UNIVERSIDADE FEDERAL DE LAVRAS

TIAGO MORALES SILVA

EFFECT OF SOIL CONTAMINATION WITH LEAD (Pb) ON THE MULTITROPHIC WEB OF KALE-ASSOCIATED INSECTS

LAVRAS - MG
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Prof. Dr. Lucas Del Bianco Faria
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EFFECT OF SOIL CONTAMINATION WITH LEAD (Pb) ON THE MULTITROPHIC WEB OF KALE-ASSOCIATED INSECTS

EFEITO DA CONTAMINAÇÃO DO SOLO COM CHUMBO (Pb) NA REDE MULTI-TRÓFICA DE INSETOS ASSOCIADOS A COUVE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Biologia e Ecologia de Insetos, para a obtenção do título de Doutor.

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Aos meus "doutores" em educação, humanidade e amor:

Lucimar (Mãe) e João (Pai)

DEDICO

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“Eu, pessoalmente, acho que ensinar é muito mais importante que pesquisar. Porque é no ensino que se aprende a pensar. E é da capacidade de pensar que surgem os pesquisadores. Se a pesquisa é um fruto, o ensino são as sementes que foram plantadas. Sem sementes não há árvores, sem árvores não há frutos.”

(Rubem Alves)

RESUMO

Atividades antrópicas como mineração, indústria, resíduos urbanos e o uso demasiado de insumos agrícolas podem resultar na contaminação do solo com metais pesados. Esses elementos podem ser absorvidos do solo pelas plantas e causar efeitos tóxicos aos organismos que se alimentam dessas plantas e às cadeias tróficas, devido ao seu potencial acumulativo nos tecidos, podendo impactar o ecossistema. Diante disso, a presente tese teve como objetivo principal investigar o efeito da contaminação do solo com metal pesado na comunidade de insetos, estruturada em uma rede trófica, que vivem na parte aérea da planta. Para isso, nós usamos como modelo experimental o chumbo (Pb), em diferentes concentrações, e a comunidade de insetos associados a couve (*Brassica oleracea* L., var. acephala). Plantas de couve foram cultivadas em solo contaminado experimentalmente com quatro concentrações de nitrato de Pb: 0 (controle), 144 (T1), 360 (T2) e 600 (T3) mg/Kg de solo. As concentrações dos tratamentos contaminados correspondem, respectivamente, ao valor de prevenção, valor limite para área agrícola e valor limite de Pb para área residencial. O experimento foi conduzido em uma casa de vegetação aberta para a colonização natural dos insetos. Os insetos foram coletados duas vezes em campo através de sacos-armadilha presos à folha da planta e por retirada direta. No primeiro artigo, nós avaliamos o efeito das concentrações de Pb na riqueza de espécies acumuladas, composição e na abundância dos diferentes grupos funcionais de insetos da comunidade. Nós encontramos que a concentração de Pb na planta aumentou com o aumento da contaminação do solo, inclusive apresentando valores acima do permitido para esse vegetal. O tratamento controle mostrou apresentar uma maior riqueza de espécies acumuladas. Além disso, os tratamentos exerceram efeito na ordenação da comunidade, tendo espécies de parasitoides como indicadoras da ausência do contaminante. A abundância de herbívoros mastigadores e sugadores, seus respectivos parasitoides, predadores e parasitoides de predadores foi afetada negativamente. Contudo, hiperparasitoides não foram afetados na abundância, mas sim na riqueza. No segundo artigo, nós avaliamos o efeito da contaminação nas propriedades estruturais e funcionais da rede trófica. Nós avaliamos o efeito das concentrações de Pb na arquitetura geral da rede trófica e nas seguintes métricas: número de espécies e de ligações, densidade de ligações, conectância, razão predador-presa e generalidade e vulnerabilidade trófica. No geral, observamos uma simplificação da rede trófica à medida que se aumentou as concentrações de Pb, refletindo em redes visualmente menores. Houve redução nas métricas da rede trófica a partir do tratamento T2. Estruturalmente, houve redução no número de espécies e na densidade de ligações. A conectância aumentou com a contaminação. Funcionalmente, houve redução na razão predador/presa, vulnerabilidade e generalidade trófica. Esse trabalho mostrou que a contaminação do solo com Pb pode afetar negativamente a comunidade de insetos associados à planta e a estrutura e função da rede trófica, mesmo em concentrações permitidas.

Palavras-chave: Metais pesados. Insetos herbívoros. Predadores. Parasitoides. Métricas de redes tróficas.

ABSTRACT

Anthropogenic activities such as mining, industry, urban waste, and the excessive use of agricultural inputs can result in soil contamination with heavy metals. These elements can be absorbed from the soil by plants and cause toxic effects to organisms that feed on these plants and to trophic chains, due to their cumulative potential in tissues, which can impact the ecosystem. Therefore, this thesis had as main objective to investigate the effect of soil contamination with heavy metal on the insect community, structured in a trophic network, which lives in the aerial part of the plant. For this, we used as an experimental model lead (Pb), at different concentrations, and the insect community associated with kale (*Brassica oleracea* L., var. acephala). Kale plants were grown in soil experimentally contaminated with four concentrations of Pb nitrate: 0 (control), 144 (T1), 360 (T2), and 600 (T3) mg/Kg of soil. The concentrations of contaminated treatments correspond, respectively, to the prevention value, limit value for agricultural soil, and limit value of Pb for residential soil. The experiment was conducted in an open greenhouse for the natural colonization of insects. Insects were collected twice in the field through trap bags attached to the plant leaf and by direct removal. In the first article, we evaluated the effect of Pb concentrations on accumulated species richness, composition, and abundance of different functional groups of insect in the community. We found that the concentration of Pb in the plant increased with the increase in soil contamination, even presenting values above those allowed for this plant. The control treatment showed a greater richness of accumulated species. In addition, the treatments had an effect on community composition, with parasitoid species as indicators of the absence of the contaminant. The abundance of chewing and sucking herbivores, their respective parasitoids, predators, and predator parasitoids was negatively affected. However, hyperparasitoids were not affected in abundance, but rather in richness. In the second article, we evaluate the effect of contamination on the structural and functional food web properties. We evaluated the effect of Pb concentrations on the overall food web architecture and on the following metrics: number of species and links, links density, connectance, predator-prey ratio, and trophic generality and vulnerability. In general, we observed a food web simplification as Pb concentrations increased, reflecting in visually smaller webs. There was a reduction in the food web metrics from treatment T2. Structurally, there was a reduction in the number of species and links density. Connectance increased with contamination. Functionally, there was a reduction in the predator/prey ratio, vulnerability, and trophic generality. This work showed that soil contamination with Pb can negatively affect the insect community associated with a plant and the structure and function of the food web, even at permitted concentrations.

Keywords: Heavy metals. Herbivorous insects. Predators. Parasitoids. Food web metrics.

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO GERAL

O uso demasiado de insumos agrícolas (corretivos, fertilizantes, pesticidas, etc.), bem como o descarte indevido de efluentes e produtos como lâmpadas, pilhas e baterias e atividades industriais e mineradoras, pode gerar a contaminação do solo com metais pesados. Esses elementos são tóxicos aos organismos em geral, pois, além de prejudicar e interromper processos biológicos importantes, não são degradáveis, acumulando-se nos tecidos e no ambiente. Com isso, uma vez estabelecidos no solo, esses elementos podem ser absorvidos pelas plantas e acumular-se nos seus tecidos.

A acumulação de metais pesados pelas plantas, as quais constituem o primeiro nível trófico (produtores), pode se tornar uma situação agravante, uma vez que esses elementos podem ser transferidos para os níveis subsequentes da cadeia trófica (consumidores primários e secundários, predadores, etc.), provocando efeitos letais e subletais a esses organismos e possíveis desequilíbrios nas interações ecológicas e no ecossistema.

Cada espécie de planta geralmente possui uma diversa comunidade de insetos associados. Essa comunidade é estruturada em uma rede trófica do tipo “*source web*”, onde há uma rede de interações entre as espécies baseada em um único recurso fonte (vegetal). Com isso, a contaminação do solo com metais pesados pode gerar a transferência desses elementos do solo para uma determinada espécie de planta e da planta para os insetos da rede trófica, sendo eles herbívoros, predadores e parasitoides primários e secundários. A transferência e acumulação desses elementos nos componentes da rede trófica, associado aos efeitos tóxicos, poderia afetar a comunidade de insetos associados a planta e prejudicar tanto a estrutura quanto o funcionamento da rede trófica, gerando uma simplificação das espécies e das interações e comprometimento das dinâmicas tróficas, como é conhecido para sistemas aquáticos, por exemplo.

Diante disso, a presente tese teve o objetivo de investigar como a contaminação do solo com metal pesado afeta a comunidade de insetos associados a uma planta e as propriedades estruturais e funcionais da rede trófica. Nós usamos como modelo experimental o chumbo (Pb), em diferentes concentrações, e a comunidade de insetos associados a couve (*Brassica oleracea* L., var. acephala).

2. REFERENCIAL TEÓRICO

2.1. Os metais pesados e seu potencial tóxico

O termo metal pesado foi introduzido no meio científico por Bjerrum (1936), definindo como metais que possuem densidade acima de 7 g.cm^{-3}. Posteriormente, várias outras definições foram elaboradas pautadas na densidade ou no número atômico, o que causava a inclusão ou exclusão de metais e outros elementos nesse grupo, sem ter, portanto, um consenso. Com isso, percebeu-se que a densidade não é de grande importância com relação à reatividade de um metal, sendo recomendado o abandono das definições baseadas na densidade (DUFFUS, 2002). Com isso, passou-se a usar o termo metal pesado para designar elementos, sendo metais ou metaloides, relacionados à toxicidade dos organismos.

Existem metais chamados essenciais que são necessários aos organismos vivos em pequenas quantidades para funções fisiológicas e bioquímicas vitais, como por exemplo: Ferro (Fe), Manganês (Mn), Cobre (Cu), Zinco (Zn) e Níquel (Ni) (CEMPEL; NIKEL, 2006; GÖHRE; PASZKOWSKI, 2006). Geralmente os organismos são capazes de regular pequenas quantidades desses metais, porém o excesso deles pode ser tóxico (CHAFFAI; KOYAMA, 2011; KABATA-PENDIAS, 2010). Já outros metais não participam de nenhuma função biológica nos organismos, sendo tóxicos mesmo em baixas concentrações, como o Cádmio (Cd), o Chumbo (Pb), o Arsênio (As), o Mercúrio (Hg) e o Cromo (Cr) (ALI; KHAN; SAJAD, 2013; COBBETT, 2003; KABATA-PENDIAS, 2010; KÄRENLAMPI et al., 2000; MERTZ, 1981; SÁNCHEZ-CHARDI; RIBEIRO; NADAL, 2009)

A toxicidade dos metais está ligada principalmente ao fato de não serem degradáveis pelos organismos, acumulando-se nos mesmos e no ambiente (ALI; KHAN; SAJAD, 2013). Os metais pesados podem causar vários efeitos no metabolismo dos organismos, sendo os principais: estresse oxidativo pela formação de formas reativas de oxigênio, podendo sobrecarregar as defesas antioxidantes das células e levando ao dano ou morte celular, interferência na função da membrana e assimilação de nutrientes, perturbação da função e atividade proteica e danos ao DNA ou em seus mecanismos de reparo (BEYERSMANN; HARTWIG, 2008; KRYSTOFOVA et al., 2009; LEMIRE; HARRISON; TURNER, 2013; SÁNCHEZ-CHARDI; RIBEIRO; NADAL, 2009; WYSOCKI; TAMÁS, 2010).

Esses metais podem estar disponíveis na natureza (metais litogênicos) ou serem adicionados ao ambiente por ações humanas (metais antropogênicos) como: resíduos urbanos, mineração, indústria automotiva e agricultura (corretivos, fertilizantes, pesticidas, etc.)

(ALLOWAY, 1995; FERNANDES et al., 2007; PEZZAROSSA et al., 1993; STERRETT et al., 1996; TAVARES, 2013).

As mudanças na forma de uso do solo em combinação com o uso exagerado de insumos agrícolas são as principais causas do rápido declínio da biodiversidade em várias paisagens (BENTON; VICKERY; WILSON, 2003; BIANCHI; BOOIJ; TSCHARNTKE, 2006; ROBINSON; SUTHERLAND, 2002). Essas práticas de modo intensivo podem causar degradação química do solo, como resultado do acúmulo de elementos e/ou compostos tóxicos, como os metais pesados, em níveis indesejáveis e, consequentemente, a incorporação desses elementos na cadeia trófica (DAR et al., 2015; GIMENO-GARCÍA; ANDREU; BOLUDA, 1996; RAMALHO; AMARAL SOBRINHO; VELLOSO, 2000).

2.2. O chumbo (Pb)

Devido ao seu alto grau de toxicidade, os metais As, Cd, Cr, Pb e Hg estão dentre os metais de grande importância para a saúde pública, sendo todos eles tóxicos sistêmicos que são conhecidos por induzir danos em múltiplos órgãos, mesmo em níveis mais baixos de exposição (TCHOUNWOU et al., 2012). O Pb é classificado como o segundo mais perigoso na lista de prioridades da Agência de Proteção Ambiental dos Estados Unidos (ATSDR, 2020).

A contaminação por Pb está associada a atividades como: fabricação de baterias, combustão de gasolina com chumbo, herbicidas e inseticidas (THANGAVEL; SUBBHURAAM, 2004; WUANA; OKIEIMEN, 2011). A intoxicação com esse metal causa problemas em crianças, como desenvolvimento prejudicado, inteligência reduzida, perda de memória de curto prazo, dificuldades de aprendizagem e problemas de coordenação; provoca insuficiência renal, aumento do risco de desenvolvimento de doenças cardiovasculares, dentre outros (IQBAL, 2012; PADMAVATHIAMMA; LI, 2007; WUANA; OKIEIMEN, 2011; ZENG et al., 2020).

2.3. A absorção e transferência de metais pesados nos organismos e cadeias tróficas

A incorporação de metais pesados pelos organismos envolve alguns conceitos, como: bioacumulação, biotransferência, biomagnificação e biodiluição.

A bioacumulação é a absorção e retenção de um metal por um organismo a partir do meio abiótico (ambiente) e/ou biótico (alimentar), onde geralmente a concentração é maior no organismo acumulador comparado com a fonte (GRAY, 2002; MANN; VIJVER;

PEIJNENBURG, 2011). A biotransferência trata-se, mais especificamente, da passagem dos metais de um nível trófico para o outro (JENSEN; TRUMBLE, 2003).

Uma vez que esses elementos se acumulam nos tecidos corporais dos organismos vivos (bioacumulação), as suas concentrações podem aumentar à medida que passam de níveis tróficos mais baixos para níveis tróficos mais elevados. Esse fenômeno é conhecido como biomagnificação (ALI; KHAN; SAJAD, 2013; GRAY, 2002). Esses metais se biomagnificam ao longo das cadeias alimentares porque os níveis tróficos sucessivos consomem grandes quantidades de alimento para obterem os recursos necessários para o funcionamento metabólico e, uma vez que a biomassa total consumida estiver contaminada, os contaminantes serão absorvidos em grande quantidade pelo consumidor (GRAY, 2002; MANN; VIJVER; PEIJNENBURG, 2011).

Alguns autores consideram bioacumulação e biomagnificação como sinônimos (CHERUIYOT et al., 2013), embora o termo bioacumulação seja mais utilizado para designar a acumulação de um metal a nível de organismo, enquanto biomagnificação é mais utilizado para designar a acumulação a nível de cadeia trófica. A biodiluição, ao contrário da biomagnificação, é a diminuição das concentrações dos metais à medida que passam de níveis tróficos mais baixos para níveis tróficos mais elevados (GRAY, 2002; MANN; VIJVER; PEIJNENBURG, 2011).

Uma vez estabelecidos nos solos, os metais pesados podem ser absorvidos pelas plantas através das raízes. Além disso, as plantas podem ainda aborver metais pesados através da deposição direta de material particulado e contaminantes da atmosfera em suas superfícies (AMATO-LOURENCO et al., 2016; DE TEMMERMAN et al., 2015). Ao absorverem metais pesados, além do estresse metabólico, as plantas podem ter seu crescimento e produtividade reduzidos (ASHRAF et al., 2017). No entanto, muitas plantas podem tolerar e acumular metais em seus tecidos em menor ou maior nível sem apresentar qualquer sintoma tóxico ou crescimento e produtividade prejudicados (LIMA et al., 2009; LIMA; NASCIMENTO; SOUSA, 2015). Quanto ao nível de absorção e acumulação de metais, as plantas podem ser classificadas como normais, acumuladoras ou hiperacumuladoras, dependendo do metal. Por exemplo, para diversos metais, como Pb, Ni ou Cu, uma planta considerda normal geralmente apresenta concentrações de metal abaixo de 5 mg / Kg em uma base de massa seca nas partes acima do solo, enquanto uma planta considerada acumuladora apresenta concentração mínimia de 100 mg / Kg , e uma planta considerada hiperacumuladora apresenta concentração mínima de 1000 mg / Kg (MAESTRI et al., 2010; REEVES; BAKER, 2000).

Como as plantas são a base das cadeias alimentares terrestres, há o risco potencial de transferência de metais pesados acumulados nos seus tecidos para os organismos em níveis tróficos superiores (GALL; BOYD; RAJAKARUNA, 2015; ZHUANG; ZOU; SHU, 2009). A transferência de metais pesados da planta para os insetos hervívoros tem sido bastante documentada nos últimos anos (DAR et al., 2015, 2017; NAIKOO et al., 2021; SHI et al., 2020; WOŹNIAK et al., 2017; ZHOU et al., 2015). Esses trabalhos mostraram que o mecanismo de acumulação nesses insetos é bastante variável, no qual os herbívoros podem apresentar concentrações de metais em seus corpos menores do que na planta (biominização) ou concentrações maiores (biomagnificação), dependendo tanto do metal quanto das espécies de planta e insetos. A exposição a esses metais provocam efeitos negativos nos insetos herbívoros, podendo assim beneficiar a planta (MARTENS; BOYD, 1994). Esses efeitos podem depender do metal e do modo de alimentação dos herbívoros. Por exemplo, a hiperacumulação de Ni em *Streptanthus polygaloides* (Brassicaceae) diminuiu significativamente a sobrevivência dos mastigadores de folhas (gafanhoto e lepidóptero) mas não teve efeito sobre insetos sugadores de tecido vascular (afídeo, cigarrinha e mosca-branca) (JHEE; BOYD; EUBANKS, 2005). Em contrapartida, tanto o desempenho da espécie lepidóptera mastigadora *Mamestra brassicae* quanto o da espécie afídea sugadora *Myzus persicae* foram reduzidos em plantas de *Arabidopsis halleri* (Brassicaceae) cultivadas em solo contaminado com Cd e Zn (STOLPE; KRÄMER; MÜLLER, 2017).

Tem sido demonstrado que insetos herbívoros que se alimentam em plantas cultivadas em meio contaminado com metais pesados, ou em dietas artificiais, transferem esses elementos para seus inimigos naturais, como insetos predadores e parasitoides (KAZIMIROVA, 2000; KAZIMÍROVÁ; SLOVÁK; MANOVÁ, 1997; NAIKOO et al., 2019, 2021; ORTEL, 1995; SHI et al., 2020; YE et al., 2009). Assim como ocorre nos herbívoros, o nível de acumulação desses elementos no corpo desses insetos pode ser maior ou menor do que nos herbívoros, dependendo do sistema. Os trabalhos mostraram que tanto os predadores quanto os parasitoides sofrem efeitos adversos devido a exposição a metais pesados via herbívoros contaminados. Por exemplo, foi observado redução na taxa de predação e na fecundidade e aumento da mortalidade larval em espécies de joaninhas predadoras (NAIKOO et al., 2019; SHI et al., 2020), enquanto para parasitoides foi observado redução na eclosão de adultos, fecundidade e taxa de crescimento populacional (KAZIMÍROVÁ; SLOVÁK; MANOVÁ, 1997; KRAMARZ; STARK, 2003; ORTEL; GINTENREITER; NOPP, 1993; VICKERMAN et al., 2004; YE et al., 2009).

2.4. O efeito dos metais pesados nas comunidades e redes tróficas

Identificar efeitos de poluentes em comunidades não é considerado algo simples. De acordo com Rosenberg & Resh (2009), as respostas fisiológicas e respostas a nível de indivíduo são mais fáceis de serem detectadas do que respostas populacionais ou respostas a nível de comunidade. Em contrapartida, as ultimas respostas são consideradas de maior relevância ecológica do que as primeiras. De fato, de acordo com a revisão de Butler and Trumble (2008) o número de estudos que não examinaram o efeito de poluentes em níveis tróficos mais elevados era alto (79.3%), e ainda continua relativamente pouco explorado, provavelmente devido à dificuldade e complexidade em projetar experimentos com vários níveis tróficos. A falta de conhecimento sobre os efeitos diretos da poluição nos diferentes compartimentos biológicos dos ecossistemas terrestres requer análises mais aprofundadas sobre o tema (SKALDINA; SORVARI, 2019).

Nas comunidades aquáticas, tem sido investigado o impacto da contaminação de metais derivada, principalmente, de atividades mineradoras. A poluição dos sistemas aquáticos por metais pesados gera o acúmulo desses elementos nos tecidos dos organismos que vivem nesse ambiente. Por exemplo, a poluição de riachos por minas gerou a tranferência de Fe, Zn e Cu do ambiente para os invertebrados (QUINN et al., 2003). Em algumas situações, as concentrações de metais nesses ivertebrados e em outros organismos, como os peixes, aumentaram com o aumento do nível trófico (BRINKMANN; RASMUSSEN, 2010; QUINN et al., 2003). Contudo, além da transferência e acumulação dos metais pesados nesses sistemas, as comunidades podem sofrer impactos tanto em sua estrutura como no seu funcionamento. Comunidades de macroinvertebrados bentônicos sofreram mudanças na composição e redução na abundância total e na riqueza de espécies com o aumento das concentrações de metais (QU et al., 2010; QUINN et al., 2003). Um fato agravante é que os efeitos de redução nessas comunidades podem ser persistentes, sendo observados em sistemas aquáticos mesmo depois de 75 anos de inatividade local de minas (LEFCORT; VANCURA; LIDER, 2010). Sobre uma abordagem de redes tróficas, essas comunidades de invertebrados e outros organismos aquáticos expostos a metais apresentaram redes tróficas simplificadas, com menos elementos, ligações entre as espécies, menor densidade de ligações por espécie e ainda redução de invertebrados predadores e ausência de peixes predadores (HOGSDEN; HARDING, 2012a, 2014; YULE; BOYERO; MARCHANT, 2010). Além da redução da predação, essas redes tróficas também foram funcionalmente alteradas porque os recursos basais são menos produtivos ou inacessíveis, o processamento microbiano da matéria orgânica é lento e muitos herbívoros e trituradores estão ausentes (HOGSDEN; HARDING, 2012b).

Nos ambientes terrestres, a contaminação do solo por metais pesados também ocasiona a transferência e acumulação desses metais em invertebrados que vivem no solo, como artrópodes e vermes, por exemplo (HEIKENS; PEIJNENBURG; HENDRIKS, 2001). Assim como nas comunidades aquáticas, as comunidades do solo também sofrem impactos em sua estrutura e funcionamento devido à contaminação por metais. Diversos estudos mostraram que as comunidades de invertebrados edáficos, no geral, sofrem mudanças na composição de espécies e redução nas densidades populacionais (BABIN-FENSKE; ANAND, 2011; MANU et al., 2017; MIGLIORINI et al., 2004; NAHMANI; LAVELLE, 2002; ŠALAMÚN et al., 2012). A riqueza de espécies de alguns grupos, como os nematoides, frequentemente é reduzida com a contaminação do solo com metais pesados (PARK et al., 2011). Tratando-se mais especificamente do impacto nas redes tróficas do solo, foi encontrado uma redução na abundância de insetos predadores e nematoides ovívoros/predadores com o aumento da contaminação (BABIN-FENSKE; ANAND, 2011; CHAUVIN et al., 2020; ŠALAMÚN et al., 2012). Essa redução de nematoides predadores levou a perturbação da rede trófica, resultando em uma superabundância de nematoides herbívoros (PARMELEE et al., 1993; SHARMA; CHENG; GREWAL, 2015).

No entanto, a contaminação do solo por metais pesados não afeta apenas as comunidades edáficas. Como mostrado anteriormente, existe um fator agravante que é a absorção e acumulação desses elementos pelas plantas e a transferência para os níveis tróficos superiores, como demonstrado em insetos herbívoros, predadores e parasitoides. Os efeitos letais e subletais encontrados para os insetos predadores e parasitoides sugerem que a acumulação de metais pesados pela planta poderia afetar toda a comunidade de insetos que vivem na parta aérea da planta, pois os herbívoros em locais poluídos poderiam atuar como sumidouros que reduzem as densidades populacionais de inimigos naturais (BUTLER; BECKAGE; TRUMBLE, 2009; GARDINER; HARWOOD, 2017). No entanto esses trabalhos se restringem a experimentos laboratoriais a nível de indivíduo ou uma cadeia trófica por vez, não sendo possível estender esses efeitos para vários grupos de espécies e níveis tróficos, a nível de comunidade natural e de redes tróficas.

Com isso, esse trabalho se propôs a investigar como a presença de um metal pesado no solo, em diferentes concentrações, pode afetar a comunidade e a rede trófica de insetos que vive na parte aérea de uma planta. Nós investigamos como as concentrações de Pb permitidas para solos brasileiros afetam a comunidade de insetos na couve (*Brassica oleracea* L., var. acephala), em um experimento em campo. No primeiro artigo, nós avaliamos como os tratamentos com as diferentes concentrações permitidas de Pb afetam a riqueza, composição e abundância de espécies da comunidade de insetos associados a couve, investigando o efeito nos seguintes grupos funcionais: herbívoros mastigadores e sugadores e seus respectivos parasitoides, hiperparasitoides, predadores e parasitoides de predadores. No segundo artigo, nós avaliamos o efeito das concentrações de Pb nas propriedades estruturais e funcionais da rede trófica estudada, avaliando o efeito nas seguintes métricas: número de espécies, número de ligações, densidade de ligações, conectância, razão predador-presa e generalidade e vulnerabilidade trófica.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 – Soil contamination with permissible levels of lead negatively affects the community of plant-associated insects: A case of study with kale

Journal Pre-proof

Soil contamination with permissible levels of lead negatively affects the community of plant-associated insects: A case of study with kale

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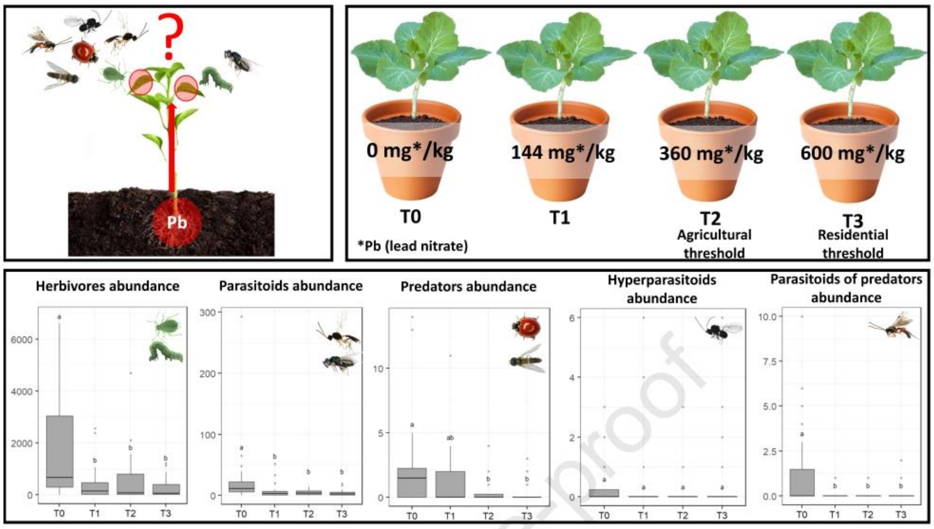
Author statement

Tiago Morales-Silva: Conceptualization, Methodology, Investigation, Data Curation, Formal analysis, Writing - Original Draft preparation **Bruna Corrêa da Silva:** Investigation, Data Curation **Lucas Del Bianco Faria:** Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Supervision


?
Pb
0 mg*/kg
144 mg*/kg
360 mg*/kg
600 mg*/kg
T0
T1
T2
Agricultural threshold
T3
Residential threshold
*Pb (lead nitrate)
Herbivores abundance
0
2000
4000
6000
a
b
b
b
T0
T1
T2
T3
Parasitoids abundance
0
100
200
300
a
b
b
b
T0
T1
T2
T3
Predators abundance
0
5
10
a
ab
b
b
T0
T1
T2
T3
Hyperparasitoids abundance
0
2
4
6
a
a
a
a
T0
T1
T2
T3
Parasitoids of predators abundance
0.0
2.5
5.0
7.5
10.0
a
b
b
b
T0
T1
T2
T3

Soil contamination with permissible levels of lead negatively affects the community of plant-associated insects: a case of study with kale.

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Abstract

This study investigated whether lead (Pb), at concentrations allowed for soil, affects the community of insects that live in the aerial part of plants. We evaluated the effect of Pb concentrations on accumulated species richness, composition, and abundance of different functional groups of insects. Kale plants were grown in soil experimentally contaminated with four concentrations of lead nitrate: 0 (control), 144 (T1), 360 (T2), and 600 (T3) mg/kg of soil. The experiment was conducted in an open greenhouse for the natural colonization of insects. Insects were collected twice using trap bags attached to the plant leaf and by direct removal. The concentration of Pb in the stem and leaf samples increased with the increased soil contamination, even showing values above the limit allowed by the legislation for this plant species. Control plants showed a higher richness of accumulated insect species. In addition, the treatments had an effect on the community composition, in which *Diaeretiella rapae* (primary parasitoid) was found as an indicator of the control+T1 treatments and the top species *Pachyneuron* sp. (parasitoid of predators) was associated with the control. The abundance of chewing and sucking herbivores, their respective parasitoids, predators, and parasitoids of predators were negatively affected. Hyperparasitoid abundance was not affected, but their accumulated species richness was. This study was innovative in demonstrating that soil contamination by different concentrations of a heavy metal (Pb) can negatively affect the community of plant-associated insects, even at concentrations allowed for soil, reflecting possible damage to the ecosystem.

Keywords: Elemental defense; Heavy metals; Herbivorous insects; Kale; Natural enemies; Population density

1 Introduction

In environmental impact studies, the term "heavy metal" is used to designate elements (metals or metalloids) that cause toxicity to organisms (Rascio and Navari-Izzo, 2011). The toxicity of these elements is mainly due to their cumulative potential, as they are not degradable by organisms, so they accumulate in tissues and in the environment (Ali et al., 2013). Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), and chromium (Cr), which have no participation in the biological functions of organisms, are considered toxic to organisms, even at low concentrations (Jan et al., 2015; Kabata-Pendias, 2010; Rascio and Navari-Izzo, 2011). Other metals such as iron (Fe), copper (Cu), and zinc (Zn) are essential for vital functions. However, when these essential metals reach high levels they also become toxic (Kabata-Pendias, 2010; Rascio and Navari-Izzo, 2011; Zoroddu et al., 2019). Heavy metals are naturally found in soils at low concentrations; however, soil contamination by these elements is often associated with activities such as industry, mining, and smelting, the release of different residues, pesticides, fertilizers, among others (Chaffai and Koyama, 2011; Wuana and Okieimen, 2011). Once established in the soil, these elements can be absorbed by plants through the roots. Thereby, heavy metals can be accumulated in plant tissues and, as these organisms are the basis of terrestrial food chains, there is a potential risk of transferring these elements to organisms at higher trophic levels (Gall et al., 2015; Zhuang et al., 2009).

Plants can tolerate and accumulate metals in their tissues without presenting any toxic symptoms or impaired growth and productivity (Lima et al., 2015, 2009). Regarding the level of absorption and accumulation of metals, plants can be classified as normal, accumulator, or hyperaccumulator, depending on the metal. For various metals such as Pb, Ni, or Cu, a normal plant generally has metal concentrations below 5 mg/kg (based on dry mass) in the above-ground parts, while an accumulator plant has a minimal concentration of 100 mg/Kg, and a hyperaccumulator plant has a minimum concentration of 1000 mg/Kg (Maestri et al., 2010; Reeves and Baker, 2000).

The transfer of heavy metals from plants to herbivorous insects has been well documented in recent years (e.g., Dar et al., 2015, 2017; Naikoo et al., 2021; Shi et al., 2020; Woźniak et al., 2017; Zhou et al., 2015). These studies showed that the insects' accumulation levels are quite variable, in which herbivores may have lower metal concentrations in their bodies than the plant (biominimization) or higher concentrations (biomagnification), depending on the metal and plant and insect species. In a review on the effects of metals and metalloids on insects, Butler & Trumble (2008) found that most herbivores (72.72%) had their fitness diminished due to contamination, including reductions in weight, growth, survival, fecundity and hatching success. The negative effects of heavy metals on herbivores are often related to the elemental defense hypothesis. This hypothesis predicts that the accumulation of metals by plants can be beneficial for them, since the presence of these elements in plant tissues works as a mechanism against herbivory, causing toxicity to the individuals that feed on them (Martens and Boyd, 1994).

The effects of elemental defense can depend on the metal and the feeding mode of herbivores. For example, hyperaccumulation of nickel in *Streptanthus polygaloides* (Brassicaceae) significantly decreased the survival of leaf chewers (grasshoppers and lepidopterans) but did not affect vascular tissue-sucking insects (aphid, leafhopper, and whitefly species) (Jhee et al., 2005). In contrast, there was a decrease in the performance of chewing Lepidoptera species and sucking aphid species in *Arabidopsis halleri* (Brassicaceae) plants grown in soil contaminated with Cd and Zn (Stolpe et al., 2017). The above-mentioned studies have been carried out in the laboratory; few studies have extended the effects of elemental defense to the field. Field research conducted by Kazemi-Dinan (2015), in which *A. halleri* plants were grown in soil contaminated with Cd and Zn, showed that control plants were visited by more herbivores than contaminated plants, with more insects per plant and more species. Thus, the herbivorous insect community associated with a metal-contaminated plant may change, resulting in possible effects on the natural enemies of these insects.

Controlled laboratory experiments have shown that herbivorous insects that feed on plants grown in soil contaminated with heavy metals, or on contaminated artificial diets, transfer these elements to their natural enemies, such as predatory insects and parasitoids (Kazimírová et al., 1997; Kazimírová and Ortel, 2000; Naikoo et al., 2021, 2019; Ortel, 1995; Shi et al., 2020; Ye et al., 2009). Some studies have shown that both predators and parasitoids suffer adverse effects due to exposure to heavy metals via herbivores. For example, ladybug species had a decrease in predation and fecundity and an increase in larval mortality (Naikoo et al., 2019; Shi et al., 2020), whereas adult hatching, fertility, and population growth rate were reduced in parasitoids (Kazimírová et al., 1997; Kramarz and Stark, 2003; Ortel et al., 1993; Vickerman et al., 2004; Ye et al., 2009). All these studies suggest that the accumulation of heavy metals by plants could affect the community of insects that live in their aerial part, as herbivores in polluted areas could act as sinks reducing population densities of natural enemies (Butler et al., 2009; Gardiner and Harwood, 2017). However, these works are restricted to laboratory experiments at the individual level or one trophic chain at a time; therefore, it is not possible to extend these effects to various groups of species and trophic levels, at the natural community level.

Thus, this study aimed to investigate how the presence of heavy metals in the soil, at different concentrations, can affect the insect community that lives in the aerial part of a plant. We investigated how soil Pb concentrations allowed in Brazil affect kale (*Brassica oleracea* L., var. *acephala*) insect community in a field experiment. We evaluated how treatments with different Pb concentrations affect the richness, composition, and abundance of species in the food web of kale-associated insects, investigating the effect on the following functional groups: chewing and sucking herbivores and their respective parasitoids, hyperparasitoids, predators, and parasitoids of predators. Based on the literature, we hypothesized that increasing soil lead levels negatively affect the insect community associated with the plant as a whole.

2 Methods

2.1 Experimental procedure

We chose kale (*Brassica oleracea* L., var. *acephala*) and its associated insects because it is a fast-developing plant and has a diverse community of associated insects (more details can be found in Supplementary Material-1). In addition, previous studies showed that kale plants absorb heavy metals from the soil, presenting levels of metals that classify this species as a normal plant, favoring this type of experiment. Kale plants grown in soils considered uncontaminated showed lead levels in their leaves of up to 0.041 mg/kg, while plants grown in soils experimentally contaminated with heavy metals showed lead levels of up to 3.2 mg/kg, on average (Lima et al., 2015, 2009; Mutua et al., 2020).

The experiment was conducted in a greenhouse on the experimental farm ("Centro de Desenvolvimento de Transferência de Tecnologia-CDTT") of the Federal University of Lavras (UFLA), located in the municipality of Ijaci (Minas Gerais, Brazil). An open greenhouse was used so that the colonization of plants by insects occurred naturally, making the experiment as close as possible to what happens in the field. Therefore, the conditions of the greenhouse (temperature, humidity and photoperiod) were natural. The kale plants were grown in 12 L-plastic pots. Each pot was connected to a PET bottle through a hose attached at the bottom of the pot, closing the water circuit and preventing local contamination. Only the upper part of the greenhouse was covered with a transparent tarpaulin, as the closed circuit pot system would not withstand heavy rains.

To prepare the soil in each pot, the following mixture was used: 2 kg of expanded clay, used for drainage, and 8 kg of soil containing sand, organic fertilizer, and limestone.

The soil used was previously analyzed for levels of heavy metals, showing a Pb concentration of 25.9 mg/Kg (more details can be found in Supplementary Material-1 and Table S1). This concentration is within the Pb concentrations assumed normal for soils in the region (Fernandes et al., 2007). The soil, in each pot, was contaminated with the following concentrations of lead nitrate (Pb $(NO_3)_2$): 0 (control, referred to T0 in this paper), 144 (T1), 360 (T2), and 600 (T3) mg/kg of soil. The concentrations of contaminated treatments correspond, respectively, to the prevention value, limit value for the agricultural area, and limit value for residential area, according to CONAMA resolution 460/2013 (Conselho Nacional do Meio Ambiente, 2013). The contamination was performed by diluting lead nitrate in two liters of water and applying this solution to the soil in the pots. For the control pots (T0), only water was applied.

Kale seedlings were planted in January 2019, three months after soil contamination. Ten pots were prepared for each treatment, totaling 10 replicates of individual plants (one kale plant per pot) for each treatment and 40 pots. The pots were kept at a distance of 1.2 m^2 and arranged randomly (Figure S1 of the Supplementary Material). We tagged plants from 1 to 40 and drew each plant position in the greenhouse using an online random number drawing service. All plants were irrigated *ad libitum* twice a week. Details on the study system and experimental procedure can be found in Supplementary Material-1.

2.2 Insect collection

Two collection methods were used due to differences in the colonization of plants by insects and their biology. Lepidoptera species (the first insects to colonize plants) and their parasitoids were collected by inspecting each plant and collecting the lepidopteran pupae attached to the plants, placing them into properly labeled plastic tubes kept in the laboratory. Two collections were performed, on March 28 and April 4, 2019.

Aphid species, as well as their parasitoids and predators, were captured after natural colonization using voile bags tied to the base of the petiole of the leaves with twine. The voile bags allowed us to preserve the aphids on the same leaf while also containing emerging parasitoids, predators, and predator parasitoids that were already on the leaf at the time of collection. In the collection, we placed a properly labeled voile bag on a leaf of each plant, randomly. The collections were performed on May 3rd and 31st, 2019. After 15 days in the field, we took the bagged leaves to the laboratory for screening and identification of insects. Details on insect collection can be found in Supplementary Material-2

2.3 Lead detection

After the field experiment, the plants were harvested, removing the tissue above the ground, and taken to the laboratory for drying in an oven, weighing on a precision analytical balance, and subsequent grinding in a knife mill. Plants were divided into stems and leaves for Pb determination in their structures. Dried and ground samples of leaves and stems were sent to the company SG "Soluções Científicas" (São Carlos, SP, Brazil) for Pb determination using an Optical Emission Spectrometer with Inductively Coupled Plasma (ICP-OES). For the determination of Pb, a sample of 1.0 g of stem and leaf of each plant was used (10 repetitions for each treatment). Details on the chemical analysis procedure can be found in Supplementary Material-3 and tables S2 and S3.

2.4 Data analysis

To assess the sampling efficiency of collections and identify differences between treatments in accumulated species richness, we constructed species accumulation curves using the rarefaction method based on interpolation/extrapolation and Chao1 asymptotic richness species estimator. Non-Metric Multidimensional Scaling (NMDS) was used to analyze the insect community composition. The similarity analysis (ANOSIM) was used to compare species composition between treatment and collection (the two time points) groups. The indicator value (IndVal) was used to assess which species in the community could be considered indicators for collection and treatments (Cáceres et al., 2020).

Generalized linear models (GLM) and post-hoc Tukey test were used to analyze the results of plant biomass at the end of the experiment and lead determination. Generalized linear mixed-effect models (GLMM) and post-hoc Tukey test were used to assess the effect of treatments on the abundance of functional groups of insects in the community and on the parasitism rate. Different structures for random effects were assessed (Rhodes et al., 2009). The best model was selected, based on the variables used as a random effect, using the Akaike information criterion corrected for small samples (AICc) (Burnham et al., 2011). Details on the statistical analysis procedure can be found in Supplementary Material-4 and Table S4. All statistical analyses were performed using the R software (R Core Team, 2020).

3 Results

3.1 Effect of treatments on plant biomass and detection of lead in samples

The total biomass of leaves and stem of kale plants at the end of the experiment did not differ statistically between treatments (for more details, please refer to Figure S2 in the Supplementary Material).

Among the ten leaf samples from the control (T0), nine had Pb content below the detection limit and one had 0.170 mg/kg^{-1}, with an average of 0.017 (±0.054) mg of Pb/kg^{-1} (dry weight). For the contaminated treatments (T1, T2, and T3), the leaf samples had averages of 0.120 (±0.036), 0.181 (±0.125), and 0.388 (±0.150) mg of Pb/kg^{-1}, respectively. Only the Pb concentrations in T1 and T2 leaf samples were not statistically different (p = 0.18) (Figure 1A; Table S5 of the Supplementary Material). Two leaf samples from T2 and five from T3 showed Pb concentrations above the limit for the studied plant species (0.3 mg/kg^{-1}), ranging from 0.386 to 0.598 mg/Kg^{-1}. The values in parentheses presented in this section refer to the standard deviation.

The stem samples from T0, T1, T2, and T3 had averages of 0.076 (±0.032), 0.198 (±0.061), 0.354 (±0.198), and 0.745 (±0.422) mg of Pb/kg^{-1}, respectively. Pb concentrations in the treatments were all statistically different (Figure 1B; Table S6).

3.2 Sampling, description, and assessment of community richness and composition

A total of 96,151 specimens, belonging to 17 species and three trophic levels, were obtained (Table S7 of the Supplementary Material). The Chao1 asymptotic species richness estimator identified 23 (17.93-55.37) species (Figure S3A). In the first collection, the species accumulation curve showed a tendency toward an increase in the number of species, with 15 observed species and 29.99 (18.27-83.60) estimated species. In the second collection, the curve showed a tendency toward stability, with 14 observed species and 16 (14.18-36.12) estimated species, (Figure S3B). The values in parentheses presented in this section refer to the confidence interval.

The T0 curve was the farthest from reaching an asymptote, with 15 observed species and 17.25 (15.26-34.03) estimated species. On plants of the T1 treatment, 14 species were observed, showing no difference of species estimated, 14.16 (14.00-17.53). On plants of the T2 and T3 treatments, 12 species were observed, and an estimated number of 12.25 (12.01-16.73) and 12.00 (12.00-13.62), respectively (Figure 2A).

Two species (*Brevicoryne brassicae*, the most abundant, and *Myzus persicae*) of herbivorous insects are suckers (aphids), representing 97.13% of the collected insects. Two species (*Plutella xylostella*, the most abundant, and *Trichoplusia ni*) are chewers (Lepidoptera), representing 0.27%. The observed richness of herbivorous species on plants of the four treatments was 4 species, not differing from the species value indicated by the estimator (4 species for all treatments; 4.00-4.83 for T0 and T1, 4.00-4.28 for T2, and 4.00-4.48 for T3) (Figure S3C).

Three species of parasitoid insects are parasitoids of sucking herbivores, and *Diaeretiella rapae* (Braconidae, Aphidiinae) was the most abundant species. One parasitoid, *Oomyzus sokolowskii* (Eulophidae, Tetrastichinae), is a gregarious parasitoid of a chewing herbivore (*P. xylostella*). The estimated richness of parasitoid species did not differ from the observed, accounted for 3 species for plants of the T0 (3.00-3.84), T2 (3.00-4.50), and T3 (3.00-4.50) treatments and 4 species for plants of the T1treatment (4.00-5.51) (Figure S3D).

The three predatory species recorded here feed on sucking herbivores, and *Allograpta exotica* (Diptera, Syrphidae) was the most abundant species. The estimated richness of predator species did not differ from the observed, comprising 2 species for plants of the T0 (2.00-3.60), T1 (2.00-2.86), and T2 treatments and 1 species for plants of the T3 treatment (1.00-1.01) (Figure S3E).

The four hyperparasitoid species found here are associated with sucking herbivores. The estimated richness of hyperparasitoid species for plants of the T0 treatment was the only one that differed from the observed richness, with 4.87 (4.07-15.80) estimated species and 4 observed species. The estimated and observed species richness on plants of the contaminated treatments (T1 – T3) was 2 (2.00-2.85 for T1, 2.00-2.89 for T2, and 2.00-2.28 for T3) (Figure 2B).

Two species of parasitoids of predators (*A. exotica*) were also found, one of them of gregarious habit (*Pachyneuron* sp.). The estimated species richness of parasitoids of predators did not differ from the observed one, with 2 species for plants of the T0 (2.00-2.00), T1 (2.00-2.51), and T3 (2.00-2.29) treatments, and 1 species for plants of the T2 treatment (1.00-1.46) (Figure S3F).

The community ordering analysis showed a clear dissimilarity between the two collections (the two time points) regarding species composition (NMDS stress = 0.04; Figure 3). Similarity analysis showed that both collection (R = 0.31, *p* = 0.001) and treatments (R = 0.04, *p* = 0.02) contributed significantly to community ordering. The indicator value (IndVal) selected two species associated with the treatments: one associated with the control treatment (*Pachneuron* sp.) and another associated with both the control and T1 (*D. rapae*). For collection, IndVal selected six species: one associated with collection 1 (*M. persicae*) and five associated with collection 2, with the highest indicator value recorded for *B. brassicae* (Table S8).

3.3 Effect of treatments on total insect and herbivore abundance

On plants of the T0, a significantly higher abundance of insects was found than on plants of the contaminated treatments. ($p < 0.05$). There was no significant difference between plants of the contaminated treatments (Figure S4 and Table S9 of the Supplementary Material).

Similar results were found for herbivorous insects as a whole, as they constitute the majority of individuals (97.39%). Plants of T0 had a significantly higher herbivore abundance than plants of the contaminated treatments ($p < 0.05$). Herbivore abundance did not differ statistically between plants of the contaminated treatments (Figure 4A; Table S10).

Plants of T0 had a higher abundance of sucking herbivores than plants of the contaminated treatments ($p < 0.05$). There was no statistical difference in abundance between plants of the contaminated treatments (Figure 4B; Table S11).

Plants of T0 also had a higher abundance of chewing herbivores. Only plants of T2 did not differ statistically from plants of T0 ($p = 0.3110$). There was no statistical difference in abundance between plants of the contaminated treatments (Figure 4C; Table S12).

3.4 Effect of treatments on parasitoid insect abundance

The parasitism rate showed no significant difference between treatments (Figure 5A and Tables S13 and S14 of the Supplementary Material). However, on plants of T0, a higher abundance of parasitoids was found than on plants of the contaminated treatments, which did not differ statistically from each other (Figure 5B; Table S15).

Plants of T0 had a higher abundance of parasitoids of sucking herbivores and did not differ statistically only from plants of the least contaminated treatment (T1) ($p = 0.4017$). Plants of the contaminated treatments did not differ statistically from each other (Figure 5C; Table S16).

Plants of T0 had a higher abundance of parasitoids of chewing herbivorous. Only plants of T2 did not differ statistically from plants of T0 ($p = 0.3872$). Plants of the contaminated treatments did not differ statistically from each other (Figure 5D; Table S17).

3.5 Effect of treatments on predatory insect abundance

On plants of T0, a significantly higher abundance of predatory insects was found. Only plants of the least contaminated treatment (T1) did not differ statistically from plants of T0 ($p = 0.1948$). Plants of the contaminated treatments did not differ statistically from each other (Figure 6; Table S18 of the Supplementary Material).

3.6 Effect of treatments on top parasitoid insect abundance

There was no significant difference between the plants of the four treatments for hyperparasitoid abundance (Figure 7A; Table S19 of the Supplementary Material). For parasitoids of predators, plants of T0 had a higher abundance, differing statistically from plants of the contaminated treatments, which did not differ from each other (Figure 7B; Table S20).

4 Discussion

4.1 Pb absorption by kale

Pb concentration in the plants increased with the increase of Pb in the soil through the treatments. The stem had a higher Pb concentration than the leaves. The allocation of Pb in brassicas grown in contaminated soils is known to occur in the following order: root > stem > leaves (Lima et al., 2009). The stem plays an important role in Pb accumulation and translocation to leaf areas through vascular flow (De Temmerman et al., 2015; Krzesłowska et al., 2010).

Although the Pb concentrations of the treatments represent allowed Pb levels for soil, kale leaf samples from T2 and T3 had Pb concentrations above the limit allowed for human consumption, which is 0.3 mg/Kg^{-1} (Agência Nacional de Vigilância Sanitária, 2021; Codex Alimentarius Commission, 2017). This result indicates potential risks to human health caused by the consumption of leafy vegetables grown in agricultural and residential soils containing Pb concentrations allowed in Brazil. In addition, kale and other vegetables, even when absorbing Pb concentrations above the tolerance limits, complete their cycle without visible symptoms of phytotoxicity and without having their dry matter production affected, as we also observed in the present work, which is an aggravating factor (Lima et al., 2009; Lima et al., 2015).

4.2 Effect of Pb on accumulated species richness and composition

The species accumulation curves showed that the contaminant Pb affects the kale-associated insect community richness. The number of species between treatments was found in the following order: T0 > T1 > T2 = T3. The effect of Pb on the insect community can also be observed through the composition of the species, in which the treatments had a significant effect on the community ordering. It is known that soil contamination by metals affects the edaphic arthropod community. Some studies have shown that the community of organisms such as Symphila, Protura, Diplura, Collembola, Arachnida, Coleoptera, among others, were negatively affected by soil contamination with various heavy metals, mainly regarding their composition (Manu et al., 2017; Migliorini et al., 2004; Nahmani and Lavelle, 2002; Syrek et al., 2006). However, regarding the influence of soil heavy metals on the insect community living in the aerial part of plants, studies are limited to herbivorous or pollinating insect species, which had their richness and composition negatively affected (Kazemi-Dinan et al., 2015; Moroń et al., 2012). Our research addresses, for the first time, the effect of heavy metal soil contamination on the insect community of parasitoids, predators, and higher levels, showing that primary parasitoid (*D. rapae*) and top parasitoid species (*Pachneuron* sp.) may be more sensitive to soil Pb contamination.

4.3 Effect of Pb on herbivorous insects

The Pb treatments caused a decrease in the total abundance of insects compared to the control treatment (T0). These results were found for Pb concentrations allowed by soil legislation, corroborating the results recorded in a review by Monchanin et al. (2021), who showed that heavy metals significantly impact invertebrate physiology and behavior, even at levels below those recommended as safe for humans.

The same was observed for herbivorous insects as a whole. This result is in accordance with the elemental defense hypothesis, which predicts that plants benefit when accumulating heavy metals from the soil, as herbivory is reduced due to the toxic effect of metals on herbivores (Martens and Boyd, 1994). Both sucking and chewing herbivores had their abundance affected by Pb, which corroborates the results found by Stolpe et al. (2017), who observed a decrease in the population of *Myzus persicae* (aphid species), also found in our research, and that *Mamestra brassicae* caterpillars fed less on *Arabidopsis halleri* (Brassicaceae) plants grown in soil contaminated with Cadmium (Cd) and Zinc (Zn).

The effect of Pb levels on elemental defense was previously investigated by Coleman et al. (2005) and Jhee et al. (2006), who used an artificial diet corrected with different metal concentrations to feed *Plutella xylostella* caterpillars, which is the main chewing species in our study and the main brassica pest. They found that Pb is toxic to caterpillars, even in diets with metal concentrations below the limit considered for a Pb accumulator plant (100 mg/kg^{-1}, based on dry weight), being 30, 17, 15, and 9.5 mg/kg, which caused a decrease in survival and percentage of pupation in all contaminated treatments. Based on this result, Coleman et al. (2005) suggested that even lower concentrations could have a defensive value for a plant, which was confirmed in our research, as kale herbivore populations decreased at mean concentrations of 0.388, 0.181, and 0.120 mg of Pb/Kg of leaf.

Other factors that act synergistically on elemental defense may be implicated in reducing the population density of herbivorous species. The oxidative stress generated by the metal in the plant is one of them. Pb indirectly affects aphids through higher amounts of reactive oxygen species formed in pea leaves (*Pisum sativum*), creating negative effects on the reproduction, longevity, and feeding of these insects, according to Woźniak et al. (2019, 2017). Another mechanism is the joint effects hypothesis, which states that metals and plant organic defenses might work in an additive or synergistic manner, amplifying the harmful effects of each form of defense individually (Boyd, 2012). Pb caused the accumulation of organic defense elements in pea leaves with higher concentrations of these elements in aphid-infested plants (Woźniak et al., 2017). Targeted studies are needed to investigate which of these factors may affect the insect communities on kale.

4.4 Effect of Pb on parasitoid insects

The parasitism rate did not differ statistically between treatments, which shows that the parasitoids possibly do not discriminate hosts in Pb-contaminated plants from those in non-contaminated plants. Laboratory tests indicated that when hosts are offered, parasitoid species do not discriminate herbivorous hosts contaminated with different concentrations of heavy metals (Pb, Cd, Cu, Ni, and Zn) by food from non-contaminated hosts, resulting in similar parasitism rates between treatments (Kazimírová et al., 1997; Ortel et al., 1993; Ruohomaki et al., 1996). There is a lack of behavioral studies of parasitoids in systems contaminated by heavy metals, making it difficult to establish relationships between parasitic behavior and the non-difference in parasitism rates. One of the few experiments with olfactometry showed that adults of the parasitoid *Cotesia marginiventris* did not discriminate alfalfa plants contaminated with the metalloid Selenium from the control plants, even though the plant had volatiles of the contaminant. (Vickerman et al., 2004).

On the other hand, parasitoid abundance was significantly negatively affected by Pb-contaminated treatments in our research. Butler et al. (2009) suggested that hosts in polluted areas could act as sinks reducing parasitoid population densities precisely because of the parasitoids’ inability to detect contaminated hosts. Studies have shown adverse effects on the development of parasitoids in hosts contaminated by various metals and metalloids, such as extended larval and pupal period, reduced number of hatched adult parasitoids, adult life shortened, and reduced percentage of female offspring and fertility (Kazimírová et al., 1997; Kramarz and Stark, 2003; Ortel et al., 1993; Vickerman et al., 2004; Ye et al., 2009). In the case of Pb, the number of hatched adults was significantly reduced when a species of parasitoid developed in caterpillars fed on a diet contaminated with 4 mg of Pb/kg (Ortel et al., 1993). Thus, the decreased population density of parasitoids of caterpillars and aphids fed on Pb-contaminated kale could be explained by the toxic effects from the contaminated hosts, thus decreasing the number of successful adult parasitoids in hatching, corroborating the idea of hosts as sinks suggested by Butler et al. (2009). This effect could cause, in the long term, a decrease in the rate of parasitism in environments contaminated by metals and, consequently, an increase in the population density of herbivores, as a space free from natural enemies is created in these environments, as demonstrated by Zvereva and Kozlov (2006).

4.5Effect of Pb on predatory insects

The abundance of predatory insects decreased in the Pb most contaminated treatments (T2 and T3). The predators found in our research are composed mostly of *Allograpta exotica* (Syrphidae) and a few ladybugs, both feed on aphids. The effect of heavy metals on predatory syrphids is still poorly known. Markova and Alexiev (2002) showed that predatory species of the genus *Sphaerophoria* accumulate higher concentrations of metals in their body than species of a detritivorous genus (*Eristalis*). *Sphaerophoria* specimens collected near a steel production plant had mean Pb concentrations ranging from 4.4 to 15.2 mg/kg^{-1} (based on dry weight) and even higher values of other metals. The possibility of accumulating high concentrations, via aphids, could cause adverse effects on the development of these insects and explain the decreased population density. The experiments by Jaworska and Gospodarek (2003, 2000), growing white fava bean and beets in soil contaminated with heavy metals, found that syrphid larvae that prey on aphids were most frequently observed in control aphid colonies. Furthermore, female Syrphidae laid more eggs on control plants than on contaminated ones, suggesting possible discrimination from plants contaminated by heavy metals.

4.6 Effect of Pb on top parasitoids

The abundance of the two top parasitoid groups had divergent results, in which the abundance of hyperparasitoids was not affected by the Pb treatments, whereas parasitoids of predators were negatively affected by all contaminated treatments equally. Given that no studies have addressed the effect of heavy metals on these higher trophic levels in plant-associated insect communities, we can think about the biology and mode of exposure of these insects to explain the effect of Pb treatments on population density. In the case of hyperparasitoids, each individual feeds on a single parasitoid larva which, in turn, feeds on a single aphid. In contrast, parasitoids of Syrphidae feed on individuals that feed on a large number of aphids throughout their larval period. *A. exotica* larvae can feed on an average of up to 23.34 aphids per day (Arcaya et al., 2017). Thus, parasitoids of predators may be more exposed to heavy metal toxicity than hyperparasitoids, which could result in a population decrease. However, some negative effect on the accumulated species richness of hyperparasitoids was still observed. This result indicated that top species in plant-associated terrestrial invertebrate systems may be more vulnerable to heavy metal pollution, as expected in other systems, such as in the case of soil-predating invertebrates (Chauvin et al., 2020) and fish and aquatic predatory invertebrates (Hogsden and Harding, 2012a, 2012b).

5 Conclusions

Our research demonstrated that soil contamination with lead, even at allowed concentrations, negatively affects the insect community in the aerial part of plants. The results found here evidence the need to consider different groups of insects in heavy metal risk analysis, as important ecosystem services such as predation and parasitism can be impacted.

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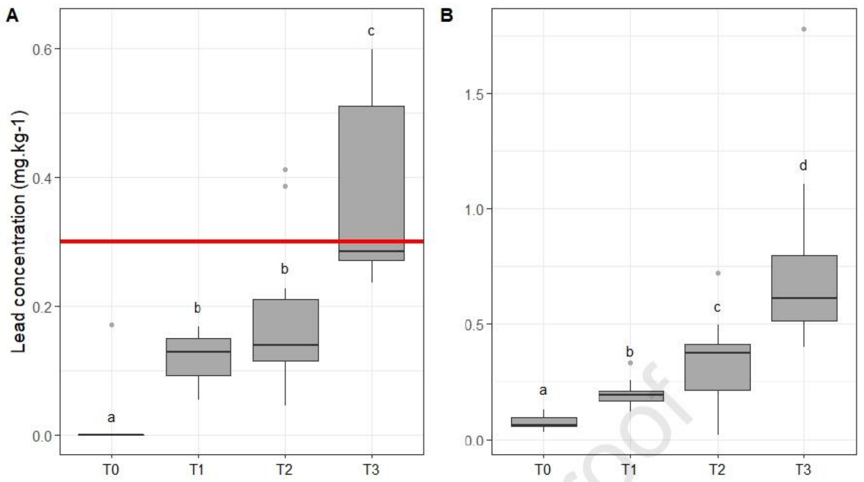

Figure 1. Lead concentration (mg.kg^{-1}, based on dry weight) in kale samples determined by ICP-OES. (A) Leaf. (B) Stem. Number of replicates per treatment = 10 samples from 10 plants. Box: interquartile range (IQR); lower and upper borders of box: the lower and upper quartiles (Q1 and Q3); line inside the box: median; whisker: minimum to maximum (Q1-1,5*IQR and Q3+1,5*IQR); points: outliers. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of $Pb(NO_3)_2$ /Kg of soil. GLMs significant difference: $p < 0.001$. Different letters indicate a significant difference by Tukey test. Red line: the maximum limit of lead allowed for kale leaf.

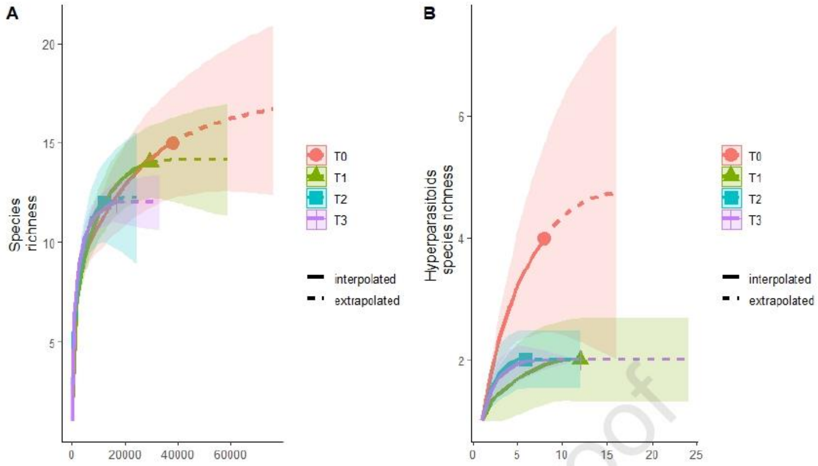

Figure 2. Species accumulation curves based on specimens sampling for kale-associated insect community. The curves were constructed using the rarefaction method based on interpolation/extrapolation and using the Chao1 species richness estimator and a 95% confidence interval. (A) Treatments. (B) Hyperparasitoids. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of $Pb(NO_3)_2$ /Kg of soil.

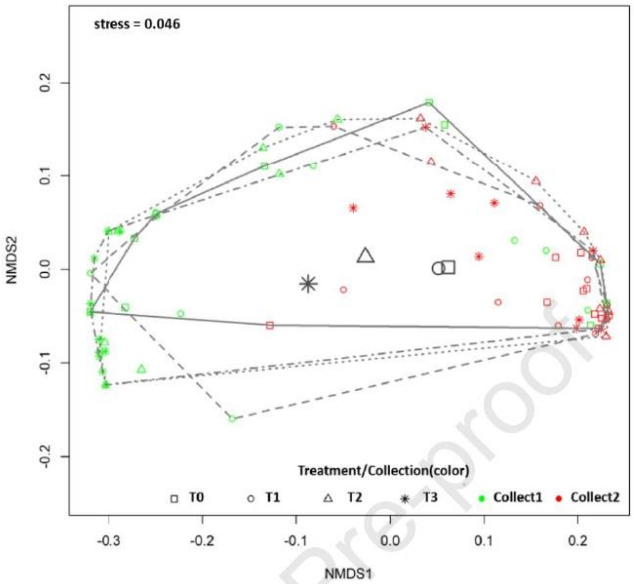

Figure 3. NMDS of the kale-associated insect community. The scores of sampling units of collections 1 and 2 (the two time points) are shown in green and red. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of $Pb(NO_3)_2$ /Kg of soil. Treatment scores are shown as their respective figures (indicated in the caption) in the centroid of the treatment positions in the ordering space.

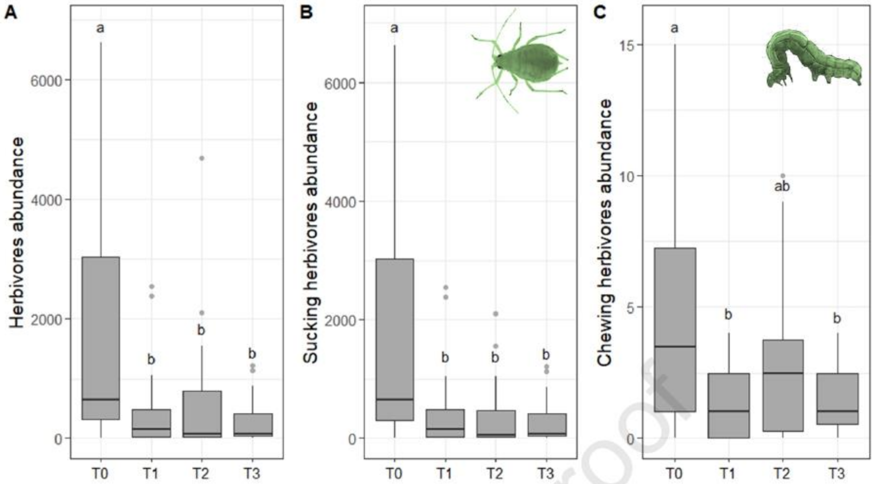

Figure 4. Effect of Pb treatments on abundance of kale-associated herbivorous insects. (A) Herbivores in total. (B) Sucking herbivores. (C) Chewing herbivores. Abundance is plotted per plant and per collection, totaling 20 replicates per treatment. Box: interquartile range (IQR); lower and upper borders of box: the lower and upper quartiles (Q1 and Q3); line inside the box: median; whisker: minimum to maximum (Q1-1,5*IQR and Q3+1,5*IQR); points: outliers. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of Pb(NO$_3$)$_2$ /Kg of soil. GLMM were significantly different from the null model (total herbivores, *p* = 0.0054, sucking herbivores, *p* = 0.0142, and chewing herbivores, *p* = 0.0065). Different letters indicate a significant difference by Tukey test.

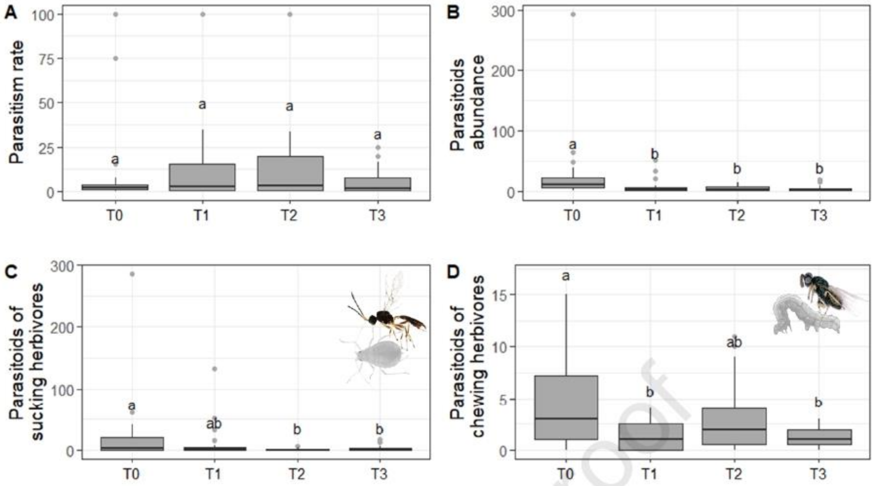

Figure 5. Effect of Pb treatments on abundance of kale-associated parasitoid insects. (A) Parasitism rate. (B) Abundance of parasitoids in total. (C) Abundance of parasitoids of sucking herbivores. (D) Abundance of parasitoids of chewing herbivores. Abundance is plotted per plant and per collection, totaling 20 replicates per treatment. Box: interquartile range (IQR); lower and upper borders of box: the lower and upper quartiles (Q1 and Q3); line inside the box: median; whisker: minimum to maximum (Q1-1,5*IQR and Q3+1,5*IQR); points: outliers. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of $Pb(NO_3)_2$ /Kg of soil. GLMM were significantly different from the null model for three models (parasitoid abundance in total, p = 0.0001245, abundance of parasitoids of sucking herbivores, p = 0.01049, abundance of parasitoids of chewing herbivores, p = 0.009339). The parasitism rate model did not differ from the null model (p = 0.8208). Different letters indicate a significant difference by Tukey test.

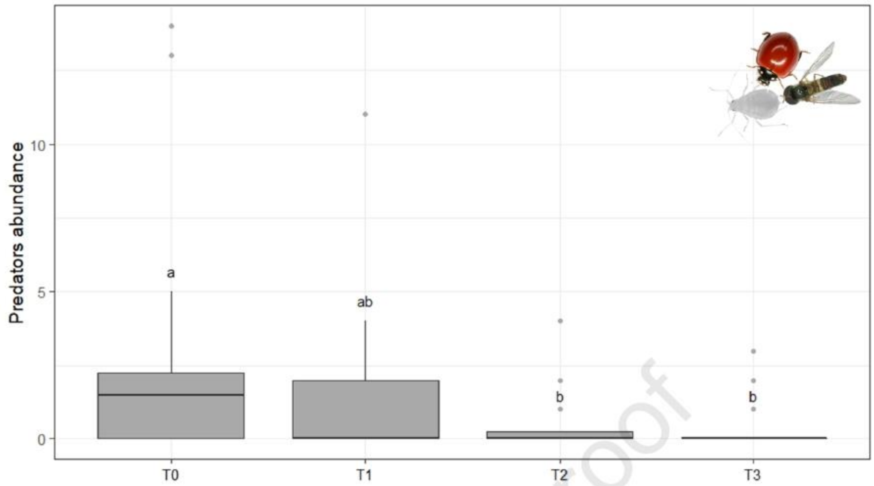

Figure 6. Effect of Pb treatments on abundance of kale-associated predatory insects of herbivores. Abundance is plotted per plant and per collection, totaling 20 replicates per treatment. Box: interquartile range (IQR); lower and upper borders of box: the lower and upper quartiles (Q1 and Q3); line inside the box: median; whisker: minimum to maximum (Q1-1,5*IQR and Q3+1,5*IQR); points: outliers. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of $Pb(NO_3)_2$ /Kg of soil. GLMM were significantly different from the null model (p = 0.009521). Different letters indicate a significant difference by Tukey test.

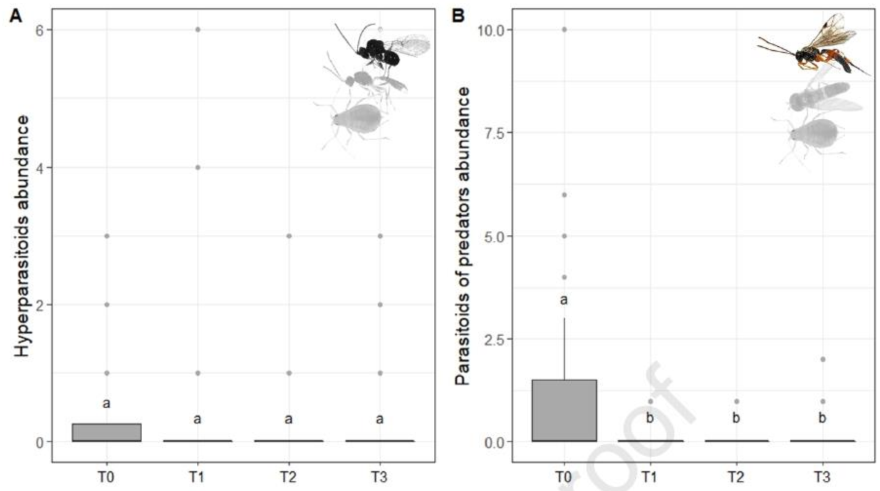

Figure 7. Effect of Pb treatments on abundance of kale-associated top parasitoid insects. (A) Abundance of hyperparasitoids. (B) Abundance of parasitoids of predators. Abundance is plotted per plant and per collection, totaling 20 replicates per treatment. Box: interquartile range (IQR); lower and upper borders of box: the lower and upper quartiles (Q1 and Q3); line inside the box: median; whisker: minimum to maximum (Q1-1,5*IQR and Q3+1,5*IQR); points: outliers. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of $Pb(NO_3)_2$ /Kg of soil. GLMM were significantly different from the null model (*p* = 0.001293) for parasitoids of predators, but not for hyperparasitoids (*p* = 0.9857). Different letters indicate a significant difference by Tukey test.

Highlights

Permissible Pb concentrations for soils affect the plant-associated insect community

Herbivores, primary and top parasitoids and predators show a reduced abundance

Soil Pb concentrations lead to Pb levels in leaves above the permitted level

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supplementary Material

1. The study system and experimental procedure

Kale-associated insects are a good study model, as their identification at the species level is relatively easy, avoiding errors related to taxonomy. Kale-associated insects have different characteristics, being mainly chewing and sucking herbivores (e.g., lepidopterans and aphids, respectively) and their natural enemies, which are represented by parasitoid and predatory insects (e.g., ladybugs and syrphids) (Celestino et al., 2015; Cividanes et al., 2020; Resende et al., 2006; Valbon et al., 2015; Zuim et al., 2015). Thus, this insect community, structured in a source-type food web, based on kale, makes possible a comparative study on the effect of environmental contamination by metals on different groups of organisms.

The lead (Pb) was chosen for this study, which is ranked the second most harmful metal on the US Environmental Protection Agency priority list (ATSDR, 2020). Environmental contamination by Pb is mainly associated with the manufacture of batteries, combustion of gasoline containing Pb, welding, paints, herbicides, and insecticides (Thangavel and Subbhuraam, 2004; Wuana and Okieimen, 2011). The survey of Contaminated Areas carried out by the State Environmental Foundation identified Pb as the heavy metal most frequent in contaminated areas in the state of Minas Gerais (Brazil), where this study was carried out (FUNDAÇÃO ESTADUAL DE MEIO AMBIENTE, 2015).

The soil used in the experiment is of the "red latosol" type, characteristic of the region. This type of soil is usually acidic, with a pH ranging from 4.0 to 6.0 (Ker, 1997). Samples of the soil used in the experiment were analyzed for levels of heavy metals before soil preparation using Flame Atomic Absorption Spectrometry (FAAS) (Table S1). For soil contamination, Pb was used in the form of nitrate (Pb $(NO_3)_2$), making possible better handling and application to the soil by diluting with water. Lead nitrate was previously weighed on a precision analytical scale and transported in individual paper bags to each pot, according to each concentration. We applied the previously weighed lead nitrate concentrations by diluting them in two liters of water in a PET bottle, which was sealed and mixed for two minutes. Then, the solution was applied to the soil of each pot. Kale seedlings were planted in January 2019, three months after soil contamination to increase the metal adherence to the soil. The general setup of the experiment can be seen in Figure S1.

Table S1. Result of the determination of heavy metals (FAAS) in the soil used in the experiment.

Heavy metal	**Mg/kg**
Fe	37896
Cu	9.1
Mn	79.58
Zn	12.6
Ni	12.1
Cr	56.74
Cd	5.4
As	46.8
Pb	25.9
Se	72.7

Figure S1. Overview of the setup of the kale cultivation experiment in an open greenhouse.

2. Insect collection

Insect collections were conducted during the non-flowering stage, with kale plants measuring about 40 cm in height and showing many leaves. The lepidopteran species *Plutella xylostella* (L.), and *Trichoplusia ni* (Hübner), were the first insects to colonize the plants. As females of these species usually lay eggs singly and individuals feed on plants in a dispersed manner, plant inspection is the most correct sampling method (Celestino et al., 2015; Zuim et al., 2015). In the laboratory, the pupae were kept in plastic tubes covered with voile cloth for completing their development and emergence, as well as for containment of adults or parasitoids.

The aphid species *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer) colonized the plants in a second moment. Unlike lepidopteran species, these insects are found feeding on the leaves in an aggregate way, forming numerous colonies (Valbon et al., 2015). For specimen identification, after their death, the insects were maintained in vials containing 70% alcohol; then the specimens were identified using identification keys and specialized literature.

3. Lead Detection

The samples were digested in a digestion system assisted by a digester block equipped with perfluoroalkoxy (PFA) tubes (Savillex, MN, USA). Approximately 1.00 g of each sample was weighed directly into the PFA tubes, using an analytical scale (model AY 220, Shimadzu, Kyoto, Japan). After weighing the samples, each one was added with an acid mixture containing 5.0 mL of HNO_3, 5.0 mL of deionized water, and 2.0 mL of H_2O_2. Together with the samples, two analytical blanks were performed. For Pb determinations in the properly digested samples, an inductively coupled plasma optical emission spectrometer (ICP-OES iCAP 7000 model, Thermo Fischer Scientific, Madison, WI, USA) was used. For details on the heating program, parameters, and operating conditions of the equipment, please refer to Tables S1 and S2. The detection limit of the used method was 1.0 ($\mu g.kg^{-1}$) and the quantification limit was 5.0 ($\mu g.kg^{-1}$).

Table S2. Heating program and operating parameters used for total digestion of samples assisted by a digester block.

Istrumental parameters	Operation conditions
Digestion temperature	100 °C
Ramp time	30 minutes
Digestion time	3 hours

Table S3. Instrumental parameters used for the determination of Pb by ICP OES.

Instrumental parameters	Operation conditions
Integration time for emission line(s)	5
Sample introduction flow rate (mL min-1)	4.2
Sample flow rate during analysis (mL min-1)	2,1

Peristaltic pump stabilization time (s)	25
Radio frequency applied power (W)	1200
Auxiliary gas flow rate (L min-1)	0.25
Nebulization gas flow rate (L min-1)	0.83
Cooling gas flow rate (L min-1)	16
Pb emission line used (nm)	220.353
Signal observation view	Axial

4. Data analyses

To construct the species accumulation curves, we used Chao1 asymptotic species richness estimator, which is based on information about rare species (singletons and doubletons) to estimate a lower bound for true species richness, and a 95% confidence interval, using the iNEXT package (Chao et al., 2014; Hsieh et al., 2016). From the total number of specimens collected, we constructed four types of rarefaction curves, as follow: (1) for the sampled community as a whole, (2) for the two collections, (3) for the four treatments, and (4) for the functional groups of insects in the community.

In Non-Metric Multidimensional Scaling (NMDS) the external variables treatment and collection were used to compare the Bray-Curtis similarity of the community composition. First, communities were standardized and the dissimilarity matrix and adequacy of treatments and collections were assessed. The community dataset was transformed by the *Hellinger* method, which is based on square roots of standardized sites for the unit total. Subsequently, species and external variables were adjusted in order, assuming maximum correlation with the corresponding variables. The similarity analysis (ANOSIM) was performed using the vegan package (Oksanen et al., 2020). The species indicator value (IndVal) was obtained with the multipatt function of the indicspecies package. This method analyzes the association between species patterns (occurrence/abundance) and combinations of local groups, in addition to testing the significance of the associations (Cáceres and Legendre, 2009).

Generalized linear models (GLM) were used to analyze the results of plant biomass at the end of the experiment. For this, we built models for leaf biomass and models for stem biomass. The Gaussian error distribution was used in both situations. GLM were also used to analyze the results of lead determination, considering the amount of lead in leaf and stem samples as a response variable and the treatments as an explanatory variable. For the model of leaf samples, Gaussian distribution was used, whereas, for the model of stem samples, Gamma distribution had the best fit. The results below the lead detection limit were considered as zero. Pb concentrations were transformed from µg/kg to mg/kg to facilitate and standardize the discussion of results.

To analyze the effect of treatment on insect abundance through Generalized Linear Mixed-Effect Models (GLMM), the insects were divided into the following functional groups: total of insects, herbivores, parasitoids, predators, hyperparasitoids, and parasitoids of predatory insects. Herbivores were classified as suckers and chewers, whereas parasitoids as sucking-herbivore and chewing-herbivore parasitoids. The models for parasitoids of chewing insects were built based on the number of parasitized chewing herbivores, as the species of the parasitoid in question (*O. sokolowskii*) has a gregarious habit, which causes an abundance overestimation. Regarding parasitoids, GLMM were also used to assess the effect of treatments on the parasitism rate, which was calculated as follows: number of herbivores parasitized/total number of herbivores*100.

Models with four random effects structures were built, using the following variables: (*i*) plant, (*ii*) collection, (*iii*) plant and collection (singly), and (*iv*) plant plus collection (together). The models were built using the glmmTMB package (Brooks et al., 2017). Subsequently, the best model was selected, based on the variables used as a random effect, using the Akaike information criterion corrected for small samples (AICc), for which the lowest value represents the best model and the Akaike weight (w), which estimates the probability of each model being selected as the best model if the process is repeated several times (Burnham et al., 2011). The model was selected using the MuMIn package (Bartoń, 2020). The models selected for each analysis are listed in Table S4. The fit of the distribution of the models was verified by simulation of residues scaled using the DHARMa package (Hartig, 2021). Significance levels between treatments were tested by the post-hoc Tukey test, using the 'emmeans' package (Lenth, 2021).

Table S4. Models selected using the Akaike information criterion corrected for small samples (AICc), according to random variables and error distribution.

Model	Selected random variables	Selected error distribution
Insects in total	Plant plus collection (together)	Poisson
Herbivores	Plant plus collection (together)	Poisson
Sucking herbivores	Plant plus collection (together)	Poisson
Chewing herbivores	Plant and collection (singly)	Negative binomial
Parasitoids	Plant plus collection (together)	Poisson
Parasitism rate	Plant and collection (singly)	Tweedie
Parasitoids of sucking herbivores	Plant plus collection (together)	Poisson
Parasitoids of chewing herbivores	Plant and collection (singly)	Negative binomial
Predators	Collection	Negative binomial
Hyperparasitoids	Plant plus collection (together)	Poisson
Parasitoids of predators	Plant plus collection (together)	Poisson

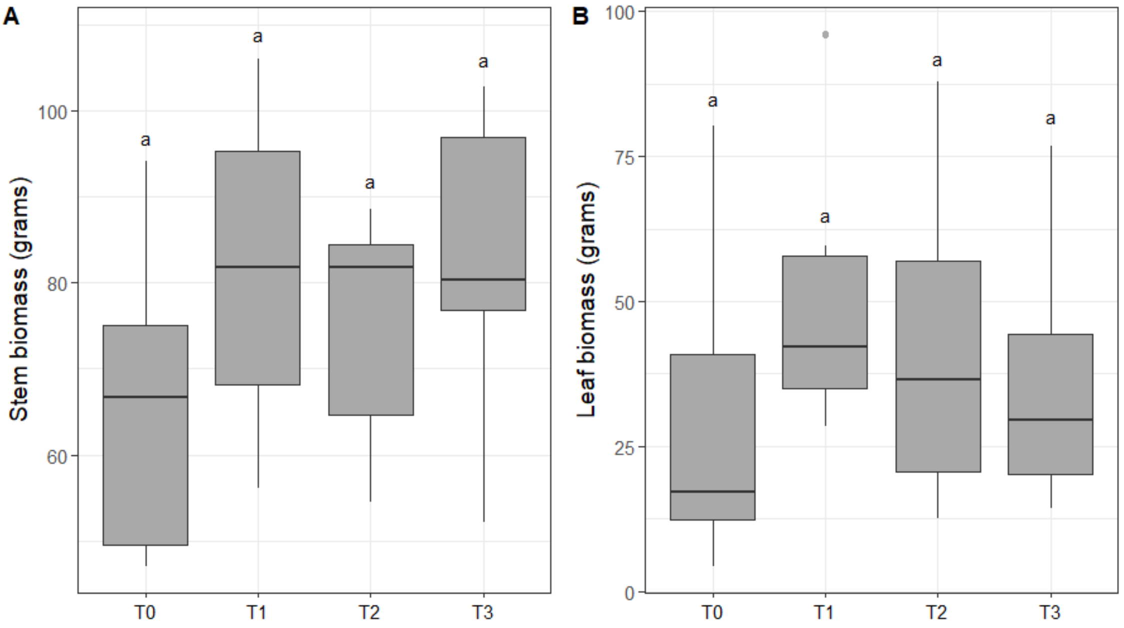

Figure S2. Total dry biomass of kale plants at the end of the experiment. (A) Leaf. (B) Stem. Number of replicates per treatment = 10 plants. Box: interquartile range (IQR); lower and upper borders of box: the lower and upper quartiles (Q1 and Q3); line inside the box: median; whisker: minimum to maximum (Q1-1,5*IQR and Q3+1,5*IQR); points: outliers. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of Pb$(NO_3)_2$ /Kg of soil. The generalized linear models (GLM) did not differ significantly from the null model (*p* = 0.09111 for the stem model and *p* = 0.1767 for the leaf model.).

Table S5. Contrast analysis between treatments (post-hoc Tukey) for the lead concentration (determined by ICP OES) in kale leaf samples.

Contrast	Estimate	Std. Error	z value	p value
t0 - t1	-0.10320	0.04533	-2.277	**0.042486***
t0 - t2	-0.16400	0.04533	-3.618	**0.000897***
t0 - t3	-0.37144	0.04657	-7.976	**3.22e-15***
t1 - t2	-0.06080	0.04533	-1.341	0.179788
t1 - t3	-0.26824	0.04657	-5.760	**2.65e-08***
t2 - t3	-0.20744	0.04657	-4.455	**3.42e-05***

*significant values, p<0.05.

Table S6. Contrast analysis between treatments (post-hoc Tukey) for the lead concentration (determined by ICP OES) in kale stem samples

Contrast	**Estimate**	**Std. Error**	**z value**	**p value**
t0 - t1	8.11760	2.1106	3.846	**0.000357***
t0 - t2	10.33660	2.0206	5.115	**1.16e-06***
t0 - t3	11.81530	1.9812	5.964	**9.99e-09***
t1 - t2	2.21900	0.8766	2.531	**0.011363***
t1 - t3	3.69770	0.7813	4.733	**9.05e-06***
t2 - t3	1.47870	0.4888	3.025	**4.85e-03***

*significant values, $p<0.05$.

Table S7. Abundance and characteristics of kale-associated insect community, according to the treatments. The treatments refer to the concentration used in the experimental soil contamination with lead nitrate: T0 = control, T1 = 144, T2 = 360 and T3 = 600 mg/kg of soil.

Species	**T0**	**T1**	**T2**	**T3**	**Total**	**RF* (%)**	**Function/ feeding mode**	**RF* (%)**
Brevicoryne brassicae	35,653	27,642	9,990	13,481	**86,766**	**90.24**	Sucking herbivore	**97.13**
Myzus persicae	1,051	1,163	1,650	2,759	**6,623**	**6.89**		
Plutella xylostella	98	41	73	34	**246**	**0.26**	Chewing herbivore	**0.27**
Trichoplusia ni	2	2	4	3	**11**	**0.01**		
Diaeretiella rapae	326	200	7	32	**565**	**0.59**	Parasitoid of sucking herbivore	**0.60**
Lysiphlebus testaceipes	0	1	0	0	**1**	**0.00**		

Aphelinus asychis	2	4	1	1	**8**	**0.01**		
Cycloneda sanguinea	0	2	2	0	**4**	**0.00**	Predator of sucking herbivore	**0.10**
Hippodamia convergens	1	0	0	0	**1**	**0.00**		
Allograpta exotica	51	23	8	12	**94**	**0.10**		
Oomyzus sokolowskii	761	271	455	225	**1,712**	**1.78**	Parasitoid of chewing herbivore	**1.78**
Alloxysta consobrina	3	10	4	8	**25**	**0.03**	Hyperparasitoid of sucking herbivore	**0.04**
Phaenoglyphis fuscicornis	1	0	0	0	**1**	**0.00**		
Syrphophagus aphidivorus	3	2	2	4	**11**	**0.01**		
Pachyneuron aphidis	1	0	0	0	**1**	**0.00**		
Diplazon laetatorius	13	5	0	4	**22**	**0.02**	Parasitoid of predator	**0.09**
Pachyneuron sp.	47	3	3	7	**60**	**0.06**		
Total	**38,013**	**29,369**	**12,199**	**16,570**	**96,151**	**100**		**100**

* Relative frequency

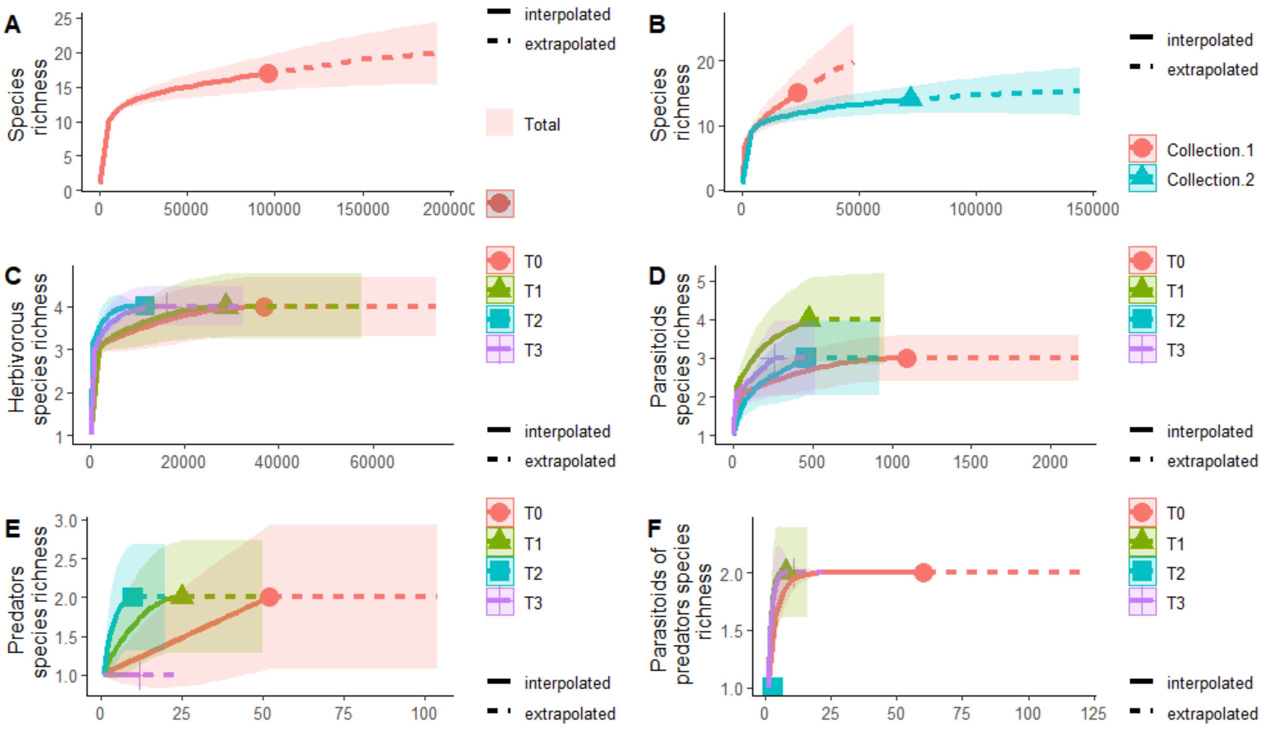

Figure S3. Species accumulation curves based on specimens sampling for kale-associated insect community. The curves were constructed using the rarefaction method based on interpolation/extrapolation and using the Chao1 species richness estimator and a 95% confidence interval. **(A)** Insects in total. **(B)** Collections. (**C**) Herbivorous species. (**D**) Parasitoids species. (**E**) Predators species. (**F**) Parasitoids of predators species. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of *Pb*$(NO_3)_2$ /Kg of soil.

Table S8. Indicator species for collection and treatment variable groups obtained by IndVal analysis.

Species	Associated group	IndVal	*p* value
Pachneuron sp.	T0	0.52	0.0041
D. rapae	T0+T1	0.61	0.0302
M. persicae	Collection 1	0.85	2e-04
B. brassicae	Collection 2	0.90	0.0001
Pachneuron sp.	Collection 2	0.49	0.0018

S. aphidivorus	Collection 2	0.45	0.0143
D. laetatorius	Collection 2	0.43	0.0159
A. consobrina	Collection 2	0.41	0.0158

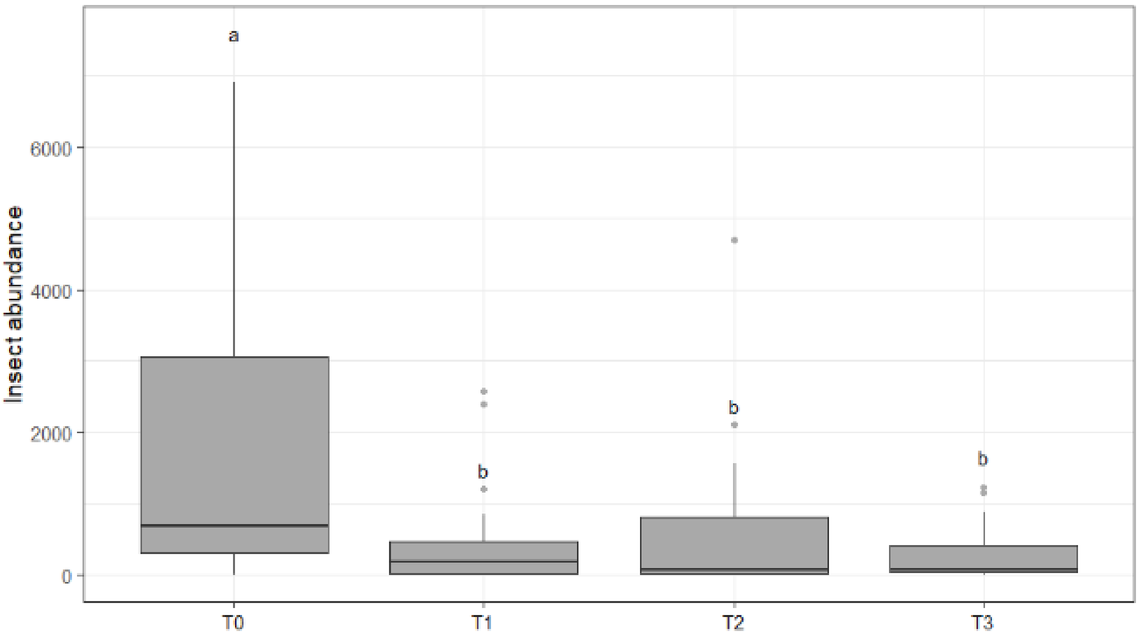

Figure S4. Total abundance of kale-associated insects. Abundance is plotted per plant and per collection, totaling 20 replicates per treatment. Box: interquartile range (IQR); lower and upper borders of box: the lower and upper quartiles (Q1 and Q3); line inside the box: median; whisker: minimum to maximum (Q1-1,5*IQR and Q3+1,5*IQR); points: outliers. T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of $Pb(NO_3)_2$ /Kg of soil. GLMM showed a significant difference in comparison with the null model (*p* = 0.0054). Different letters indicate a significant difference by Tukey test.

Table S9. Contrast analysis between treatments (post-hoc Tukey) for the the total abundance of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	1.8263	0.6135	2.977	**0.0175***
t0 - t2	1.8893	0.5953	3.174	**0.0118***
t0 - t3	1.7133	0.6114	2.802	**0.0232***
t1 - t2	0.0629	0.6149	0.102	0.9765
t1 - t3	-0.1130	0.6307	-0.179	0.9765
t2 - t3	-0.1759	0.6129	-0.287	0.9556

*significant values, p<0.05.

Table S10. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated herbivorous insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	1.85901	0.64039	2.903	**0.0218***
t0 - t2	1.93127	0.62115	3.109	**0.0142***
t0 - t3	1.69008	0.63772	2.65	**0.0345***
t1 - t2	0.07226	0.64194	0.113	0.9525
t1 - t3	-0.16893	0.6582	-0.257	0.9525
t2 - t3	-0.24119	0.63941	-0.377	0.9246

*significant values, p<0.05.

Table S11. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated sucking herbivores.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	1.889972	0.681773	2.772	**0.0304***
t0 - t2	1.981053	0.676539	2.928	**0.0234***
t0 - t3	1.881733	0.695138	2.707	**0.0304***
t1 - t2	0.091081	0.702101	0.13	0.9896
t1 - t3	-0.008239	0.70904	-0.012	0.9908
t2 - t3	-0.099319	0.719705	-0.138	0.9896

*significant values, p<0.05.

Table S12. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated chewing herbivores.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	1.12071	0.36315	3.086	**0.0128***
t0 - t2	0.52897	0.33414	1.583	0.3110
t0 - t3	1.14911	0.36399	3.157	**0.0122***
t1 - t2	-0.59174	0.38554	-1.535	0.3110
t1 - t3	0.02839	0.41005	0.069	0.9450
t2 - t3	0.62013	0.38586	1.607	0.3110

*significant values, p<0.05.

Table S13. Data referring to the parasitism rate (%) of each treatment.

Treatment	Minimum	Maximum	Median	Mean	Standard deviation
T0	0.09	100	1.90	11.21	26.63
T1	0.00	100	2.59	12.72	23.55
T2	0.00	100	3.40	13.93	23.06
T3	0.00	25	1.39	5.26	7.47

Table S14. Contrast analysis between treatments (post-hoc Tukey) for the parasitism rate of kale-associated parasitoids.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	-0.17750	0.64651	-0.275	0.959
t0 - t2	-0.17336	0.63860	-0.271	0.959
t0 - t3	0.35961	0.63323	0.568	0.918
t1 - t2	0.00415	0.63876	0.006	0.995
t1 - t3	0.53712	0.64200	0.837	0.835
t2 - t3	0.53297	0.63456	0.84	0.835

*significant values, p<0.05.

Table S15. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated parasitoid insects.

Contrast	Estimate	Std. Error	t value	p value

t0 - t1	1.6938	0.4147	4.085	**0.000568***
t0 - t2	1.2257	0.3761	3.259	**0.006268***
t0 - t3	1.8157	0.3963	4.582	**8.73e-05***
t1 - t2	-0.4681	0.4504	-1.039	0.475110
t1 - t3	0.1219	0.4340	0.281	0.779608
t2 - t3	0.5900	0.4263	1.384	0.354460

*significant values, p<0.05.

Table S16. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated parasitoids of sucking herbivores.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.8798	0.7229	1.217	0.4017
t0 - t2	2.3137	0.7671	3.016	**0.0184***
t0 - t3	2.0415	0.7635	2.674	**0.0389***
t1 - t2	1.4339	0.7829	1.832	0.2112
t1 - t3	1.1617	0.7784	1.492	0.3265
t2 - t3	-0.2722	0.8154	-0.334	0.7395

*significant values, p<0.05.

Table S17. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated parasitoids of chewing herbivores.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	1.09366	0.38066	2.873	**0.0231***
t0 - t2	0.4273	0.34387	1.243	0.3872
t0 - t3	1.16651	0.38601	3.022	**0.0179***
t1 - t2	-0.66635	0.39503	-1.687	0.2340
t1 - t3	0.07286	0.42927	0.170	0.8657
t2 - t3	0.73921	0.39772	1.859	0.2015

*significant values, p<0.05.

Table S18. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated predatory insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.8327	0.4452	1.871	0.1950
t0 - t2	1.3692	0.5198	2.634	**0.0422***
t0 - t3	1.5208	0.5602	2.715	**0.0390***
t1 - t2	0.5365	0.5776	0.929	0.5497
t1 - t3	0.6882	0.6165	1.116	0.5061
t2 - t3	0.1516	0.6661	0.228	0.8206

*significant values, p<0.05.

Table S19. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated hyperparasitoids.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.037948	0.950771	0.04	0.999
t0 - t2	0.034945	0.964159	0.036	0.999
t0 - t3	-0.259959	0.914869	-0.284	0.988
t1 - t2	-0.003003	0.980333	-0.003	0.999
t1 - t3	-0.297908	0.92857	-0.321	0.988
t2 - t3	-0.294905	0.933299	-0.316	0.988

*significant values, p<0.05.

Table S20. Contrast analysis between treatments (post-hoc Tukey) for the abundance of kale-associated parasitoids of predators.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	3.249	1.1592	2.803	**0.0262***
t0 - t2	2.7454	0.8991	3.054	**0.0153***
t0 - t3	1.8264	0.7094	2.574	**0.0413***
t1 - t2	-0.5036	1.3456	-0.374	0.7093
t1 - t3	-1.4226	1.2292	-1.157	0.4782
t2 - t3	-0.9191	0.9898	-0.929	0.5550

*significant values, p<0.05.

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ARTIGO 2 - Effect of lead soil contamination on the structure and function of a food web of plant-associated insects[#]

[#]Artigo formatado de acordo com as normas do periódico *Environmental Science & Technology*, no qual foi submetido.

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Abstract

Activities such as mining, industry, urban waste production, and the overuse of agricultural inputs may result in the contamination of the environment by heavy metals, which are toxic to organisms mainly due to their potential to accumulate in tissues. This contamination causes the simplification of aquatic and edaphic food webs, which show impaired structure and function. We investigated, for the first time, whether soil contamination with heavy metal at the permitted concentrations affects the structural or functional properties of a terrestrial food web of insects living in the aerial part of a plant. As study models we used lead (Pb) and kale-associated insects. In general, we observed a simplification of the food web as the Pb concentrations increased, resulting in visually smaller food webs. The food web metrics decreased starting with treatment T2 (the Pb limit for agricultural soils). Structurally, the number of species and link density fell. Connectance increased with Pb concentration. Functionally, the predator/prey ratio and trophic vulnerability and generality became lower.

Keywords: heavy metals, kale, herbivorous insects, predators, parasitoids, food web metrics

Synopsis

This study investigates the effect of different allowable concentrations of lead in the soil on the structural and functional properties of a food web of plant-associated insects.

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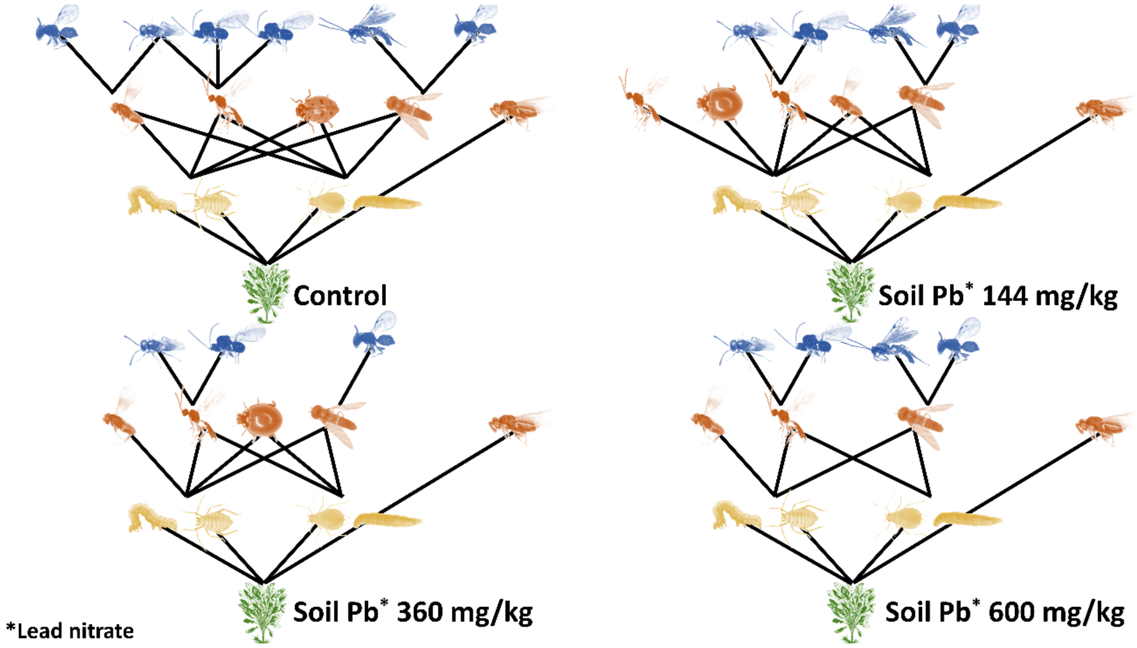

1. Introduction

The term “heavy metal” designates a group of elements, including metals or metalloids, that are highly toxic to organisms. The toxicity of these elements is mainly due to their cumulative potential, as they are not degradable by organisms, so they accumulate in tissues and in the environment [1–3]. Heavy metals can cause the inactivation of enzymes and functional groups of various molecules and lead to the production of reactive oxygen species, thus interrupting important biological processes and harming the health of organisms (Rascio & Navari-Izzo, 2011). Some of these elements are considered essential metals because they participate in vital biological processes, such as iron (Fe), zinc (Zn), and copper (Cu). Other elements, such as lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), and mercury (Hg), do not participate in biological functions and are toxic even at low concentrations. Even when essential metals reach levels above what is considered safe, they become equally toxic to organisms [1–3]. Heavy metals are present in nature in soils and rocks, for example, but environmental contamination with these elements is often associated with anthropogenic activities, such as industry, mining and smelting, waste disposal, and the discharge of wastewater, sewage sludge, pesticides, and fertilizers [4,5]. Thus, both aquatic and terrestrial environments may suffer from heavy metal contamination.

The impact of metal contamination, derived mainly from mining activities, has been investigated in aquatic environments. The pollution of aquatic systems by heavy metals causes their accumulation in the tissues of organisms living there. For example, the pollution of streams by mines generated the transfer of Fe, Zn, and Cu from the environment to invertebrates [6,7]. In addition to the transfer and accumulation of heavy metals in these systems, communities can experience impacts both on their structure and on their function. Benthic macroinvertebrate communities undergo changes in composition and reduction in total abundance and species richness with increasing metal concentrations [6,8]. An aggravating factor is that the reduction effects in these communities may persist, being observed in aquatic systems even 75 years after the last activity of local mines [9]. From a food web approach, communities of aquatic invertebrates and other organisms exposed to metals present simplified food webs, with fewer elements and links between species, lower link density per species, and fewer predatory invertebrates and an absence of predatory fish [10–12]. These food webs are also functionally altered because the basal resources are less productive or inaccessible, the microbial processing of organic matter is slow, and many herbivores and shredders are absent [13].

In terrestrial environments, soil contamination by heavy metals also causes the transfer and accumulation of these metals to invertebrates living in the soil, such as arthropods and worms [14]. Like aquatic communities, soil communities experience impacts on their structure and function from metal contamination. In general, edaphic invertebrate communities undergo changes in species composition and reductions in population densities [15,16,18–20]. The species richness of some groups, such as nematodes, is often reduced in soil contamination by heavy metals [21]. In other groups, such as arthropods, the increase in the concentration of these contaminants does not always decrease their species diversity. Sometimes an increase in the diversity of some groups can be observed, caused by the dominance of species more tolerant to pollution in the most contaminated environments, which take over the niche of the more sensitive species [15,16,22]. Specifically regarding the impact on soil food webs, a lower abundance of predatory insects and ovivorous/predatory nematodes has been found with increased contamination [18,20,23]. This reduction in predatory nematodes led to disruption of the food web, resulting in an overabundance of herbivorous nematodes [24,25].

Soil contamination by heavy metals affects not only edaphic communities. There is an aggravating factor: the absorption and accumulation of these elements by plants and, as plants are the basis of terrestrial food chains, the risk of transfer to organisms at higher trophic levels [26,27]. The transfer of heavy metals from plants to herbivorous insects has been widely reported in recent years (e.g., Dar et al., 2015, 2017; Naikoo et al., 2021; Shi et al., 2020; Woźniak et al., 2017; L. Zhou et al., 2015). Herbivorous insects that feed on plants grown in environments contaminated with heavy metals, or on artificial diets, transfer these elements to their natural enemies, such as predatory insects and parasitoids [30,32,34–38]. The level of accumulation of these elements in the body of these insects is quite variable, and may be higher or lower than that at the previous trophic level, depending both on the specific detoxification mechanisms of the species and on the elements involved. Both predators and parasitoids experience lethal and sublethal adverse effects from exposure to heavy metals via herbivores contaminated with them [30,38,39]. The accumulation of heavy metals by plants seems to affect the entire insect community living in the aerial part of plants, because herbivores in polluted sites can act as sinks that reduce the population densities of natural enemies [40,41].

In fact, we recently demonstrated that experimental soil contamination with different permitted concentrations of Pb, in addition to affecting the species composition of the insect community in the aerial parts of plants, reduces the population density of the following functional groups: herbivores, primary parasitoids, predators, and top parasitoids (hyperparasitoids and parasitoids of predators) [42]. However, there are still no studies showing how soil contamination by metals structurally and functionally affects these natural and agriculturally important terrestrial food webs.. We investigated how the Pb concentrations that are allowed in Brazilian soils affect the structural and functional properties of the food web of kale-associated insects (*Brassica oleracea* L., var. *acephala*), in a field experiment. We evaluated the effect of Pb concentrations on the overall food web architecture and on the following structural metrics: number of species, number of links, link density and connectance, and on the following functional metrics: predator-prey ratio and trophic generality and vulnerability.

2. Materials and methods

2.1. Study system

We chose kale (*Brassica oleracea* L. var. *acephala*) as a model study plant, mainly because it is a fast-growing plant that has a diverse community of associated insects, whose identification at the species level is relatively easy, preventing taxonomy-related errors. The insects associated with kale have different characteristics. They are mainly chewing and sucking herbivores (lepidopterans and aphids, respectively) and their natural enemies, which are represented by parasitoid and predatory insects (mainly ladybugs and syrphids) [43–47]. In addition, previous studies showed that kale plants absorb heavy metals from the soil, presenting levels of metals that classify this species as a normal plant, favoring this type of experiment [48–50].

The metal chosen for the study was Pb. This metal is classified as the second most dangerous on the list of priorities of the United States Environmental Protection Agency [51].

Environmental contamination by Pb is mainly associated with battery manufacturing, leaded gasoline combustion, welding, paints, and herbicides, and insecticides [4,52]. The Contaminated Areas Inventory conducted by the State Environment Foundation identified Pb as the most common heavy metal in contaminated areas in the state of Minas Gerais, Brazil, where this study was conducted (FUNDAÇÃO ESTADUAL DE MEIO AMBIENTE, 2015).

2.2. Experimental procedure

The experiment was conducted in a greenhouse on the experimental farm (Technology Transfer Development Center) of Federal University of Lavras (Universidade Federal de Lavras), located in the municipality of Ijaci, Minas Gerais, Brazil. We used an open greenhouse so insects could colonize the plants naturally, making the experiment resemble what happens in the field as closely as possible. Therefore, the conditions of the greenhouse (temperature, humidity and photoperiod) were natural. The plants were grown in 12-L plastic pots. We connected each pot to a plastic bottle through a hose installed at the bottom of the pot, closing the water circuit and preventing local contamination. Only the upper part of the greenhouse was covered with a transparent tarpaulin, as the closed circuit pot system would not withstand heavy rains.

To prepare the soil in each pot, we used the following mixture: 2 kg of expanded clay, used for drainage, and 8 kg of soil containing sand, organic fertilizer, and lime. (more details can be found in Supplementary Information - Appendix A and Table S1). To contaminate the soil, we used Pb in the form of lead nitrate ($Pb(NO_3)_2$), allowing better handling and application to the soil by dilution in water. We previously weighed the lead nitrate on a precision analytical balance and transported it in individual paper bags to each pot, according to the concentration. We contaminated the soil of each pot with the following concentrations of $Pb(NO_3)_2$: 0 (control, referred to as T0), 144 (T1), 360 (T2), and 600 (T3) mg/kg of soil. The concentrations of the contaminated treatments (T1, T2, and T3) corresponded, respectively, to the prevention value, the limit for the agricultural area, and the limit for residential locations, according to CONAMA resolution 460/2013 [53]. We performed the contamination by diluting the $Pb(NO_3)_2$ in 2 L of water and applying it to the soil present in the pots. Only water was applied to the control pots (T0).

We planted kale seedlings in January 2019, 3 months after soil contamination, to increase the adherence of the metal to the soil. We prepared 10 pots for each treatment, making for 10 replicates of individual plants for each treatment and 40 pots in total. The pots were kept at a distance of 1.2 m^2 and were randomly arranged. The plants were watered twice a week.

2.3. Insect collection

It was necessary to perform two collection methods due to differences in plant colonization by the insects and their biology. The first insects to colonize the plants were the lepidopteran species *Plutella xylostella* (L.), popularly known as the diamondback moth, and *Trichoplusia ni* (Hübner), known as the cabbage looper. As females of these species usually lay eggs in isolation and individuals feed on plants in a dispersed manner, the most appropriate sampling method is plant inspection [45,46]. We performed two collections, on March 28 and April 4, 2019, inspecting each plant and collecting the pupae found into plastic tubes properly labeled with the plant and treatment. In the laboratory, the pupae were kept in plastic tubes covered with voile to finish developing, go through emergence, and be contained as adults or parasitoids.

The aphids colonized the plants in a second wave. Unlike the lepidopteran species, these insects feed in an aggregate manner on the leaves, forming numerous colonies [47]. These insects were collected by trapping in voile bags, which let us keep the aphids feeding on the same plant and collect mummies and their emergent parasitoids and predators, which were contained within the bags. For this purpose, we made voile bags sewn with a string at their base, which allowed them to be tied at the base of the leaf petiole. We performed two collections, on May 3 and May 31, 2019. For collection, we placed a labeled voile bag on a leaf of each plant at random. After 15 days in the field, we transported the bagged leaves to the laboratory for screening, identification, and quantification of insects and their interactions. To identify the specimens, we kept the insects after death in vials containing 70% ethanol, and then the individuals were identified by using identification keys and consulting the specialized literature. The interactions between species were determined based on the literature, which is well known for this agrosystem, and by laboratory observations.

2.4. Data analysis

For analysis of the food web, we calculated the metrics using two approaches: (1) metrics for the total food web of each of the four treatments, with the objective of describing the food webs; (2) metrics per plant and collection, with the objective of analyzing the effects of the treatments on the metrics of the food webs. We calculated the following structural metrics: number of species, number of links (i.e. the total number of interactions between species), link density (i.e. the mean number of links per species), and connectance (i.e. the proportion of observed links in relation to the possible total), and the following functional metrics: predator/prey ratio (i.e. the ratio between the mean number of predators and prey), and trophic generality (i.e. the mean number of prey of a species) and vulnerability (i.e. the mean number of predators of a species). The plant was considered a species in these analyses. The metrics of the food webs were extracted using the *cheddar* package [54,55]. To evaluate possible groupings of the metrics relative to the treatments, we performed principal component analysis (PCA) using the *factoextra* [56], *tidyverse* [57], and *ggplot2* packages [58].

To analyze the effects of treatments on food web metrics, we used generalized linear mixed-effect models (GLMMs). To construct the models, we evaluated different structures for the random effects [59]. We constructed models with four random-effects structures using the following variables: (*i*) plant; (*ii*) collection; (*iii*) plant and collection; and (*iv*) plant plus collection. The models were constructed with the *glmmTMB* package [60]. The best model was selected based on the variables used as random effects, using the Akaike information criterion corrected for small samples (AICc), in which the lowest value represents the best model, and the Akaike weight (w), which estimates the probability that each model will be selected as the best model if the process is repeated several times [61]. The description of the models selected by each metric is shown in Table S2. Model selection was performed with the *MuMIn* package [62]. The fit of the error distribution of the models was checked using simulated scaled residuals with the *DHARMa* package [63]. Significant differences between treatments were tested by Tukey's post hoc test with the *emmeans* package [64]. All statistical analyses were performed in R [65].

3. Results

3.1. Description of food webs

We collected a total of 96,151 individuals belonging to 17 species and three trophic levels (Table S3). The food web was characterized by species of herbivorous insects (second trophic level), parasitoids and predators (third trophic level), and hyperparasitoids and parasitoids of predators (fourth trophic level). Of the herbivorous insects, two species were suckers (aphids), together representing 97.13% of the insects collected, namely, *Brevicoryne brassicae*, the most abundant, and *Myzus persicae*. Two species were chewers (lepidopterans), *Plutella xylostella*, the most abundant, and *Trichoplusia ni*. Of the parasitoid insects, three species were parasitoids of the sucking herbivores, *Diaeretiella rapae* (Braconidae, Aphidiinae) being the most abundant. One species of parasitoid, *Oomyzus sokolowskii* (Eulophidae, Tetrastichinae), is a parasitoid of a chewing herbivore (*P. xylostella*). The three predatory species found feed on the sucking herbivores, *Allograpta exotica* (Diptera, Syrphidae) being the most abundant. The four hyperparasitoid species found are associated with the sucking herbivores. Two parasitoids of the predator *A. exotica* were also found.

The apparent architecture of the food web changed with the treatment of the soil with lead (Pb), with simplification of the system as the Pb concentration increased (Figure 1). The food web of the control treatment (38,013 individuals in total) had a total of 15 species and 20 links (Figure 1A). The food web of the contaminated treatment T1 (29,369 individuals) had a total of 14 species and 17 links (Figure 1B). The food web of treatment T2 (12,199 individuals) had a total of 12 species and 15 links (Figure 1C). Finally, the food web of treatment T3 (16,570 individuals) had a total of 12 species and 14 links (Figure 1D). The other metrics for each treatment are shown in Table 1.

Table 1. General food web metrics of kale-associated insects for each soil treatment with Pb. T0 = Control, T1 = 144, T2 = 360 and T3 = 600 mg of Pb nitrate/kg of soil. S = total number of species, L = total number of links, z = mean density of links per species, P:P = predator/prey ratio, G = mean trophic generality and V = mean trophic vulnerability.

Treatment	S	L	L/S	z	P:P	G	V
T0	15	20	1.250	0.078	2.143	1.333	2.858
T1	14	17	1.133	0.076	2.333	1.214	2.833
T2	12	15	1.154	0.089	2.000	1.250	2.500
T3	12	14	1.077	0.083	2.000	1.167	2.333

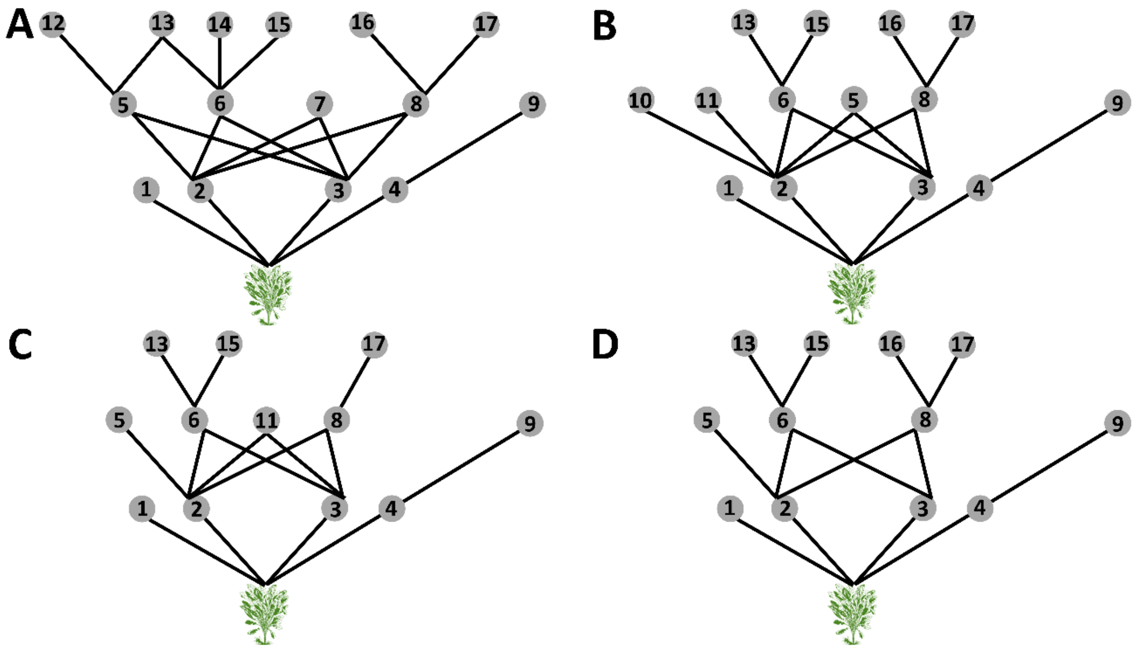

Figure 1. Food webs of insects associated with kale, according to soil treatment with Pb. (A) 0 (control), (B) T1 = 144, (C) T2 = 360, (D) T3 = 600 mg of $Pb(NO_3)_2$/kg of soil. Species 1 to 4 are herbivores: 1 = *Trichoplusia ni* (Lepidoptera: Noctuidae), 2 = *Brevicoryne brassicae,* 3 = *Myzus persicae* (Hemiptera, Aphididae), and 4 = *Plutella xylostella* (Lepidoptera, Plutellidae). Species 5 to 11 are primary parasitoids and predators: 5 = *Aphelinus asychis* (Hymenoptera, Aphelinidae), 6 = *Diaeretiella rapae* (Hymenoptera, Braconidae), 7 = *Hippodamia convergens* (Coleoptera, Coccinellidae), 8 = *Allograpta exotica* (Diptera, Syria), 9 = *Oomyzus sokolowskii* (Hymenoptera, Eulophidae), 10 = *Lysiphlebus testaceipes* (Hymenoptera, Braconidae), and 11 = *Cycloneda sanguinea* (Coleoptera, Coccinellidae). Species 12 to 17 are hyperparasitoids and parasitoids of predators: 12 = *Pachyneuron aphidis* (Hymenoptera, Pteromalidae), 13 = *Syrphophagus aphidivorus* (Hymenoptera, Encyrtidae), 14 = *Phaenoglyphis fuscicornis,* 15 = *Alloxysta consobrina* (Hymenoptera, Figitidae), 16 = *Diplazon laetatorius* (Hymenoptera, Ichneumonidae), and 17 = *Pachyneuron* sp. (Hymenoptera, Pteromalidae).

The PCA did not show a distinguishable grouping of the four groups by the food web metrics analyzed. However, the highest values for number of species, number of links, and link density were observed in the control group, while the lowest were observed in the most contaminated group. In contrast, the highest connectance values were found in the most contaminated groups (Figure 2).

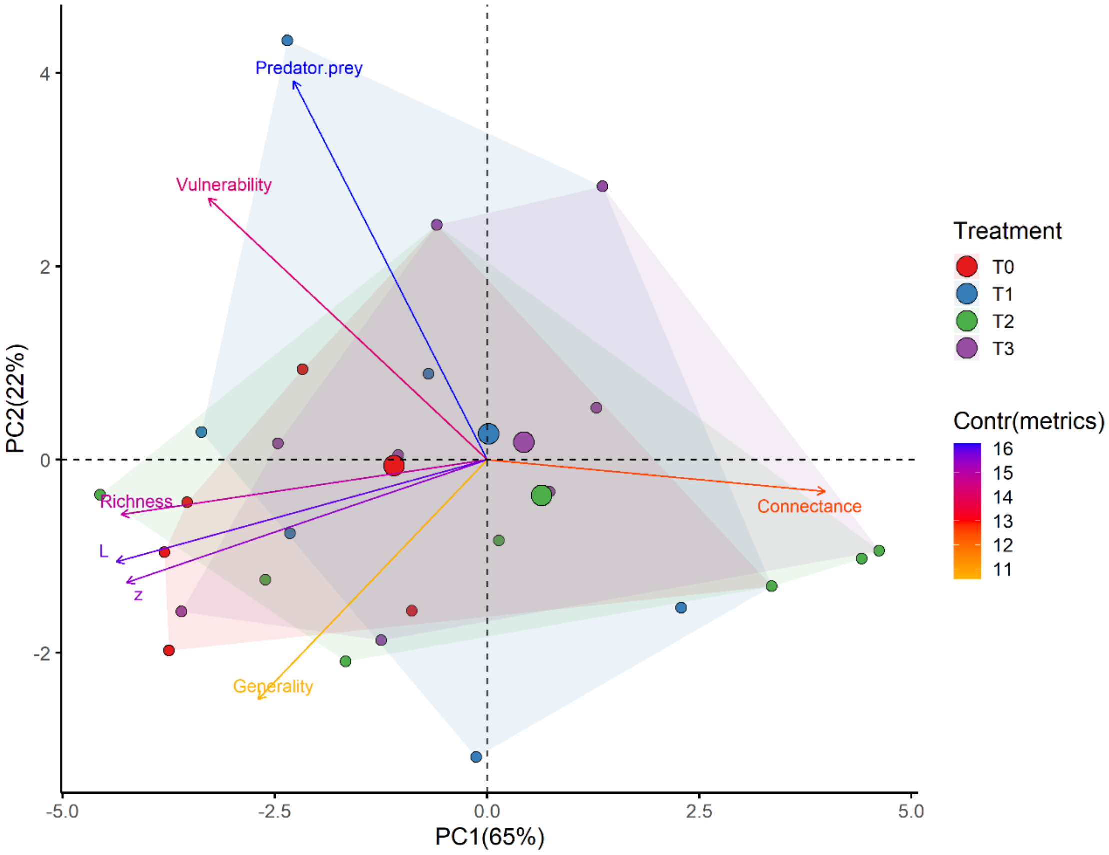

Figure 2. Principal component analysis (PCA) of the following metrics of the food web of insects associated with kale, according to soil treatments with Pb: Total number of species (Richness), Total number of links (L), Mean density of links by species (z), Connectance, Predator/Prey Ratio, Generality and Trophic Vulnerability. T0 = Control, T1 = 144, T2 = 360 and T3 = 600 mg of Pb nitrate/kg of soil. The arrow colors represent the representation of quality (e.g. contribution) that the food web metrics have on the component axes. The contribution is a scaled representation of the square correlation between the variables and the component axes.

3.2. Effect of Pb treatment on food web metrics

The control group had the highest number of species per plant, with a mean of 6.5 (7 ± 2.01). The mean under contaminating treatment T1 was 5.32 (6 ± 1.53), for T2 it was 4.9 (4 ± 2.07), and for T3 it was 4.95 (4.5 ± 1.64). Only T1 did not differ significantly from the control (p = 0.1097). The number of species did not differ significantly between the three contaminated groups (Figure 3A, Table S4). The values presented in parentheses in this section correspond to the median and standard deviation, respectively.

The mean number of links per plant was 5.9 (6 ± 2.38) in the control group, 4.47 (5 ± 1.71) in group T1, 4.25 (3 ± 2.45) in group T2, and 4.1 (3.5 ± 1.86) in group T3. The treatments showed no difference in the number of links per plant, but the *p* value of the comparison between group T3 and the control was 0.0631 (Figure 3B, Table S5).

The mean link density per plant was 0.881 (0.857 ± 0.117) for the control, 0.820 (0.833 ± 0.100) for T1, 0.790 (0.750 ± 0.177) for T2 and 0.767 (0.750 ± 085) for T3. Despite the progressive decrease with increasing Pb concentration, only T3 differed significantly from the control in link density. The three contaminated treatments did not differ from each other (Figure 3C, Table S6).

In general, the connectance per plant increased with the Pb concentration of the treatments. The mean connectance was 0.145 (0.140 ± 0.036) in the control group, 0.163 (0.143 ± 0.035) in T1, 0.178 (0.188 ± 0.044) in T2, and 0.175 (0.188 ± 0.031) in T3. Only T1 did not differ significantly from the control (p = 0.3335). The three contaminated treatments did not differ from each other (Figure 3D, Table S7).

The mean predator/prey ratio per plant was 1.55 (1.5 ± 0.159) in the control, 1.52 (1.5 ± 0.366) in T1, 1.36 (1.45 ± 0.273) in T2, and 1.48 (1.5 ± 0.225) in T3. Although the T2 and

T3 groups had lower values, only T2 differed significantly from the control. The three contaminated treatments did not differ from each other (Figure 3E, Table S8).

The mean trophic vulnerability per plant was 1.65 (1.67 ± 0.157) in the control, 1.63 (1.5 ± 0.327) in T1, 1.43 (1.5 ± 0.329) in T2, and 1.43 (1.5 ± 0.143) in T3. T2 and T3 showed the lowest trophic vulnerability, differing significantly from the control. The three contaminated treatments did not differ from each other (Figure 3F, Table S9).

Trophic generality was highest in the control group, with a mean of 1.06 (1 ± 0.094). The mean for T1 was 1.02 (1 ± 0.050), for T2 it was 1.03 (1 ± 0.069), and for T3 it was 1 (1 ± 0). Only T3 differed significantly from the control. The three contaminated treatments did not differ from each other (Figure 3G, Table S10).

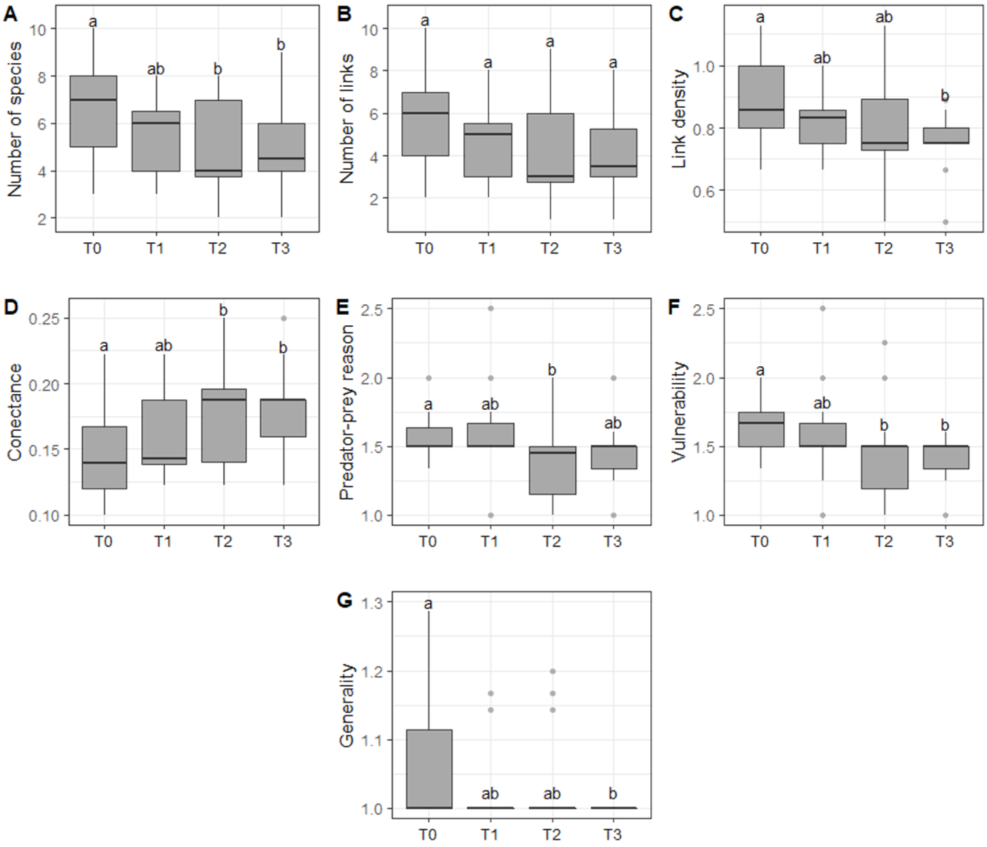

Figure 3. Effect of soil treatment with Pb on the metrics of the food web of kale-associated insects. The metrics are plotted per plant and collection. (A) Number of species. (B) Number

of links. (C) Link density. (D) Connectance. (E) Predator/prey ratio. (F) Trophic vulnerability. (G) Trophic generality. The *x*-axis represents the treatments according to the experimental contamination of the soil with the amount of $Pb(NO_3)_2$ to which they were subjected: T0 = Control, T1 = 144, T2 = 360, and T3 = 600 mg/kg of soil. Different letters indicate significant differences between the treatments by Tukey's post hoc test applied to generalized linear mixed-effect models.

4. Discussion

This study is the first to demonstrate how soil contamination by a heavy metal at different concentrations affects the structural and functional properties of a food web of insects in the aerial part of a plant. In general, we observed a simplification of the food web as the Pb concentration increased, resulting in visually smaller food webs. The highest values for number of species, number of links, and link density were associated with the control, according to the PCA, while the lowest values of these metrics were associated with the most contaminated treatment. The GLMMs, together with the comparison analysis, showed a reduction in the food web metrics starting at the T2 treatment (the Pb limit for agricultural soils). Structurally, there were fewer species and lower link density but greater connectance with more contamination. Functionally, there was a reduction in the predator/prey ratio and in trophic vulnerability and generality.

We observed a general simplification in the food web architecture as the Pb concentration increased, with visually smaller food webs, fewer species, and fewer links. Previous studies have shown that aquatic food webs were simplified by metal contamination. Yule et al. [10] found food webs of invertebrates with fewer elements and links in sites polluted by gold mines. Similarly, Hogsden and Harding [11,12] found smaller food webs in streams contaminated by mining, with the absence of predatory fish and with invertebrates being the main predators. To the best of our knowledge, our study is the first to investigate the effect of soil contamination by different concentrations of a heavy metal on the structure of a terrestrial food web. As found in aquatic environments, food webs of insects living in the aerial part of a plant grown in soil contaminated with Pb became simplified.

We found fewer species in the studied food web in groups T2 and T3 than the control. This result is in agreement with our previous findings, which showed a higher estimated species richness in the control and lower richness in the groups contaminated with Pb [42]. Soil contamination with heavy metals can negatively affect the diversity and richness of food webs of soil invertebrates, reducing mite, collembolan, beetle, and nematoid communities, for example, by suppressing more sensitive species [16,22,23,66]. Here, we show that this effect can also occur in terrestrial food webs in the aerial part of plants.

The heavy metals in the soil are absorbed by the plants, concentrating in their tissues. These elements are transferred to herbivorous insects and from them to predators or parasitoids, causing lethal and sublethal effects on these organisms, which may explain the reduction in the number of species [30,34,38]. A reduction in the number of herbivore species has been documented in plants grown in soil contaminated with heavy metals [67], and we extended this result to the food web. Furthermore, we previously showed that the parasitoids of this food web do not discriminate between herbivorous hosts in plants contaminated with Pb vs. uncontaminated plants. However, the abundance of adult parasitoids was reduced in the contaminated treatments, the herbivores contaminated with Pb acting as a type of parasitoid sink [40,42]. This mechanism could also explain the lower species richness of the system.

Although the food webs of the contaminated treatments showed an apparent simplification in the number of total links between species, the models showed no significant difference between treatments in the number of links per plant. Instead, the models of link density, i.e., the mean number of links per species per plant, showed a reduction, which was significant in group T3, suggesting that this metric may be important in detecting effects on interactions. Conversely, the connectance showed an increasing trend as the contamination with Pb grew, being significantly different in groups T2 and T3 compared to the control. The low values of connectance in the control and less contaminated treatments (T1) indicate that the insect species of the kale food web are naturally less interconnected, that is, they share fewer resources, being a more specialized food web. The increase in soil contamination with Pb and the consequent decrease in the food web (lower number of species and link density) generated an increase in connectance, signaling that the species in the food web became more interconnected, that is, they share more resources, being webs less specialized. These results are in agreement with the effects of heavy metals found for aquatic food webs, in which fewer total links and lower link density per species have been observed, while the connectance increased with the simplification of food webs by the metals [10,11].

The models for the predator/prey ratio, trophic vulnerability (i.e., the mean number of predators of a species), and trophic generality (i.e., the mean number of prey of a species) showed a reduction in these metrics under the most contaminated treatments. These results demonstrate that the functional properties of the studied food web were impaired by soil contamination with Pb, as the relationship between predators and primary and top parasitoids (hyperparasitoids and parasitoids of predators) and their prey/hosts are weakened by decreased consumption. The reduction in these metrics can be explained by the effect of the contaminant on the abundance of the functional groups of this food web, as we previously showed that Pb reduces the population density of predators and primary and top parasitoids [42]. A reduction or even absence of predators caused by heavy metals has been identified in insects [20] and predatory nematodes in contaminated soil [23], in predatory benthic macroinvertebrates [10], and in fish living in water contaminated by metals [11–13]. In the case of soil predator insects, a reduction in abundance has been observed even 40 years after the decommissioning of the smelter complex that caused the metal contamination [20], indicating that the impact of such elements on these organisms can last a long time.

The impact of heavy metal contamination on the function of food webs can go beyond the reduction in the density of predators. For example, Kwan et al. [68] experimentally showed that water contamination with copper impairs the effects of predator consumption, altering the density-dependent interactions between prey conspecifics. They found that the reduction of whelks by crab predation in the contaminated system did not trigger the expected normal increase in the rate of barnacle consumption by whelks, which was observed in the absence of the contaminant. These effects still need to be explored in terrestrial food webs.

Our findings show that soil contamination with Pb negatively affects the structure and function of insect food webs in the aerial part of a plant, even at Pb concentrations allowed for agricultural and residential soils. The results shed light on the impact of anthropogenic activities that cause environmental contamination by heavy metals in terrestrial ecosystems and on the need for studies on this topic. We observed that soil Pb concentrations considered safe for humans and allowed by law are not safe for organisms such as insects, so they negatively affect the ecosystem. In this sense, we emphasize the need to consider other organisms in efforts to identify impacts and determine safe levels of metals for the ecosystem because they may harm important ecosystem services, such as the biological control performed by predators and parasitoids.

Support Information

Details about the type of soil used in the experiment; previous quantification of heavy metals in used soil; the soil contamination procedure; the experiment configuration; the models selected by the Akaike information criterion corrected for small samples (AICc), according to the random variables and error distribution; the list of species with their respective characteristics, abundances and relative frequencies; and the tables with the contrast analysis between treatments for each metric model.

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Supporting Information

Effect of lead soil contamination on the structure and function of a food web of plant-associated insects

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References

Appendix A. Methodological details

The soil used in the experiment is of the "red latosol" type, characteristic of the region. This type of soil is usually acidic, with a pH ranging from 4.0 to 6.0 [1]. Samples of the soil used in the experiment were analyzed for levels of heavy metals before soil preparation using Flame Atomic Absorption Spectrometry (FAAS) (Table S1). The soil used in the experiment showed a Pb concentration of 25.9 mg/Kg. This concentration is within the Pb concentrations assumed normal for soils in the region [2].

For soil contamination, Pb was used in the form of nitrate ($Pb(NO_3)_2$), making possible better handling and application to the soil by diluting with water. We applied the previously weighed lead nitrate concentrations by diluting them in two liters of water in a PET bottle, which was sealed and mixed for two minutes. Then, the solution was applied to the soil of each pot. We tagged plants from 1 to 40 and drew each plant position in the greenhouse using an online random number drawing service.

Insect collections were conducted during the non-flowering stage, with kale plants measuring about 40 cm in height and showing many leaves.

Table S1. Result of the determination of heavy metals (FAAS) in the soil used in the experiment.

Heavy metal	Mg/kg
Fe	37896
Cu	9.1
Mn	79.58
Zn	12.6
Ni	12.1
Cr	56.74
Cd	5.4
As	46.8
Pb	25.9
Se	72.7

Table S2. Models selected based on the Akaike information criterion corrected for small samples (AICc), according to the random variables and error distribution.

Model	Selected random variables	Selected error distribution
Number of species	Collection	Gaussian
Number of links	Collection	Poisson
Link density	Collection	Gaussian
Connectance	Plant	Gamma
Predator/prey reason	Collection plus plant (together)	Gaussian
Trophic vulnerability	Collection plus plant (together)	Tweedie
Trophic generality	Collection plus plant (together)	Gamma

Table S3. Abundance and characteristics of the food web of kale-associated insects, according to the treatments. The treatments refer to the concentration used in the experimental soil contamination with lead nitrate: T0 = control, T1 = 144, T2 = 360 and T3 = 600 mg/kg of soil.

Species	T0	T1	T2	T3	Total	FR* (%)	Function/ feeding mode	RF* (%)
Brevicoryne brassicae	35,653	27,642	9,990	13,481	**86,766**	**90.24**	Sucking herbivore	**97.13**
Myzus persicae	1,051	1,163	1,650	2,759	**6,623**	**6.89**		
Plutella xylostella	98	41	73	34	**246**	**0.26**	Chewing herbivore	**0.27**
Trichoplusia ni	2	2	4	3	**11**	**0.01**		
Diaeretiella rapae	326	200	7	32	**565**	**0.59**	Parasitoid of sucking herbivore	**0.60**
Lysiphlebus testaceipes	0	1	0	0	**1**	**0.00**		
Aphelinus asychis	2	4	1	1	**8**	**0.01**		

Cycloneda sanguinea	0	2	2	0	**4**	**0.00**	Predator of sucking herbivore	**0.10**
Hippodamia convergens	1	0	0	0	**1**	**0.00**		
Allograpta exotica	51	23	8	12	**94**	**0.10**		
Oomyzus sokolowskii	761	271	455	225	**1,712**	**1.78**	Parasitoid of chewing herbivore	**1.78**
Alloxysta consobrina	3	10	4	8	**25**	**0.03**	Hyperparasitoid of sucking herbivore	**0.04**
Phaenoglyphis fuscicornis	1	0	0	0	**1**	**0.00**		
Syrphophagus aphidivorus	3	2	2	4	**11**	**0.01**		
Pachyneuron aphidis	1	0	0	0	**1**	**0.00**		
Diplazon laetatorius	13	5	0	4	**22**	**0.02**	Parasitoid of predator	**0.09**
Pachyneuron sp.	47	3	3	7	**60**	**0.06**		
Total	**38,013**	**29,369**	**12,199**	**16,570**	**96,151**	**100**		**100**

* Relative frequency

Table S4. Contrast analysis between treatments (post-hoc Tukey) of Generalized Linear Mixed-Effect Models (GLMM) for the number of species in the food web of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	1.2018	0.5573	2.156	0.1097
t0 - t2	1.6000	0.5500	2.909	**0.0242***
t0 - t3	1.5500	0.5500	2.818	**0.0264***
t1 - t2	0.3982	0.5573	0.715	0.7557

t1 - t3	0.3482	0.5573	0.625	0.7581
t2 - t3	-0.0500	0.5500	-0.091	0.9278

*significant values, p<0.05.

Table S5. Contrast analysis between treatments (post-hoc Tukey) of Generalized Linear Mixed-Effect Models (GLMM) for the number of links in the food web of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.28052	0.14230	1.971	0.1595
t0 - t2	0.32803	0.14226	2.306	0.0927
t0 - t3	0.36396	0.14377	2.532	0.0631
t1 - t2	0.04751	0.15343	0.310	0.9327
t1 - t3	0.08344	0.15482	0.539	0.8524
t2 - t3	0.03593	0.15479	0.232	0.9327

*significant values, p<0.05.

Table S6. Contrast analysis between treatments (post-hoc Tukey) of Generalized Linear Mixed-Effect Models (GLMM) for the link density in the food web of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.06194	0.03924	1.578	0.3256
t0 - t2	0.09118	0.03874	2.354	0.0842
t0 - t3	0.11464	0.03980	2.881	**0.0262***
t1 - t2	0.02924	0.03924	0.745	0.6793
t1 - t3	0.05270	0.04029	1.308	0.3955
t2 - t3	0.02346	0.03980	0.589	0.6793

*significant values, p<0.05.

Table S7. Contrast analysis between treatments (post-hoc Tukey) of Generalized Linear Mixed-Effect Models (GLMM) for the connectance in the food web of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.73766	0.47135	1.565	0.3335
t0 - t2	1.24837	0.45436	2.748	**0.0370***
t0 - t3	1.18680	0.46028	2.578	**0.0491***
t1 - t2	0.51071	0.43742	1.168	0.4762
t1 - t3	0.44913	0.44499	1.009	0.4996
t2 - t3	-0.06157	0.42744	-0.144	0.8859

*significant values, p<0.05.

Table S8. Contrast analysis between treatments (post-hoc Tukey) of Generalized Linear Mixed-Effect Models (GLMM) for the predator-prey ratio in the food web of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.11599	0.09113	1.273	0.4148
t0 - t2	0.24650	0.08808	2.798	**0.0332***
t0 - t3	0.04985	0.08623	0.578	0.7088
t1 - t2	0.13051	0.08627	1.513	0.3615
t1 - t3	-0.06614	0.09463	-0.699	0.7088
t2 - t3	-0.19665	0.09063	-2.170	0.1259

*significant values, $p<0.05$.

Table S9. Contrast analysis between treatments (post-hoc Tukey) of Generalized Linear Mixed-Effect Models (GLMM) for the trophic vulnerability in the food web of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	0.01197	0.05234	0.229	0.9673
t0 - t2	0.14233	0.05154	2.761	**0.0365***
t0 - t3	0.14049	0.05383	2.610	**0.0461***
t1 - t2	0.13036	0.05310	2.455	0.0564
t1 - t3	0.12852	0.05534	2.322	0.0623
t2 - t3	-0.00184	0.05453	-0.034	0.9733

*significant values, $p<0.05$.

Table S10. Contrast analysis between treatments (post-hoc Tukey) of Generalized Linear Mixed-Effect Models (GLMM) for the trophic generality in the food web of kale-associated insects.

Contrast	Estimate	Std. Error	t value	p value
t0 - t1	-0.03616	0.01674	-2.160	0.1290
t0 - t2	-0.02139	0.01648	-1.298	0.4380
t0 - t3	-0.05076	0.01667	-3.045	**0.0170**
t1 - t2	0.01477	0.01699	0.869	0.5910
t1 - t3	-0.01460	0.01715	-0.851	0.5910
t2 - t3	-0.02937	0.01690	-1.738	0.2500

*significant values, $p<0.05$.

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