer's guidelines, the sprayer was calibrated to a speed of 5 km$\cdot$h^{-1}, with the spray boom positioned 0.75 m above the pots, delivering an application volume of 156 L ha^{-1}. Environmental conditions during spraying were monitored, with a temperature of 27.2 °C and relative humidity of 63%. The control treatments without tebuthiuron received an equivalent volume of water to maintain consistency.

Seven days following herbicide application, three seeds of either *C. ensiformis* or *M. pruriens* were sown in each pot, according to the treatment design. Although three seeds were initially sown per pot, thinning was performed to retain only one plant per pot to ensure uniform growth conditions. These plants were cultivated for 70 days after sowing (DAS). Three days after harvesting the leguminous plants, three seeds of sunn hemp (*C. juncea*) were sown in each pot and cultivated for an additional 70 days, following the same thinning procedure.

All plants were cultivated in a greenhouse equipped with an automated irrigation system to maintain optimal growth conditions. Irrigation was performed daily to maintain the soil at 60% of its field capacity. The system was programmed via a digital timer to execute up to four irrigation cycles per day, each lasting approximately 40 ± 10 min. A micro-sprinkler located at the top of the greenhouse provided irrigation at a flow rate of approximately 80 L h^{-1} under a pressure of 2 bar, as indicated by the irrigation manometer. This controlled environment facilitated the investigation of the tolerance and phytoremediation capabilities of *C. ensiformis* and *M. pruriens*, as well as the influence of microbial inoculants in tebuthiuron-contaminated soil.

2.4. Plant Growth and Development Evaluation

Plant growth was monitored weekly by recording plant height in centimeters. At 70 DAS, the plants were harvested, the roots were gently cleaned to remove soil, and the samples were dried in a forced-air oven at 65 °C for 72 h. Dry biomass was then weighed and recorded in grams.

2.5. Ecotoxicological Bioassays

The ecotoxicological potential of the treatments was assessed at specific time points: 0, 20, 40, 60, 70 DAS (end of *M. pruriens* and *C. ensiformis* cultivation), and 140 DAS (end of *C. juncea* cultivation). Bioassays were conducted following the methodologies described in NBR 10006 [16] and Sobrero and Ronco [17].

For each treatment, five replicates of aqueous soil extracts were prepared. Superficial soil samples (approximately 2 cm deep) were collected from the edges of different pots within each treatment group. A 25 g sample of soil was mixed with 100 mL of deionized water in a 250 mL Erlenmeyer flask. The flasks were sealed with PVC plastic film, shaken at 120 rpm for five minutes, and then incubated in a Biochemical Oxygen Demand (BOD) chamber without lighting at 20 ± 2 °C for 7 days.

After incubation, 2.0 mL of the solubilized soil extract was applied to Petri dishes lined with filter paper and containing 10 seeds of *L. sativa*. The dishes were sealed with PVC plastic film to prevent moisture loss and incubated in a BOD chamber at 20 ± 2 °C with a 12 h photoperiod for 5 days. The positive control (CP) and negative control (CN) treatments were prepared using 0.05 M zinc sulfate solution and deionized water, respectively, to test seed sensitivity.

During the bioassays, seed germination, hypocotyl elongation, and root elongation were measured. These parameters were used to calculate the germination index (GI) of *L. sativa* seeds, as described in Equation (1) [18]:

$$\mathrm{GI} = \frac{(\mathrm{G\%} \times \mathrm{R\%})}{100} \tag{1}$$

This equation includes the following:
GI represents the germination index;
G% denotes the percentage of seed germination;
R% indicates the percentage of root elongation.

2.6. Statistical Data Analysis

Prior to conducting statistical analyses, the data were evaluated to ensure compliance with the assumptions of homogeneity of variances and normality. Homoscedasticity was assessed using Bartlett's test, while the Shapiro–Wilk test was employed to verify the normal distribution of residuals. Once these assumptions were confirmed, an Analysis of Variance (ANOVA) was performed at a 5% significance level ($p < 0.05$) to identify significant differences among treatment groups. When the ANOVA indicated significant effects, Tukey's Honest Significant Difference (HSD) post hoc test was utilized for pairwise comparisons to determine which specific means differed.

To model the growth dynamics of plant height and the germination index over time, we applied the Gompertz sigmoid function (Equation (2)). This nonlinear model is particularly suitable for describing sigmoidal growth patterns and has been widely used in ecological and biological studies due to its flexibility and interpretability [12,19]. The Gompertz function allowed us to estimate key growth parameters, such as the maximum attainable value (asymptote), growth rate constant, and inflection point, providing insights into the developmental processes under different treatment conditions. By fitting the Gompertz model to our data, we could predict growth trends beyond the observed time frames of 70 and 140 days after sowing (DAS), offering a more comprehensive understanding of the long-term effects of the treatments.

$$f_x = \alpha e^{-\beta e^{-kx}} \tag{2}$$

This equation includes the following:

fx represents the plant height in centimeters or the germination index;

x denotes the time in days after sowing (DAS);

α represents the upper asymptote or the maximum height development and germination index that the plants can reach;

β indicates the inflection point, which corresponds to the time when the growth rate starts to decrease;

k represents the exponential decay of the specific growth rate, indicating how quickly the growth rate decreases over time;

e represents Euler's constant, a mathematical constant approximately equal to 2.71828.

$$\gamma = \alpha + \beta + \sum \left((t_i \times f_{i1}) + (t_i \times f_{i2}) \right) + \varepsilon \tag{3}$$

This equation includes the following:

γ represents the dependent variable (e.g., plant height or germination index);

α denotes the global intercept of the model;

β represents the random effect (tebuthiuron);

ti indicates the time in days after sowing (DAS);

f_{i1} represents the fixed effect 1 (green manure);

f_{i2} represents the fixed effect 2 (inoculant);

ε represents the residual term.

In cases where time was a significant variable, we extended the model to include time as a continuous covariate or as repeated measures, depending on the data structure. This allowed us to capture temporal trends and assess how the treatments influenced growth trajectories over time.

All statistical analyses were conducted using R software 4.2.2. for its advanced statistical capabilities and flexibility. The nlme package was utilized for fitting mixed-effects models (Equation (3)), while the nls function facilitated nonlinear regression for the Gompertz function. GraphPad Prism 9 and Microsoft Excel® 2019 were employed for data visualization, preliminary analyses, and to generate graphical representations of the results. These tools collectively enabled efficient data management, rigorous statistical testing, and clear presentation of findings.

2.6.1. Model Validation and Goodness-of-Fit and Consideration of Multiple Comparisons

Model adequacy was evaluated through diagnostic plots and goodness-of-fit statistics. For the Gompertz function, the coefficient of determination (R^2) and residual analysis were used to assess how well the model described the observed data. The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were also considered for model selection and comparison. In the mixed-effects models, the significance of fixed effects was tested using likelihood ratio tests, and random effects were assessed through variance component analysis. Residuals were examined for homoscedasticity and normality to validate model assumptions. Given the multiple treatment groups and comparisons, we employed the Tukey–Kramer method in the post hoc analysis to control for Type I error rates associated with multiple testing. This approach ensures that the overall family wise error rate remains at the desired significance level, enhancing the reliability of the statistical conclusions.

2.6.2. Statistical Significance and Reporting

All statistical findings were interpreted in the context of the biological and ecological implications for phytoremediation practices; therefore, the results were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Soil's Properties: Enhancing Fertility

As discussed in the preceding section, the soil used in this study was classified as a Dystrophic Red-Yellow Oxisol, a common soil type in tropical regions characterized by low natural fertility, acidic pH, and high levels of iron and aluminum oxides, which can influence nutrient and contaminant retention [20]. These properties make it an ideal substrate for simulating real-world scenarios of pesticide-contaminated agricultural soils.

Therefore, according to Table 1, significant improvements in the soil's chemical attributes were observed following amendments with lime and fertilizers, as well as the cultivation of *M. pruriens* and *C. ensiformis*. Various nutritional parameters increased numerically, indicating enhanced soil fertility. Notably, the concentration of exchangeable aluminum (Al^{3+}) did not increase, which is advantageous since high levels of aluminum can inhibit root growth and impair plant development [21]. The application of lime and fertilizers contributed to balancing soil pH and improving nutrient availability, thereby enhancing overall soil conditions.

Implementing appropriate agricultural practices, such as soil chemical correction and the cultivation of leguminous plants, can have multiple positive effects on soil health. Additionally, the presence of plants and soil microorganisms, combined with soil amendments, can lead to the release of organic acids. These organic acids aid in the solubilization of essential nutrients like phosphorus and potassium, making them more available for plant uptake. Furthermore, they contribute to an increase in the soil's cation exchange capacity, which helps reduce toxic levels of aluminum, ultimately benefiting plant growth and development [22].

Enhancing soil fertility through these practices creates a more conducive environment for plant growth, which is essential for successful phytoremediation efforts. Improved soil conditions can enhance plant vigor, allowing for more effective uptake and degradation of contaminants such as tebuthiuron.

3.2. Production of Leguminous Plants with Microbial Inoculation: Unveiling the Biocatalytic Phytoremediation

3.2.1. Growth Dynamics

The growth dynamics of *C. ensiformis* and *M. pruriens* exhibited distinct patterns (Figure 2). *M. pruriens* demonstrated a faster growth rate, indicated by a steeper growth curve compared to *C. ensiformis*. Table 2 presents the parameters of the growth curves for each treatment, including the maximum height (α), the inflection point (β), and the specific growth rate (k). A higher k value signifies a faster growth rate; both *M. pruriens* (k = 0.113) and *C. ensiformis* (k = 0.075) showed considerable growth rates.

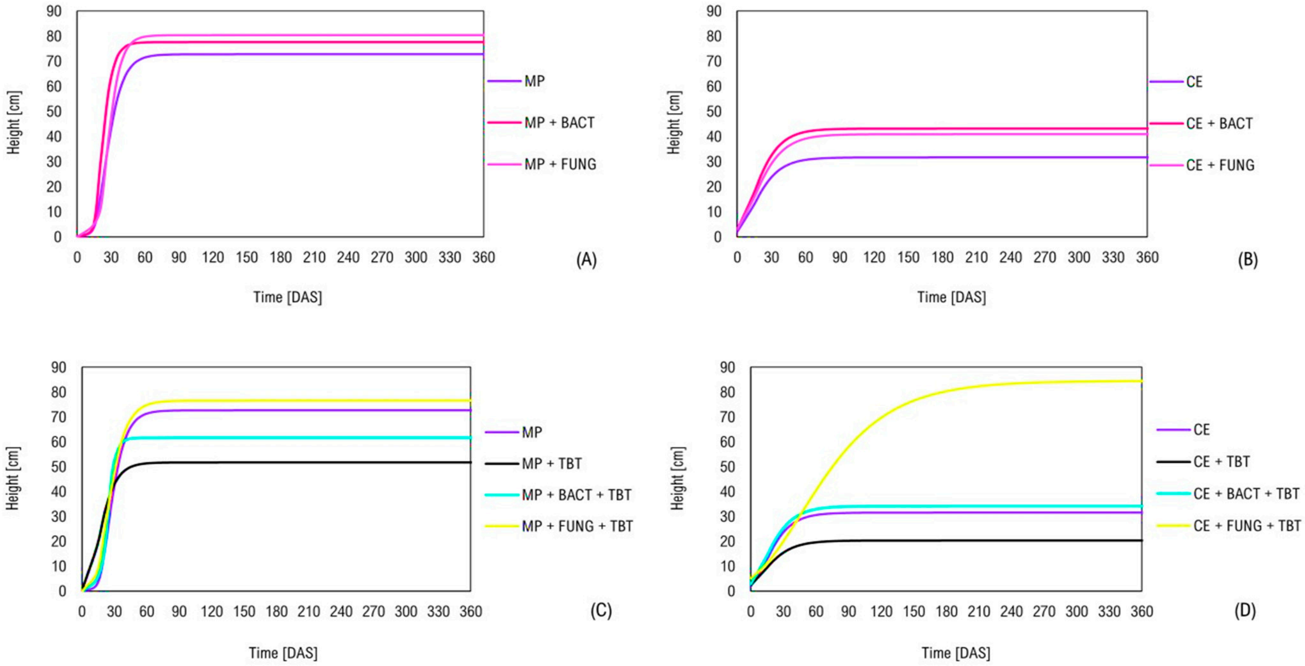

Figure 2. Kinetic growth of *M. pruriens* (MP) and *C. ensiformis* (CE) as potential phytoremediators of the herbicide tebuthiuron (TBT) in soil with fungal (FUNG) or bacterial (BACT) inoculants from the Gompertz model. (**A**,**B**) Treatments without TBT. (**C**,**D**) Treatments with TBT.

Table 2. Parameters of Gompertz kinetic models for the height of *M. pruriens* (MP) and *C. ensiformis* (CE) as potential phytoremediators of tebuthiuron (TBT) with fungal (FUNG) or bacterial (BACT) inoculants.

Treatments	Complexity			Adequacy		
	α	β	k	R^2	AIC	BIC
MP	72.72	15.49	0.1127	0.72	63.96	65.17
MP + TBT	51.69	4.76	0.1087	0.94	63.96	65.17
MP + BACT	77.66	32.71	0.1695	0.97	69.10	70.31
MP + FUNG	80.31	38.14	0.1425	0.99	56.33	57.54
MP + BACT + TBT	61.58	144.19	0.2272	0.89	78.80	80.01
MP + FUNG + TBT	76.63	9.15	0.0997	0.93	75.82	77.04
CE	31.64	2.72	0.0748	0.97	46.29	47.50
CE + TBT	20.27	2.13	0.0686	0.93	44.71	45.92
CE + BACT	43.10	2.73	0.0751	0.97	53.67	54.88
CE + FUNG	40.90	2.62	0.0679	0.97	50.41	51.62
CE + BACT + TBT	34.26	2.51	0.0707	0.96	49.03	50.24
CE + FUNG + TBT	84.52	2.81	0.0224	0.94	60.08	61.29

Parameters α, β, and k denote the superior asymmetry, the inflection point, and the exponential decay of the specific growth rate, respectively; $\beta = 1$ keeps the relative decrease with time constant; $\beta > 1$ accelerates the relative decrease with time; $\beta < 1$ slows the relative decrease with time; R^2: coefficient of determination; AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion.

The application of microbial inoculants influenced the growth dynamics of both species. Within the *M. pruriens* group, the fungal inoculant treatment (MP + FUNG) resulted in faster growth compared to the bacterial inoculant (MP + BACT) and the control (MP alone). Similarly, for *C. ensiformis*, the bacterial inoculant (CE + BACT) promoted faster growth than the fungal inoculant (CE + FUNG) and the control (CE alone). These results suggest that the type of microbial inoculant can differentially affect plant growth, potentially due to specific interactions between the inoculant microorganisms and the plant species.

The introduction of tebuthiuron had a noticeable impact on plant growth, reducing plant height compared to the control treatments without the herbicide. As shown in Table 2, the CE + TBT treatment exhibited a greater reduction in height-related parameters, including maximum height (α) and specific growth rate (k), compared to MP + TBT. This indicates that *C. ensiformis* is more sensitive to tebuthiuron than *M. pruriens*. Previous studies by Ferreira et al. [7] and Belo et al. [23] also reported the sensitivity of *C. ensiformis* to tebuthiuron, suggesting that this species may not be ideal for the phytoremediation of soils contaminated with this herbicide.

Conversely, Mendes et al. [6] demonstrated that *C. ensiformis* can tolerate and even degrade tebuthiuron, potentially due to specific microorganisms present in its rhizosphere. This highlights the importance of considering plant–microbe interactions when selecting species for phytoremediation. For *M. pruriens*, Ferreira et al. [7] observed a 15% decrease in plant height when exposed to tebuthiuron. However, they found that the phytotoxicity of tebuthiuron was mitigated by the addition of vinasse (an industrial by-product) at 150 m^3 ha^{-1}, allowing the plant to develop even at high herbicide concentrations (2 L ha^{-1}).

Discrepancies between our results and previous studies may be attributed to factors such as differences in experimental conditions, soil types, moisture levels, environmental temperatures, and light exposure. These variables can directly influence phytoremediation efficiency and should be carefully considered when selecting plants for remediating tebuthiuron-contaminated soils [24].

An important finding in our study was the mitigating effect of microbial inoculants on plant sensitivity to tebuthiuron in contaminated soil. The inoculants appeared to enhance plant growth and increase height, particularly in *C. ensiformis*. For instance, the CE + BACT + TBT treatment showed a higher specific growth rate ($k = 0.071$) compared to CE + TBT without inoculants, although only the CE + FUNG + TBT treatment achieved the maximum height ($\alpha = 84.52$). This suggests that fungal inoculants may be more effective in promoting growth under herbicide stress in *C. ensiformis*.

In *M. pruriens*, the MP + FUNG + TBT treatment displayed a steeper growth curve than MP + BACT + TBT, reaching a greater maximum height ($\alpha = 76.63$). Although the bacterial inoculant (MP + BACT + TBT) exhibited a higher specific growth rate ($k = 0.227$ vs. $k = 0.100$), the overall growth performance was better with the fungal inoculant. These results could be explained by several factors: (a) Fungal inoculants may enhance plant resilience to herbicide-induced stress by improving nutrient uptake or producing growth-promoting substances, allowing plants to maintain or increase growth under adverse conditions. (b) The fungal inoculant may have the ability to degrade or metabolize tebuthiuron, reducing its toxicity in the soil environment. (c) The inoculants may stimulate beneficial soil microorganisms, creating a more favorable rhizosphere environment for plant growth.

Previous studies have demonstrated the effectiveness of such microbial combinations. Zhang et al. [25] reported successful remediation of soils contaminated with pentachloronitrobenzene using a fungal–bacterial inoculum in association with *Panax notoginseng*. Similarly, Madariaga-Navarrete et al. [26] observed substantial atrazine removal from soil within 40 days using *Trichoderma* sp. combined with *Phaseolus vulgaris*. These findings support the potential of microbial inoculants in enhancing phytoremediation efficiency.

3.2.2. Phytomass Accumulation

The application of microbial inoculants in the absence of tebuthiuron led to a significant increase in biomass production in both *M. pruriens* and *C. ensiformis* compared to the control treatments (Figure 3). This positive impact underscores the efficacy of microbial inoculants in promoting plant growth and enhancing phytomass accumulation, which is pivotal for the plants' capacity to tolerate and effectively remediate contaminated soils.

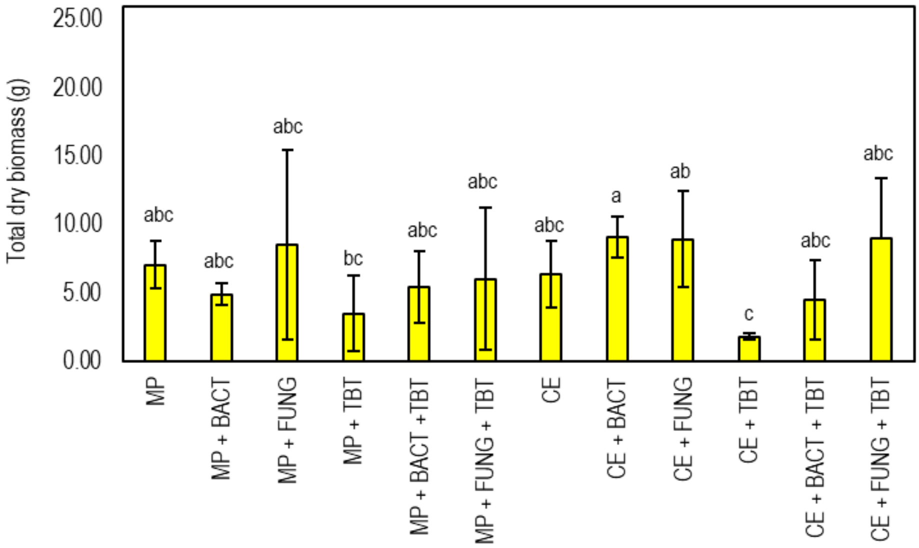

Figure 3. Production of total dry biomass of *M. pruriens* (MP) and *C. ensiformis* (CE) in soil associated or not with tebuthiuron (TBT) and/or fungal (FUNG) or bacterial (BACT) inoculants after 70 DAS. Different lowercase letters indicate statistical differences by the Tukey test ($p < 0.05$).

In contrast, the introduction of tebuthiuron had an adverse effect on biomass production in both species. The most pronounced reduction was observed in the MP + TBT treatment, indicating that *M. pruriens* was particularly affected by the herbicide. Tebuthiuron's negative influence on photosynthesis during plant development can impair biomass production and compromise the overall efficiency of phytoremediation processes [27], even though *M. pruriens* and *C. ensiformis* are not target plants for this herbicide in agroecosystems. Efficient biomass production is crucial for facilitating the transformation of pollutants into less toxic substances, a process optimized when plants grow without intense stress [28].

Further examination revealed that, in the presence of tebuthiuron, the bacterial inoculant did not significantly improve biomass production in *M. pruriens*. For *C. ensiformis*, microbial inoculation did not result in a substantial increase in phytomass when tebuthiuron was present, despite contributing to increased plant height. These observations emphasize the limitations of relying solely on variables like height and biomass accumulation to evaluate phytoremediation efficiency.

Moreover, the rhizospheric interactions between plants and microorganisms play a key role in the degradation of soil contaminants. Root exudates from leguminous plants like *M. pruriens* and *C. ensiformis* can enhance microbial activity by providing essential nutrients and signaling molecules that stimulate the growth and metabolic functions of degradative microbes [29]. These exudates may increase the bioavailability of tebuthiuron by altering soil pH and releasing chelating agents, thereby facilitating its uptake and degradation. Understanding the synergistic relationships within the rhizosphere is crucial, as it can lead to optimized phytoremediation strategies that harness both plant and microbial capabilities for more efficient contaminant removal.

Multiple uncontrolled factors can influence the bioavailability and environmental behavior of the herbicide, including soil properties, microbial community dynamics, and environmental conditions [30]. Therefore, relying exclusively on plant growth parameters may not provide a comprehensive assessment of phytoremediation effectiveness. Complementary approaches, such as cultivating bioindicator plants and implementing ecotoxicological bioassays, are indispensable for thoroughly evaluating environmental reclamation efforts [31].

In summary, our findings highlight the critical aspect of biomass accumulation in the context of phytoremediation. While microbial inoculants positively influenced biomass production in the absence of tebuthiuron, the presence of the herbicide negated these benefits.

This underscores the significance of considering multiple parameters and employing complementary assessment methods to accurately evaluate the efficiency of phytoremediation practices, thereby facilitating sustainable and effective soil remediation strategies.

3.3. Production of C. juncea: Evaluating Ecotoxicity and Phytoremediation Efficiency

3.3.1. Growth Dynamics

The cultivation of *C. juncea* as a bioindicator provided valuable insights into the residual phytotoxicity of tebuthiuron and the effectiveness of the phytoremediation treatments. In the reference treatment (Ref), which involved soil without prior plant cultivation (*CE* or *MP*), no inoculants (BACT or FUNG), and no herbicide (TBT), *C. juncea* exhibited slow height development (Figure 4). This baseline serves as a control for comparing the effects of various treatments.

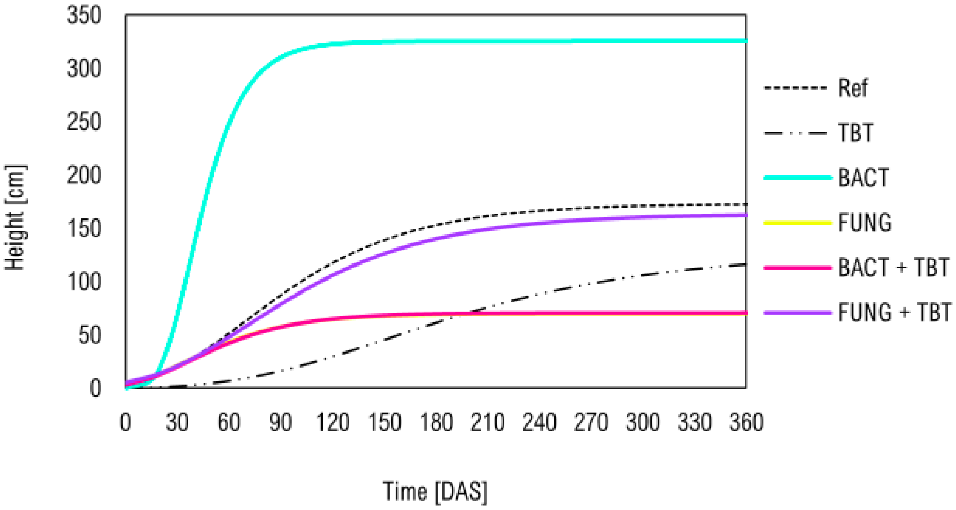

Figure 4. Kinetic growth of *C. juncea* height as bioindicator species in soil with tebuthiuron (TBT) and fungal (FUNG) or bacterial (BACT) inoculants from the Gompertz model. Ref—reference control soil without leguminous plants, inoculants, and tebuthiuron.

Interestingly, soil treated with the bacterial inoculant (BACT) without prior leguminous cultivation or herbicide application showed a faster increase in *C. juncea* height, with a higher specific growth rate (k = 0.0582) and greater maximum height (α = 324.99) around 60 DAS (Table 3). In contrast, the fungal inoculant (FUNG) had a minimal contribution to *C. juncea*'s height development, indicated by a lower growth rate (k = 0.0302) and lower maximum height (α = 69.95). This suggests that the bacterial inoculant may promote the growth of *C. juncea* in uncontaminated soils more effectively than the fungal inoculant.

The presence of tebuthiuron in the soil demonstrated a significant phytotoxic effect on *C. juncea*, evidenced by a lower specific growth rate (k = 0.0115) (Table 3 and Figure 5). Despite the slower growth rate, *C. juncea* in soil with tebuthiuron alone (without inoculants or prior cultivation) reached a maximum height exceeding 100 cm, which was higher than in treatments with FUNG or BACT + TBT. This indicates that while tebuthiuron adversely affects growth, *C. juncea* can still attain considerable height in its presence.

Remarkably, the phytotoxic effect of tebuthiuron was mitigated by the fungal inoculant in certain treatments, suggesting the involvement of microorganisms in bioremediation. Previous studies have associated microbial genera such as *Methylobacterium*, *Microbacterium*, *Paenibacillus*, and *Streptomyces* with tebuthiuron degradation [32,33]. The long-term presence of the fungal inoculant may contribute to the dissipation of tebuthiuron, reducing its toxicity to subsequent plantings.

Table 3. Parameters of the Gompertz kinetic models for the height of *C. juncea* as a bioindicator species in tebuthiuron (TBT) soil with *M. pruriens* (MP) and *C. ensiformis* (CE) and bacterial (BACT) or fungal (FUNG) inoculants. Ref—reference control soil without leguminous plants, inoculants, and tebuthiuron.

Treatments	Complexity			Adequacy		
	α	β	k	R^2	AIC	BIC
MP	156.35	5.94	0.01061	0.98	49.51	50.30
MP + TBT	86.91	3.29	0.03295	0.98	45.17	45.96
MP + BACT	181.96	3.76	0.01741	0.98	45.39	46.18
MP + FUNG	42.01	3.23	0.05290	0.98	41.15	41.94
MP + BACT + TBT	147.18	3.75	0.02018	0.99	41.67	42.46
MP + FUNG + TBT	248.45	8.52	0.06591	0.98	54.98	55.77
CE	178.13	3.82	0.01486	0.96	47.42	48.20
CE + TBT	115.67	3.34	0.02142	0.98	46.18	45.39
CE + BACT	78.74	3.00	0.02500	0.97	45.81	45.02
CE + FUNG	66.01	2.91	0.02667	0.96	47.20	47.98
CE + BACT + TBT	115.76	5.60	0.09303	0.97	47.70	48.49
CE + FUNG + TBT	199.88	4.11	0.01602	0.98	43.88	44.67
Ref	172.93	3.78	0.01897	0.98	44.72	45.51
TBT	324.99	8.91	0.05819	0.98	51.98	52.77
BACT	69.95	2.95	0.03017	0.97	48.00	47.21
FUNG	127.47	5.76	0.01148	0.99	44.82	45.61
BACT + TBT	70.50	3.08	0.02991	0.98	42.20	42.99
FUNG + TBT	163.15	3.47	0.01731	0.97	50.26	51.05

Parameters α, β, and k denote the superior asymmetry, the inflection point, and the exponential decay of the specific growth rate, respectively; $\beta = 1$ keeps the relative decrease with time constant; $\beta > 1$ accelerates the relative decrease with time; $\beta < 1$ slows the relative decrease with time; R^2: coefficient of determination; AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion.

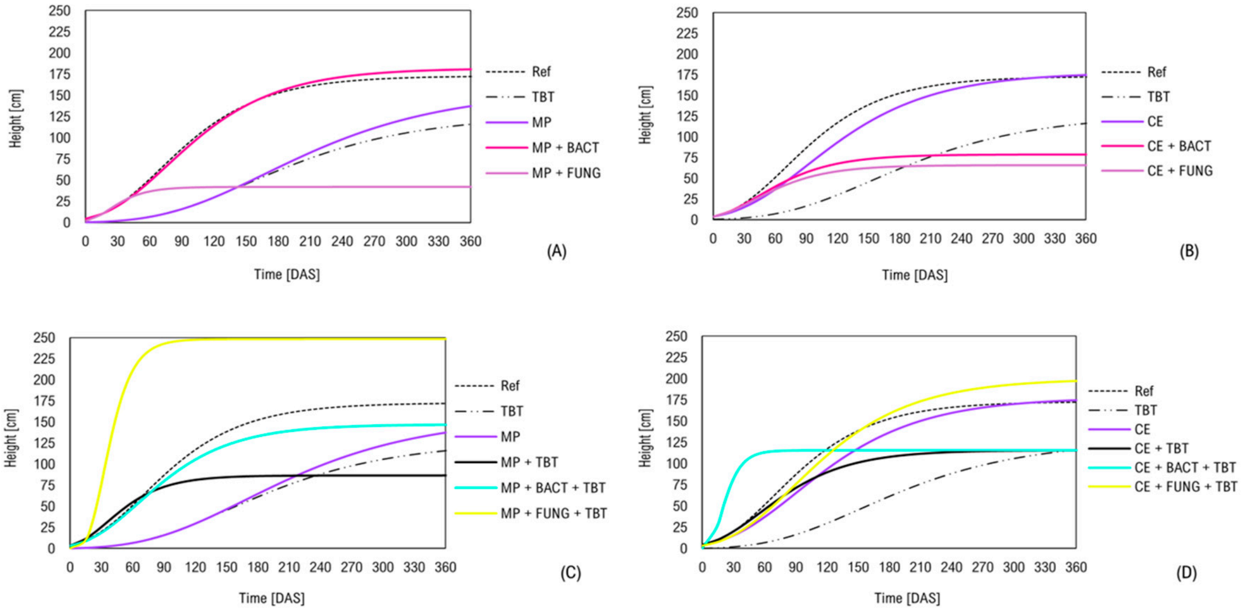

Figure 5. Kinetic growth of *C. juncea* height as bioindicator species in tebuthiuron (TBT) soil with *M. pruriens* (MP) and *C. ensiformis* (CE) and fungal (FUNG) or bacterial (BACT) inoculants. Ref—reference control soil without leguminous plants, inoculants, and tebuthiuron. (**A**,**B**) Treatments without TBT. (**C**,**D**) Treatments with TBT.

Prior cultivation of *M. pruriens* and *C. ensiformis* significantly influenced the growth of *C. juncea*. Soil previously cultivated with *C. ensiformis* had a more positive effect on *C. juncea*'s height compared to soil with *M. pruriens*. As shown in Table 3, the parameters

α and k for *C. ensiformis* were greater than those for *M. pruriens* (α = 156.35 vs. 78.13; k = 0.0149 vs. 0.0106). While *M. pruriens* is beneficial for soil health due to nitrogen fixation and nutrient cycling, it may exhibit allelopathic effects that inhibit the growth of nearby plants through the production of bioactive compounds [34].

Interactions between the green manure species and microbial inoculants yielded distinct results in *C. juncea*'s growth. For instance, the negative effect of *M. pruriens* on *C. juncea* was alleviated when associated with the bacterial inoculant (MP + BACT), resulting in a higher specific growth rate (k = 0.0174) and maximum height (α = 181.96 cm) compared to MP alone. Conversely, the association of *C. ensiformis* with the bacterial inoculant (CE + BACT) had an antagonistic effect on *C. juncea*'s growth, yielding a smaller growth curve compared to soil with *CE* alone. Similarly, the association of fungal inoculants with either phytoremediation species was generally detrimental to *C. juncea*, particularly in the MP + FUNG treatment, where the maximum height was the lowest (α = 42.01 cm), despite a higher growth rate (k = 0.0529).

When analyzing treatments involving tebuthiuron and prior cultivation with *M. pruriens* or *C. ensiformis*, high phytotoxicity and severe limitations in *C. juncea* height were observed compared to the control treatments without the herbicide. Nevertheless, the height growth rates after prior cultivation with the herbicide remained higher than those in the control tests, ranging from k = 0.0330 for MP + TBT to k = 0.0214 for CE + TBT. The presence of phytotoxic compounds in the soil and the natural senescence of the phytoremediator and sentinel species may contribute to these adverse effects [7].

Notably, the presence of the fungal inoculant reduced the phytotoxic effect of tebuthiuron in certain treatments. In the MP + FUNG + TBT treatment, *C. juncea* exhibited a rapid height increase (k = 0.0659), resulting in a steep growth curve and an impressive maximum height (α = 248.45 cm). Similar results were observed in the CE + FUNG + TBT treatment, where the maximum height (α = 199.88 cm) was higher compared to other treatments with the same species. These findings indicate the potential of the fungal inoculant in mitigating tebuthiuron toxicity, possibly through microbial degradation of the herbicide.

The bacterial inoculant also exhibited a mitigating effect, though it was generally less pronounced than that of the fungal inoculant. In the CE + BACT + TBT treatment, *C. juncea*'s growth rate was higher (k = 0.0930), and in the MP + BACT + TBT treatment, the maximum height (α = 147.18 cm) was higher compared to other treatments with the same species. This suggests that the bacterial inoculant can aid in tebuthiuron remediation in soil, albeit with some limitations [35].

Overall, these findings provide a detailed assessment of the growth dynamics of *C. juncea* as a bioindicator plant. The results elucidate the impact of microbial inoculants and the herbicide tebuthiuron on the development of the bioindicator, highlighting the potential of certain inoculants—particularly the fungal inoculant—in mitigating the herbicide's phytotoxic effects. Additionally, the efficacy of fungal inoculants in mitigating tebuthiuron toxicity may be attributed to their robust enzymatic systems capable of degrading complex organic pollutants. Fungi such as *Trichoderma* spp. produce a variety of extracellular enzymes, including laccases and peroxidases, which can oxidize and break down persistent herbicides [36]. These enzymes facilitate the cleavage of chemical bonds within the tebuthiuron molecule, transforming it into less toxic metabolites that are more amenable to further microbial degradation or assimilation by plants. The deployment of such fungi in bioaugmentation strategies not only enhances the degradation of recalcitrant compounds but also improves soil health by suppressing pathogenic microorganisms and promoting plant growth [37]. This contributes valuable knowledge to the field of phytoremediation, informing strategies for effectively addressing tebuthiuron-contaminated soils.

3.3.2. Phytomass Accumulation

The accumulation of dry biomass in *Crotalaria juncea* varied significantly across treatments, revealing critical insights into the interplay between microbial inoculants, herbicide presence, and phytoremediation efficacy (Figure 6). Notably, the treatment combining tebuthiuron with bacterial inoculants (TBT + BACT) resulted in the highest dry biomass production, reaching approximately 18 g. Despite being higher, there was no statistical significance compared to the other treatments, which suggested that the addition of specific bacterial strains effectively mitigated the herbicide's phytotoxic effects on *C. juncea*. The bacteria likely facilitated enhanced degradation or transformation of tebuthiuron, reducing its toxicity and promoting plant growth. This finding underscores the potential of bacterial inoculants as a pivotal component in phytoremediation strategies for tebuthiuron-contaminated soils.

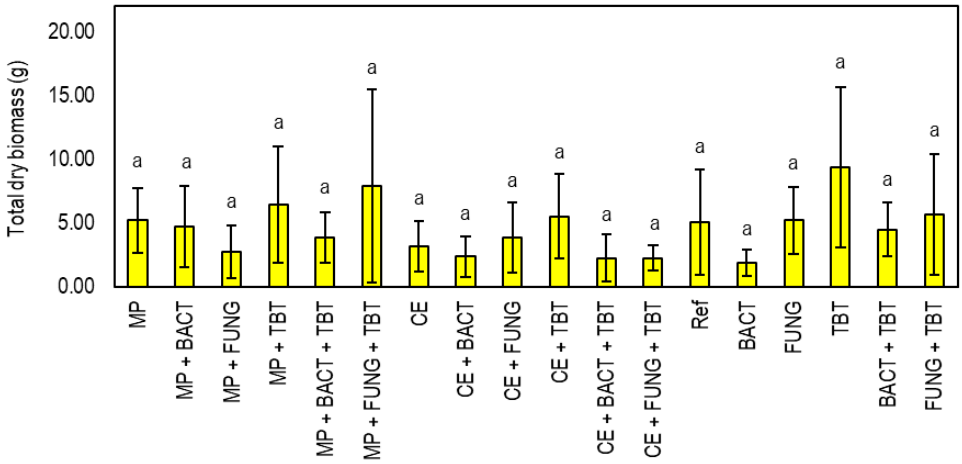

Figure 6. Production of total dry biomass of *C. juncea* in soil associated or not with tebuthiuron (TBT), bacterial (BACT), or fungal (FUNG) inoculants and/or the different plants *M. pruriens* (MP) and *C. ensiformis* (CE) after 70 DAS. Ref—reference control soil without leguminous plants, inoculants, and tebuthiuron. Same lowercase letters did not indicate statistical differences by the Tukey test ($p < 0.05$).

In stark contrast, the treatment with tebuthiuron alone (TBT) without any inoculants resulted in significantly lower biomass accumulation, around 7 g. This substantial reduction reflects the negative impact of the herbicide on the growth of *C. juncea*, confirming its phytotoxicity in the absence of bioremediation interventions. The persistence and toxicity of tebuthiuron in this treatment could be attributed to its chemical stability and the substantial organic matter content in the soil, which may enhance herbicide adsorption and reduce its bioavailability for degradation [30].

Fungal inoculants (FUNG), when applied in combination with tebuthiuron (TBT + FUNG), were less effective than bacterial inoculants in mitigating the herbicide's phytotoxicity, resulting in dry biomass of approximately 9 g. While fungi are known to play roles in biodegradation and plant growth promotion, their efficacy in this context was inferior to that of bacteria. This suggests that the specific fungal species used may not have possessed the necessary metabolic pathways to effectively degrade tebuthiuron or may have had less synergistic interactions with *C. juncea* compared to the bacterial strains.

The combinations involving prior cultivation of *M. pruriens* (MP) or *C. ensiformis* (CE) with microbial inoculants and tebuthiuron yielded variable biomass outcomes. Generally, these treatments did not achieve biomass values as high as the TBT + BACT treatment. Biomass production in these groups ranged between 5 and 12 g, indicating that the interactions between the leguminous plants, microbial inoculants, and tebuthiuron are complex. Possible antagonistic effects, such as competition for nutrients, allelopathic interactions, or microbial community shifts, may have limited the growth of *C. juncea* in these scenarios [7,34].

These findings align with previous studies emphasizing the critical role of microorganisms in enhancing plant tolerance to soil contaminants. The superior efficacy observed with bacterial inoculants may be attributed to their ability to metabolize toxic compounds, produce plant growth-promoting substances, or enhance nutrient availability, thereby creating more favorable conditions for plant development [10,32]. In contrast, the lower efficacy of fungal inoculants could be due to less efficient degradation pathways for tebuthiuron or weaker interactions with the phytoremediator and bioindicator plants.

Overall, the results highlight the importance of selecting appropriate microbial inoculants in phytoremediation strategies. The significant increase in biomass with bacterial inoculation demonstrates its potential application in agricultural practices to mitigate tebuthiuron contamination. The enhanced biomass production not only indicates improved plant health but also suggests a greater capacity for phytoremediation, as higher biomass is often correlated with increased pollutant uptake and degradation [27,28].

3.4. Bioassays with L. sativa: Validating Ecotoxicity and Phytoremediation Efficiency

Ecotoxicity testing with *L. sativa* is a pivotal tool for evaluating soil quality, particularly in environments potentially affected by herbicides like tebuthiuron. This indirect method not only detects the presence of herbicide but also verifies reductions in soil toxicity. Multiple researchers have conducted ecotoxicity tests using *L. sativa* following biological pesticide remediation experiments [7,19].

The germination index (GI) of *Lactuca sativa* served as a sensitive indicator of soil ecotoxicity and the effectiveness of phytoremediation treatments over time. In the uncultivated treatments (Figure 7), the reference soil (Ref) initially exhibited a significantly higher GI compared to other treatments without tebuthiuron from 0 to 40 DAS. However, by the end of the experimental period, the bacterial inoculant treatment (BACT) surpassed both the Ref and fungal inoculant (FUNG) treatments in final GI values (0.95 > 0.92 > 0.89, respectively) (Table 4). The higher growth rate (k value) observed for BACT indicates its positive influence on the germination and early development of *L. sativa* seeds, possibly through enhanced nutrient availability or the production of growth-promoting substances.

Table 4. Parameters of the Gompertz kinetic models for the germination index of *L. sativa* in ecotoxicity bioassays in tebuthiuron (TBT) soil with *M. pruriens* (MP) and *C. ensiformis* (CE) and bacterial (BACT) or fungal (FUNG) inoculants. Ref—reference control soil without leguminous plants, inoculants, and tebuthiuron.

Treatments	**Complexity**			**Adequacy**		
	α	**β**	**k**	**R^2**	**AIC**	**BIC**
MP	1.00	6.98	0.04774	0.98	−11.64	−12.47
MP + TBT	0.97	10.09	0.06319	0.98	−13.20	−14.04
MP + BACT	1.00	7.06	0.04656	0.98	−13.15	−13.99
MP + FUNG	0.97	10.09	0.06319	0.98	−13.20	−14.04
MP + BACT + TBT	1.00	6.81	0.04618	0.98	−11.94	−12.77
MP + FUNG + TBT	1.00	7.49	0.05091	0.98	−11.67	−12.51
CE	1.00	5.94	0.04001	0.98	−11.13	−11.97
CE + TBT	1.00	7.25	0.03861	0.99	−21.18	−22.02
CE + BACT	1.00	6.96	0.04861	0.98	−10.72	−11.56
CE + FUNG	1.00	7.03	0.04880	0.98	−10.94	−11.77
CE + BACT + TBT	1.00	6.46	0.04224	0.98	−12.94	−13.77
CE + FUNG + TBT	1.00	7.06	0.04797	0.98	−11.94	−12.77
Ref	1.00	3.27	0.02642	0.99	−19.10	−19.93
TBT	1.00	3.65	0.03053	0.99	−24.09	−24.92
BACT	1.00	2.84	0.02250	0.96	−8.90	−9.73
FUNG	1.00	3.01	0.03141	0.99	−15.20	−16.03
BACT + TBT	1.00	3.28	0.03499	0.99	−18.46	−19.29
FUNG + TBT	1.00	3.33	0.02641	0.99	−19.68	−20.51

Parameters α, β, and k denote the superior asymmetry, the inflection point, and the exponential decay of the specific growth rate, respectively; $\beta = 1$ keeps the relative decrease with time constant; $\beta > 1$ accelerates the relative decrease with time; $\beta < 1$ slows the relative decrease with time; R^2: coefficient of determination; AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion.

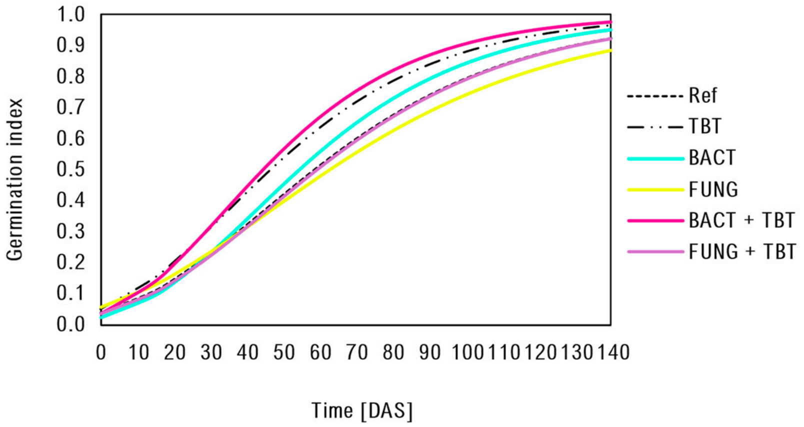

Figure 7. Kinetic evolution from the Gompertz model of the germination index of *L. sativa* in ecotoxicity bioassays in soil with tebuthiuron (TBT) and fungal (FUNG) or bacterial (BACT) inoculants from the Gompertz model. Ref—reference control soil without leguminous plants, inoculants, and tebuthiuron.

Intriguingly, the treatment containing only tebuthiuron (TBT) without any inoculants showed a higher final GI (0.96) and faster growth rate (k = 0.0314) compared to the Ref. This unexpected result suggests that natural attenuation processes were at play, whereby indigenous soil microorganisms gradually degraded the herbicide over time, reducing its phytotoxicity [38,39]. As tebuthiuron concentrations decreased, the inhibitory effects on seed germination diminished, allowing *L. sativa* to achieve higher GI values.

The impact of microbial inoculants in tebuthiuron-contaminated soil differed between bacteria and fungi. The BACT + TBT treatment exhibited the highest final GI (0.98) and growth rate (k = 0.0350), indicating that bacterial inoculants effectively enhanced the dissipation of tebuthiuron's toxic effects, facilitating seed germination and growth. In contrast, the FUNG + TBT treatment had a lower final GI (0.92) and growth rate (k = 0.0264), suggesting that the fungal inoculant was less effective in mitigating the herbicide's ecotoxicity.

The prior cultivation of *M. pruriens* (MP) and *C. ensiformis* (CE) also influenced the GI of *L. sativa* (Figure 8A,C). Initially, the GI in these treatments was lower than the Ref between 0 and 40 DAS, possibly due to residual allelopathic compounds from the leguminous plants or incomplete degradation of tebuthiuron. Over time, however, the GI increased, reaching 0.99 in the MP treatment (Table 4), indicating a reduction in soil toxicity. The higher growth rate (k = 0.0477) compared to the Ref (k = 0.0264) suggests that the prior cultivation of *M. pruriens* improved soil conditions, possibly through enhanced microbial activity, nutrient cycling, and the degradation of residual herbicides.

The combination of microbial inoculants with leguminous plants further affected the GI. In the MP + BACT treatment without tebuthiuron, the growth rate increased (k = 0.0632) compared to MP alone, although the final GI was slightly lower (0.97 < 0.99). This indicates that while bacterial inoculants can accelerate the reduction in ecotoxicity, they may also introduce competitive dynamics that slightly affect germination rates. In treatments with tebuthiuron, microbial inoculation appeared to reduce the negative ecotoxicological impact, as evidenced by higher GI values compared to treatments without inoculants.

Overall, the GI of *L. sativa* provided valuable insights into the temporal dynamics of soil ecotoxicity and the effectiveness of phytoremediation strategies. The results highlight the potential of combining leguminous plants with specific microbial inoculants to enhance the degradation of tebuthiuron and reduce its phytotoxic effects. However, the complexity of interactions among plants, microorganisms, and contaminants underscores the need for careful selection and optimization of phytoremediation components to achieve effective soil remediation [38,39].

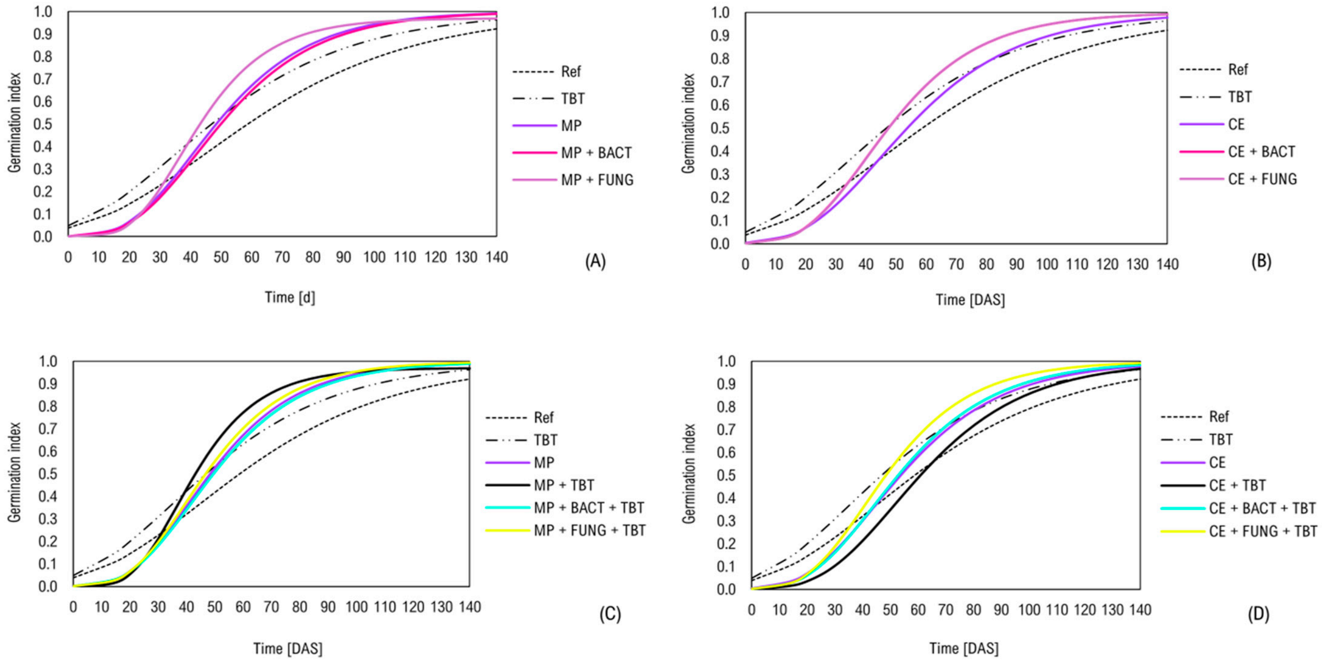

Figure 8. Kinetic evolution from the Gompertz model of the germination index of *L. sativa* in ecotoxicity bioassays in tebuthiuron (TBT) soil with *M. pruriens* (MP) and *C. ensiformis* (CE) and bacterial (BACT) or fungal (FUNG) inoculants. Ref—reference control soil without leguminous plants, inoculants, and tebuthiuron. (**A**,**B**) Treatments without TBT. (**C**,**D**) Treatments with TBT.

Furthermore, the physicochemical properties of the soil, such as pH, organic matter content, and cation exchange capacity, significantly influence the bioavailability and persistence of tebuthiuron [40]. Soils with high organic matter can adsorb greater amounts of herbicides, potentially reducing their immediate bioavailability to plants and microbes but also prolonging their environmental persistence [41]. Adjusting soil conditions through amendments like compost or biochar can enhance microbial activity and modify sorption characteristics, thereby improving degradation rates [42]. Tailoring phytoremediation strategies to account for these soil properties is essential for optimizing contaminant removal and ensuring the sustainability of remediation efforts [43].

3.5. The Impact of the Soil–Herbicide–Plant–Microbe Nexus on L. sativa's GI: A Deeper Understanding of Bioremediation

A multivariate response analysis using a mixed linear model (Figure 9) from 0 to 140 DAS elucidated the intricate interactions among tebuthiuron, leguminous plants, and microbial inoculants on the germination index of *L. sativa*. This analysis revealed that the combined effects of these factors played a crucial role in determining seed germination rates and seedling development.

When leguminous plants and microbial inoculants were combined, the treatments displayed a less steep slope in the model, indicating a strong interdependence between these factors. This synergistic interaction resulted in higher germination rates of *L. sativa*, even in the presence of tebuthiuron (accounted for as a random effect in the model). The combination of potential phytoremediators and bioaugmentation appeared to mitigate the phytotoxic effects of the herbicide more effectively than either factor alone.

In contrast, the individual effects of leguminous plants or microbial inoculants exhibited steeper slopes, suggesting a lower impact on reducing tebuthiuron toxicity when applied independently. This implies that microbial inoculants alone may not sufficiently alleviate the herbicide's phytotoxicity, and similarly, the cultivation of leguminous species without microbial augmentation may have limited efficacy.

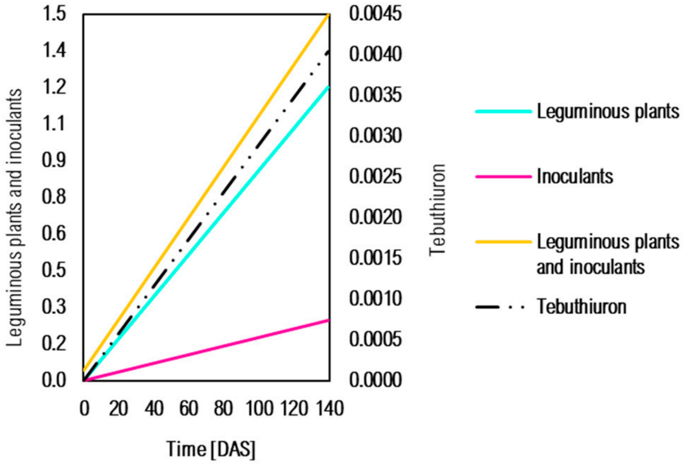

Figure 9. Dynamics of random (tebuthiuron) and fixed (leguminous plants—*M. pruriens* and *C. ensiformis*; and microbial inoculants—fungal and bacterial) effects on the specific rate of the germination index (GI) of *L. sativa* in ecotoxicity bioassays.

The enhanced performance of the combined treatments can be attributed to several mechanisms. The leguminous plants likely improved soil health by increasing organic matter content, enhancing nutrient availability through nitrogen fixation, and stimulating microbial activity [21,22]. The introduced microbial inoculants may have possessed specific degradative capabilities for tebuthiuron or facilitated the proliferation of indigenous degrader populations, leading to accelerated herbicide dissipation [9,10].

These findings underscore the importance of integrated phytoremediation strategies that leverage synergistic interactions between plants and microorganisms. By combining phytoremediators with bioaugmentation, it is possible to enhance the degradation of persistent contaminants like tebuthiuron, improve soil quality, and reduce ecotoxicity more effectively than with single-factor approaches.

3.6. Limitations and Directions to Improve the Credibility and Practicality of Phytoremediation

While this study demonstrates the potential of combining leguminous plants and microbial inoculants for the phytoremediation of tebuthiuron-contaminated soils, several limitations warrant consideration for future research and practical application.

The experiments were conducted under controlled greenhouse conditions, which may not fully capture the complexities of field environments. Factors such as soil heterogeneity, climatic variations, and ecological interactions can significantly influence phytoremediation outcomes. Field trials are essential to validate the effectiveness of the proposed strategies under real-world conditions, accounting for spatial and temporal variability.

Assessing the long-term sustainability of phytoremediation efforts is crucial. Continuous monitoring of contaminant levels, soil health indicators, and ecological impacts over extended periods will provide insights into the persistence of remediation effects and potential rebound of contaminant concentrations. In addition, agricultural soils are often contaminated with a mixture of pesticides and other pollutants. Future studies should investigate the efficacy of phytoremediation strategies in the context of multiple contaminants to develop comprehensive remediation approaches. Additionally, understanding how soil properties such as texture, organic matter content, and microbial diversity influence remediation processes will enable more tailored interventions. A deeper understanding of the microbial community dynamics is also essential. Metagenomic and metatranscriptomic analyses can identify key microbial taxa involved in contaminant degradation and elucidate functional pathways. This knowledge can inform the selection or engineering of more effective microbial consortia for bioaugmentation.

Economic analyses comparing phytoremediation to conventional remediation methods are necessary to assess cost-effectiveness. Factors such as the cost of microbial inoculants, plant cultivation, timeframes for remediation, and potential economic benefits from biomass utilization should be considered. Developing scalable and economically viable phytoremediation models will facilitate broader adoption in agricultural practices. The effective implementation of phytoremediation strategies requires supportive policies, regulatory frameworks, and stakeholder engagement. Educating farmers, land managers, and policymakers about the benefits and limitations of phytoremediation will promote its integration into sustainable land management practices.

3.7. Future Perspectives

Advancements in phytoremediation research hold promise for enhancing the efficiency and applicability of this eco-friendly remediation method. Future directions include (a) plant–microbe interactions, exploring novel symbiotic relationships, and co-cultivation techniques can optimize contaminant degradation. Genetic studies may reveal plant traits that enhance microbial colonization and activity; (b) genetic engineering, developing transgenic plants with enhanced metabolic capabilities to degrade specific contaminants offers potential, though ecological risks and ethical considerations must be addressed; (c) integrated remediation strategies, combining phytoremediation with other remediation technologies, such as biostimulation, chemical oxidation, or nanotechnology, may overcome limitations associated with single-method approaches; (d) urban and aquatic applications, extending phytoremediation to urban settings and aquatic environments requires adaptation to unique challenges, such as space constraints and pollutant dispersion dynamics; and (e) policy development and public awareness, establishing clear guidelines, safety protocols, and public education initiatives will facilitate acceptance and trust in phytoremediation practices.

As environmental concerns escalate globally, the practical application of phytoremediation on a larger scale becomes increasingly significant. By addressing the current limitations and capitalizing on emerging research, phytoremediation can evolve into a more robust and versatile tool for mitigating environmental pollution, promoting ecosystem health, and supporting sustainable agricultural practices.

4. Conclusions

This study has demonstrated that integrating leguminous plants with microbial inoculants offers a promising and sustainable strategy for the phytoremediation of tebuthiuron-contaminated agricultural soils. *M. pruriens* exhibited a faster growth rate than *C. ensiformis*, but both species experienced growth inhibition due to the herbicide's phytotoxic effects. The application of microbial inoculants significantly mitigated these negative impacts, with fungal inoculants particularly enhancing plant performance in *M. pruriens*. This suggests that specific fungi may possess metabolic pathways capable of degrading or transforming tebuthiuron into less toxic compounds, thereby promoting plant growth even in contaminated conditions. The use of *C. juncea* as a bioindicator validated the effectiveness of the remediation process. Treatments combining tebuthiuron with bacterial inoculants resulted in the highest biomass production of *C. juncea* and a marked reduction in herbicide toxicity, highlighting the potential of bacterial strains in enhancing tebuthiuron degradation. Ecotoxicological bioassays with *L. sativa* further confirmed that the combination of leguminous plants with microbial inoculants accelerated soil detoxification, achieving higher germination indices and growth rates compared to treatments without inoculants or prior cultivation. These findings accentuate the synergistic effects of plant–microbe interactions in enhancing the degradation of persistent herbicides, improving soil health, and reducing environmental toxicity.

While the results are promising, it is important to recognize that the experiments were conducted under controlled greenhouse conditions, which may not fully replicate the complexities of field environments. Field trials are essential to validate these phytoremediation strategies in real-world settings and to assess their long-term sustainability and effectiveness under diverse environmental conditions. Future research should focus on scaling up these approaches, analyzing microbial community dynamics to identify key degraders of tebuthiuron, optimizing plant–microbe combinations for specific soils and climates, evaluating economic feasibility, and developing supportive policies to facilitate the integration of phytoremediation into sustainable agricultural practices. In conclusion, the integration of leguminous plants with specific microbial inoculants presents a viable and eco-friendly approach for remediating tebuthiuron-contaminated soils. This strategy not only contributes to the restoration of soil quality and protection of environmental health but also advances sustainable land management practices, promoting healthier agricultural ecosystems and benefiting society as a whole.

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Data Availability Statement: The authors confirm that the data supporting the findings of this study are available in an online repository (UNESP-Brazil) and on request from the corresponding author (PRML).

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5.6. Anexo VI

Bruno Rafael de Almeida MOREIRA; Victor Hugo CRUZ; Marcelo Rodrigues BARBOSA JÚNIOR; Leonardo Gomes de VASCONCELOS; Rouverson Pereira da SILVA; Paulo Renato Matos LOPES.

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ORIGINAL ARTICLE

Adsorption of tebuthiuron on hydrochar: structural, kinetic, isothermal, and mechanistic modeling, and ecotoxicological validation of remediative treatment of aqueous system

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Abstract

Tebuthiuron ($C_9H_{16}N_4OS$) offers farmers a cost-effective chemical solution to control weeds. Nevertheless, it can manifest as a hazardous organic compound to society and the environment as it escapes from agroecosystems into the surroundings via leaching and running off, polluting surface and underground water bodies. Hence, research was designed to analyze whether hydrochar can develop an adsorbent to remove it from an aqueous solution. Food waste was reacted with subcritical water at a stoichiometric 1:4 ratio ($m\ v^{-1}$) and 1.5 M potassium hydroxide (KOH) at 10 g L^{-1} at 250 °C and 1.5 MPa for 2 h to produce porous hydrochar via simultaneous hydrothermal carbonization and chemical activation. The product at 25, 50, and 100 mg L^{-1} was tested for its ability to adsorb tebuthiuron (TBT) at 0.5, 1, and 1.5 mg L^{-1} by spectrophotometry. In addition, kinetic and isothermal models were applied to experimental data to describe the separation of the pollutant from the liquid-phase analytical environment. Equally significant, an ecotoxicological assay was developed to investigate its remediative potential; *Lactuca sativa* was employed as a testing organism, as it is responsive to TBT at phytotoxic residual quantity. Hydrochar significantly separated TBT from aqueous media. Such honeycomb-structured mesoporous carbonaceous matrix developed approximately 1420.1 $m^2\ g^{-1}$ specific surface area and 0.05 $cm^3\ g^{-1}$ total pore volume; hence, at the highest concentration, it adsorbed 98.65% of TBT at 1.5 mg L^{-1} through physical (e.g., pore filling and interparticle diffusion) or chemical (e.g., H-bonding, π-stacking, and metal-adsorbate complex) forces. In addition, it allowed seven adsorption-desorption cycles with 80% efficiency, supporting excellent regenerability. Equally significant, *L. sativa* germinated 76.6% on plates containing residual solution from sorption testing, validating the hydrochar for environmental bioremediation. Hence, it can advance the field's prominence in treating TBT by bioadsorption. It can offer stakeholders across agroindustries possibilities to remediate such a compound in aquatic environments, such as water and wastewater.

Keywords Bioremediation · Hazardous organic compound · Municipal solid waste · Phenyl-urea herbicide · Porous material

Bruno Rafael de Almeida Moreira, Victor Hugo Cruz, and Marcelo Rodrigues Barbosa Júnior contributed equally to this work and share the first authorship.

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Abbreviations

BET	Brunauer-Emmett-Teller
BJH	Barret-Joyner-Halenda
CBN	Carbon black nanoparticle
EFM	Electric force microscopy
FTIR	Fourier-transform infrared
HTC	Hydrothermal carbonization
IUPAC	International Union of Pure and Applied Chemistry
LCA	Life-cycle assessment
LD_{50}	Median lethal dose
MOF	Metal-organic framework
pH_{pzc}	Point of zero charge
POF	Porous organic framework

SEM	Scanning electron microscopy
TBT	Tebuthiuron
TEA	Techno-economic analysis
XRD	X-ray diffractometry
ZIF	Zeolite imidazolate framework

1 Introduction

Tebuthiuron ($C_9H_{16}N_4OS$) offers farmers a cost-effective chemical solution to control weeds [1]. Nevertheless, it can manifest as a hazardous organic compound to society and the environment [2] as it is significantly soluble in water (2.5 g L^{-1}, 25 °C) and can escape from agroecosystems into the surroundings via leaching and running off [1]. As a result, it can occur at an approximate concentration of 6.5 µg L^{-1} in water bodies [3], disturbing aquatic life [4]. Equally significant, it can negatively impact beneficial organisms, such as honey bees and fishes ($LD_{50} > 100$ mg L^{-1}), and human health ($LD_{50} > 500$ mg kg^{-1}) through bioaccumulation in food webs [5], driving the necessity for developing a remediative solution to balance its techno-economic benefits and trade-offs to agriculture and sustainable development [2].

Studies on the remediation of tebuthiuron are emerging topics. They base their methodologies on electrocatalytic systems [6], plants [7, 8], and microorganisms [9, 10]. Photoelectro-Fenton reactions can efficiently remove such a persistent compound from industrial and environmental effluents [11–14]. Nevertheless, they may require applying an activator metal, such as Fe^{2+}, or ultraviolet-C radiation to establish a functional treatment. As a result, a significant quantity of ferrous iron sludge can occur as hazardous waste, opening the opportunity to investigate eco-friendlier solutions, such as phytoremediation [7, 8] and microbial biodegradation [9, 10].

Mendes et al. [8] and Ferreira et al. [7] independently tested *Crotalaria spectabilis*, *Canavalia ensiformis*, *Mucuna pruriens*, *Pennisetum glaucum*, and *Stilizobium aterrimum* for the removal of tebuthiuron from soils. Such biological models significantly extracted or stabilized the herbicide through the root system. As a result of decreasing ecotoxicity, they allowed *L. sativa* to germinate, grow, and develop phenotypically normal seedlings on plates containing residual media. Nevertheless, species of green manure may require introducing a source of metabolizable carbon, such as vinasse, or implementing specific management into the system to function, limiting its reproduction at an industrial scale. Microbial biodegradation may offer an alternative to phytoremediation [15]. Bacteria can degrade tebuthiuron in marine waters through hydrolysis [16]. Nevertheless, they demand strict environmental conditions to develop and sustain efficient treatment. In addition, they may imbalance natural ecosystems by introducing foreign genes and creating unpredictable and uncontrollable antagonistic interactions with autochthonous microbiota, opening the opportunity of analyzing the remediative potential of pyrolytic and hydrothermal chars [17].

As a product of HTC of biomass or organic waste, hydrochar can develop an efficient adsorbent for agricultural, industrial, and environmental applications. It consists of functional pores and chemical groups on the outer layer and aromatic skeleton [18]; hence, it can treat dyes, such as crystal blue [19] and rhodamine B [20], as effectively as biochar. Nevertheless, it can develop a higher utility, as the catalytic phase (e.g., subcritical water with or without an acidic or alkaline activator) of the system allows a reactor to convert biomass to a pore-functional matrix at 180–260 °C; the reference interval for the processing of biochar is 300–800 °C. In addition, it may remove post-synthesis activation, further increasing the operating cost-effectiveness [19]. Equally significant, simultaneous HTC and chemical activation of organic material with H_2SO_4, H_3PO_4, HNO_3, or NaOH can improve the physicochemical and textural properties of the product, such as surface area and active binding forces. Nevertheless, it can decrease conversion, opening the opportunity for improvements [21].

The remediative treatment of tebuthiuron is a relevant topic to sustainable agriculture. Nevertheless, its research is at an early stage of development. Especially for bioadsorption, results and conclusions are inconsistent, and the analytical characterization of absorbing carbonaceous matrices is insufficient to understand and improve the process. Hence, a comprehensive, multi-objective study was designed to (i) analyze whether hydrochar can develop functional solid-phase separation of tebuthiuron from an aqueous solution; (ii) investigate the impact of varying adsorbent/adsorbate ratio on the capabilities and rates of the system; and (iii) characterize the bioproduct to inform its theoretical mechanisms for tunable and controllable application in prospective aquatic environments.

2 Material and methods

Research (Figure S1, Supplementary material) was designed to convert food waste to a functional porous carbonaceous adsorbent for the solid-phase separation of TBT from an aqueous solution. The technical quality of the hydrothermal bioproduct was tested for static adsorptive capability. In addition, it was comprehensively characterized for standard morphological, textural, and spectral properties. Equally significant, an ecotoxicological assay was conducted to validate its remediative potential for treating water and wastewater. Methods that are well-established in the literature were summarized and indicated by a reference to ensure the eligibility and reproducibility of our approach.

2.1 Acquisition of food waste and chemical activator

Food waste were acquired from restaurants and potassium hydroxide (KOH) from Sigma Aldrich (Burlington, MA, USA). The food waste consisted of vegetables and fruits (Figure S1, Supplementary material). The analytical purity for KOH (anhydrous) was 99.95%. Materials were stored in sterile receptacles in the laboratory at ambient temperature and without light to avoid photodecomposition.

2.2 Simultaneous synthesis and chemical activation of hydrothermal bioadsorbent

A one-step synthesis and chemical activation (Figure S1, Supplementary material) was planned to address a resource-saving approach. Food waste and subcritical water at a stoichiometric 1:4 ratio (m v^{-1}) and 1.5 M potassium hydroxide (KOH) at 10 g L^{-1} were introduced into the reactor for the treatment. The equipment was programmed to convert the material to a pore-functional hydrochar at 250 °C and 1.5 MPa for 2 h [22]. Such a solid by-product was physically recovered from it and, immediately, cooled to room temperature in a freezing solution to cease catalytic coalification. Subsequently, it was rinsed with ultrapure water to remove soluble inorganic ions [23]. Afterward, it was separated from the liquid phase by filtration in a cellulose nitrate membrane. Finally, it was heated in an oven at 75 ± 2.5 °C to constant mass, obtaining a dry and pure powder for sorption testing. The percentage yield of the process was calculated by the gravimetric difference in quantities of product and starting material.

2.3 Technical assessment

The hydrochar was comprehensively assessed for standard indicators of technical quality, such as potential removal, kinetic and isothermal capability, recyclability, and relative efficiency. In addition, the aqueous solution remaining in the sorption testing was tested for its toxicity to *L. sativa*, which is TBT-sensitive organism and can establish a bioindicator of remediative performance [9].

2.3.1 Potential removal

The efficiency of hydrochar at 25, 50, and 100 mg L^{-1} in removing TBT at 0.5, 1, and 1.5 mg L^{-1} from an aqueous solution was quantified by spectrophotometry [23]. Pesticide-containing analytical solutions were prepared by pipetting 1, 2, and 3 μL was from Combine® (Dow AgroSciences, 500 g TBT L^{-1}) and adding to 1 L ultrapure water. The previous components were introduced into glass containers with 100 mL of ultrapure water. They were stirred in a magnetic table at 40 rpm and room temperature until obtaining a homogeneous solution. Aliquots of 1 mL were centrifugated at 12,000 rpm for 5 min to separate the solid from the liquid phase. The analytical concentration of TBT remaining in the liquid phase of the system was quantified by the difference in absorbance in a UV-Vis spectrophotometer at 540 rpm. Such a sorption testing was conducted for 24 h with a sampling resolution of 1 h to collect sufficient data for kinetic and equilibrium adsorption modeling. The percentage removal of TBT by hydrochar was calculated through Eq. (1). Importantly, 50 and 1 mg L^{-1} were established as the respective reference concentrations of adsorbent and adsorbate to analyze the effect of varying their relationship. Equally significant, tests were triplicate to reduce systematic errors and ensure consistent results.

$$R = \left({}^{C_0 - C_t}\!\big/_{C_0} \right) \times 100 \tag{1}$$

R: potential removal, %;
C_0: initial concentration of the analyte, ppm;
C_t: concentration of the analyte at time t, ppm.

2.3.2 Kinetic adsorption modeling

Lagergren's and Ho's pseudo-functions (Eqs. (2) and (3)) were applied to spectrophotometric data to model kinetic adsorption [24].

$$q_t = q_e\left(1 - e^{-k_1 t}\right) \tag{2}$$

$$q_t = {}^{k_2 {q_e}^2 t}\!\big/_{1 + k_2 {q_e}^2 t} \tag{3}$$

q_t: adsorptive capacity, mg g^{-1};
k_1: Lagergren's constant, h^{-1};
k_2: Ho's constant, mg g^{-1} h^{-1}.

2.3.3 Equilibrium adsorption modeling

The adsorptive capability of the TBT-water-hydrochar system at equilibrium was estimated by fitting spectrophotometric data for Freundlich's, Langmuir's, and Temkin's isothermal functions (Eqs. (4)–(6)) [25].

$$q_e = {}^{q_m K_L P}\!\big/_{1 + K_L P} \tag{4}$$

$$q_e = K_F P^{\,{}^{1}\!/_{n}} \tag{5}$$

$$q_e = {}^{\ln(K_T P) RT}\!\big/_{b} \tag{6}$$

q_e: adsorption at equilibrium, mg g^{-1};
q_m: maximum adsorption, mg g^{-1};
K_L: Langmuir's constant, L mg^{-1};

K_F: Freundlich's constant; mg g^{-1} atm^{-1};
K_T: Temkin's constant, L mg^{-1};
P: pressure, atm;
R: universal gas constant, 8.314×10^{-3} J K^{-1} mol^{-1};
T: temperature, K;
b: sorption energy, J mol^{-1}.

2.3.4 Cycling performance

The TBT-water-hydrochar system was investigated for regenerability over multiple adsorption-desorption cycles. Hence, it was exposed to ten long-term batches of 24 h; the quantities of hydrochar were 25, 50, and 100 mg L^{-1}, while the concentration of TBT was the standard 1 mg L^{-1}. After adsorption, solid material was separated from the bulk aqueous environment by filtration in a cellulose nitrate membrane. Subsequently, it was eluted in 50 mL of ethanol at 0.1 M for regeneration for 3 h. Afterward, it was centrifugated and rinsed with ultrapure water to remove excess humidity and inorganic elements. Finally, it was dried in an oven at 62 ± 1.5 °C for 4 h for a new repetition [26].

2.3.5 Relative efficiency and textural quality

The hydrochar was compared to conventional pesticide-adsorbing matrices available in the literature, such as CBN, graphene, ZIF, MOF, and POF, by removal potential and textural quality (Table S1, Supplementary material) to determine whether it can offer a high-throughput solution for treating aqueous systems.

2.4 Ecotoxicological bioassay

An ecotoxicological bioassay was conducted to validate the efficiency of hydrochar in removing TBT from the aqueous environment. Aliquots of 2 mL were collected from the liquid phase of the experimental units remaining in the sorption testing. They were homogeneously distributed on sterile glass plates with ten seeds of *L. sativa* as a bioindicator of toxic residual TBT [9]. The experimental units were transferred to a climatic chamber at 25 ± 2 °C, 60 ± 5%, and 12-h photoperiod. The growth and development of the biological model were monitored for five days [9]. Its percentage germination was quantified visually (Figure S1 and S2, Supplementary material).

2.5 Characterization

Materials and products were analytically and comprehensively characterized for morphological, textural, physical, chemical, and electrostatic indicators of scalable functionality.

2.5.1 N_2 adsorption-desorption capability, textural quality, pH, and ζ-potential

A gravimetric sorptometry was conducted on hydrochar to determine N_2 adsorption-desorption capability and the pore's volume and diameter. Samples of 0.1 g were loaded on an ultra-sensitive micro-thermobalance in a sorptometer (Q5000 SA, TA Instruments). They were outgassed at 150 °C for 10 h prior to programming the analyzer to measure alterations in mass under a pure atmosphere of N_2 at − 196 °C and with unit relative pressure for the system [25]. The BET surface area and BJH total pore volume were calculated from data on isothermal N_2 adsorption-adsorption [27]. In addition, the ζ-potential were determined from data on electric potential and pH and by a Zetasizer (DLS-ZP, Malvern Panalytical) to validate an electrokinetic modeling.

2.5.2 External morphology, surface chemistry and functionality, and thermal stability

A SEM (EVO LS15, Carl Zeiss) was conducted to produce high-resolution micrographs of the morphological and textural aspects of the KOH-activated hydrochar. Its elemental composition was quantitatively and qualitatively analyzed by XRD with copper radiation at λ equals 0.2 nm [23]. A diffractometer device (Ultima IV, Rigaku) was attached to the SEM system, where imaging capability identifies elements across an area of a sample. In addition, FTIR spectroscopy (Thermo Nicolet NEXUS 670, GMI Inc.) was performed to profile its functional groups at 4000–400 cm^{-1} [28] and hypothesize mechanisms for adsorption.

2.6 Statistical data analysis

A descriptive analysis was performed to calculate summary statistics of potential removal and textural quality between hydrothermal bioadsorbent and conventional pesticide-adsorbing matrices. A bar chart with a histogram was designed to present them. In addition, data-to-viz diagrams were elaborated on to better illustrate and make it smoother for prospective readers to identify and interpret patterns of static (isocurve plot) and kinetic (scattering plot) adsorption, regenerability (scattering plot), the ecotoxicological effect of residual TBT on *L. sativa* (contour plot), sorption and pore's volume and diameter (scattering plot), liquid-phase electroconductivity (scattering plot) and pH (isocurve plot), crystallographic structure and elemental composition (micrograph), and absorption of infrared energy (Fourier-transform spectrogram). Analyses were ran in software R for statistical computing and graphs.

3 Results and discussion

3.1 N_2 adsorption-desorption capability, morphology, and textural quality of hydrochar

Hydrochar developed an IV isotherm and a H_3 hysteresis loop (Figure S3, Supplementary material). These are characteristics of a mesoporous ($2 \leq Ø \leq 50$ nm) material (Fig. 1), as per IUPAC. Type H_3 predicts an inherent mesoporosity and aggregation of plate-like particles to slit-shaped pores (Figure S4, Supplementary material) without a limiting adsorptive capability at high relative pressure. Its slope results from pulling force. Typically, it occurs for N_2 between 0.4 and 0.45 relative pressure [29]. Phosphorus-doped biochar [30] and hydroxyl-functional POF with an amphiphilic feature [31] can develop an IV isotherm and a H_3 hysteresis loop, concording to analytical trends in this study. Their mesopores and micropores ($Ø < 2$ nm), which generally are not accessible by SEM [32], can support a satisfactory removal of pesticides in aqueous systems.

In contrast, a bionic MOF for the magnetic solid-phase separation of organophosphorus pesticides from vegetables can rapidly adsorb N_2 up to 0.85 relative pressure. Nevertheless, its adsorptive capability sharply decreased at higher P/P_o [33]. Those authors described it as a macroporous material with a convex downward trend (III isotherm). In addition, they obtained approximately 68.8 $m^2\ g^{-1}$ BET surface area and 0.2 $cm^3\ g^{-1}$ BJH total pore volume, supporting a conducive structure to adsorbing organic compounds. Another nanometer biomaterial can develop 129 $m^2\ g^{-1}$ and 0.05 $cm^3\ g^{-1}$, respectively, making it functional for removing thiamethoxam from an aqueous system [34]. The size of its particles fluctuated from 12 to 26 nm, while the value estimated in this study was 40 µm at a 5×10^{-3} $cm^{-3}\ g^{-1}$ volume (Figure S3, Supplementary material).

Textural quality affects the adsorptive capability of a porous matrix as effectively as typology and morphology. Haq et al. [35] developed a ZnO-doped composite for the high-throughput adsorption of metribuzin. The authors characterized it by SEM. Hence, they detected pores and cavities on the surface, which can positively impact adsorption.

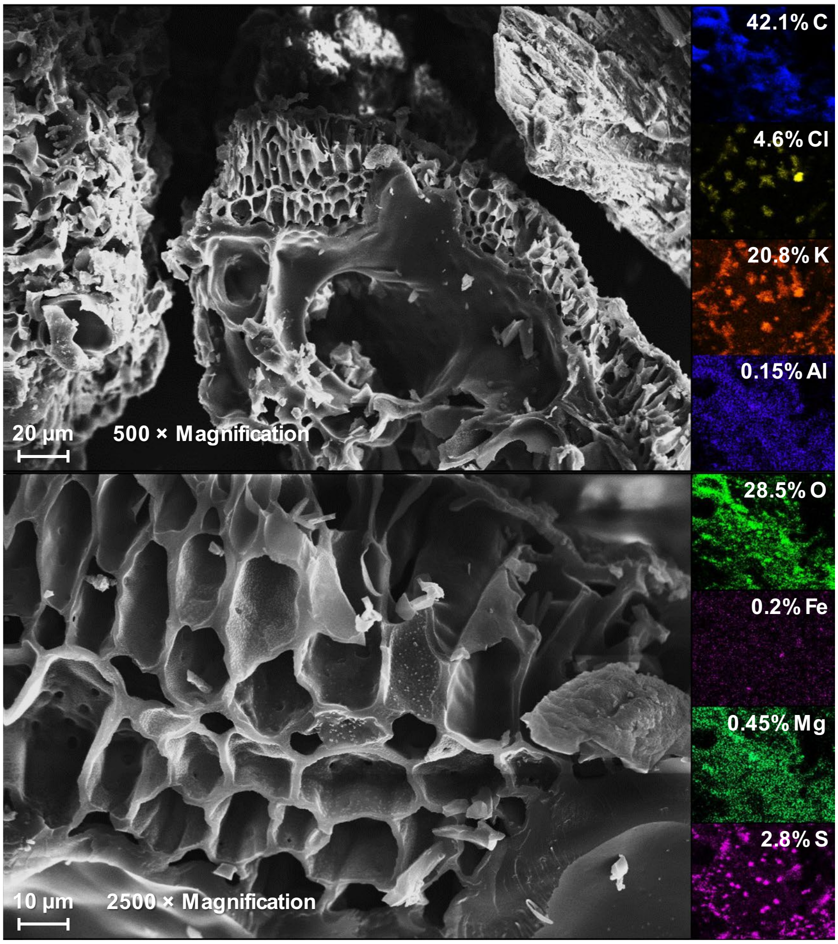


Fig. 1 High-resolution micrographs and elemental mapping of hydrochar

Nevertheless, as ZnO impregnated the material, it filled into pores and decreased roughness. An elemental mapping supported such a statement by those authors since it detected ZnO immobilized on samples. In addition, it allowed them to confirm biosorption. As metribuzin contains sulfur, such an element appeared on the surface of the composite. In this study, S was an indicator of tebuthiuron on hydrochar. Approximately 2.8% S occurred in the bioproduct, which developed an irregular structure containing honeycomb-like and sponge-like arrangements covered by ellipsoidal particles (Fig. 1).

The previous morphological patterns can enhance crystallinity (Table 1) and adsorbability by increasing available surface area [36]. In addition, flat-shaped sheets (Figure S4, Supplementary material) across smooth regions of the adsorbent may interact with the adsorbate through π–π stacking. Such an orbital overlap is conducive to the adsorption of aromatic compounds [37], such as phenyl-urea herbicides [38]. Wang et al. [31] identified a broad peak at 20° in a diffractogram for a POF, supporting a predominately amorphous specimen and, more importantly, the ability of the hydrochar from this study to develop a higher crystallinity (Table 1 and Figure S5, Supplementary material).

3.2 Technical efficiency of hydrochar in removing tebuthiuron from aqueous solution

The hydrochar developed a functional solid-phase separation of TBT from an aqueous solution (Fig. 2). Such an adsorbent at 50 mg L^{-1} removed 91.2 ± 2.5% of adsorbate at 1 mg L^{-1}. At 100 mg L^{-1}, it further adsorbed 97.4 ± 0.6%. Nevertheless, at 25 mg L^{-1}, it separated a lower portion of 91.6 ± 2.3% of the analyte at a standard concentration. Hence, the higher the adsorbent concentration, the more adsorbate it removes. Its technical efficiency increases while maintaining a recoverable feature. A similar upward trend was identified for adsorbate concentration. A 92.6 ± 2.9% efficiency was calculated at 0.5 mg L^{-1} TBT. The removal increased to 97.8 ± 0.85% at 1.5 mg L^{-1}. As the available adsorbate increases in a liquid environment, it enhances effective retention on a functional mesoporous biocarbon until saturation.

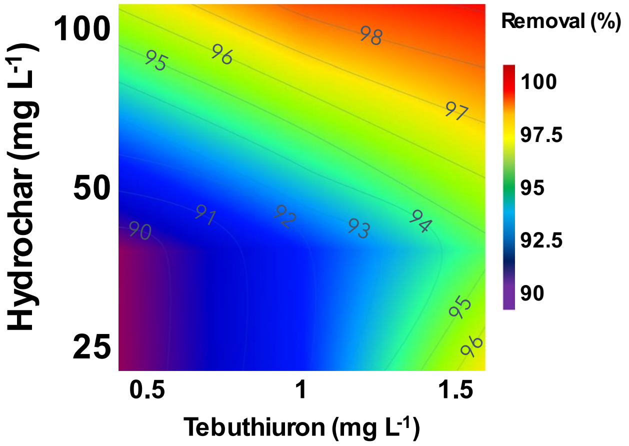


Fig. 2 Technical efficiency of hydrochar in removing tebuthiuron from aqueous solution

Table 1 Technical features of the remediative adsorptive tebuthiuron-water-hydrochar system

Indicator	Unit
	Adsorbent
Yield	29.8%
Potential removal (%)	84.95 ± 5.3
BET surface area	1420.1 ± 18.9
BJH total pore volume	0.5 ± 1.5e-02
Crystallinity	1.8% < 2.1% (precursor hydrochar) < 3.7% (food waste)
Zeta potential	− 40.2 ± (− 1.5) mV
Operating concentration	25–100 mg L^{-1}
	Tebuthiuron
Solubility in water at 25 °C	2.5 g L^{-1}
pKa	1.2
$\log K_{OW}$	1.9 mL g^{-1}
pH	3.1 ± 0.4
Analytical concentration	0.5–1 mg L^{-1}
	Bulk aqueous solution
pH	8–12

The previous trends concur with those of studies in the literature. Boumaraf et al. [39] tested the quality of a porous biocarbon for removing 2,4-D from an aqueous solution. The bioproduct adsorbed such a compound more effectively as its dose increased. An increasing dose enhanced the available specific surface area on the material for adsorption [40]. Nevertheless, its efficiency decreased as the matrix saturated, supporting a maximum adsorbing capability. Usually, no significant modification occurs in an adsorptive system at equilibrium [39]. Liu et al. [26] demonstrated the ability of a bionic MOF to develop a finite quantity of active pesticide-binding sites. Such a bioproduct could not adsorb organophosphorus compounds as their concentration exceeded the systematic adsorbing capacity. A nanometer adsorbent removed the highest quantity of thiamethoxam from an aqueous solution at the lowest dose of 0.1 g [34]. Increasing it to 0.4 g limited its efficiency as excess particles aggregated, decreasing the availability of active sites for adsorption. In addition, as adsorbate concentration increased from 10 to 200 mg L^{-1}, it increased the adsorptive capability from 3.65 to 43.15 mg g^{-1}. Nevertheless, it decreased effective removal from 82.8 to 52.7% [34]. Increasing analytical concentration boosts a systematic adsorptive initially. As the process continues, it exceeds removal capability. As a result, efficiency decreases [41].

Other significant variables for adsorbing capability and removal efficiency than sorbent or sorbate dose are morphology, elemental composition (Fig. 1), pH, and electric potential (Table 1 and Figure S6, Supplementary material). The honeycomb-arranged hydrothermal bioadsorbent from this study developed up to 1420.1 $m^2 g^{-1}$ BET surface area and 0.5 $cm^3 g^{-1}$ BJH total pore volume (Table 1 and Table S1, Supplementary material). Its mesopores (Fig. 1) acted as paths for interparticle diffusion of TBT to micropores [32]. They modulated functional hydraulic properties, which are significant for the solid-phase separation of hydrophilic herbicides from aqueous systems [30]. Such a bioproduct can offer stakeholders scalable functionality and technical competitiveness for substituting adsorbing frameworks, such as ZIF, MOF, and POF (Figure S6, Supplementary material), in high-performance adsorptive systems. El-Kammah et al. [34] obtained evidence for BET surface area enhancing the adsorbing capability and removal efficiency of an adsorbent. It can develop a lower specific surface area than usual. Nevertheless, π-stacking can balance its technical performance as it intensifies retention across relatively smoother areas of its irregular and heterogeneous structure. As a result, it can efficiently treat atrazine in water.

The primary hypothesis for the adsorption of pesticides on porous material is hydrophobic partition [42]. As pesticides partition into hydrophobic fractions of an adsorbent, such as polyaromatic backbone and pyrolytic granular charcoal, they bind to its surface. Such a mechanism frequently occurs in physical structures with a significant quantity of C and high adsorptive capability, such as the hydrothermal bioadsorbent from this study. It contained 42.1% C (Fig. 1) and adsorbed up to 99.2% of TBT at the highest analytical concentration (Fig. 2) while maintaining a functioning feature at high pH (Figure S7, Supplementary material). As for carbon-dense materials with low adsorptive potential, other mechanisms than hydrophobic partition may occur, such as H-bonding and adsorbate-metal complex (Graphical abstract). These indicate contribution of morphological structure and elemental composition to the adsorptive process [30].

3.2.1 Comparative and cycling performance of hydrothermal bioadsorbent

The hydrothermal bioadsorbent at 100 mg L^{-1} can remove up to 99.2% of TBT at 1.5 mg L^{-1} from an aqueous solution. As a result of its high efficiency, it can outperform traditional pesticide-adsorbing matrices available in the literature [43–47] for treating water, wastewater, and terrestrial ecosystems (Figure S6 and Table S1, Supplementary material). Comparatively, woody and nonwoody biocarbons can remove up to 95% of TBT from soil [30]. A nano-carbon black can separate up to 80% of such a compound from a synthetic liquid medium [38]. As these products could develop neither BET surface area nor BJH total pore volume as significantly as the hydrothermal bioadsorbent from this study (Table 1, Figure S6, and Table S1, Supplementary material), it offers stakeholders a texturally and technically superior option for bioremediation in synthetic and natural streams. Nevertheless, its efficiency can sharply decrease over multiple adsorption-desorption cycles (Fig. 3).

At a standard 50 mg L^{-1} concentration, hydrothermal adsorbent maintained at least 80% efficiency up to the third cycle. At 100 mg L^{-1}, it removed at least 80% of TBT at 1 mg L^{-1} up to the seventh cycle, significantly enhancing regenerability. In contrast, at 25 mg L^{-1}, it could resist only two batches without losing more than 20% efficiency in removing such an analyte. In the last cycle, 25, 50, and 100 mg L^{-1} developed 64.9, 68.5, 75.2, and % efficiency, respectively, further supporting the positive impact of adsorbent concentration on recyclability. A graphene matrix separated up to 97.4% of atrazine from an aqueous solution up to the second cycle. At the third repetition, its efficiency decreased to 95%, supporting excellent reusability [37]. After five times of adsorption and desorption, the efficiency of pyrolytic biochar in removing 2,4-D decreased to 94.8%; hence, it maintained its textural quality, which is crucial for repeatability [30]. A nanometer adsorbent removed approximately 88% of thiamethoxam up to the third cycle. Beyond the fifth cycle, its effective retention signifcantly decreased to 60% [34], supporting trends in this study. Nevertheless, the hydrothermal adsorbent at 100 mg L^{-1} can allow more cycles with at least 80% efficiency.

The regenerability of an adsorbent determines whether its operation is stable; hence, it offers a restriction for its potential application in treating water and wastewater [30]. Another limiting factor is elution. Wang et al. [31] regenerated a hydroxyl-functional POF by washing it with

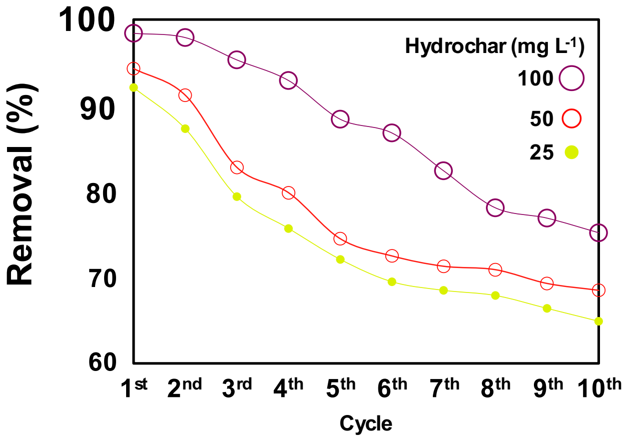


Fig. 3 Cycling performance of hydrothermal bioadsorbent

methanol. Similarly, the adsorptive TBT-hydrochar system from this study was eluded in an alcoholic solvent. The non-polar region of TBT can develop a significant affinity to ethanol. They attract each other with a higher force than those occurring between such an herbicide and functional groups and atoms on the surface of hydrothermal bioadsorbent, facilitating desorption. Ignoring the previous statement can make the regeneration testing methodologically challenging and unreproducible.

3.3 Dependency of hydrothermal bioadsorbent on pH and electric potential

Hydrothermal bioadsorbent separated a higher quantity of TBT from an aqueous solution as pH increased (Figure S7, Supplementary material). Such bioproduct at 50 mg L^{-1} removed 87 ± 2.8% of the adsorbate at 1 mg L^{-1} at pH 8. Its efficiency increased to 96.8 ± 2.1% at pH 12. Hence, the solid-phase separation positively responded to pH. In addition, a negative electric potential (Table 1) supported the occurrence of oppositely charged components in the adsorptive TBT-water-hydrochar system. While TBT appeared predominately electropositive, hydrochar manifested as alkaline for the process. Usually, such a pesticide assumes a cationic form at a pH lower than pKa. Nevertheless, it develops and maintains a molecular structure as pH surpasses pKa [48]. As a result of no ionization or dissociation into an anionic form (Figure S9, Supplementary material), its adsorption on an electronegative surface (Table 1) occurs effectively at high pH (Figure S7, Supplementary material). In contrast, as pH decreases, creating an acidic environment, it limits the adsorption of an electropositive analyte on an oppositely charged surface, such as the hydrochar in this study; hence, co-occurrence of high-concentration competing ions, such as H^+ and Na^+ [49], can decrease the availability of active sites on a surface to retain TBT.

The isoelectric point (i.e., pH at a ζ-potential equals zero) of a hydroxyl-functional POF was approximately 2.5; hence, it effectively adsorbed positively charged sulfonamides at a pH below pKa (1.3 mg L^{-1}). Nevertheless, as the pH increased and surpassed pKa, it electrostatically repulsed those analytes [31]. Those authors demonstrated the significance of ζ-potential for adsorption. A negative ζ-potential indicates an anionic adsorbent, implying a solution to treating a cationic compound. The higher its magnitude, the higher the tendency for attraction or repulsion between particles. A ζ-potential of − 45.2 ± (− 3.8) mV was estimated to a colloidal TBT-water-hydrochar system based on its electric potential (Figure S8, Supplementary material). Such a trend further supported the opposite electric character of adsorbent and adsorbate and their propensity for developing attraction rather than repulsion at the shear (slipping) plane of a potentially stable suspension. A zeta potential analyzer measured a magnitude of − 40.2 ± (– 1.5) mV from samples for sorption testing (Table 1, Supplementary material); hence, the electrokinetic model in this study can provide a valid and reliable prediction and interpretation of critical stages and potentials (Figure S8, Supplementary material).

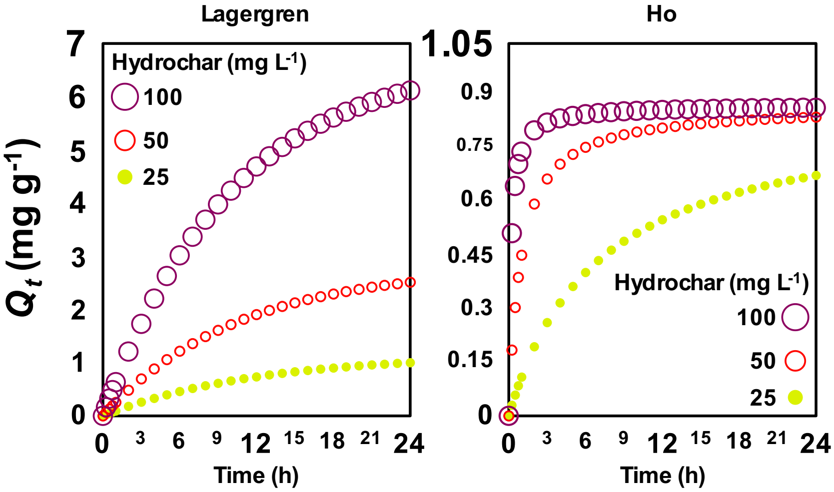
As charges occurred on hydrochar, they developed a theoretical surface potential of – 190 ± (– 30) mV, allowing it to attract and retain TBT electrostatically. As TBT was electropositive, it formed a relatively immobile (stern) layer of ions charged oppositely to the surface of the adsorbent. The distance between the external boundary of the stern layer and the bulk environment may create a potential of – 125 ± (– 25) mV. Next to the previous layer may occur a diffusive layer. In such a region, electrical forces and thermal motions act on ions, controlling their dynamics and intensity of repulsion and attraction [50]. Zeta potential occurs at the shear (slipping) plane, an imaginary surface separating the thin layer of liquid bound to the solid surface of an adsorptive system. Such an interface develops a lower electric potential than the surface and diffusion medium since it occurs farther from the surface than the previous layers [50]. Nevertheless, its potential may depend not only on the distance but also on the adsorbent concentration. The more hydrochar in a suspension, the higher the magnitude of ζ-potential, facilitating collision and attraction between particles until they approach a stationary phase. In such a stage, the adsorption ceases as the pores saturate. Nevertheless, the system remains physically active while maintaining a regenerable feature [50].

3.4 Kinetic adsorption of tebuthiuron on hydrothermal bioadsorbent

Lagergren's and Ho's kinetic models predicted a time-dependent adsorption of TBT at 1 mg $L–^{1}$ on hydrochar at varying concentrations (Fig. 4). Nevertheless, the latter more accurately described such a phenomenon since it developed a higher value of r_{adj}^2 (Table S2, Supplementary material). Lagergren's model predicts a directly proportional time-dependent adsorption to the quantity of analytical uptake; hence, it offers a better trend to describe an adsorptive system initially. In contrast, Ho's approach assumes a rate-limiting chemisorption while predicting the behavior of a solute over the entire range of adsorption [51]. Hence, mechanisms such as H-bonding, aromatic-aromatic interaction, and metal-adsorbate complex (Graphical abstract) controlled the adsorption of TBT on hydrochar.

At 50 mg L^{-1}, such a bioproduct adsorbed 3.2 mg g^{-1} of TBT (Table S2, Supplementary material) until pore saturated within 6 h. At 100 mg^{-1}, it retained a higher quantity of 6.9 mg g^{-1} within only 2 h. Increasing concentration accelerated and increased the efficiency of the process. As a result, it developed the most intensive adsorption

Fig. 4 Kinetic adsorption of tebuthiuron on hydrothermal bioadsorbent



possible. In contrast, 25 mg L^{-1} limited the removal to 1.2 mg g^{-1} and postponed the equilibrium. Nevertheless, precisely determining such a condition from a pseudo-second-order breakthrough curve could be challenging. Perhaps, it occurs after 24 h, implying a more gradual remediative solution.

The previous trends concur with those of studies available in the literature. Ho's model outperformed Lagergren's function in describing the adsorption of 2,4-D on a porous matrix in the kinetic investigation by Liu et al. [26]. Although 2,4-D and tebuthiuron are disparate compounds, their retention on pyrolytic [33] and hydrothermal granular charcoals can similarly occur through surface adsorption (stage I) and interparticle or membrane diffusion (stage II) until entering stationary condition (stage III) [52]. As interparticle diffusion determined the adsorption of TBT on hydrothermal bioadsorbent, k_2 was different from zero independently on concentration (Table S2, Supplementary material). Nevertheless, 100 mg L^{-1} maximized it to 0.12 mg g^{-1} h^{-1}, supporting its ability to enhance available energetically active pesticide-bonding sites and the positive effect of a boundary layer on adsorption [52].

Pierri et al. [30] demonstrated the capability of nano-carbon black to adsorb 41.4 mg g^{-1} of TBT from an aqueous system. Such advanced adsorbent rapidly retained the analyte within only 45 min, developing a 34.8 mg g^{-1} equilibrium adsorption. In addition, it approached the stationary phase earlier for diuron at 30 min (q_e = 36.6 mg g^{-1}) and later for ametryn at 80 min (q_e = 43.2 mg g^{-1}) while adsorbing such compounds at approximately 35.8 and 34.5 mg g^{-1}, respectively. Hence, another significant factor for adsorption than the quantity and quality of sorbent is the nature of sorbate. For instance, a hydroxyl-functional POF can more rapidly adsorb sulfachlorpyridazine than sulfamerazine, sulfamethazine, and sulfamethoxazole [31]. A relatively higher $\log K_{ow}$ for such a sulfonamide enhanced its retention on an organic surface containing electrostatically active pesticide-binding sites, such as phenolic groups.

A $\log K_{ow}$ equals 1.9 mL g^{-1} for TBT (Figure S6, Supplementary material) offered sufficient hydrophobicity for developing 97.8 ± 0.85% removal on a hydrothermal adsorbent at 100 mg L^{-1}, which is technically comparable with the efficiency (92–99%) of a hydroxyl-functional POF with an amphiphilic feature [31]. In addition, a high water-solubility (2.5 g L^{-1}) may contribute to its high removal potential. A complete dissolution of TBT in liquid at ambient temperature was obtained, facilitating its adsorption on a hydrophobic hydrochar. As a result, it would not require agitating a solution to promote contact between adsorbent and adsorbate. Stakeholders must consider the hydrophilicity and solubility of pesticides to control technical limitations, such as the occurrence of electrostatic repulsion and formation of supernatant, in treating water and water by thermally fabricated biocarbons.

3.5 Adsorption of tebuthiuron on hydrothermal bioadsorbent at equilibrium

Freundlich's, Langmuir's, and Temkin's isothermal functions predicted the adsorption of TBT at 1 mg L^{-1} on hydrochar at rising concentrations at equilibrium (Fig. 5). Nevertheless, the earlier most precisely described the type of adsorption and nature of contact between adsorbent and adsorbate since it developed higher values of r_{adj}^2 (Table S2, Supplementary material). The process developed a reversible multilayer rather than a single-layer adsorption of the adsorbate on a heterogeneous surface. An R_L between 0 and 1 was estimated from Langmuirian fitting parameters, confirming favorable adsorption with a high affinity between TBT and hydrochar. Nevertheless, it could assume an irreversible character (R_L equals zero) as the adsorbent concentration increases. For instance, R_L at 25 mg L^{-1} was

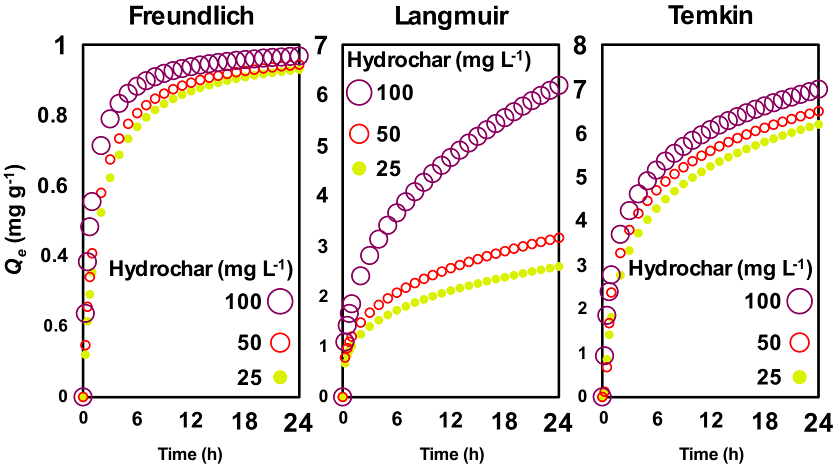


Fig. 5 Adsorption of tebuthiuron on hydrothermal bioadsorbent at equilibrium

0.16. It decreased to 0.14 and 0.09 at 50 and 100 mg L^{-1}, respectively.

Boumaraf et al. [39] most accurately described the solid-phase separation of 2,4-D from an aqueous solution by a biocarbon by fitting data for Langmuir's function. The authors estimated an R_L of approximately 0.03, concording with the trends for favorable and increasing adsorption with rising adsorbent concentration in this study. Such a model allowed El-Kammah et al. [34] to most accurately predict the adsorption of thiamethoxam on a nanometer adsorbent. The authors estimated 1/n of approximately 0.54. The values in this study varied from 0.295 to 0.38 among the lowest and highest quantities of hydrochar (Table S2, Supplementary material). A 1/n below 1 implies the occurrence and contribution of chemisorption to the process [34]. Hence, TBT and hydrochar developed energetically sufficient interactions for chemical sorption in addition to physisorption, making it bifunctional. Cheng et al. [37] and Pierri et al. [30] demonstrated the tendency of graphene matrix and pyrolytic hydrochar for physisorption and chemisorption. Functional groups, such as COOH, OH$^-$, C–O–C, and carbonyl, allowed them to interact with and retain atrazine and 2,4-D on their active heterogeneous surfaces through H-bonding and π–π stacking. The previous mechanisms were predicted for the adsorption of TBT on hydrochar (Graphical abstract).

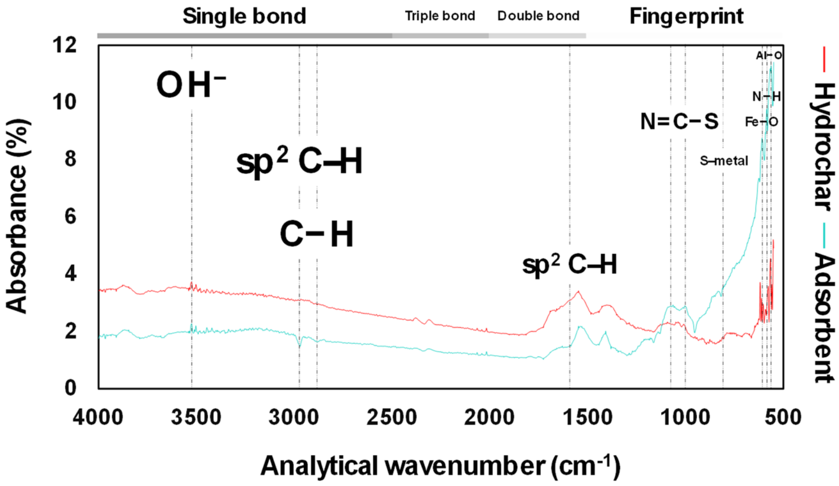
A lower accuracy of Temkin's model validated predominant neither physisorption nor chemisorption in the process, which could develop 1.8–1.915 J mol^{-1} binding energy irrespective of the adsorbent concentration (Table S2, Supplementary material). Hence, morphology and chemistry effectively contributed to the functionality and capability of an adsorptive TBT-water-hydrochar system. Morphologically, pores enabled molecules of TBT to fill into the hydrothermal bioadsorbent until saturation (Fig. 4). Chemically, functional groups and elements (e.g., Al and Fe) in the hydrochar interacted with the adsorbate at low-affinity (e.g., double bonds at aromatic rings) and high-affinity (e.g., ketone) sites, as predicted by a Langmuir-Freundlich model in Prete et al. [38]. At high concentrations, pesticides interact with adsorbents at low-affinity sites; hence, the adsorption assumes a Langmuirian curve. At high concentrations, low-affinity pesticide-binding energies predominate, developing a Freundlich-type isotherm [53], as evidenced by an equilibrium adsorption modeling in this study.

3.6 Functional groups in hydrothermal bioadsorbent and theoretical mechanisms for adsorption

A characteristic peak corresponding to a vibrational OH$^-$ stretching was detected at 3500 cm^{-1} (Fig. 6). As such a functional group occurred in hydrochar, it absorbed a portion of radiant energy. As a result, it reduced transmittance from sample to analyzer. In contrast, its intensity decreased in the adsorbent. Suppose H-bonding occurs between an electronegative donor atom (e.g., N) or group (e.g., carbonyl) in adsorbate and another electronegative element bearing a lone pair of e$^-$ in adsorbent, such as OH$^-$ (Graphical abstract). As a result of such theoretical electrostatic interaction, a specimen may absorb a lower quantity of radiant energy; hence, absorptive intensity in a specific band of its spectral profile decreases. Wang et al. [31] demonstrated the significance of OH$^-$ in developing an amphiphilic PFO for high-performance adsorption of pesticides in aqueous systems.

Another characteristic peak appeared at 3000 cm^{-1}. Such a feature was ascribed to as a skeletal vibration of sp^2 C–H. The hydrothermal pretreatment converted food waste to hydrochar through dehydration, devolatilization, and decarboxylation [30]; hence, it developed a hybrid carbon network while removing volatile organic matter from starting material (Figure S10, Supplementary material). As a result, hydrochar developed a barely visible spectral pattern of sp^2 C–H. In addition, its intensity decreased in the adsorbent. Such a hybrid orbital possibly acted as a precursor of π bond

Fig. 6 Adsorption of infrared energy by functional groups in hydrochar and adsorbent



for the adsorption of TBT on an active surface through an aromatic-aromatic interaction; hence, its occurrence and ability to adsorb infrared energy decreased, increasing transmittance from sample to analyzer. A π bond at an isomeric heterocyclic arrangement in TBT, namely thiadiazole, may interact with an available π acceptor site in the adsorbent, resulting in a π–π interaction [34]. Nevertheless, such a presumptive attractive, noncovalent π-stacking (orbital overlap) may not develop a high-affinity mechanism for the solid-phase separation of TBT from aqueous by a functional mesoporous matrix solution as a primarily electrostatic force of attraction between H covalently bound to an electronegative donor O in a ketone (R–C=O–R′) in adsorbate and another electronegative acceptor OH^- in the hydrothermal bioadsorbent [54].

Another characteristic peak appeared at 820 cm^{-1}. Such a pattern was assigned for a vibrational S-metal stretching. As hydrothermal bioadsorbent retained TBT on its surface, it absorbed a higher quantity of radiant energy than its precursor through an S–metal bond. Hence, metallic bonding was supposed to be another mechanism for the remediative adsorption of TBT from an aqueous solution by a carbonaceous matrix. Aluminum (Al^{3+}) and Fe^{3+} in such a bioproduct may interact with N, S, or O in TBT; hence, they may originate an active adsorbent-metal-adsorbate complex. As Fe–O, N–H, and Al–O occurred at 610, 580, and 560 cm^{-1} (Fig. 6), they supported a metallic bonding [55] and an H-bonding involving an electronegative donor N. In addition, a polar covalent S–Fe bond may provide insight into the reactivity and magnetism of hydrothermal adsorbent. Another S–Al bond may offer an indicator of ionic attraction. Liu et al. [33] developed a bionic MOF for the magnetic solid-phase separation of organophosphorus pesticides from vegetables, such as Chinese cabbage and green onion. The authors extensively mapped it to infrared-absorbing functional groups by FTIR spectroscopy at 4000–480 cm^{-1}. A characteristic peak occurred at 580 cm^{-1}; hence, they referred to it as a vibrational Fe–O stretching. Such an ionic bonding and a bending vibration of Fe–OH at 3405 cm^{-1} permitted them to validate a magnetic feature and an interaction between Fe_3O_4 and graphitic carbon nitride (g–C_3N_4). In addition, the authors stressed the significance of Fe in developing a magnetically recoverable MOF.

The pH (10 ± 2) of the solution surpassed pKa (1.2) and pH (3.1 ± 0.4) of TBT (Table 1 and Figure S6, Supplementary material). In such a condition, S may occur as an S^{2-}, further donating e^- for interaction with an available metallic cation from an adsorbent. Nevertheless, it may develop an electronegative region of TBT, limiting its retention on an alkaline surface [56]. Although such an ionization may occur at a pH higher than pKa or pH_{pzc} for S-containing organic compounds [34], it could determine neither a synergism to adsorption nor a repulsive electrostatic relationship between TBT and hydrothermal adsorbent, as its effective removal increased with increasing pH. Tebuthiuron manifests as a predominately cationic compound at pH below pKa. Nevertheless, it neither ionizes nor dissociates at pH above pKa or pH_{pzc} (Figure S9, Supplementary material). It maintains a molecular form even though an environment modifies from acidic to alkaline [48]. As a result, its adsorption on an anionic surface occurs independently of pH. Such a statement can be visualized on an isoline chart illustrating the removal efficiency as a function of electroconductivity and pH (Figure S7, Supplementary material).

A characteristic peak at 1040–995 cm^{-1}, corresponding to a vibrational N=C–S stretching, provided the most reliable insight into the adsorption of TBT on a functional porous biocarbon. The previous atoms occur in an aromatic arrangement of the analyte's molecular structure. As hydrothermal bioadsorbent removed such a compound from the aqueous solution, it developed a sharper peak than its precursor. Haq et al. [35] detected C=N–S at 1040 cm^{-1}. Nevertheless, they assigned such a spectral pattern for metribuzin occurring on a ZnO-impregnated composite adsorbent. In addition, El-Kammah et al. [34] confirmed the physisorption of thiamethoxam by the appearance of C=S at 1900 cm^{-1} in the profile of the adsorbent. Such an insecticide contains S in its molecular structure; hence, its adsorption on a nanometer matrix developed a new absorptive peak. Hydroxyl, C–O, O–Al–O, and ferrous hydroxide contributed as effectively as C=S in developing mechanisms for the retention of thiamethoxam on a functional porous surface, such as electrostatic interaction and metallic bonding between N in adsorbate and Al or Fe in the adsorbent [34].

Summarily, Fourier-transform spectroscopy can offer a rapid, accurate, and nondestructive analytical technique for identifying, qualifying, and legitimating organic, inorganic, and polymeric pesticide-adsorbing matrices. As the previous functional groups and theoretical mechanisms may represent enablers of solid-phase separation for treating water and wastewater, stakeholders across research centers and industries must further study them for validation and a controllable application. Addressing EFM and analytical electrostatic modeling may assist them in developing knowledge for comprehensively understanding how charges and attractive (repulsive) forces distribute and interact across liquid and solid phases and boundary interfaces of a remediative adsorptive TBT-water-hydrochar system.

3.7 Ecotoxicological validation of remediative adsorptive tebuthiuron-water-hydrochar system

A significant quantity of seedlings occurred on plates containing residual analytical solution at rising concentrations (Fig. 7) validating the remediative feature of the physical model for TBT-adsorbing hydrochar. Hydrothermal bioadsorbent removed the pesticide from a synthetic liquid medium, decreasing its toxicity to *L. sativa*; hence, such a sensitive organism germinated and developed phenotypically normal individuals on sterile Petri dishes (Figures S1 and S2, Supplementary material).

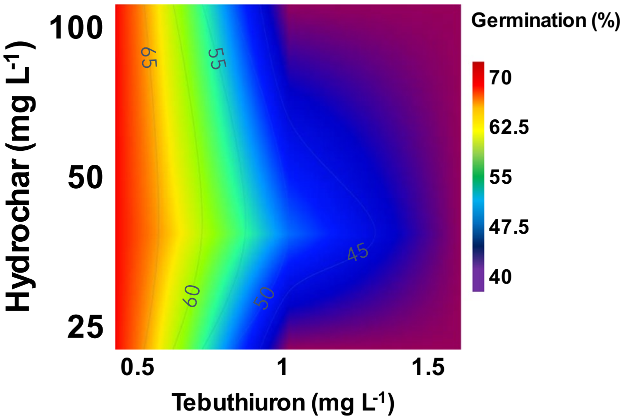


Fig. 7 Phytotoxic effect of the residual aqueous solution on the development of *L. sativa*

A 46.7% germination was calculated at a standard condition involving 1 mg L^{-1} TBT and 50 mg L^{-1} hydrochar. At 0.5 mg L^{-1} adsorbate, the previous adsorbent concentration allowed *L. sativa* to 76.7% germination. Comparable percentages were determined from ecotoxicological units with 0.5 mg L^{-1} TBT and 25 or 100 mg L^{-1} hydrochar. Hence, *L. sativa* germinated more effectively on plates containing the lowest quantity of pesticide independently of hydrochar concentration. In contrast, it developed only 33.3–36.6% germination at 1.5 mg L^{-1} TBT, irrespective of adsorbent quantity. The highest quantity of seedlings was counted on experimental units without TBT and hydrochar (Figures S1 and S2, Supplementary material), supporting a consistent ecotoxicological assay.

The previous downward trend supported an incomplete solid-phase separation. Hydrothermal bioadsorbent at 100 mg L^{-1} removed 97.8 ± 0.85% of TBT at 1.5 mg L^{-1} from liquid; hence, the portion of pesticide remaining in solution from the sorption testing limited vegetable germination and development. Nevertheless, the occurrence of phenotypically normal seedlings on plates with residual TBT confirmed a partial detoxification of the medium by hydrochar, supporting its potential application for remediative bioadsorption in treating water and wastewater.

Researchers base their inferences and technical recommendations for pesticide-adsorbing matrices on removal potential, kinetic rates and capabilities, cycling performance, and morphological, textural, and elemental properties. They do not investigate them for ecotoxicological potential. A pesticide-sensitive organism, such as *L. sativa*, can assist them in testing the efficiency of an adsorbent for detoxification. Such a resource can play a significant role in determining whether the process can dissipate the quantities and qualities of a toxic substance, such as TBT. As represented in an isoline chart (Fig. 2), its retention on hydrothermal bioadsorbent was higher than 90%. Nevertheless, it was not sufficient to effectively detoxify the medium. As a result, the biological model for ecotoxicological assay could develop 76.6% germination (Fig. 7). As the environment can remain toxic after adsorption, stakeholders must consider analyzing its ecotoxicological aspects; otherwise, they cannot develop knowledge for accurate control, biomonitoring, and optimization.

3.8 Implications to progress the field's prominence in adsorbing tebuthiuron from aqueous systems

As emerging topics, studies on removing TBT from artificial and natural systems focus on phytoremediation by green manure [7] and biodegradation by rhizospheric microbiota [9]. Researchers emphasize treating agricultural soils; hence, they do not delve into investigating its potential remediative treatment in liquid media. Such tendency stresses the novelty and significance of this study to advance the field's prominence in remediating such a hazardous compound. A TBT-adsorbing biocarbon represents technical progress in treating aqueous environments. It can offer stakeholders across industries a high-throughput functional mesoporous bioproduct to physically separate such a pesticide from water and wastewater.

Such an approach integrates HTC and KOH, removing post-synthesis activation; hence, it can offer stakeholders an alternative for resource-saving manufacturing lines [19]. In addition, it can yield 29.8% of hydrochar (Table 1), which is highly capable of physisorption and chemisorption. At optimal adsorbate/adsorbent ratio, it can separate up to 98.65% of the analyte from an artificial aqueous system (Fig. 2), allowing a TBT-sensitive organism to germinate 76.6% of its seeds on a plate containing residual herbicide (Fig. 7). In addition to its ability to remove toxic substances and quantities, making it a remediative bioproduct, it can outperform those traditional pesticide-adsorbing frameworks available in the literature, such as ZIF, MOF, and POF, in developing solid-phase separation (Figure S6 and Table S1, Supplementary material). Its surface contains sufficient attractive forces and binding sites to interact with and retain molecules of TBT (Graphical abstract). The process is reversible, allowing up to seven adsorption-desorption cycles with at least 80% efficiency at the highest adsorbent concentration (Fig. 3); hence, it may streamline workflow, reduce solvent demand for elution, and, ultimately, increase the cost-effectiveness of an adsorbing unit.

The hydrothermal bioadsorbent was comprehensively analyzed and characterized for (bio)indicators of technical viability. Hence, this study provides fundamental knowledge for establishing and understanding significant relationships between materials, products, and processes for scaling. Nevertheless, it is at an early stage of development, driving the need to conduct further research for independent validation, optimization, and reproduction on commercial water-treating plants. For instance, stakeholders must investigate food waste for its reproducibility. Its composition is highly heterogeneous and may limit the resembling of (controlled) conditions and efficiency of its TBT-adsorbing product in a real-world application. In addition, they must apply TEA and LCA to determine and delve deeper into the ramifications of the techno-performance and environmental footprint of a remediative adsorptive TBT-water-hydrochar system through all stages of its fabrication, operation, assessment, and monitoring.

4 Conclusion

Hydrochar developed a functional mesoporous carbonaceous matrix for the solid-phase separation of tebuthiuron from an aqueous solution. Such honeycomb-structured bioproduct consisted of a 1420.1 ± 18.9 BET surface area and − 40.2 ± (− 1.5) mV zeta potential; hence, it efficiently removed 84.95 ± 5.3% of the adsorbate, depending on its analytical concentration, from an artificial liquid substratum of pH 8–12. In addition, it maintained 80% efficiency for seven adsorption-desorption cycles. As a result of its significant potential removal and excellent regenerability, it may offer stakeholders an eco-compatible alternative to address a superior treatment of industrial and environmental streams compared to pesticide-adsorbing biocarbons available in the literature. An ecotoxicological assay supported its ability to detoxify a medium, allowing *L. sativa* to develop 76.6% germination and visually normal seedlings on plates containing residual herbicide from the sorption testing. Such a remediative feature is crucial to creating conditions for environmental protection in agroecosystems, such as those areas producing sugarcane, where tebuthiuron occurs as a hazardous organic compound to the surroundings when leaching and running off. This study provides sufficient fundamental and practical knowledge for advancing the field's prominence in developing high-throughput bioadsorption for the bioremediation of tebuthiuron. Nevertheless, as the base of hydrochar was food waste, it may represent a technical limitation to its scalability. The composition of such a resource is considerably heterogeneous, compromising the reproducibility of its conversion and product. Hence, developing further research to overcome this restrictive aspect is crucial to improve the remediative adsorptive TBT-water-hydrochar system.

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Author contribution Bruno Rafael de Almeida Moreira: conceptualization, data curation, methodology, formal analysis, writing—original draft, writing—review and editing; Victor Hugo Cruz: conceptualization, investigation, methodology; Marcelo Rodrigues Barbosa Júnior: investigation; Leonardo Gomes de Vasconcelos: methodology; Rouverson Pereira da Silva: funding acquisition, supervision; Paulo Renato Matos Lopes: writing—review and editing, funding acquisition, supervision.

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Data availability Experimental data and materials will be available from the corresponding authors on reasonable request.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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