UNIVERSIDADE FEDERAL DE LAVRAS

JÉSSICA CRISTINA TEODORO

LANTHANUM ECOTOXICOLOGY AND EFFECTS ON CROP SPECIES GROWN IN TROPICAL SOILS

LAVRAS - MG
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ECOTOXICOLOGIA E EFEITOS DO LANTÂNIO SOBRE CULTURAS CULTIVADAS EM SOLOS TROPICAIS

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“Porque aqui está o que disse o Senhor Deus, o Santo de Israel: É na
conversão e na calma que está a vossa salvação; é no repouso e na
confiança que reside a vossa força”.
Is 30, 15

RESUMO

Devido às suas propriedades únicas, como maleabilidade e ductilidade, os elementos terras raras (ETR), dentre os quais o lantânio (La), são fundamentais para as indústrias de alta tecnologia. Assim, nas últimas décadas houve um expressivo aumento das concentrações destes elementos no ambiente, devido ao descarte de resíduos industriais e também por causa da aplicação de fertilizantes fosfatados, uma vez que esses podem conter quantidades expressivas de ETR. Diante disso, torna-se necessário avaliar quais são os riscos a que os organismos estão sujeitos em um ambiente com concentrações de ETR acima das naturais, principalmente em áreas agrícolas que exigem altas aplicações de fertilizantes fosfatados, como é o caso de solos tropicais. Nesse sentido, os objetivos do presente estudo foram: (i) avaliar o risco ecotoxicológico do La e propor valor limite de adição do elemento em solos tropicais para prevenção de fitotoxicidade; e, (ii) avaliar o efeito do La sobre os teores de nutrientes na parte aérea de espécies de plantas cultivadas em solos tropicais. Foram realizados ensaios ecotoxicológicos seguindo protocolos padrão em que quatro espécies de plantas (milho, sorgo, soja e girassol) foram expostas a concentrações crescentes de La aplicadas em três solos, dois solos naturais (Latossolo distrófico Vermelho - LVd e Latossolo distrófico Vermelho-Amarelo - LVAd) e um solo artificial tropical (SAT). Os resultados variaram de acordo com a espécie vegetal e o solo, evidenciando como as propriedades físico-químicas dos solos determinam os efeitos do La sobre as plantas e como estas apresentam diferenciadas respostas ao elemento. Os efeitos prejudiciais foram mais pronunciados nas plantas cultivadas em solos naturais, principalmente em LVd, para as quais foram encontrados os menores valores dos índices de toxicidade. Além disso, para a parte aérea das plantas cultivadas nestes solos, constatou-se, em geral, maior ocorrência de redução dos teores de nutrientes e aumento dos teores de La. Por outro lado, para as plantas cultivadas em SAT foi observada menor fitotoxicidade do La, o que, em geral, ocorreu a partir da dose de 614 mg La kg^{-1} de solo seco. Além disso, os teores de nutrientes na parte aérea destas plantas, em geral, aumentaram ou não apresentaram efeitos significativos com relação aos controles. Neste solo foi observado ainda o fenômeno conhecido como hormese, principalmente para as plantas de milho, com leves efeitos estimulantes na matéria seca da parte aérea e fotossíntese. De modo geral, considerando todos os solos e espécies, o girassol foi a espécie mais sensível ao La. Além disso, a matéria seca da parte aérea foi a variável mais afetada, com exceção do milho cultivado em LVd, para o qual a fotossíntese apresentou os menores valores dos índices de toxicidade. Baseando-se nos valores de EC_{20} (concentração que causou 20% de efeito na variável) encontrados para as plantas cultivadas em solos naturais, foi proposto o valor limite de La de 147.6 mg kg^{-1} de solo seco, valor preventivo de fitotoxicidade e, possivelmente, de ecotoxicidade nos solos estudados.

Palavras-chave: Fertilizante fosfatado. Elementos terras raras. Contaminante emergente. Fitotoxicidade. Efeito hormese.

ABSTRACT

The rare earth elements (REE), among which lanthanum (La), are essential for high-tech industries due to their unique properties as malleability and ductility. Thus, the concentrations of those elements in the environment have increased in recent decades due to industrial waste disposal and the application of phosphate fertilizers, since these could contain significant concentrations of REE. Therefore, it is necessary to assess the risks that the organisms are subject to in an environment with concentrations of REE above natural ones, mainly in agricultural areas requiring high applications of phosphate fertilizers, as is the case of tropical soils. In this sense, we aim in this study: (i) to evaluate the ecotoxicological risk of La and to propose a limit value of the addition of the element in tropical soils to prevent phytotoxicity (ii) to evaluate the La effect on the nutrient contents in the shoot of plant species grown in tropical soils. Ecotoxicological tests following standard protocols were carried out in which four plant species (maize, sorghum, soybean, and sunflower) were exposed to increasing concentrations of La applied in three soils, two natural soils (Dystrophic Red Oxisol - LVd and Dystrophic Red-Yellow Oxisol - LVAd) and the other a tropical artificial soil (SAT). The results varied according to the plant species and the soil, evincing how the physicochemical properties of the soil determine the effects of La on plants and how these present different responses to the element. The harmful effects were more pronounced in plants grown in the natural soils than in SAT, mainly in the LVd, for which the lowest toxic endpoints were found. Also, for the shoot of the plants grown in these soils, it was observed, in general, the higher occurrence of reduction of nutrient contents and an increase of La contents. On the other hand, for plants grown in SAT, less phytotoxicity of La was observed, which in general occurred from 614 mg La kg^{-1} of dry soil. Moreover, in general, the nutrients' levels in the shoot of those plants increased or did not show significant effects concerning the controls. Also, in the SAT, the phenomenon known as hormesis was observed, mainly for maize plants, which presented slight stimulating effects on the shoot dry matter and photosynthesis. In general, considering all soils and species, the sunflower was the most sensitive species to La. Furthermore, the shoot dry matter was the most affected biological endpoint, except for maize grown in the LVd because, for this specie, the photosynthesis showed the lowest values of toxic endpoints. Based on the EC_{20} values (concentration of La that caused 20% of effect on biological endpoint) found for plants grown in the natural soils, the La limit value of 147.6 mg kg^{-1} of dry soil was proposed, a preventive value for phytotoxicity and possibly ecotoxicity in the studied soils.

Keywords: Phosphate fertilizer. Rare earth elements. Emerging contaminant. Phytotoxicity. Hormesis effect.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Os elementos terras raras (ETR) compreendem um conjunto de 17 elementos químicos, dentre os quais 15 são da série dos lantanídeos (cujos números atômicos vão de 57 ao 71), e os outros dois são ítrio (Y) e o escândio (Sc), com números atômicos 39 e 21 respectivamente, agrupados no mesmo conjunto por apresentarem propriedades físico-químicas semelhantes aos lantanídeos. Os 15 elementos da série dos lantanídeos são: lantânio (La), cério (Ce), praseodímio (Pr), neodímio (Nd), promécio (Pm), samário (Sm), európio (Eu), gadolínio (Gd), térbio (Tb), disprósio (Dy), hólmio (Ho), érbio (Er), túlio (Tm), itérbio (Yb) e lutécio (Lu) (IUPAC, 2005).

Um equívoco relacionado aos ETR decorre do termo “rara”, que pode levar à interpretação de que estes elementos sejam pouco abundantes (STEGEN, 2015). De fato, no século passado achava-se que os ETR fossem uma raridade (STEGEN, 2015). No entanto, posteriormente foi descoberto que a maioria dos lantanídeos tem abundância similar ou ainda são mais abundantes do que muitos outros elementos bem conhecidos. Alguns ETR, como Tb, Tm, Lu e Eu são realmente pouco abundantes, mas outros como Ce e Y, são mais abundantes do que todos os metais preciosos (CHAKHMOURADIAN; WALL, 2012). Além disso, as abundâncias médias de Y, La e Ce na crosta terrestre são 20, 30 e 60 mg kg^{-1}, respectivamente, as quais são comparáveis àquelas de outros metais conhecidos como o Zn (70 mg kg^{-1}), Cu (55 mg kg^{-1}), Co (30 mg kg^{-1}) e P (10 mg kg^{-1}) (ROMERO-FREIRE et al., 2018; TYLER, 2004). Até mesmo os ETR mais escassos, Tm (0,5 mg kg^{-1}) e Lu (0,8 mg kg^{-1}) são mais abundantes do que a prata, o bismuto, o cádmio e o selênio (GREENWOOD; EARNSHAW, 1984; MARTINS; ISOLANI, 2005).

O termo “rara”, atualmente, está relacionado à dificuldade de se separar os ETR. Esses elementos apresentam-se unidos na estrutura do mineral, devido à similaridade de raio iônico e do estado de oxidação, ou seja, nenhum mineral apresenta apenas um, mas vários ETR associados (STEGEN, 2015; TYLER, 2004). Os ETR são amplamente explorados, sendo a China o país detentor do maior número de reservas e da maior parte da produção (STEGEN, 2015). Por serem metais maleáveis, dúcteis e macios, os ETR constituem matéria-prima fundamental para as indústrias de alta tecnologia. A maior parte da produção destes elementos destina-se a catalisadores utilizados em refinarias de petróleo, sendo também utilizados em ímãs de alta resistência em equipamentos eletrônicos, em turbinas eólicas e veículos elétricos, em computadores, equipamentos de áudio e automóveis, e em várias tecnologias de energia nuclear e defesa. Além disso, os ETR são aplicados na medicina e na agricultura. O Gd, por exemplo, é o agente de contraste mais comumente usado em imagens de ressonância magnética para o diagnóstico de tumores (PAGANO et al., 2015). Cério, La, Pr e Nd são componentes de fertilizantes chineses aplicados para o aumento da produtividade de culturas (HU et al., 2004; PANG et al., 2002).

A ocorrência natural de ETR em solos é altamente influenciada pelo material de origem (HU et al., 2006). Da Silva et al. (2016), ao avaliarem diferentes solos de referência no Brasil, encontraram menores concentrações de ETR em sedimentos arenosos e maiores em sedimentos de basalto, biotita gnaisse e argilosos. Estes elementos são encontrados em mais de 270 minerais (primários e secundários), em variadas concentrações (CHAKHMOURADIAN; WALL, 2012). Os minerais mais explorados são monazita (La, Ce-PO_4), bastnasita (CeF-CO_3) e xenotímio (YPO_4). Em muitos casos, devido à semelhança de raios iônicos, os ETR podem substituir Na, Ca, Th e U na estrutura dos minerais, sendo que a substituição do Ca é a mais comum (KANAZAWA; KAMITANI, 2006).

Os processos de formação do solo, aliados à redução de pH, causam perdas de ETR por lixiviação, sendo que as concentrações totais destes elementos na camada superior de solos nativos são muitas vezes menores que no material de origem (TYLER, 2004). Os ETR são distribuídos e redistribuídos durante o intemperismo em função da estabilidade e natureza mineralógica do mineral em que estão presentes, bem como das diferentes propriedades que apresentam na solução do solo.

Como é de conhecimento, a biodisponibilidade, a toxicidade e a deficiência de qualquer elemento no ambiente dependem das propriedades do próprio elemento e suas concentrações no meio, bem como das propriedades físico-químicas e mineralógicas do solo, tais como textura, pH, conteúdo de matéria orgânica, capacidade de troca de cátions (CTC), quantidade de óxidos de Fe amorfos, de fosfatos e de sulfetos (HU et al., 2006). Com relação às suas características, os ETR são química e fisicamente muito semelhantes, devido à natureza de sua configuração eletrônica, geralmente alcançando um estado de oxidação +3, particularmente estável, e uma pequena redução, mas constante, do raio iônico, com o aumento do número atômico, o que é conhecido por "contração do lantanídeo" (DINALI et al., 2019; MIGASZEWSKI; GALUSZKA, 2014). No entanto, também podem ocorrer formas tetravalentes e bivalentes, como é o caso do Ce^{+4} e Eu^{+2}, respectivamente (MIGASZEWSKI; GALUSZKA, 2014). Sendo assim, a concentração destes elementos na solução do solo é influenciada principalmente por cargas negativas no solo, cujas principais fontes são argilas e matéria orgânica.

A matéria orgânica é uma importante fonte de sítios de ligação de ETR, por apresentar grupos funcionais carregados negativamente, com alta capacidade de adsorção ou quelação de cátions (PANG et al, 2002). Os ETR podem ainda ser complexados com ligantes inorgânicos, como carbonato e sulfato (PANG et al., 2002). Assim como observado para outros elementos-traço, a adsorção de ETR é dependente do pH, da força iônica da solução, do tipo de argila e das concentrações de óxidos de Fe e de Mn, sendo que estes possuem elevada capacidade de adsorção. Em condições tropicais, como as do Brasil, a alta precipitação e os solos altamente intemperizados com elevada acidez podem promover maior solubilidade de ETR, aumentando, assim, sua biodisponibilidade ou perdas por lixiviação. Por outro lado, os solos brasileiros são ricos em óxidos de Fe e Al amorfos, o que pode contribuir para a adsorção destes elementos (DINALI et al., 2019; VILELA, 2015). De Sá Paye et al. (2016) observaram que os óxidos de Fe, Mn e Ti, assim como a matéria orgânica, foram as principais variáveis correlacionadas significativamente com os teores de ETR em solos brasileiros.

Além disso, a ocorrência de ETR em solos também pode ser decorrente de ações antrópicas, por meio da aplicação via fertilizantes e ainda pelo mal descarte de resíduos industriais. Na China, aplicações intencionais de ETR na agricultura têm sido realizadas desde a década de 80, via fertilizantes enriquecidos com estes elementos, predominantemente La e Ce (HU et al., 2004; PANG et al., 2002). Estes fertilizantes, denominados Changle e Nongle, conhecidos por promover aumentos na produção de culturas, são amplamente utilizados naquele país. Os ETR também podem estar presentes na agricultura de outros países, como constituintes de fertilizantes fosfatados porque podem substituir o Ca na estrutura da apatita, principal fonte para a fabricação destes insumos. Considerando-se os solos tropicais, especialmente os do Brasil, em que a aplicação de fertilizantes fosfatados é intensa, conclui-se que de maneira não intencional, grandes quantidades de ETR vêm sendo adicionadas a esses solos agrícolas (RAMOS et al., 2016b; TURRA et al., 2011). A concentração de La, por exemplo, em superfosfatos simples (SSP) produzidos no Brasil, encontra-se na faixa de 673 a 1926 mg La kg^{-1} (RAMOS et al., 2016b; TURRA et al., 2011).

Como é ampla a aplicação de ETR na indústria, a procura no mercado por estes elementos é alta, e com isso, houve um rápido aumento na exploração de recursos minerais que os contêm e, assim a geração de resíduos é cada vez mais elevada, tanto de processos de mineração e beneficiamento, quanto de produtos de alta tecnologia. O descarte destes resíduos no ambiente, que em geral é o solo, assim como a aplicação, intencional ou não, de fertilizantes em solos agrícolas, podem aumentar as concentrações de ETR em solos e águas subterrâneas, o que pode resultar em impactos do ponto de vista ecológico ou de saúde pública. Diante disso, percebe-se a necessidade de se conhecer quais são os possíveis riscos aos seres vivos do solo associados a estes elementos.

Comparados aos estudos com outros elementos-traço, os estudos acerca dos efeitos de ETR sobre os seres vivos ainda são incipientes e realizados apenas em alguns países, principalmente na China. Os estudos são relativamente recentes já que a maior parte das aplicações tecnológicas destes elementos tem se concentrado nas últimas duas décadas. Segundo levantamento realizado por Ramos et al. (2016a), poucos são os trabalhos envolvendo ETR, solos e plantas, assim como são escassos estudos de avaliação de risco de ETR em solo. A maioria dos estudos envolvendo plantas e ETR foi realizada em solução nutritiva, sendo que, os poucos trabalhos realizados em solos ou em campo são restritos a regiões específicas do hemisfério norte, o que impossibilita qualquer generalização para as condições tropicais, especialmente as brasileiras. Sabe-se que, devido às características dos solos, a capacidade de adsorção de ETR e, consequentemente, sua disponibilidade em solos tropicais intemperizados em comparação a solos de regiões do hemisfério norte é extremamente diferenciada, o que foi relatado em estudo conduzido por Dinali et al (2019). Além disso, existe a dificuldade de acesso às pesquisas devido ao idioma, já que grande parte dos trabalhos realizados foi publicada em Chinês, dificultando, assim, o acesso aos detalhes experimentais e resultados (POŠĆIĆ et al., 2017).

De acordo com as pesquisas realizadas com plantas até o momento, os ETR não são considerados essenciais. No entanto, eles têm se mostrado benéficos, podendo estimular a fisiologia vegetal, de uma forma dependente da dose aplicada, uma vez que estes elementos apresentam o fenômeno conhecido como hormese, caracterizado por efeitos benéficos sob baixas concentrações e efeitos prejudiciais sob altas concentrações (AGATHOKLEOUS; KITAO;

CALABRESE, 2018). Baixas concentrações de La em solução nutritiva e em substrato resultaram em aumento dos teores de nutrientes, taxa fotossintética, conteúdo total de clorofila e transcrição das subunidades da ATPase do cloroplasto (DE OLIVEIRA et al., 2015; HU et al., 2016). Por outro lado, altas concentrações do elemento causaram redução de todas as variáveis citadas e do crescimento devido a modificações ultraestruturais na parede celular, tilacoides e cloroplastos (DE OLIVEIRA et al., 2015; HU et al., 2016).

Nesse sentido, o La, assim como outros ETR, pode desencadear efeitos tanto antioxidantes como pró-oxidantes dependendo do tempo e concentração aos quais as plantas são expostas, podendo, assim, modificar o metabolismo, incluindo a biossíntese de biomoléculas bem como o *turnover* proteico (GARCÍA-GIMÉNEZ et al., 2017). De forma geral, os efeitos visíveis observados em plantas expostas aos ETR, sejam eles benéficos ou prejudiciais, são possivelmente explicados pela influência que estes elementos exercem sobre a atividade enzimática, conteúdo de clorofila, taxa fotossintética, estabilidade de membrana, absorção de nutrientes, função metabólica e estrutural do Ca, metabolismo hormonal e, ainda, sobre a resposta a estresses ambientais (EL-RAMADY, 2000; HU et al., 2004; SALGADO et al., 2019).

Os ETR podem interferir em processos biológicos que envolvam cátions, principalmente o Ca^{2+}, uma vez que podem substituí-los, devido à similaridade de raio iônico. Assim, os efeitos de La no nível bioquímico são principalmente devido à substituição do Ca, bloqueio dos canais de Ca e interferência no metabolismo do Ca (LI et al., 2018). De fato, os ETR exercem importantes efeitos sobre a estabilidade da membrana e interagem fortemente com o Ca (HU et al., 2004). Com isso, a absorção de elementos inorgânicos pode ser regulada pelos ETR e há relatos de que o metabolismo de nutrientes pode ser intensificado na presença destes elementos (REZAEE et al., 2018; ZHANG et al., 2013). A atividade da redutase do nitrato foi aumentada em plantas submetidas a aplicação de La, aumentando a taxa de conversão de N inorgânico para orgânico, contribuindo para a síntese de proteínas e regulação do balanço nutricional (CAO et al., 2007; PANG et al., 2002).

Embora os ETR ainda não se configurarem como um problema ambiental, e sejam considerados de baixa toxicidade, com o crescente aporte destes elementos em solos como resultado das atividades antrópicas, surge a preocupação para o controle da adição destes elementos no ambiente, conforme já realizado para vários outros elementos-traço, por meio de valores orientadores. Estes valores são definidos como concentrações de substâncias/elementos que fornecem orientação sobre a qualidade e as alterações de solos e águas subterrâneas (CONAMA, 2009). São utilizados pelos órgãos ambientais na avaliação e diagnóstico daqueles recursos ambientais, permitindo, assim, ações de prevenção ou controle da poluição. A legislação brasileira estabelece três valores orientadores (CONAMA, 2009), englobando desde aqueles que exprimem as concentrações naturais dos elementos nos solos, Valor Orientador de Referência de Qualidade (VRQ), acima dos quais haverá contaminação, até aqueles valores relacionados aos riscos ecotoxicológicos e vias de exposição de plantas, humanos e animais, Valor de Prevenção (VP) e Valor de Intervenção (VI) (FERNANDES, 2011).

Os VI indicam os valores limite de contaminação do solo acima do qual, existe risco potencial à saúde humana. Já os VP orientam sobre o valor limite de adição de um elemento/substância tóxica ao solo, tal que este seja capaz de manter suas principais funções, garantindo assim a proteção de receptores ecológicos e da água subterrânea (CONAMA, 2009). Valores de VP são obtidos por meio da avaliação de risco do elemento/substância para os organismos, neste caso a biota do solo, realizada por meio de ensaios ecotoxicológicos. Estes ensaios são conduzidos sob condições controladas onde organismos-teste, pertencentes a uma ou mais espécies, são submetidos a concentrações crescentes de um determinado contaminante. As plantas são importantes receptores ecológicos para uso nestes ensaios, já que apresentam diferentes graus de susceptibilidade e, principalmente, por representarem o primeiro nível trófico (THOMAS et al., 2014).

Os ensaios ecotoxicológicos podem ser agudos, crônicos, ou ainda subcrônicos, consoante a sua duração e o efeito observado. Os resultados podem ser expressos em uma série de índices de toxicidade, como aqueles cujos valores são exatamente iguais aos das concentrações utilizadas no ensaio, NOEC (do Inglês No Observed Effect Concentration, que é a maior concentração testada que não causa efeitos comparados ao controle) e LOEC (do Inglês, Lowest Observed Effect Concentration, que é a menor concentração que causa efeitos comparados ao controle) ou ainda aquele cujos valores são estimados, EC_x (do Inglês Effect Concentration, que corresponde à concentração de um contaminante que causa x% de redução a uma variável resposta dos organismos-teste). O EC_x comumente utilizado é aquele em que a concentração do elemento afeta 50% das variáveis biológicas, ou seja, EC50. Os EC_x obtidos para um grupo de organismos podem ser expressos graficamente em uma curva de distribuição de sensibilidade das espécies (DSE – no Inglês, Species Sensitivity Distributions Curve – SSD curve), a partir da qual pode ser obtido o HC_y (do Inglês Hazardous Concentration, ou seja, a concentração do elemento perigosa para y % das espécies avaliadas). O índice mais utilizado é o que protege 95% das espécies, ou seja, o HC_5.

Como são poucos os estudos dos efeitos de ETR sobre os organismos, ainda não estão bem estabelecidos valores orientadores para estes elementos. A Holanda, país pioneiro na elaboração de relatórios e resoluções para o controle do aporte de substâncias tóxicas no ambiente, baseados em criteriosos estudos (eco)toxicológicos, mais uma vez foi o primeiro a atentar-se para os impactos que o crescente acúmulo de ETR podem causar ao ambiente, estabelecendo as concentrações máximas admissíveis (o mesmo que VP) de alguns destes elementos para águas superficiais, sedimentos e solos (KUCERA et al., 2007; SNELLER et al., 2000). No Brasil, as concentrações naturais de ETR em diferentes solos do país foram determinadas (DE SÁ PAYE et al., 2016; DA SILVA et., 2016), contribuindo, assim, para os possíveis primeiros VRQ para estes elementos, sendo que para o La o teor médio encontrado foi 38,06 mg kg^{-1} de solo seco. No entanto, estudos visando o estabelecimento de valores de ETR que sejam preventivos são raros no Brasil, sendo registrado apenas o estudo ecotoxicológico conduzido por Moreira et al. (2019) com plantas expostas ao Ce.

Assim, tendo-se em vista que a grande aplicação de fertilizantes fosfatados em solos tropicais tem elevado as concentrações de ETR em solos agrícolas, e que são escassos os estudos envolvendo ETR, solos, plantas e análise de risco, bem como não há nenhum registro de avaliação ecotoxicológica do La em solos tropicais, o presente trabalho de tese foi desenvolvido. Este trabalho foi dividido em duas partes, sendo a primeira a presente parte, constituída por uma introdução geral e a segunda parte constituída pelos artigos. Conforme visto, a presente parte enfoca os principais tópicos necessários para a justificativa do desenvolvimento da tese, descritos com mais detalhes do que na introdução apresentada em cada um dos artigos. O foco do primeiro artigo foi a avaliação de risco do La para as plantas cultivadas em solos tropicais. Para isso, foram realizados ensaios ecotoxicológicos com quatro espécies de plantas cultivadas em três solos tropicais, sendo avaliadas diferentes variáveis biológicas com o objetivo de gerar índices de toxicidade, a partir dos quais concentrações limite de La nos solos puderam ser estimadas. Considerando-se que tais índices podem ser obtidos apenas a partir de variáveis para as quais são observadas predominantemente reduções e, ainda, que os teores de nutrientes são muito variáveis e que os ETR apresentam diferentes influências sobre estes, optou-se por contemplar e apresentar os resultados da composição de nutrientes em outro artigo. Assim, o foco do segundo artigo foi a influência do La sobre os teores de nutrientes nas quatro espécies vegetais testadas, sendo discutido ainda os possíveis casos de efeito hormese observados.

Espera-se que o presente trabalho contribua para o banco de dados sobre as respostas de plantas aos ETR quando cultivadas em solos, especificamente os tropicais, incluindo variáveis como os teores de nutrientes, considerando-se que grande parte dos estudos realizados até o momento foi conduzida em solução nutritiva. Além disso, este trabalho provê a primeira avaliação de risco do La em solos tropicais, possibilitando, assim, a geração de preliminares valores preventivos do elemento nestes solos. Espera-se, então, que os dados gerados possam contribuir para o banco de dados de ecotoxicologia terrestre em solos tropicais e que os valores preliminares estimados possam vir a ser considerados futuramente por órgãos ambientais brasileiros, assim como de outros países cujos solos também sejam tropicais, para o estabelecimento legal de VP para o La.

REFERÊNCIAS

AGATHOKLEOUS, E.; KITAO, M.; CALABRESE, E. J. The rare earth element (REE) lanthanum (La) induces hormesis in plants. **Environmental Pollution**, v. 238, p. 1044–1047, 2018.

CHAKHMOURADIAN, A. R.; WALL, F. Rare earth elements: minerals, mines, magnets (and more). **Elements**, v. 8, p. 333–40, 2012.

CONSELHO NACIONAL DO MEIO AMBIENTE. **Resolução nº 420**, de 28 de dezembro de 2009. Dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas por essas substâncias em decorrência de atividades antrópicas. Publicada no Diário Oficial da União, Brasília, n. 249, p. 81-84, 30 dez. 2009. Alterada pela Resolução CONAMA nº 460/2013. Disponível em: <http://www.mma.gov.br/port/conama/res/res09/res42009.pdf>. Acesso em: 15 fev. 2017.

DE OLIVEIRA, C. et al. Bioaccumulation and effects of lanthanum on growth and mitotic index in soybean plants. **Ecotoxicology and Environmental Safety**, v. 122, p. 136–144, 2015.

DA SILVA, Y. J. A. B. et al. Rare earth element concentrations in Brazilian benchmark soils. **Revista Brasileira de Ciência do Solo**, v. 40, p. 1–13, 2016.

DINALI, G.S. et al. Rare earth elements (REY) sorption on soils of contrasting mineralogy and texture. **Environment International,** v. 128, p. 279–291, 2019.

GARCÍA-JIMÉNEZ, A. et al. Lanthanum affects bell pepper seedling quality depending on the genotype and time of exposure by differentially modifying plant height, stem diameter and concentrations of chlorophylls, sugars, amino acids, and proteins. **Frontiers in Plant Science**, v. 8, p. 1–14, 2017.

GREENWOOD, N. M.; EARNSHAW, A.; **Chemistry of the Elements**, Pergamon Press: Great Britain, 1984.

HU, H. et al. Insight into mechanism of lanthanum (III) induced damage to plant photosynthesis. **Ecotoxicology and Environmental Safety,** v.127, p. 43-50, 2016.

HU, Z. et al. Physiological and biochemical effects of rare earth elements on plants and their agricultural significance: a review. **Journal of Plant Nutrition**, New York, v. 27, n. 1, p. 183–220, Dez. 2004.

HU, Z. et al. Rare earth elements in soil. **Communications in Soil Science and Plant Analysis**, New York, v. 37, n. 9/10, p. 1381-1420, Jun. 2006.

IUPAC. NOMENCLATURE OF INORGANIC CHEMISTRY-IUPAC. **Recommendations**, 2005. [s.l: s.n.]. p. 366.

KANAZAWA, Y.; KAMITANI, M. Rare earth minerals and resources in the world. **Journal of Alloys and Compounds**, v. 408–412, p. 1339–1343, 2006.

KUCERA, J. et al. Pollution of agricultural crops with lanthanides, thorium and uranium studied by instrumental radiochemical neutron activation analysis. **Journal of Radioanalytical and Nuclear Chemistry**, v. 271, n. 3, p. 581-587, 2007.

LI, J.; VERWEIJ, R. A.; VAN GESTEL, C. A. M. Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil. **Chemosphere**, v. 193, p. 412–420, 2018.

MARTINS, T. S.; ISOLANI, P. C. Terras Raras: Aplicações Industriais e Biológicas. **Química Nova**, v. 28, n. 1, p. 111–117, 2005.

MIGASZEWSKI, Z. M.; GAŁUSZKA, A. The characteristics, occurrence, and geochemical behavior of rare earth elements in the environment: a review. **Critical Reviews in Environmental Science and Technology**, v. 45, p. 429–71, 2014.

PANG, X. et al. Application of rare-earth elements in the agriculture of China and its environmental behavior in soil. **Environmental Science Pollution Research**, v. 9, p. 143-148, 2002.

DE SÁ PAYE, H. S. et al. Distribution and fractionation of the rare earth elements in Brazilian soils. J**ournal of Geochemical Exploration,** v. 161, p. 27–41, 2016.

POŠĆIĆ, F.; SCHAT, H.; MARCHIOL, L. Cerium negatively impacts the nutritional status in rapeseed. **Science of the Total Environment**, v. 593–594, p. 735–744, 2017.

RAMOS, S. J. et al. Rare Earth Elements in the Soil Environment. **Current Pollution Report**, v. 2, p. 28–50, 2016a.

RAMOS, S. J. et al. Rare earth elements in raw materials and products of the phosphate fertilizer industry in South America: Content, signature, and crystalline phases. **Journal of Geochemical Exploration**, v. 168, p. 177–186, 2016b.

REZAEE, A. et al. Accumulation and toxicity of lanthanum and neodymium in horticultural plants (*Brassica chinensis* L. and *Helianthus annuus* L.). **Canadian Journal of Chemical Engineering**, v. 96, n. 10, p. 2263–2272, 2018.

ROMERO-FREIRE, A. et al. Assessment of baseline ecotoxicity of sediments from a prospective mining area enriched in light rare earth elements. **Science of the Total Environment**, v. 612, p. 831–839, 2018.

SALGADO, O. G. G. et al. Cerium alleviates drought-induced stress in *Phaseolus vulgaris.* **Journal of Rare Earths**, v. 38, n. 3, p. 324–331, 2020.

SNELLER, F. E. C., KALF, D. F., WELTJE L., VAN WEZEL A. P. **Maximum Permissible Concentrations and Negligible Concentrations for Rare Earth Elements**, RIVM Report 601501011, Bilthoven, The Netherlands, Jun. 2000.

STEGEN, K. S. Heavy rare earths, permanent magnets, and renew able energies: An imminent crisis. **Energy Policy**, v. 79, p. 1-8, 2015.

THOMAS, P. J. et al. Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. **Chemosphere**, v. 96, p. 57-66, 2014.

TURRA, C. et al. Evaluation on rare earth elements of Brazilian agricultural supplies. **Journal of Environmental Chemistry and Ecotoxicology**, v. 3, p. 86-92, 2011.

TYLER, G. Rare earth elements in soil and plant systems: a review. **Plant and Soil**, The Hague, v. 267, n.1/2, p. 191-206, Dez. 2004.

ZHANG, C. et al. Effects of rare earth elements on growth and metabolism of medicinal plants. **Acta Pharmaceutica Sinica B**, v. 3, n. 1, p. 20–24, 2013.

SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - Lanthanum phytotoxicity: an approach to assess ecological risk in tropical soils

Artigo formatado de acordo com as normas específicas do periódico *Ecotoxicology and Environmental Safety* (ISSN: 0147-6513) – versão preliminar.

Lanthanum phytotoxicity: an approach to assess ecological risk in tropical soils

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Abstract

The rare earth elements (REE), including lanthanum (La), are essential for high-tech industries and, therefore, the exploration, production, and use of these elements have increased considerably in recent years. Furthermore, those elements are present in apatite, the primary source of phosphate fertilizers. Consequently, the concentrations of REE in the environment are increasing, becoming it necessary to investigate if there are risks for organisms, especially in tropical soils. Thus, this study aimed to assess the ecotoxicological risk of La to determine an acceptable concentration of the element in tropical soils that prevent toxicity, thus contributing to improve the database of soil screening levels in the Brazilian legislation. Experiments were carried out with four plant species (sunflower, soybean, sorghum, and maize) exposed to increasing concentrations of La applied in three soils. These soils were two typical tropical soils - Red-Yellow Oxisol (LVAd) and Red Oxisol (LVd) -, and a tropical artificial soil (SAT). The biological endpoints evaluated were emergence, shoot dry matter, stomatal conductance, transpiration, photosynthesis and relative chlorophyll content. The risk of La causing phytotoxicity in the LVd was higher than other soils, and in this soil, the photosynthesis of maize was affected from low doses of the element ($\leq$ 150 mg kg^{-1} dry soil). The shoot dry matter was the most affected endpoint for most species, with sunflower being the most sensitive species in all soils. We propose the limit value of 147.6 mg La kg^{-1} of dry soil, which is representative and protective of the evaluated crops/endpoints, anticipating that other soil organisms may also be protected.

Keywords: Rare earth element**,** soil ecotoxicology, plants, Hazardous concentration, tropical agroecosystem.

Highlights

- The physicochemical properties of soils are determinants of La phytotoxicity
- The shoot dry matter and photosynthesis are very sensitive endpoints to La
- Lanthanum concentrations $\leq$ 150 mg kg^{-1} dry soil resulted in photosynthesis impairment

1. Introduction

Interest in rare earth elements (REE) has been growing in recent years due to their varied applications, mainly in high-tech industries. Rare earth elements encompass a group of seventeen chemical elements with similar physicochemical properties, fifteen of which belong to the lanthanide series, and the others are scandium (Sc) and yttrium (Y) (IUPAC, 2005). Despite the term rare, the abundance of REE in the earth's crust and soils is significant, with cerium (Ce) being the most abundant REE, followed by lanthanum (La) (Hu et al., 2006; Ramos et al., 2016a). The La average natural concentration in Brazilian tropical soils is 38.08 mg kg-1 (0.10 - 197.63) (de Sá Paye et al., 2016). Rare earth elements are present in more than 270 minerals, mainly monazite, bastnasite, and xenotime (Gupta et al., 2005). Due to the ionic radius similarity with Ca^{2+}, REE can replace it in the crystalline structure of minerals such as apatite, the primary source for the exploration of phosphate fertilizers, and bind strongly to P, thus being able to be constituents of these inputs (Kanazawa; Kamitani, 2006; Ramos et al., 2016b). As most of the typical tropical soils found in Brazil have low fertility and high P retention (Lopes and Guilherme, 2016; Withers et al., 2018), intensive applications of fertilizers, especially phosphates, are necessary. Thus, over many years, significant REE amount has been applied, unintentionally, to Brazilian agricultural soils (Ramos et al., 2016b; Turra et al., 2011).

Rare earth elements have also been present in Chinese agriculture since the 1980s, being deliberately applied via REE fertilizers to the seed treatment, foliar fertilization, and soil fertilization (Hu et al., 2004; Pang et al., 2002). As China has the most massive production and the biggest REE reserves (Ramos et al., 2016a; Smith Stegen, 2015), most studies with these elements were performed in that country. According to Chinese research, the application of REE-based fertilizers promoted plant growth, with increases in the order of 5 to 15% in crop productivity (Xiong et al., 2000). However, other studies have shown phytotoxicity, mainly under high concentrations, since REE present hormetic effect, i.e., under low concentrations, they can be beneficial, but under high concentrations, they can be toxic (Agathokleous et al., 2018; de Oliveira et al., 2015; Ramos et al., 2016a).

The widespread use of REE in the high-tech industries and agriculture, as well as the mining and refining processes, have increased the concentrations of them in the environment, which can result in the contamination or even pollution of soils, rivers, and air. Although these elements are not yet an environmental problem and present low toxicity (Thomas et al., 2014), considering the increase in generation and the inappropriate disposal of waste that contains them, whose final destination is usually the soil, their concentrations in soils can become high. Furthermore, the intensive REE application via phosphate fertilizers has also contributed to the increase in the concentrations of these elements in the soil (Ramos et al., 2016b; Turra et al., 2011). The La levels in single superphosphates (SSP) produced in Brazil range from 673 to 1926 mg La kg^{-1} (Ramos et al., 2016b; Turra et al., 2011). High contents of REE in soils can lead to overexposure of organisms and, therefore, increases ecological risks. In this context, there is a great need for studies focusing on the risk assessment of REE in soils to characterize their effects and establish acceptable concentrations that prevent toxicity to most of the living organisms that are relevant in our ecosystems.

Through so-called ecotoxicological tests, the effects of different concentrations of a chemical element/substance on organisms of one or more species can be evaluated, aiming to estimate the limit concentrations of addition to the soil so that it could be possible to prevent harmful effects. Among these organisms, plants are valuable ecological components since they are the primary producers and have different degrees of susceptibility (Thomas et al., 2014), and are the first miner of these elements from soil. Researches with plants exposed to REE have shown that, in general, the effects observed could be related to REE influence on enzymatic activity, chlorophyll content, photosynthesis, membrane stability, nutrient absorption, function metabolic and structural Ca, hormonal metabolism and also on the response to environmental stresses (El-Ramady, 2000; Hu et al., 2004; Salgado et al., 2019).

The ecotoxicological tests are part of the preliminary stages of the ecological risk assessment, and the estimated concentrations could be subsidies to the database of the specific environmental agency and legislation. These concentrations can be established by normative acts, being classified as guiding or soil screening values, i.e., levels of chemical element that orient about the quality and changes in environmental resources (CONAMA, 2009), thus allowing prevention or control actions of pollution. The use of guiding values is well established in countries in the northern hemisphere (European Union, United States, Canada) and Australia, which have consolidated methodologies for risk assessments concerning most chemicals of potential concern (Checkai et al., 2014).

In Brazil's resolution No. 420/2009 (CONAMA, 2009), there are guiding values for a considerable number of chemical elements/substances, but many have been revised, and new tests must be carried out. It has focused on the revision of the limit concentrations, called in Brazil as a "prevention value" (PV), considering that the PV values established in that resolution were not based on tests with Brazilian natural soils (Niva et al., 2016). In addition to the PV, there are two other guiding values, which are the Quality Reference Value (QRV) and the Investigation Value (IV).

Quality reference values represent the natural occurrence of the element (background concentration). The background concentrations of REE in different soils in Brazil were firstly assessed by de Sá Paye et al. (2014) and da Silva et al. (2016), thus contributing to generating a QRV database for these elements. However, studies aiming to establish preventive REE values (PV) are scarce just not in Brazil, but in the world. As most of the technological applications of REE occurred in recent decades, research involving the biological effects of these elements is relatively recent and performed only in some countries (Ramos et al., 2016a). The great majority of the studies involving plants and REE were carried out in a nutrient solution (Gong et al., 2019; Hu et al., 2004; Ramos et al., 2016a). A few studies performed in soils or in the field are restricted to regions of the northern hemisphere, mainly in China, making it impossible to generalize results to tropical conditions.

According to the review of Ramos et al. (2016a), there are few studies involving REE, soils, and plants, or risk assessment of these elements in soils. One of the few studies involving the application of REE in soil and, still, with an ecotoxicological approach, is that of Thomas et al. (2014). These authors exposed native and crop species to increasing concentrations of La, Y, and Ce. The REE were applied in a standard artificial soil recommended by the OECD, consisting of peat, kaolinite, and sand. Carpenter et al. (2016) carried out similar experiments using the same species and soil to evaluate other REE, including praseodymium (Pr), neodymium (Nd), samarium (Sm), terbium (Tb), dysprosium (Dy) and erbium (Er).

Thomas et al. (2014) and Carpenter et al. (2015), however, carried out ecotoxicological tests only with artificial soil, although an essential aspect of ecotoxicological studies is the use of a standard natural soil (Niva et al., 2016). Also, as previously reported, data referring to studies conducted under conditions in temperate regions cannot be generalized to tropical environments. The artificial soil used by Thomas et al. (2014) and Carpenter et al. (2015) was a standard matrix composed of peat, a component of restricted occurrence in the tropics (Niva et al., 2016). An alternative for that is the one adopted by Moreira et al. (2019), who used a tropical artificial soil (an adaptation of the standard artificial soil) and also two natural soils (typical tropical), the Red-Yellow Oxisol - LVAd and the Cambisol, for ecotoxicological assessment of Ce for plant species. The authors proposed the value of 281.6 mg Ce kg^{-1} of dry soil, calculated from the data found for LVAd and Cambisol, as a PV for Ce in such soils, thus contributing for setting the first limit concentration of Ce in tropical soils.

Considering La, the studies developed with an ecotoxicological focus involved soils from regions of the northern hemisphere and the standard artificial (Chu et al., 2003; Li et al., 2018; Thomas et al., 2014; Zeng et al., 2006). Li et al. (2018) joined their data obtained in the ecotoxicological study with soil invertebrate species, with the data available in the literature for plants and bacteria. They determined the limit value of 54 mg La kg^{-1} of dry soil. However, so far, there is no record of an ecotoxicological study for La in tropical soils.

Thus, considering: i) the massive application of phosphate fertilizers in tropical soils - mainly in Brazilian agroecosystems -, which, consequently, adds a large amount of REE to agricultural soils; ii) the scarcity of studies on the evaluation of the effects of REE on plants grown in soils; and, iii) the lack of an ecotoxicological study for La in tropical soils, we carried out ecotoxicological tests with plants and tropical soils representative of Brazil, aiming to establish/suggest an acceptable/limit concentration that may be preventive and also a subsidy to improve the current database concerning soil screening values of REE in the Brazilian legislation.

2. Materials and methods

2.1 Soils and plant species

The natural soils were collected in the 0-20 cm layer in areas that showed minimal human interference. Soils used were a Dystrophic Red-Yellow Oxisol - LVAd (Typic Hapludox - USDA Soil Taxonomy) and a Dystrophic Red Oxisol - LVd (Rhodic Hapludox - USDA Soil Taxonomy), collected in the cities of Itumirim and Lavras, respectively (State of Minas Gerais, Brazil).

In addition to natural soils, a tropical artificial soil (SAT) was also used. This soil was formulated based on instructions contained in the OECD 207 guideline (OECD, 1984), with an adaptation of the use of coconut fiber instead of sphagnum peat (Silva and van Gestel, 2009), and was composed of 10% coconut fiber, 20% kaolinite and 70% of sand. Following their collection, the natural soils were air-dried, homogenized, and sieved. Next, all soils (including the SAT) were analyzed chemically and physically, according to the Brazilian Agricultural Research Corporation (EMBRAPA, 2011) (Table 1).

Table 1. Physical and chemical attributes of tested soils (tropical artificial soil – SAT, Dystrophic Red-Yellow Oxisol – LVAd and Dystrophic Red Oxisol – LVd).

	SAT	**LVAd**	**LVd**
pH (H_2O)	6.0	5.1	4.8
K (mg dm^{-3})	176.5	38.0	24.7
P (mg dm^{-3})	10.74	1.15	0.88
Ca ($cmol_c$ dm^{-3})	1.37	1.05	0.19
Mg ($cmol_c$ dm^{-3})	0.63	0.26	0.10
Al ($cmol_c$ dm^{-3})	0.09	0.35	0.65
CEC ($cmol_c$ dm^{-3})	4.19	1.40	0.36
MO (dag kg^{-1})	6.85	1.69	2.70
Sand (%)	70	71	22
Silt (%)	-	6	17
Clay (%)	20	23	61

CEC: cation-exchange capacity; MO: organic matter.

The plant species used were the monocotyledons *Zea mays* (maize) and *Sorghum bicolor* (sorghum) and the eudicotyledons *Glycine max* (soybean) and *Helianthus annuus* (sunflower). They were selected from the list of species of the OECD-208 guideline (OECD, 2006).

2.2 Experimental procedure

The experiments were conducted in a greenhouse under natural light and temperatures between 23 ± 4°C, adapting the procedures described in the OECD guideline n° 208 (OECD, 2006). The experimental design was completely randomized, with seven doses of La and three types of soils, with four replications, considering independents the experiment for each species. Each experimental unit consisted of a 500 ml pot and the plants, which varied according to the specie: three sorghum plants and two maize, soybean, and sunflower plants. Sorghum plants were kept in higher numbers, as this experiment was the first to be carried out. From this experiment, it was observed that a smaller number of plants per pot would allow more space for their growth, as well as be sufficient for the evaluations of the plants' endpoints.

The treatments consisted of following La doses in mg kg^{-1} of dry soil: 0, 150, 240, 384, 614, 983, and 1573, using the $LaCl_3.7H_2O$ reagent (Sigma-Aldrich, St. Louis, MO, USA). The initial dose (150 mg kg^{-1} of La) was based on data available in the literature (Thomas et al., 2014), and preliminary tests. From the results of these tests, it was defined as the first dose, and the others were calculated by the geometric progression of 1.6. To exclude the osmotic effect of the accompanying ion (chlorine, Cl), parallel treatments, called saline controls, were also carried out. The reagent used in the saline controls was $CaCl_2$ $2H_2O$ (Sigma-Aldrich, St. Louis, MO, USA), whose applied amounts were those necessary to obtain the same osmolarity as the last three doses of La (614, 983 and 1573 mg kg^{-1} of dry soil).

Initially, for each experiment, 500 g of each soil were stored in plastic bags adequately identified according to the treatment and replicate. Then, the soils were fertilized, according to Malavolta (1980) recommendations, homogenized, and kept at rest for three days. The plastic bags were used to facilitate soils' homogenization, being kept until the end of the experiment. Soils were not limed (i.e., were maintained at their natural pH during the experiments), as this would reduce the concentrations of the REE in the soil solution (Tyler et al., 2001), in addition to the fact that the aim of this study was to expose plants to soils with characteristics, mainly chemical, as close as possible to those in their natural environment.

After three days of fertilization, the treatments were applied in the respective plastic bags, and the soils were again homogenized and kept at rest for 24 hours. Only distilled water was applied as a treatment for the controls (0 mg La kg^{-1} dry soil). The volume of water applied in solutions and, throughout the experimental period for maintaining moisture, was calculated so that the soils reached 60-70% of the water holding capacity

In order to validate the La concentrations applied, as well as the procedure for applying the treatments, soil samples were collected for analysis of the total La levels. The analyzes were performed in triplicates for each nominal concentration tested. The samples were digested according to the USEPA 3051A method (USEPA, 2007). Blank samples and a reference material - Calcareous Soil ERM-CC690® - were also digested throughout the performed batteries to guarantee analyses accuracy. The extracts were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Also, soil samples (duplicates for each nominal concentration) were collected after applying treatments for the analysis of pH values (EMBRAPA, 1997).

Twenty-four hours after applying the treatments, each bag of soil was placed in its respective pot. Next, each species was sown with a variable amount of seeds (i.e., six sorghum and soybean seeds, five sunflower seeds, and four maize seeds), with subsequent thinning for three sorghum seedlings and two seedlings of other species.

Each experiment lasted 21 days after the germination of 50% of the control group (0 mg La kg^{-1} dry soil). In the last days of the experimental period, several shoot non-destructive analyses were performed as follows.

2.3 Biological endpoints

After planting the seeds, daily seedling emergence counts were performed (Maguire, 1962) until stabilization, to calculate the emergence percentage (%E) and the Emergence Speed Index (ESI).

At four days before the end of each experiment, non-destructive physiological analyzes were performed. The estimation of the relative chlorophyll content (atLEAF) was obtained using a portable chlorophyll meter (atLEAF® CHL Plus). A total of 24 readings were performed per treatment: two mature leaves per replicate and three readings per leaf were evaluated (in the apical, median, and basal regions, considering the mean of three readings for each region). Measurements of gas exchange were performed on one leaf by replicate, standardizing the fourth mature leaf for sorghum and the third for maize. These analyzes were performed between 9 am-11 am, using an infrared gas-exchange analyzer, IRGA (Li-6400, Li-Cor, Lincoln, NE, USA). The equipment's LED chamber was programmed for a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1} for assessing the following data: photosynthesis (A: μmol CO_2 m^{-2} s^{-1}), transpiration (E: mmol H_2O m^{-2} s^{-1}), and stomatal conductance (g_s: mol H_2O m^{-2} s^{-1}).

Due to the irregular shapes of the leaves of the eudicotyledons, it was not appropriate to consider their dimensions for changing the leaf area values in the IRGA, since the measurements could be over or underestimated. That was the case mainly for sunflower and soybean plants grown in the natural soils, which showed a significant reduction in biomass, including a reduction in leaf area, from the 150 and 614 mg kg^{-1} doses of La, respectively. Therefore, the leaves did not appropriately fill the IRGA chamber, being not possible to correctly measure the dimension of them in order to obtain reliable gas exchange measurements. Thus, even though the leaves of eudicotyledons grown in SAT were large enough for the analysis, it was decided not to consider the gas exchange endpoints for soybean and sunflower. On the other hand, the measurements could be performed in monocotyledons, even when the leaves were tiny.

That happened thanks to a more regular leaf shape that allowed a correct measurement of their dimensions.

At the end of the experimental period, the shoot was harvested and oven-dried at 60°C until constant weight to determine the shoot dry matter (SDM) on a precision scale.

2.4 Data analysis

The data were analyzed for normality and homogeneity, being transformed when necessary. The toxic endpoint LOEC (Lowest Observed Effect Concentration: first concentration in which there was an effect when compared with the control treatment) was determined by the Dunnett's test ($p < 0.05$).

The values of the toxic endpoint EC_x (Effect Concentration: concentration that caused x% of effect relative to the control treatment) were also estimated, for x equal to 20 and 50%. The EC_{20} and EC_{50} were estimated using non-linear regressions, considering the models: Gompertz, Hormesis, Exponential, and Logistic. From the EC_{20} and EC_{50} values, Species Sensitivity Distribution (SSD) curves were constructed from which the HC_5 values (Hazardous Concentration for 5% of the species) were estimated.

The software STATISTICA and SPEED Stat were used for statistical analysis. The software R (R Development Core Team, 2017) was used to generate the graphics.

3. Results

3.1 Lanthanum concentration and pH values in the soils

Lanthanum concentrations and pH of the soils are showed in table 2. The percentages of recovery for La concentrations were satisfactory, ranging from 86 to 128%. It can be observed that the control treatments showed low concentrations of La, being the highest value found for SAT, followed by LVAd and LVd. Besides, a gradual reduction in pH values occurred in all soils after applying the treatments.

Table 2. Nominal and measured concentrations of La in tested soils (tropical artificial soil – SAT, Dystrophic Red-Yellow Oxisol – LVAd and Dystrophic Red Oxisol – LVd), respective recovery percentages, and soil pH.

	Nominal concentration	**Measured concentration**	**% recovery**	**Soil pH_{H2O} ***
	(mg La kg dry soil^{-1})			
SAT	0	8.24 ± 0.04	-	5.93
	150	169.0 ± 4.55	107	5.58
	240	232.73 ± 9.11	94	5.47
	384	372.27 ± 9.39	95	5.33
	614	595.50 ± 14.75	96	5.39
	983	939.20 ± 6.92	95	4.9

	1573	1651.67 ± 41.23	104	4.77
LVAd	0	4.38 ± 1.15	-	5.10
	150	158.30 ± 19.43	103	4.87
	240	261.23 ± 40.64	107	4.72
	384	370.80 ± 20.25	95	4.33
	614	778.57 ± 70.69	126	4.33
	983	899.53 ± 55.67	91	4.18
	1573	1651.53 ± 96.97	105	3.62
LVd	0	2.14 ± 0.18	-	4.71
	150	194.67 ± 37.87	128	4.42
	240	272.43 ± 41.34	113	4.38
	384	490.20 ± 30.52	127	4.09
	614	690.27 ± 96.90	112	4.04
	983	953.67 ± 82.79	97	3.81
	1573	1355.50 ± 77.25	86	3.73

* pH was measured after treatments application (n=2). The concentration values are mean ± standard error (n=3)

3.2 Ecological risk assessment of La: determination of toxic endpoints

Table 3 shows the toxicity values calculated from the biological endpoints for which a reduction was observed. In general, a considerable part of the toxicity indicators, mainly EC_{50}, could not be calculated, either because there was no 20 or 50% reduction or because any of the nonlinear regression models did not accept the data. It can be observed that most of those endpoints that could not be calculated were related to plant grown in SAT, and when calculated, the values were high, i.e., higher than 614 mg La kg^{-1} of dry soil.

In general, the toxic endpoints calculated for plants grown in the LVd were the lowest, being in some instances, such as for maize's gas exchange (photosynthesis and transpiration) and sunflower's dry matter, equal to or less than the first dose of La (150 mg kg^{-1}). The sunflower was the most sensitive species, even when grown in the SAT. Seedlings that emerged in the natural soils at a dose of 614 mg kg^{-1} presented reduced growth, with a SDM about 70% less than those of controls. For the 983 mg kg^{-1} dose, the harmful effect was even more intense, as the seedlings did not survive. Moreover, there was no germination in the last dose, so the evaluation of any other biological endpoint was infeasible. Furthermore, for soybean plants grown in the three soils for the last dose of La, it was impossible to measure AtLEAF due to the reduced leaf size.

In addition to the low occurrence of harmful effects in plants grown in the SAT, there were also some stimulating effects in maize, sorghum, and sunflower plants. Maize and sorghum plants showed slight increases in SDM, at doses 384 and 614 mg kg^{-1}, respectively (Figure S3). Also, for maize and sunflower plants, there were slight increases in AtLEAF, in treatments with 983 and 150 mg La kg^{-1}, respectively (Figure S4). Finally, at doses 983 and 1573 mg kg^{-1}, there were increases in the photosynthesis (A) in maize plants (Figure S5).

From the SSDs, HC_5 values were estimated (Table 4). Because of the small number of acceptable EC_x and EC_{50} values (<8) obtained for SAT and LVd, respectively, it was not appropriated to generate SSDs and, consequently, estimate HC_5 values for these soil individually. SSDs generated from the estimated E_{20}, and EC_{50} values for plants grown in the natural soils are shown in figures 2 and 3, respectively.

Table 3. Values, in mg La kg^{-1} dry soil, of EC_{20} and EC_{50} (with respective 95% confidence intervals), and LOEC estimated from endpoints of plant species grown in three different soils (tropical artificial soil – SAT, Dystrophic Red-Yellow Oxisol – LVAd and Dystrophic Red-Oxisol - LVd) with increasing concentrations of La.

Specie	Soil	Endpoint	LOEC	EC_{20}	EC_{50}
Maize	**SAT**	**%E**	#	NE	NE
		ESI	614	NE	NE
		SDM	#	NE	NE
		atLEAF	#	NE	NE
		A	#	NE	NE
		gs	#	NE	NE
		E	#	NE	NE
	LVAd	**%E**	983	NE	NE
		ESI	240	313.3 (258.3-368.3)	743.8 (671.8-815.8)
		SDM	614	392.7 (309.9-473.5)	656.3 (577.2-735.3)
		atLEAF	240	798.0 (603.4-992.7)	NE
		A	384	391.7 (339.8-443.6)	625.2 (574.8-675.6)
		gs	384	372.9 (307.1-438.6)	611.4 (546.0-676.8)
		E	384	385.8 (323.6-447.9)	642.0 (580.1-703.9)
	LVd	**%E**	983	NE	NE
		ESI	614	NE	NE
		SDM	384	289.1 (173.9-404.4)	630 (489.6-770.3)
		atLEAF	240	555.6 (431.9-679.4)	NE
		A	150	< 150	NE
		gs	150	NE	NE
		E	150	NE	< 150
Sorghum	**SAT**	**%E**	#	NE	NE
		ESI	#	NE	NE
		SDM	1573	NE	NE
		atLEAF	#	NE	NE
		A	1573	1551.6 (1527.9-1575.2)	NE
		gs	1573	1544.8 (1520.5-1569.1)	NE
		E	1573	NE	NE

	LVAd	**%E**	614	NE	NE
		ESI	614	491.0 (423.0-559.0)	623.8 (575.3-670.2)
		SDM	983	684.7 (571.3-798.1)	810.6 (715.0-906.3)
		atLEAF	983	NE	NE
		A	983	NE	NE
		gs	983	427.0 (184.7-669.3)	755.9 (516.0-995.7)
		E	983	320.0 (57.5-582.4)	874.0 (492.1-1256.0)
	LVd	**%E**	614	NE	NE
		ESI	614	477.2 (66.0-888.5)	NE
		SDM	#	NE	NE
		atLEAF	983	NE	NE
		A	983	647.3 (197.5-1097.2)	NE
		gs	983	520.1 (44.0-996.1)	NE
		E	983	458 (36.4-879.5)	1192.0 (698.6-1685.4)
Soybean	**SAT**	**%E**	#	NE	NE
		ESI	1573	159.8 (685.7-1633.8)	NE
		SDM	614	NE	1067.7 (983.2-1152.2)
		atLEAF	384	NE	NE
	LVAd	**%E**	983	NE	NE
		ESI	614	426.7 (314.0-539.3)	697.1 (595.9-798.4)
		SDM	614	520.2 (387.7-652.6)	928.0 (820.5-1035.5)
		atLEAF	614	NE	NE
	LVd	**%E**	614	NE	NE
		ESI	614	742.2 (566.1-918.1)	1141.2 (1011.6-1270.5)
		SDM	150	323.3 (75.3-571.4)	1177.3 (746.6-1608.0)
		atLEAF	614	NE	NE
Sunflower	**SAT**	**%E**	#	NE	NE
		ESI	1573	NE	NE
		SDM	384	473.7 (372.7-574.7)	1202.0 (1094.9-1309.2)
		atLEAF	614	NE	NE
	LVAd	**%E**	614	NE	NE
		ESI	240	218.2 (169.3-267.1)	369.2 (319.5-418.9)

	SDM	150	170.6 (137.2-204.0)	369.0 (333.0-405.1)
	atLEAF	384	NE	NE
LVd	**%E**	384	NE	NE
	ESI	384	236.6 (30.9-440.3)	510.1 (252.8-767.4)
	SDM	150	< 150	250.6 (162.3-339.0)
	atLEAF	150	NE	NE

%E: Emergence percentage; ESI: Emergence Speed Index; SDM: Shoot Dry Matter; AtLEAF: Relative Chlorophyll Content; *A*: Photosynthesis; g_s: Stomatal Conductance; *E*: Transpiration; LOEC: lowest observed effect concentration; EC_{20} and $EC_{50:}$ effect concentration that resulted in a 20 and 50% reduction in biological endpoint, respectively.

#: there was no La treatment statistically different compared with the control treatment (Dunnett's test, $p<0.05$);

NE: EC_{20} or EC_{50} value could not be estimated because of the inexistence of concentration that resulted in a 20 or 50% reduction in endpoint, respectively, or the data did not present the statistical requirements to be accepted by none of the nonlinear regression models.

Table 4. Values of HC_5 (with respective 95% confidence intervals) estimated from SSDs based on EC_{20} and EC_{50} values (Table 3) obtained from biological endpoints of soybean, sunflower, sorghum, and maize. The plants were grown in three different soils (tropical artificial soil – SAT, Dystrophic Red-Yellow Oxisol – LVAd, and Dystrophic Red-Oxisol – LVd) with increasing concentrations of La.

Data group	**HC_5**	
	EC_{20}	**EC_{50}**
	(mg La kg^{-1} dry soil)	
Endpoints of plants grown in SAT	NE	NE
Endpoints of plants grown in the LVAd	238 (167.7-319)	440.5 (334.7-543.4)
Endpoints of plants grown in the LVd	98.1 (50.6-177.6)	NE
Endpoints of plants grown in the LVAd + Endpoints of plants grown in the LVd	147.6 (101-215.9)	301 (213.5-422.9)
Endpoints of plants grown in the LVAd + Endpoints of plants grown in the LVd + Endpoints of plants grown in SAT	150.4 (115-221.3)	313.2 (225.4-435.9)

$HC_{5:}$ Hazardous Concentration to 5% of the species.

SSDs: Species Sensitivity Distribution.

EC_{20} and $EC_{50:}$ effect concentration that resulted in a 20 and 50% reduction in the biological endpoint, respectively.

NE: the HC_5 value could be not estimated because there was not a minimum number of EC_x values (=8).

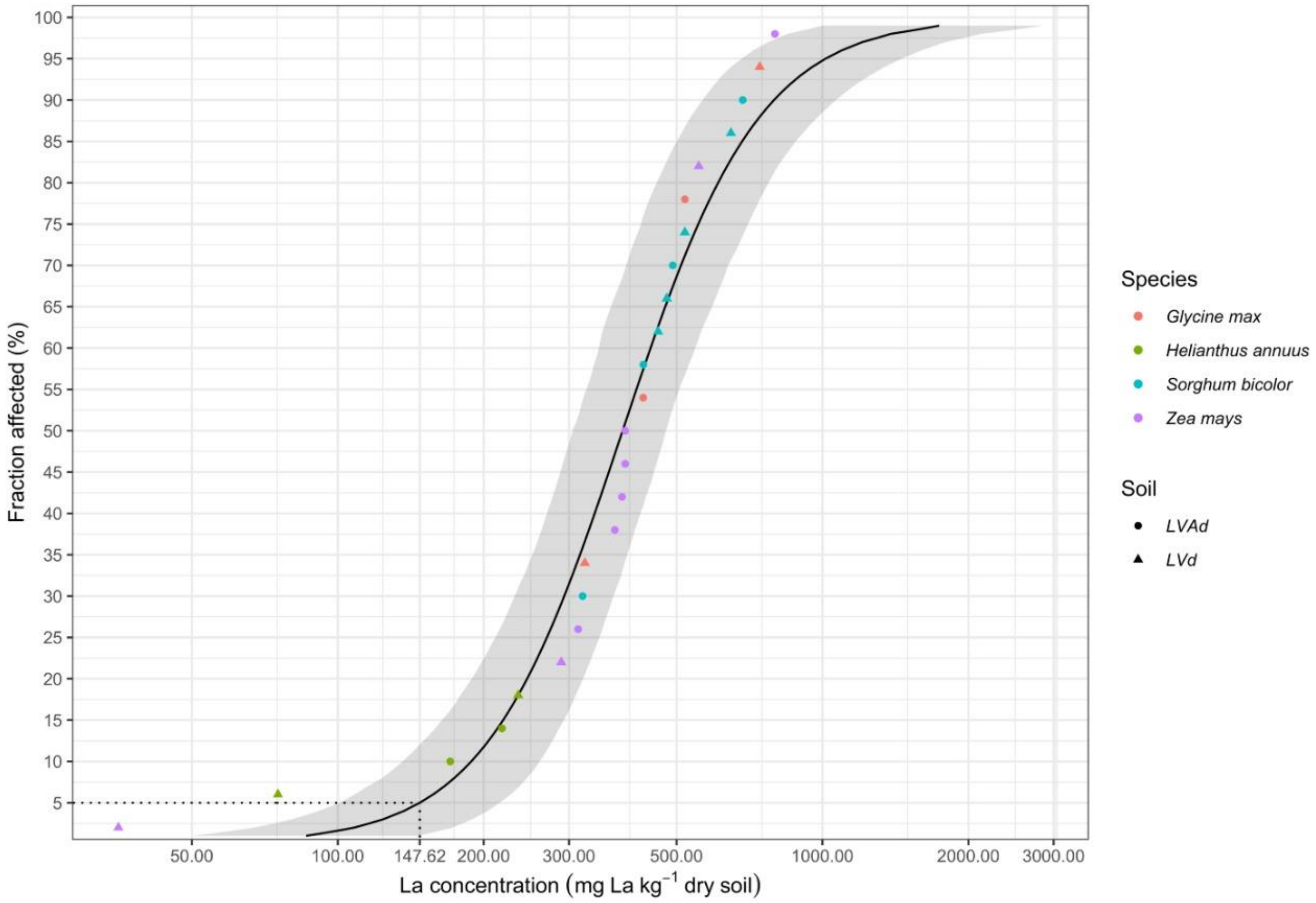

Fig. 2. Species sensitivity distribution (SSD) for lanthanum toxicity to plant species. The SSD was generated from the EC_{20} values shown in Table 3, estimated from the endpoints of soybean (*Glycine max),* sunflower *(Helianthus annuus),* sorghum *(Sorghum bicolor),* and maize *(Zea mays)* grown in the Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red-Oxisol (LVd). The 95% confidence interval (shaded region) and the 5th percentile of the distribution, the hazardous concentration to 5% of the species (HC_5, dotted area), are shown in the graph.

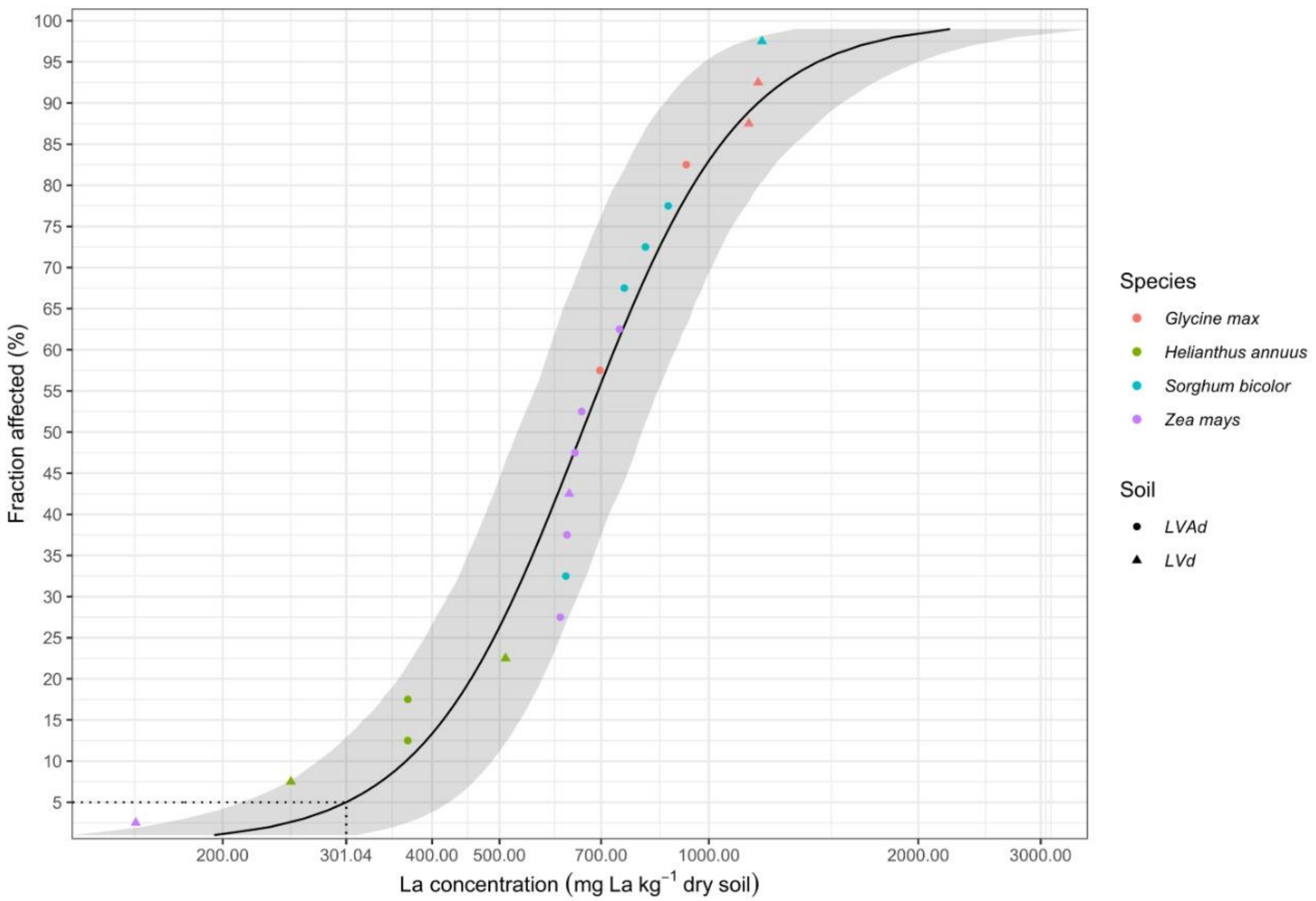

Fig. 3. Species sensitivity distribution (SSD) for lanthanum toxicity to plant species. The SSD was generated from the EC_{50} values shown in Table 3, estimated from the endpoints of soybean (*Glycine max),* sunflower *(Helianthus annuus),* sorghum *(Sorghum bicolor),* and maize *(Zea mays)* grown in the Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red-Oxisol (LVd). The 95% confidence interval (shaded region) and the 5th percentile of the distribution, the hazardous concentration to 5% of the species (HC_5, dotted area), are shown in the graph.

4. Discussion

Given the results obtained, we observed that the effects of La on biological endpoints varied according to the soil type and plant species used in ecotoxicological tests, as reported in other studies with REE (Carpenter et al., 2015; Moreira et al., 2019; Thomas et al., 2014). The influence of the soil's physicochemical properties and the plants' absorption capacities are two of the main factors that govern the phytoavailability of an element (Thomas et al., 2014).

The mean concentration of La in the earth's crust is 30 mg kg^{-1} (Taylor and Mclennan, 1995), and the world background concentration is in the range of 6.6 - 50 mg kg^{-1} (Ramos et al., 2016a). The mean background concentration found for Brazilian soils is 38.08 mg kg^{-1} (0.10 - 197.63) (de Sá Paye et al., 2016). The natural soils evaluated in the present study had levels of La lower than the national mean, and slightly higher than the smaller interval (Table 2). These results are consistent with the wide variability found by de Sá Paye et al. (2016), which is explained by the diversity of parent materials of Brazilian soils, since the natural concentrations of REE are highly influenced by the underlying geological material in which soil horizons form (Hu et al., 2006).

We created the SAT following the guidelines for the production of an artificial standard soil, with the adaptation of coconut fiber, considering the tropical conditions (Garcia, 2004; Silva and van Gestel, 2009). In general, when using this standard soil, the plants are exposed to the worst conditions (Stephenson et al., 1997), but we observed the opposite in the present study. Considering the high organic fraction and higher CEC value of the SAT (Table 1), the element (La^{3+}, which is a cation) was probably adsorbed and, then, a large part was unavailable. Also, the pH of this soil was favorable to the lower La availability since it is inversely proportional to pH (Aide et al., 2019; Dinali et al., 2019). It should be emphasized that the bioavailability of chemical compounds for plants is one of the most critical issues in environmental studies (Thomas et al., 2014).

The pH was probably one of the main factors responsible for the increase in the bioavailability of La and consequent adverse effects observed. The pH values were reduced after the addition of treatments. Thus, soils, especially the “natural” ones, which are naturally acidic, became gradually and slightly more acidic as the treatments were applied. Chu et al. (2003) also observed that the $LaCl_3$ application reduces soil pH. The authors suggest that this may occur due to the exchange of adsorbed H^+ by La^{+3} and the release of H^+ as a result of the formation of metal-organic chelates.

As a consequence of the pH reduction, the bioavailable concentrations of La in the studied soils probably increased. Moreover, these concentrations may also have been influenced by other chemical characteristics of each soil, dependent or not on the pH, e.g., the levels of organic matter and oxides (Fe, Mn, and Ti) (Ling et al., 2015; da Silva et al., 2016; de Sá Paye et al., 2014), as well as phosphorus (P) (Diatloff et al., 1993; Diatloff et al., 1996). The influence of pH on the results that we obtained for plants grown in the SAT can also be considered, since, in general, we observed adverse effects for doses 614, 983, and 1573 mg kg^{-1}, in which the pH values were lower.

Furthermore, the observed effects, especially for the last dose of La, may have been aggravated by the osmotic stress exerted by Cl ions. In some cases, it was not possible to establish statistical differences between the effects on plants treated with La and those of saline controls.

Additionally, the visual effects in both treated plants and those in saline controls were chlorosis followed by leaf necrosis, typical effects of toxicity caused by Cl^- (Geilfus, 2018)

Therefore, plants grown in SAT were less affected than those in the natural soils, mainly due to the physicochemical characteristics of that soil, which contributed to lower bioavailability of La, thus representing the mildest condition of exposure to treatments. We observed toxicity effects in SAT, but in general, from the highest doses of La (≥614 mg kg^{-1}), therefore with high toxic endpoints, in some cases with intervals over the highest dose of La applied. For most of the biological endpoints, we could not calculate the EC_x values, mainly because there was no 20% or 50% effect, which was also found by Moreira et al. (2019) for the germination, considering Ce concentrations that reduced 10 (EC_{10}) and 50% (EC_{50}) of this biological endpoint.

On the other hand, when the plants were grown in the natural soils, which present lower pH, CEC, organic matter, and P content, the bioavailability levels of La were probably higher, and the harmful effects were more frequent and intense. The toxic endpoints calculated for plants grown in these soils, mainly in the LVd, were, in some instances, equal to or less than the lowest applied La dose (≤150 mg kg^{-1}). We observed this for shoot dry matter (SDM) and gas exchange, endpoints that, depending on the species, proved to be very responsive to the treatments.

With the increase of its bioavailability in the soil, La can be absorbed more quickly, depending on the absorption capacity of the plant species, either by the seed or by the roots. However, it must be considered that the element's penetration capacity depends on its forms in the soil solution. There is evidence that under low pH there is no barrier in the Casparian strip for the transport of La^{+3} (Oross and Thomson, 1982), although at higher pH values there might be an increase in the amount of available colloidal forms, such as $La(OH)_3$, which can reach the cytoplasm (Brown et al., 1990) and trigger off the toxicity effects. We observed that La reached the plants' shoot (Table S1), but we did not perform chemical speciation on the soil solution or, still, on the plant tissues.

For the emergence percentage (%E), it was impossible to calculate the EC_x values, mainly because there was no reduction of 20 or 50%. However, based on the LOEC values, it is clear that this biological endpoint was not very responsive to initial concentrations of La, with the majority of the values being equal to or greater than 614 mg kg^{-1}. The emergence speed index (ESI), in most cases, was also little affected, but in some specific cases, the effects occurred under low concentrations of La, such as what was observed for maize and sunflower grown in the LVAd, whose LOEC was 240 mg kg^{-1}. There is still little research on the effects of REE on the early stages of plants grown in soils (Thomas et al., 2014; Zhao et al., 2019). However, until now, most of the reports have indicated that these elements have little effect on germination and emergence. Thomas et al. (2014), Moreira et al. (2019), and Zhao et al. (2019) report that germination was not a sensitive endpoint to La, Ce, and Nd/Y, respectively.

In general, the effects of treatments on SDM were more expressive, which is a trend in studies with REE (Carpenter et al., 2016; Moreira et al., 2019; Thomas et al., 2014). We found low values of toxic endpoints, mainly for LOEC and EC_{20} (Table 3), which for natural soils was in the range of La initial doses applied (150, 240, and 384 mg kg^{-1}). Carpenter et al. (2015) evaluated other REE applied on artificial soil and found EC_{25} values between 100 and 300 mg kg^{-1} of dry soil, but for native species.

The values for crops were higher than 400 mg kg^{-1}, being the majority higher than 700 g kg^{-1}. In the present study, the toxic endpoints for the species grown in the SAT were also higher (≥ 614 mg La kg^{-1} of dry soil).

Rezaae (2018) found an EC_{50} ≥ 194 mg kg^{-1} for sunflower biomass grown in a solid medium (agar) with increasing concentrations of La. In the present study, sunflower, which proved to be the most sensitive species, had an EC_{50} of 250.6 mg kg^{-1} when grown in the LVd. This comparison reinforces how much the risk in the LVd was higher than in other soils, since exposure conditions are much higher in the agar medium.

We observed that the gas exchanges proved to be sensitive to maize grown in the LVd, whose toxic endpoints were equal to or less than 150 mg La kg^{-1}. Thus, one of the explanations for the reduction of SDM, at least for maize plants, could be the effects on gas exchange. Interferences in stomata opening cause reductions in the transpiration, and the photosynthesis (Zhang et al., 2018). Moreover, damages to the chloroplasts' structures (De Oliveira et al., 2015; Hu et al., 2016) might have occurred, with La bonding on their membranes, thus decreasing the activity of enzymes such as ATP synthase and Rubisco (Sun et al., 2016).

The effects of La toxicity on the photochemical phase may also have occurred, at least in maize grown in the natural soils. Although an endpoint little affected by La treatments, the relative chlorophyll content (AtLEAF) showed low values of LOEC, 240, and 150 mg kg^{-1}, considering maize and sunflower grown in the natural soils. Chlorophyll plays a fundamental role in capturing light energy during the photochemical phase of photosynthesis. Thus, reductions in this pigment can directly interfere with the efficiency of the photosynthetic process. Such reductions may occur due to interferences in the synthesis or due to the degradation of the pigment.

Effects on growth can also be a result of the influence that La can have on cell division and chromosomes (De Oliveira et al., 2015). Also, due to the affinity with P, La can form complexes with nucleic acids that are insoluble within the physiological pH range, which should affect the mitotic index and the cell cycle (Das et al., 1988). Moreover, La and other REE can interfere in the plant nutritional balance, as they compete with nutrients for the same binding sites, especially cationic ones due to the ionic radius similarity. Nutritional imbalance can also be one of the causes of other observed phytotoxicity symptoms. All species grown in the natural soils from the dose of 614 mg La kg^{-1} of dry soil presented one (or more) of the following symptoms: chlorosis, necrosis, dwarfism, and, still, purple color of the leaves and stems (Figure S8). These symptoms are signs of nutritional deficiencies, such as nitrogen, iron, and potassium, or similar to those resulting from the phytotoxicity of other elements, such as copper (Rezaee et al., 2018).

The experiments carried out in the present study are considered chronic, that is, of prolonged exposure time. The higher was the La treatment, and the more the physicochemical characteristics of each soil type were favorable to La bioavailability, the higher was the hazard to which the plants were exposed. Therefore, in the natural soils, the risks of La were higher, especially in the LVd, which were confirmed by the lower values of toxic endpoints determined for these soils. On the other hand, considering the SAT, in which the bioavailable concentrations were probably lower, the risk was lower and, thus, the toxic endpoints showed higher values.

Additionally, plants play an essential role in the risk that a chemical element represents since the absorption capacity of each plant will also influence the phytoavailability of that element (Thomas et al., 2014). This statement explains the variability of the results found for the species when cultivated in the same soils and exposed to the same concentrations of La. In general, monocotyledons presented higher values of toxic endpoints than eudicotyledons, thus were considered more tolerant to La in this study. The adverse effects of La on monocotyledons were more pronounced when the species were grown in the LVd, but even so, from La concentrations close to the dose of 384 mg kg^{-1}, except for maize, for which the toxic endpoints for gas exchange were very low.

Pošćić; Schat; Marchiol (2017), in a study with plants grown in a hydroponic system with Ce treatments, also observed higher tolerance of monocotyledons. The authors suggest that tolerance can be explained by the high efficiency of restricting the element translocation from the roots to the shoot and by the efficient antioxidant mechanism compared with that of eudicotyledons. Also, Vilela et al. (2018) reported low La translocation in maize grown in a tropical soil. Although, in general, the La concentrations found in the maize shoot were slightly lower than those found in the shoot of the soybean and sunflower, the concentrations found in the maize shoot cultivated in the LVd were considerably high. This result leads to a hypothesis that there was some direct La effect on gas exchanges in maize plants (Zhang et al., 2018) since the photosynthesis and transpiration showed low values of EC_{20} and EC_{50}, respectively. As for sorghum plants, although there was no analysis of La content for their shoot, these plants probably have restricted the translocation of much of La since the toxic endpoints found for gas exchange were much higher.

In addition to phytotoxicity, we also observed stimulating effects in this study, for plants grown in SAT, which is indicative of hormesis effect, a phenomenon of recognized occurrence for REE (Ramos et al., 2016a) and recently described in detail for La by Agathokleous et al. (2018; 2019). Maize and sorghum showed slight significant increases in SDM at doses 384 and 614 mg La kg^{-1}, respectively. Also, maize showed significant increases for AtLEAF and photosynthesis (A) at doses 983 and 1573 mg kg^{-1}, respectively. Sunflower plants showed an increase in AtLEAF in the treatment with 150 mg kg^{-1}. Despite this, sunflower grown in this soil showed reductions on endpoints from the dose of 384 mg La kg^{-1}. This specie proved to be the most sensitive species in all three soils, presenting the lowest toxic endpoints, which was also reported by Moreira et al. (2019) in an ecotoxicological study with cerium (Ce) in tropical soils.

In agreement with the values found for toxic endpoints, the HC_5 values varied depending on the EC_x values of the soil (s) considered for the generation of SSD. The HC_5 values varied between 98.1-440.5 mg La kg^{-1} of dry soil, and the SSDs generated with the addition of EC_{20} and EC_{50} calculated for LVd resulted in the lowest HC_5 values. Also, the SSDs generated with the addition of EC_{20} and EC_{50} calculated for SAT resulted in the highest HC_5 values. According to figures 2 and 3, maize and sunflower can be considered the species to be much more affected (5%) by the hazardous concentration (HC_5). More specifically, this means that under the HC_5 of 147.6 mg La kg^{-1} of dry soil, there is a risk that endpoints A of maize and SDM of sunflower may be reduced by 20%. Also, it means that under the HC_5 of 301 mg La kg^{-1} of dry soil, there is a risk that endpoints E of maize and SDM of sunflower may be reduced by 50%.

Given the HC_5 values found, the value of 148 (101-216) mg La kg^{-1} dry soil is the most suitable to be defined as the acceptable or limit value of La in the studied soils. It is a representative value resulting from joining data from the two different types of tropical natural soil and it is also protective, as it was based on the EC_{20} values. Even though some biological endpoints of the plants grown in the LVd are under acceptable risk (5%), the effects are expected to be small.

Moreover, a more restrictive HC_5 value allows other soil organisms to be protected, especially invertebrates, which are generally the most sensitive (Li et al., 2018). Li et al. (2018) in an ecotoxicological study with soil invertebrates, found a mean HC_5 value of 54 (27-107) mg La kg^{-1} of dry soil. The authors estimated this value from the EC_{10} values of their study, in which they used the standard soil LUFA2.2 (LUFA-Speyer, Germany), and data available in the literature for plants and bacteria, resulting from studies with subtropical natural soils (Chu et al., 2003; Zeng et al., 2006).

It should be considered that the limit concentrations estimated in the present study were based on total concentrations of La, and, therefore, the toxicity of the element may be lower in the natural environment, considering the bioavailable concentrations. On the other hand, it should consider the massive increase in the total concentrations of La and other REE in the environment. As previously reported, REE have become widely used, and with this, the addition of these elements in soils, as a result of anthropic actions, has increased. The areas with the highest REE are probably those where agriculture is intense and those that are close to large industrial centers (Ramos et al., 2016a).

The application of phosphate fertilizers is responsible for a substantial addition of REE in soils, as proven by Ramos et al. (2016b) and Turra et al. (2011). The authors found high concentrations of these elements in fertilizer samples, among which many NPK formulations that are widely applied in Brazil. Turra et al. (2011) also point out that the intense and continuous application of phosphate fertilizers in soils can raise the amount of REE to levels that can result in environmental and human effects.

Considering the intense application of phosphate fertilizers over time that has been occurring in tropical soils, mainly in Brazil, the increase in REE concentrations in these soils deserves attention. Moreira (2014) evaluated the REE concentrations in Brazilian agricultural soils that received high doses of phosphate fertilizers. The results showed that the applications of these inputs for an extended period increased the levels of La and Ce by 89 and 44%, respectively, in cotton cultivation soils. The contents were even higher in soils cultivated with potato, with La, 81%, and Ce, 111%. Given these scenarios, we then reinforce the importance of the development of studies like the current one, which considers the environmental consequences of agricultural activities, pondering the risks that plants and other soil organisms may be subject to in the exogenous REE occurrence in the ecosystem.

We consider, however, that in agricultural soils the chemical characteristics are improved, and the conditions become favorable to the lower bioavailability of La and, therefore, the ecotoxicological risk may be lower. However, considering that in the natural environment of agricultural soils, REE coexist, in even higher proportions after the addition of phosphate fertilizers, the risk of the elements together can be potentially high even in soils that have improved physicochemical properties (Zhao et al., 2019).

Our study is the first that carried out an ecological risk assessment of La in tropical soils and estimated an HC_5 value for these soils. Such HC_5 was derived from ecotoxicological tests involving two natural soils, with different physicochemical characteristics, belonging to the Oxisols class, the most representative of Brazil's soils (31%) (Santos et al., 2006), the country that has the largest area of tropical soils in the world. We emphasized the importance of ecotoxicological studies with natural soils and the need for further studies with La, involving different soil classes, given the physicochemical differences they present. Furthermore, according to Niva et al. (2016), the knowledge about the suitability of natural tropical soils, specifically from Brazil, for ecotoxicological tests is still scarce. Then, we expect that this study may contribute to the definitions of which soil(s) is(are) most representative and appropriate.

We also hope that the results presented here may contribute for improving the database on La ecotoxicity in tropical soils and the continuity of ecotoxicological assessment of this element, including other soil classes, different plant species, as well as others organisms. We look forward that with the expansion of the database it will finally become possible to legally establish a robust PV for La so that there may be a risk-based, soil screening level of this element in the Brazilian legislation, as it is already being developed for other trace elements.

5. Conclusions

(1) The risk of La causing phytotoxicity in the LVd was higher, with most of the toxic endpoints determined for plants grown in this soil lesser than those of other soils; (2) sunflower was the most sensitive species in the three soils, but specifically in the LVd, the photosynthesis of maize was the most affected endpoint, from low doses of La ($\leq$ 150 mg kg^{-1} dry soil); (3) we propose the limit value of 147.6 mg La kg^{-1} of dry soil for tropical soils, which is representative and protective of the evaluated crops/endpoints, anticipating that other soil organisms may also be protected; (4) La is an element that deserves attention in tropical agroecosystems, mainly in Brazil, considering the extensive application of phosphate fertilizers that has been occurring over the years. This agricultural practice has been increasing La concentrations (and other REE) to levels that may, in a long term, exceed the limit value determined in the present study and so cause ecotoxicity.

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6. References

Aide, M., Ladonin, D., Vitová, M. et al., 2019. Lanthanide Soil Chemistry and Its Importance in Understanding Soil Pathways: Mobility, Plant Uptake, and Soil Health, in: Awwad, N.S., Mubarak, A.T. (Eds.), Lanthanides. IntechOpen, London. http://dx.doi.org/10.5772/intechopen.79238

Agathokleous, E., Kitao, M., Calabrese, E.J., 2018. The rare earth element (REE) lanthanum (La) induces hormesis in plants. Environ. Pollut. 238, 1044–1047. https://doi.org/10.1016/j.envpol.2018.02.068

Brown, P.H., Rathjen, A.H., Graham, R.D., Tribe, D.E., 1990. Chapter 92 Rare earth elements in biological systems, in: Handbook on the Physics and Chemistry of Rare Earths. pp. 423–452. https://doi.org/10.1016/S0168-1273(05)80135-7

Carpenter, D., Boutin, C., Allison, J.E., Parsons, J.L., Ellis, D.M., 2015. Uptake and effects of six rare earth elements (REEs) on selected native and crop species growing in contaminated soils. PLoS One 10, 1–21. https://doi.org/10.1371/journal.pone.0129936

Checkai, R., Van Genderen, E., Sousa, J.P., Stephenson, G., Smolders, E., 2014. Deriving site-specific clean-up criteria to protect ecological receptors (plants and soil invertebrates) exposed to metal or metalloid soil contaminants via the direct contact exposure pathway. Integr. Environ. Assess. Manag. 10, 346–357. https://doi.org/10.1002/ieam.1528

Chu, H.Y., Zhu, J.G., Xie, Z.B., Cao, Z.H., Li, Z.G., Zeng, Q., 2001. Effects of lanthanum on soil microbial biomass carbon and nitrogen in red soil. J. Rare Earths 19, 63–66.

Conselho Nacional do Meio Ambiente – CONAMA, 2009. Resolução CONAMA N°420, Diário Oficial da União n° 249. Ministério do Meio Ambiente, Brasília, Brasil. Available online at. http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=620 (Verified on 23 Jan 2019).

da Silva, Y. J. A. B., do Nascimento, C.W.A., da Silva, Y. J. A. B., Biondi, C.M., Silva, C.M.C.A.C., 2016. Rare earth element concentrations in Brazilian benchmark soils. Rev. Bras. Cienc. do Solo 40, 1–13. https://doi.org/10.1590/18069657rbcs20150413

Das, T., Sharma, A., Talukder, G., 1988. Effects of Lanthanum in Cellular Systems In the Environment. Biol. Trace Elem. Res. 18, 201–228.

de Oliveira, C., Ramos, S.J., Siqueira, J.O., Faquin, V., de Castro, E.M., Amaral, D.C., Techio, V.H., Coelho, L.C., e Silva, P.H.P., Schnug, E., Guilherme, L.R.G., 2015. Bioaccumulation and effects of lanthanum on growth and mitotic index in soybean plants. Ecotoxicol. Environ. Saf. 122, 136–144. https://doi.org/10.1016/j.ecoenv.2015.07.020

de Sá Paye, H., de Mello, J.W.V., de Magalhães Mascarenhas, G.R.L., Gasparon, M., 2014. Distribution and fractionation of the rare earth elements in Brazilian soils. J. Geochemical Explor. 161, 27–41. https://doi.org/10.1016/j.gexplo.2015.09.003

De Silva, P.M.C.S., van Gestel, C.A.M., 2009. Comparative sensitivity of *Eisenia andrei* and Perionyx excavatus in earthworm avoidance tests using two soil types in the tropics. Chemosphere 77, 1609–1613. https://doi.org/10.1016/j.chemosphere.2009.09.034

Diatloff, E., Asher, C.J., Smith, F.W., 1993. Use of geochem-PC to predict rare-earth element (REE) species in nutrient solutions. Plant Soil 156, 251–254.

Diatloff, E., Asher, C.J., Smith, F.W.J., 1996. Concentrations of rare earth elements in some Australian soils. Aust. J. Soil Res. 34, 735–747.

El-Ramady, H.R.H., 2010. Ecotoxicology of rare earth elements: ecotoxicology of rare earth elements within soil and plant environments, VDM Verlag Dr. Muller Aktiengsellsellshaft & Co. KG, Saarbrücken.

Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, 1997. Manual de métodos de análises de solo, second ed. Ministério da Agricultura e do Abastecimento, Rio de Janeiro Available online at.https://www.agencia.cnptia.embrapa.br/Repositorio/Manual+de+Metodos_000fzvhotqk02wx5ok0q43a0ram31wtr.pdf (Verified on 10 Jan 2019).

Geilfus, C.M., 2018. Review on the significance of chlorine for crop yield and quality. Plant Sci. 270, 114–122. https://doi.org/10.1016/j.plantsci.2018.02.014

Gong, B., He, E., Qiu, H., Li, J., Ji, J., Zhao, L., Cao, X., 2019. Phytotoxicity of individual and binary mixtures of rare earth elements (Y, La, and Ce) in relation to bioavailability. Environ. Pollut. 246, 114–121. https://doi.org/10.1016/j.envpol.2018.11.106

Gupta, C., Krishnamurthy, N., 2005. Extractive metallurgy of rare earths, CRC Press, Florida.

IUPAC, 2005. Nomenclature of Inorganic Chemistry, IUPAC Recommendations 2005 (the “RedBook”). In: Connelly, N., Damhus, T., Harshorn, R.M. (Eds.), RSC Publishing, Cambridge, UK.

Hu, Z., Haneklaus, S., Sparovek, G., Schnug, E., 2006. Rare earth elements in soils, Communications in Soil Science and Plant Analysis. https://doi.org/10.1080/00103620600628680

Hu, Z., Richter, H., Sparovek, G., Schnug, E., 2004. Physiological and Biochemical Effects of Rare Earth Elements on Plants and Their Agricultural Significance: A Review. J. Plant Nutr. 27, 183–220. https://doi.org/10.1081/PLN-120027555

Li, J., Verweij, R.A., van Gestel, C.A.M., 2018. Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil. Chemosphere 193, 412–420. https://doi.org/10.1016/j.chemosphere.2017.11.040

Ling, S., Wu, X., Ren, Y., Sun, C., Liao, X., Li, X., Zhu, B., 2015. Geochemistry of trace and rare earth elements during weathering of black shale profiles in Northeast Chongqing, Southwestern China: Their mobilization, redistribution, and fractionation. Chemie der Erde 75, 403–417. https://doi.org/10.1016/j.chemer.2015.07.004

Lopes, A.S., Guilherme, L.R.G., 2016. A career perspective on soil management in the Cerrado region of Brazil. Adv. Agron. 137, 1–172.

Maguire, J.D., 1962. Speed of germination: aid in selection and evaluation for seedling emergence and vigour. Crop Sci. 2, 176–177.

Malavolta, E., 1980. Elementos da nutrição mineral de plantas, Agronômica Ceres, Piracicaba.

Moreira, C.G., Carvalho, T.S. de, de Oliveira, C., Abreu, L.B. de, Castro, A.C.S. de, Ribeiro, P.G., Bispo, F.H.A., Boutin, C., Guilherme, L.R.G., 2019. Ecological risk assessment of cerium for tropical agroecosystems. Chemosphere 221, 124–131. https://doi.org/10.1016/j.chemosphere.2018.12.195

Niva, C.C., Niemeyer, J.C., Júnior, F.M.R.D.S., Nunes, M.E.T., De Sousa, D.L., Aragão, C.W.S., Sautter, K.D., Espindola, E.G., Sousa, J.P., Römbke, J., 2016. Soil ecotoxicology in Brazil is taking its course. Environ. Sci. Pollut. Res. 23, 11363–11378. https://doi.org/10.1007/s11356-016-6597-1

Organisation for Economic Co-operation and Development – OECD, 2006. Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing https://doi.org/10.1787/9789264070066-en.

Oross, J.W., Thomson, W.W., 1982. The ultrastructure of Cynodon salt glands: the apoplast. Eur. J. Cell Biol. 28, 257–263.

Pang, X., Li, D., Peng, A., 2002. Application of rare-earth elements in the agriculture of China and its environmental behavior in soil. Environ. Sci. Pollut. Res. 9, 143–148. https://doi.org/10.1007/BF02987462

Ramos, S.J., Dinali, G.S., Oliveira, C., Martins, G.C., Moreira, C.G., Siqueira, J.O., Guilherme, L.R.G., 2016a. Rare Earth Elements in the Soil Environment. Curr. Pollut. Reports 2, 28–50. https://doi.org/10.1007/s40726-016-0026-4

Ramos, S.J., Dinali, G.S., de Carvalho, T.S., Chaves, L.C., Siqueira, J.O., Guilherme, L.R.G., 2016b. Rare earth elements in raw materials and products of the phosphate fertilizer industry in South America: Content, signature, and crystalline phases. J. Geochemical Explor. 168, 177–186. https://doi.org/10.1016/j.gexplo.2016.06.009

Salgado, O.G.G., Teodoro, J.C., Alvarenga, J.P., de Oliveira, C., de Carvalho, T.S., Domiciano, D., Marchiori, P.E.R., Guilherme, L.R.G., 2020. Cerium alleviates drought-induced stress in Phaseolus vulgaris. J. Rare Earths 38, 324–331. https://doi.org/10.1016/j.jre.2019.07.014

Santos, H.G. dos, Jacomine, P.K.T., Anjos, L.H.C. dos, Oliveira, V.A. de, Oliveira, J.B. de, Coelho, M.R., Lumbreras, J.F., Cunha, T.J.F. (Ed.), 2006. Sistema Brasileiro de Classificação de Solos, second ed. Embrapa Solos, Rio de Janeiro.

Smith Stegen, K., 2015. Heavy rare earths, permanent magnets, and renewable energies: An imminent crisis. Energy Policy 79, 1–8. https://doi.org/10.1016/j.enpol.2014.12.015

Stephenson, G., Solomon, K., Hale, B., Greenberg, B., Scroggins, R. 1997. Development of Suitable Test Methods for Evaluating the Toxicity of Contaminated Soils to a Battery of Plant Species Relevant to Soil Environments in Canada, in: Dwyer, F., Doane, T., Hinman. M. (Eds.), Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment Sixth Volume. West Conshohocken, ASTM International, 474-489.

Taylor, S.R., McLennan, S.M., 1995. The geochemical evolution of the continental crust. Rev. Geophys. 33, 241.

Team, R.C., 2017. R: a language and environment for statistical computing. R Found. Stat. Comput.

Thomas, P.J., Carpenter, D., Boutin, C., Allison, J.E., 2014. Rare earth elements (REEs): Effects on germination and growth of selected crop and native plant species. Chemosphere 96, 57–66. https://doi.org/10.1016/j.chemosphere.2013.07.020

Teodoro, J.C., De Oliveira, C., Ribeiro, I.C.A., Salgado, O.G.G., Guilherme, L.R.G., 2020b. Nutritional composition of plants species grown in tropical soils spiked with lanthanum. Chemosphere, Submitted.

Turra, C., Fernandes, E.A.N., Bacchi, M.A., Energia, C. De, Paulo, U.D.S., 2011. Turra et al_2011_REE in fertiliser 3, 86–92.

Tyler, G., Olsson, T., 2001. Concentrations of 60 elements in the soil solution as related to the soil acidity. Eur. J. Soil Sci. 52, 151–165

United States Environmental Protection Agency – USEPA, 2007. Method 3051A (SW- 846): microwave assisted acid digestion of sediments, sludges, and oils, revision 1. Washington, DC. Available online at. https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf (Verified on 10 Jan 2019).

Vilela, L.A.F., Ramos, S.J., Carneiro, M.A.C., Faquin, V., Guilherme, L.R.G., Siqueira, J.O., 2018. Cerium (Ce) and Lanthanum (La) promoted plant growth and mycorrhizal colonization of maize in tropical soil. Aust. J. Crop Sci. 12, 704–710. https://doi.org/10.21475/ajcs.18.12.05.PNE754

Withers, P.J. a., Rodrigues, M., Soltangheisi, A., de Carvalho, T.S., Guilherme, L.R.G., Benites, V.D.M., Gatiboni, L.C., de Sousa, D.M.G., Nunes, R.D.S., Rosolem, C. a., Andreote, F.D., Oliveira, A. De, Coutinho, E.L.M., Pavinato, P.S., 2018. Transitions to sustainable management of phosphorus in Brazilian agriculture. Sci. Rep. 8, 2537. https://doi.org/10.1038/s41598-018-20887-z

Zeng, Q., Zhu, J.G., Cheng, H.L., Xie, Z.B., Chu, H.Y., 2006. Phytotoxicity of lanthanum in rice in haplic acrisols and cambisols. Ecotoxicol. Environ. Saf. 64, 226–233. https://doi.org/10.1016/j.ecoenv.2005.03.016

Zhang, F., Li, Y., Shi, Y., Wang, L., Zhou, Q., Huang, X., 2018. A novel evaluation of the effect of lanthanum exposure on plant populations. Chemosphere 202, 377–386. https://doi.org/10.1016/j.chemosphere.2018.03.086

Zhao, C.M., Shi, X., Xie, S.Q., Liu, W.S., He, E.K., Tang, Y.T., Qiu, R.L., 2019. Ecological Risk Assessment of Neodymium and Yttrium on Rare Earth Element Mine Sites in Ganzhou, China. Bull. Environ. Contam. Toxicol. 103, 565–570. https://doi.org/10.1007/s00128-019-02690-2

Supplementary material

Table S1. Lanthanum concentrations (µg g^{-1} DW) in the shoot of sunflower, maize, and soybean were grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil - SAT). The values represent the mean ± standard error (n = 4) (data are taken from Teodoro et al., 2020b - submitted).

Soil	**La dose (mg kg^{-1} dry soil)**	**Sunflower**	**Maize**	**Soybean**
LVd	**0**	2.87±0.32	<LOQ	1.09±0.21
	150	46.73±3.33*	32.63±2.43*	28.06±3.49*
	240	28.78±2.05*	54.23±1.38*	38.49±1.16*
	384	64.55±3.14*	52.30±4.74*	34.45±1.21*
	614	-	52.60±2.14*	30.60±2.23*
	983	-	52.87±6.12*	---
	1573	-	---	154.01±7.36*
LVAd	**0**	2.05±0.32	<LOQ	2.45±0.63
	150	33.24±2.23*	5.93±0.35*	27.38±4.05*
	240	40.68±0.54*	10.58±0.70*	25.37±1.52*
	384	66.92±4.91*	20.63±1.43*	28.83±4.26*
	614	123.35±8.04*	47.98±1.47*	61.19±7.37*
	983	-	---	179.91±9.81*
	1573	-	-	-
SAT	**0**	2.49±0.15	---	1.29±0.07
	150	8.00±0.15*	---	8.97±0.34*
	240	9.28±0.88*	---	18.00±0.47*
	384	14.21±0.11*	---	---
	614	19.54±1.41*	---	22.81±1.13*
	983	21.04±1.05*	---	33.92±1.47*
	1573	40.01±3.34*	---	53.97±0.13*

LOQ: limit of quantification; asterisk (*) indicate significant difference ($p<0.05$) according to Dunnett's test, ---: the La concentrations were not shown because the values presented wide variation.

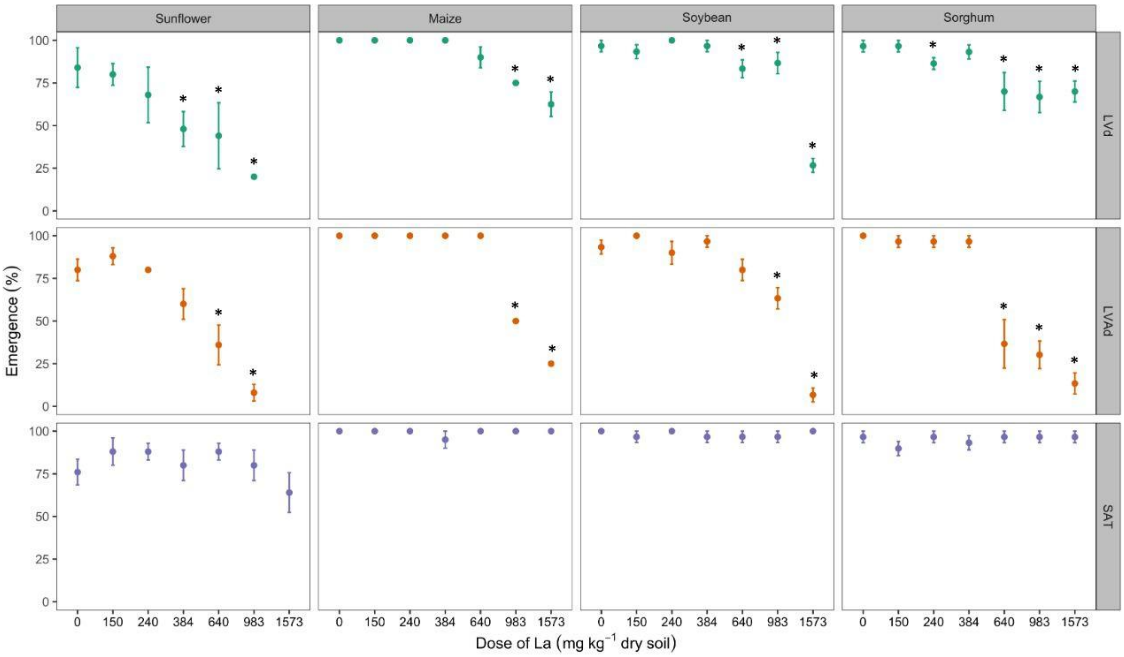

Figure S1. Effects of lanthanum on the emergence percentage (%E) of sunflower, maize, soybean, and sorghum grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil – SAT). Error bar represents standard error; * significant at p<0.05 compared with the control treatment (Dunnett's test).

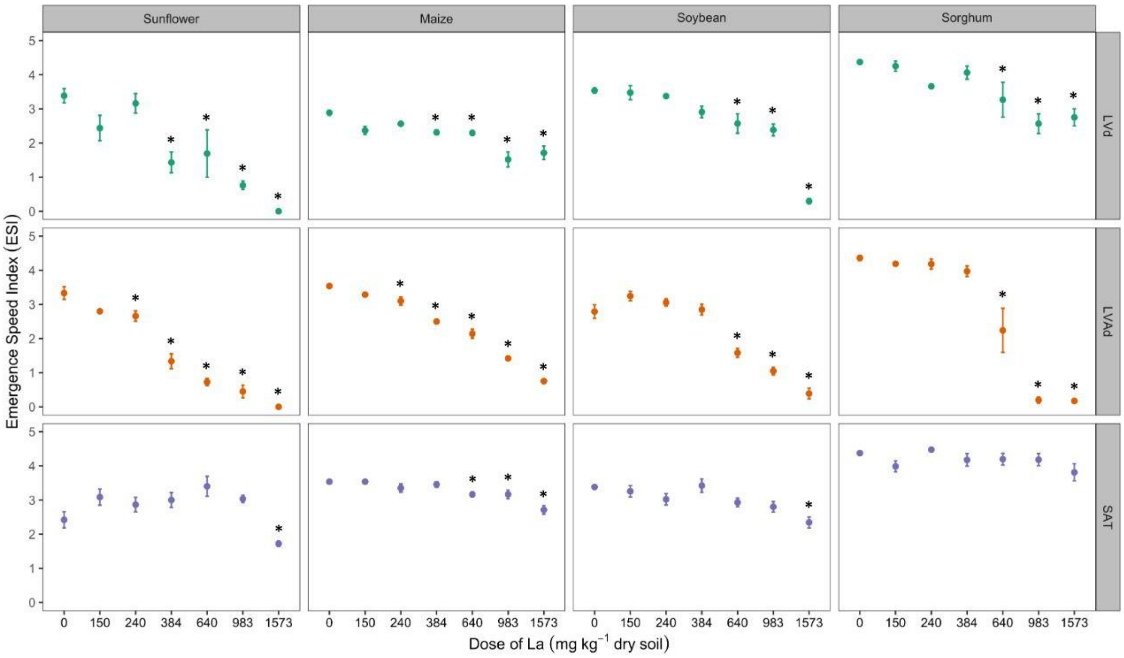

Figure S2. Effects of lanthanum on the speed emergence index (ESI) of sunflower, maize, soybean, and sorghum grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil – SAT). Error bar represents standard error; * significant at p<0.05 compared with the control treatment (Dunnett's test).

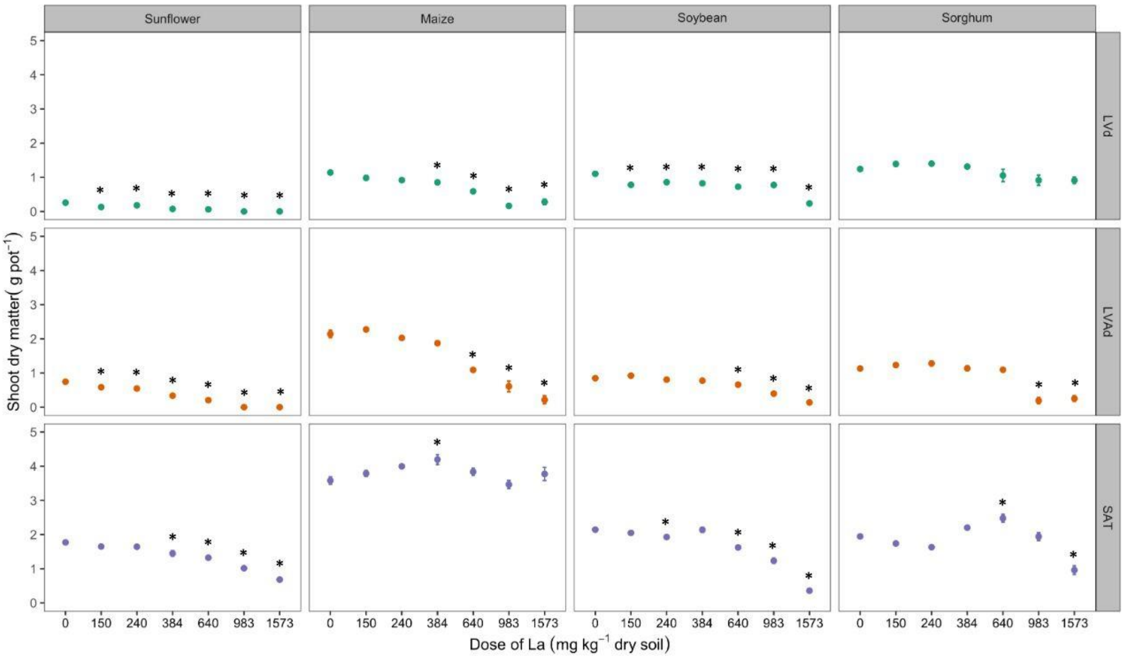

Figure S3. Effects of lanthanum on the shoot dry matter (SDM) of sunflower, maize, soybean, and sorghum grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil – SAT). Error bar represents standard error; * significant at p<0.05 compared with the control treatment (Dunnett's test).

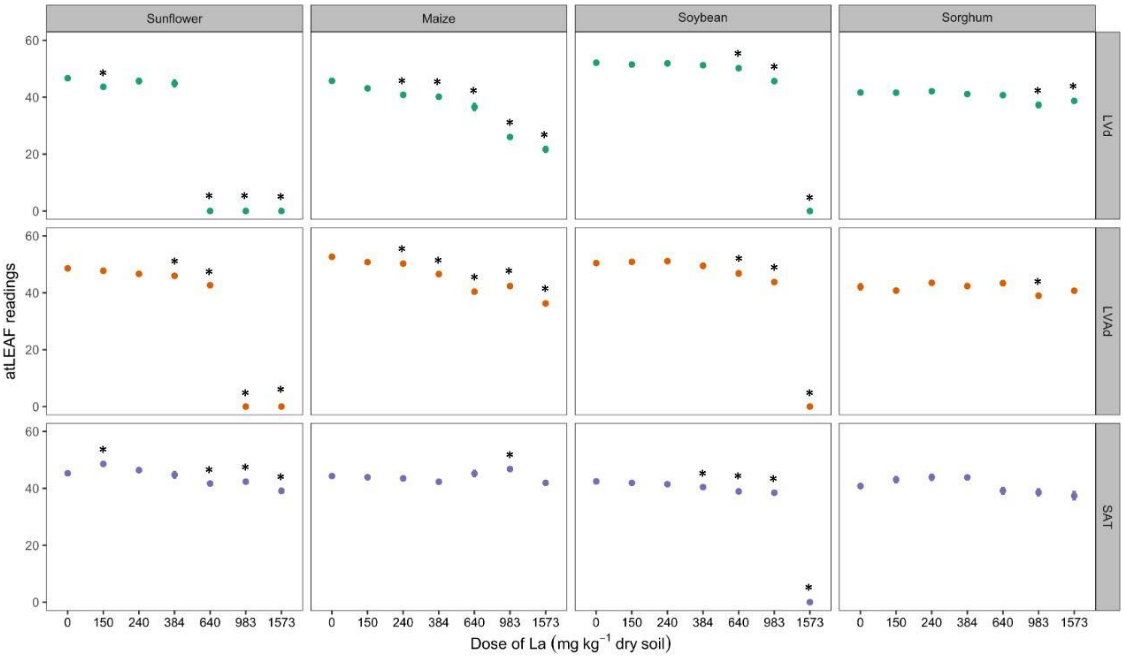

Figure S4. Effects of lanthanum on the relative chlorophyll content (AtLEAF readings) of sunflower, maize, soybean, and sorghum grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil – SAT). Error bar represents standard error; * significant at p<0.05 compared with the control treatment (Dunnett's test).

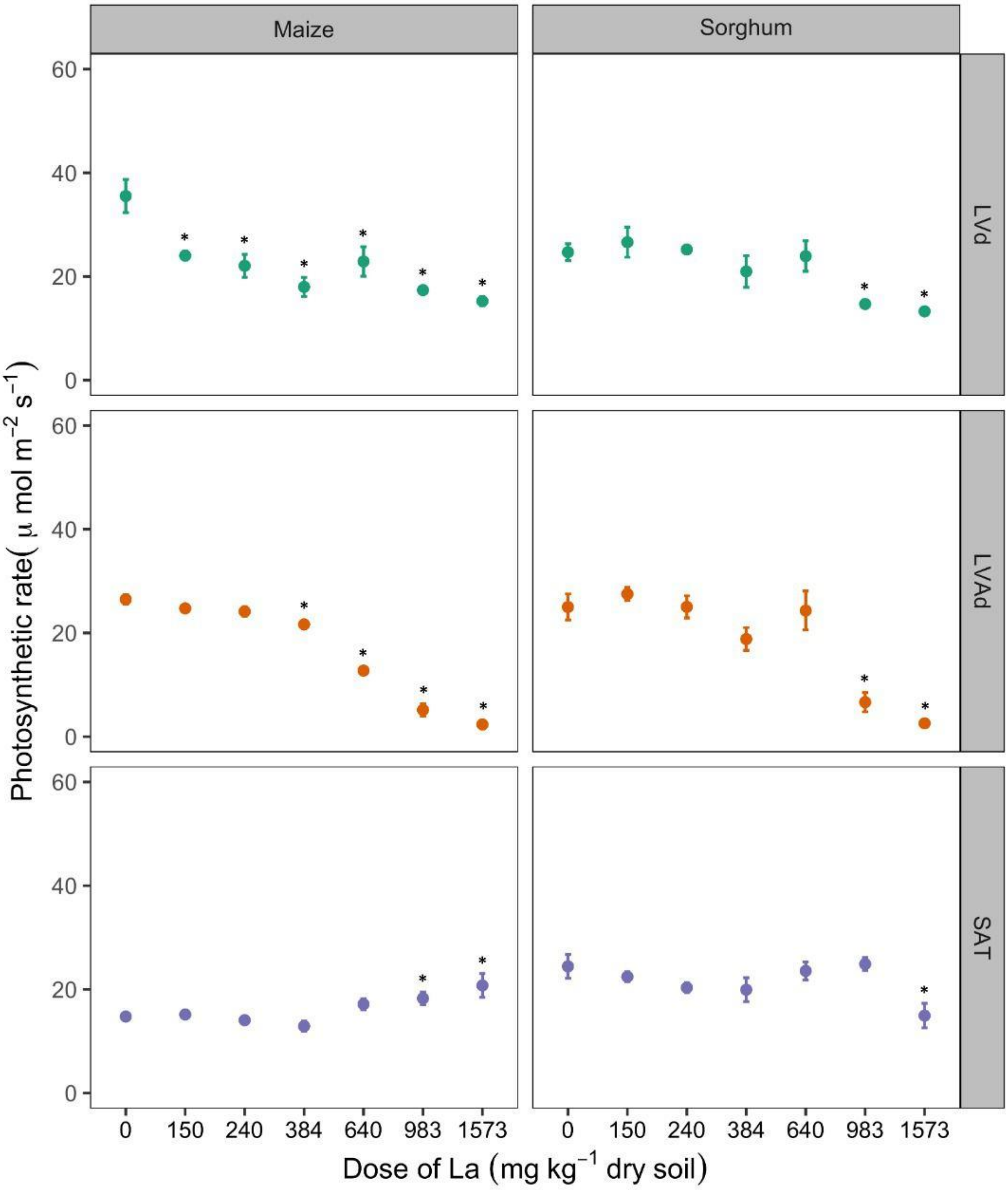

Figure S5. Effects of lanthanum on the photosynthesis (*A*) of sunflower, maize, soybean, and sorghum grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil – SAT). Error bar represents standard error; * significant at $p<0.05$ compared with the control treatment (Dunnett's test).

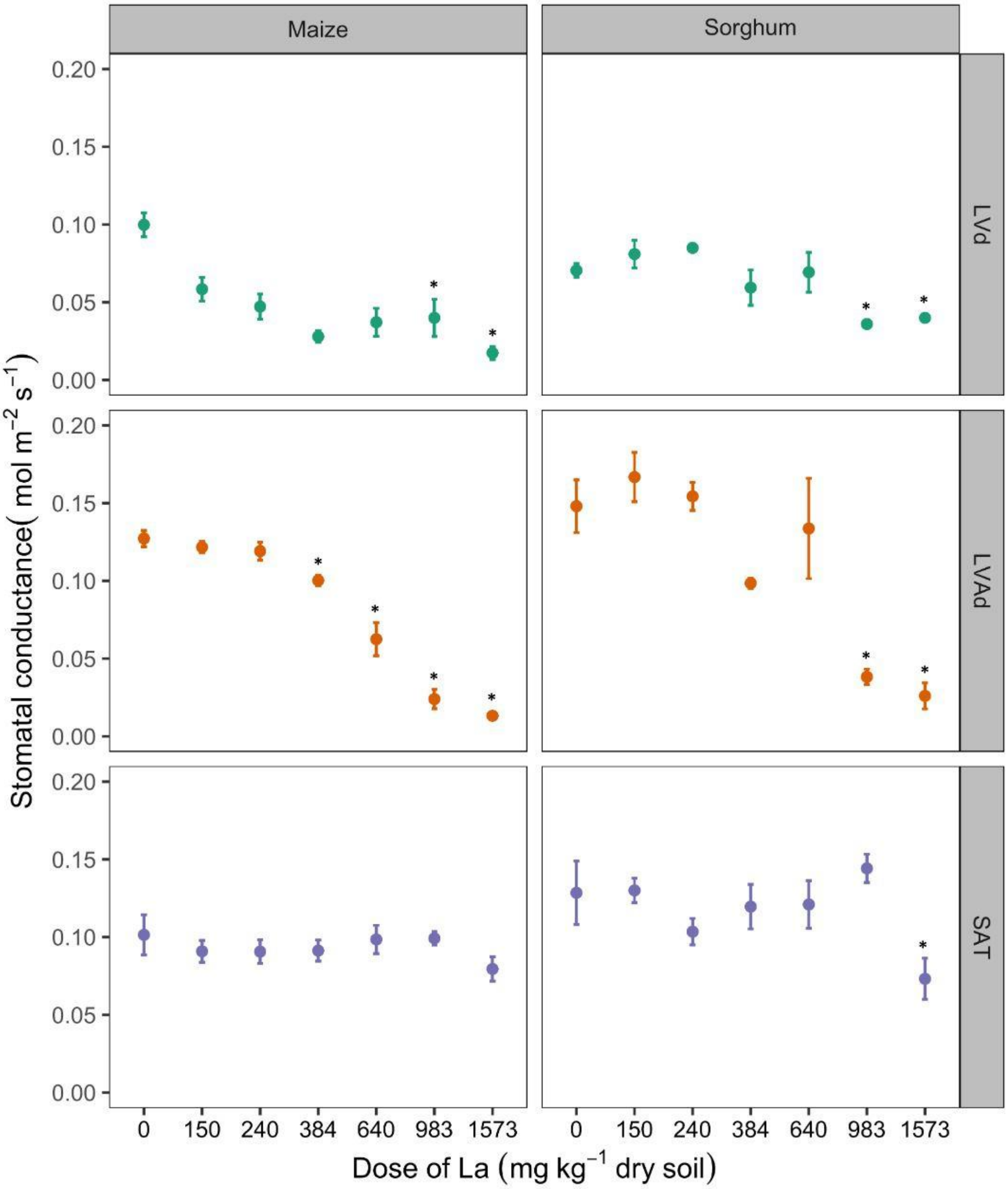

Figure S6. Effects of lanthanum on the stomatal conductance (*g*) of sunflower, maize, soybean, and sorghum grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil – SAT). Error bar represents standard error; * significant at p<0.05 compared with the control treatment (Dunnett's test).

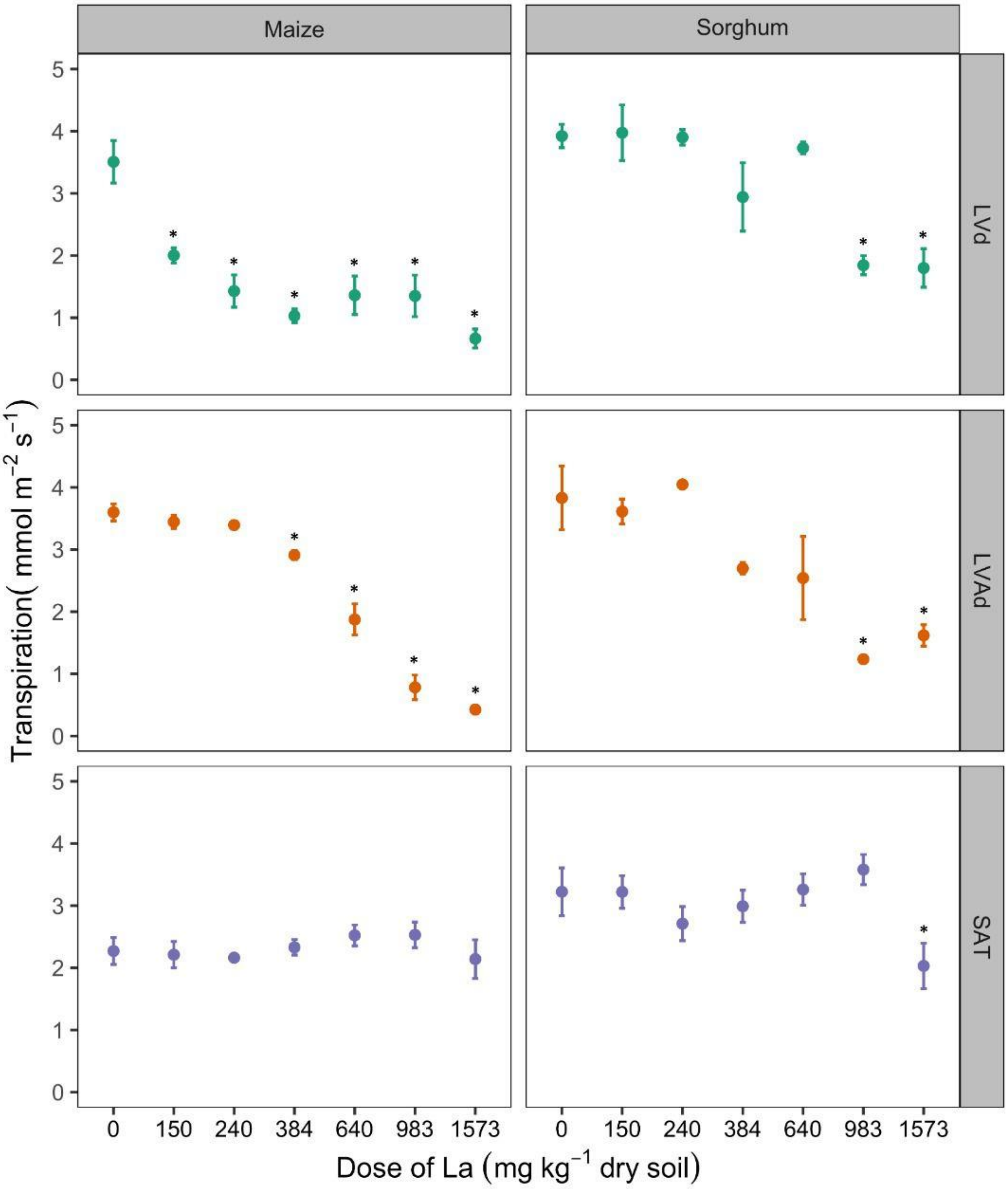

Figure S7. Effects of lanthanum on the transpiration (*E*) of sunflower, maize, soybean, and sorghum grown in three different soils (Dystrophic Red Oxisol - LVd, Dystrophic Red-Yellow - LVAd and tropical artificial soil – SAT). Error bar represents standard error; * significant at $p<0.05$ compared with the control treatment (Dunnett’s test).

Figure S8. Phytotoxicity symptoms in plants grown in Dystrophic Red Oxisol (LVd) and Dystrophic Red-Yellow (LVAd) with increasing doses of La. (A) chlorosis in soybean leaves grown in the LVd at a dose of 1573 mg kg^{-1}; (B) necrosis in leaves and dwarfism in maize grown in the LVAd at dose 1573 mg kg^{-1}; (C) chlorosis and purple coloration of leaves and stems in maize plants grown in the LVAd at dose 983 mg kg^{-1}; (D) chlorosis in sorghum leaves grown in the LVAd at dose 614 mg kg^{-1}; (E) chlorosis in sunflower leaves grown in the LVAd at dose 983 mg kg^{-1}; (F) necrosis in sunflower leaves grown in the LVd at dose 614 mg kg^{-1}.

ARTIGO 2 - Nutritional composition of plants species grown in tropical soils spiked with lanthanum

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Nutritional composition of plants species grown in tropical soils spiked with lanthanum

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Abstract

Lanthanum, as well as other rare earth elements, can affect plant growth by influencing their nutritional balance. There are a considerable number of studies that have evaluated the nutritional composition of plants grown in nutrient solutions with concentrations of La. However, few studies have been carried out on soils. Thus, we aim to evaluate the effect of La on nutrient content in plants grown in tropical soils. Maize, sorghum, soybean, and sunflower plants were exposed to increasing concentrations of La applied in three soils, two natural, Red-Yellow Oxisol (LVAd), and Red Oxisol (LVd), and an artificial one (SAT). The experiments lasted 21 days, and the shoot of the species was collected, ground, dried to constant weight, and conducted for the analysis and determination of the contents of N, P, K, Ca, Mg, S, Cu, Fe, Mn, Zn, and La. In general, in one or more doses of La, the levels of N, K, Ca, Mg, Mn, Zn increased, and P, S, Cu, and Fe decreased. Specifically, for plants grown in SAT, in general, most nutrient contents increased or did not have significant differences compared with controls, and a hormetic effect was observed. On the other hand, the reductions of nutrients for plants in the natural soils were more pronounced, in addition to that the levels of La being higher than for plants in the SAT. These results show how the effects of La on plants, whether beneficial or harmful, depend on the soil's physicochemical properties.

Keywords: lanthanide, nutritional balance, growth, soil.

Highlights

- The soil properties determine if the La effects on plants will be stimulating or harmful

- The La effects on the nutrient content are dependent on the soil properties and plant species

- Lanthanum trigger off hormetic effects on shoot dry matter and photosynthesis of maize

1. Introduction

The group known as Rare Earth Elements (REE) is composed of 17 chemical elements that have similar physicochemical properties, encompassing those of the lanthanide series and also yttrium (Y) and scandium (Sc). Among all REE, cerium (Ce) and lanthanum (La) are the most abundant in the earth's crust and soils (Aide et al., 2019). The average natural concentration of La in soils in Brazil is 38.08 mg kg^{-1} (0.10 - 197.63) (de Sá Paye et al., 2014). The concentrations of REE in soils vary depending on the pedogenetic processes and the mineralogy of the REE carrier phases in the bedrock and in the soil (Brioschi et al., 2013; Dinali et al., 2019). In addition to the fact that human activities have also contributed to the change in the levels of these elements.

Because of the diverse applications of REE, including flat panel displays (electronic devices), automobile catalysts and petroleum refining, permanent magnets and rechargeable batteries for hybrid and electric vehicles, many medical devices, and in agriculture (Ramos et al., 2016), the concentration of these elements in the environment has increased in recent decades (El-Ramady, 2010). Considering soil specifically, the increase in REE concentrations deserves special attention. Firstly, because the soil is the destination of a large part of the waste resulting from human activities, including electronic waste. Secondly, because the REE have been applied together with phosphate fertilizers, due to the high affinity of these elements to P-compounds (Tyler, 2004). The latter is of more concern, especially in the case of agricultural soils that require higher amounts of phosphate fertilizers, as is the case in Brazil (Lopes and Guilherme, 2016; Turra et al., 2011; Withers et al., 2018).

Research on the effects of REE on organisms is still scarce, and, in the case of plants, most studies have been carried out under hydroponic conditions. Most of these studies report that REE are beneficial, contributing to plant growth and development (García-Jiménez et al., 2017; Hu et al., 2004). However, these elements present a hormetic effect and, therefore, can be stimulators or toxic, depending on the dose and time that the plants are exposed (De Oliveira et al., 2015; García-Jiménez et al., 2017; Ramos et al., 2016).

Studies with plants exposed to REE have shown that these elements are directly or indirectly involved in ionic fluxes, thus interfering in the nutritional balance and, consequently, in various plant processes (Pošćić; Schat; Marchiol, 2017; Ramírez-Olvera et al.; 2018; Zhang et al., 2013). Such effects occur because REE affect membrane stability and interact actively with calcium (Ca^{2+}) (Hu et al., 2004). As La and Ce ionic radii are similar to the one of the Ca, these REE can compete with this macronutrient and block its channels, thus reducing its absorption (Huang et al., 1994; Paoli et al., 2014; Pošćić; Schat; Marchiol, 2017). Furthermore, La can interfere in the metabolic functions exerted by Ca or even replace it structurally in the cell wall (Liu et al., 2013; Tyler, 2004).

Lian et al. (2019) reported that the application of 0.4 mM of $LaCl_3$ in adzuki beans increased the acquisition of P in nutrient solution under limited macronutrient concentration. In a study with soybean grown in nutrient solution with increasing concentrations of La, De Oliveira et al. (2015) observed for the treated plants significant increases in the levels of Mg, Ca, P, K and Mn and a reduction in the levels of Cu and Fe. On the other hand, few studies have evaluated the effects of REE on the nutritional composition of plants grown in soils, specially for tropical soils. Most studies involving soils were carried out in China, a pioneer country in the studies and use of REE as fertilizers, considering that it is the largest producer and holder of reserves (Smith Stegen, 2015). As most of these studies were published in Chinese, there is a particular difficulty in accessing information about experiments carried out in that country (Pošćić; Schat; Marchiol, 2017).

Thus, we carried out pot experiments to evaluate the effect of La on the nutritional composition of maize, sorghum, sunflower, and soybean plants grown in tropical soils.

2. Material and methods

2.1 Soils and plants tested and experimental procedure

Four independent experiments were carried out, one for each plant species tested, in a completely randomized experimental design, with seven doses of La and three types of soil, with four replications.

Zea mays, *Sorghum bicolor*, *Glycine max*, *and Helianthus annuus* were tested in three types of soil (tropical artificial soil - SAT, Dystrophic Red-Yellow Oxisol - LVAd and Dystrophic Red Oxisol - LVd). The LVAd and LVd were collected in the cities of Itumirim and Lavras, respectively, under vegetation of the sub-perennial rainforest, in a 0-20 cm deep layer. After collection, the soils were air-dried, homogenized, sieved, and characterized chemically and physically (EMBRAPA, 1997), as shown in table1.

Table 1. Physicochemical characterization of the tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) used in the study (data taken from Teodoro et al., 2020a - submitted).

Soil	pH	K	P	Ca	Mg	Al	CEC	MO	Clay	Silt	Sand
	(H_2O)	---mg dm^{-3}----		-----------$cmol_c$ dm^{-3}-------------				dag kg^{-1}	----------g kg^{-1}-----------		
LVAd	5.1	38.0	1.15	1.05	0.26	0.35	1.40	1.69	230	60	710
LVd	4.8	24.73	0.88	0.19	0.1	0.65	0.36	2.7	610	170	220
SAT	6.0	176.5	10.74	1.37	0.63	0.09	4.19	6.85	20	-	70

CEC: cation-exchange capacity; MO – organic matter.

Lanthanum treatments were applied to soils in a soluble form such as heptahydrate chloride, $LaCl_3.7H_2O$, (Sigma-Aldrich, St. Louis, MO, USA) in the following doses, in mg La kg^{-1} of dry soil: 0, 150, 240, 384, 614, 983 and 1573.

The experiments were conducted in a greenhouse, under natural light and temperatures (23±4°C), according to adaptations to OECD-208 standard procedures (OECD, 2006). For each experiment, 500 g of each soil type was weighed and stored in plastic bags representative of each replicate. Then, nutrient solutions for fertilization were added to each plastic bag, according to Malavolta (1980), and each one was homogenized and kept at rest. After three days, the treatments were applied in their plastic bags, and these were homogenized again and kept at rest. After 24 h, the bags were accommodated in their pots, and sowing was carried out, with subsequent thinning. The duration of each experiment was 21 days, counted from the day when 50% of germination was observed in the controls (0 mg La kg^{-1} of dry soil).

2.2 Nutritional analysis

At the end of the experimental period, the shoot of each species was harvest and dried at 60°C until constant weight. Then, the dry material was ground in a bench mill for posterior nutritional and La levels analysis.

The maize and sorghum samples were submitted to nitric-perchloric digestion to obtain an extract and determine the contents of P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn, and La (this last just for maize) by plasma optical emission spectrometry (ICP OES). Regarding the analysis of nutrient and La contents for soybean and sunflower, the dry matter was digested according to the USEPA 3051A method (USEPA, 2007) and the extracts were taken for measurements via ICP OES. The analysis and determination of total N levels for all species were carried out by the Kjeldahl method (Malavolta, 1997).

The accuracy of the results from all digestion methods was verified by analyzing samples of a standard reference material = NIST-1573a Tomato Leaves -, as well as of blank samples.

2.3 Statistical analysis

The results were analyzed for variance (ANOVA) and by Dunnett's test ($p < 0.05$) to verify the occurrence of treatment with a different mean (higher or lower) than that found for the control treatment.

3. Results

The levels of nutrients in the shoot of maize, sorghum, soybean, and sunflower grown in the three soils are shown, respectively, in tables 2, 3, 4, and 5. The treatments' effects varied, with the occurrence of both increases and reductions of nutrient levels, which depended on the dose of La, the plant species, and the tested soils. Because for the last treatments (983 and 1573 mg La kg^{-1} of dry soil) in the natural soils, occurred a significant reduction in plant growth (Table S1), in some cases, the shoot dry matter was not enough to analyze the contents of all macro and micronutrients. In the case of sunflower, the seeds did not germinate in the last treatments (983 and 1573 mg La kg^{-1} of dry soil) and the seedlings did not survive. Specifically, for sunflowers grown in the LVd, the dry matter was sufficient only for analyzes up to the dose of 384 mg La kg^{-1} of dry soil, except for N, for which there was not enough material for the analysis of any treatment, which also occurred in the LVAd (Table 5).

In most cases, the levels of nutrients in plants grown in the SAT showed significant increases or no statistical difference compared with controls. The exceptions comprised the reductions of P in all species, K in sorghum (Table 3) and soybean (Table 4), S in soybean and sunflower (Table 5), Cu and Fe in maize (Table 2), and Fe in sunflower.

The N levels increased in maize plants grown in both natural soils and soybean grown in the LVd and decreased in sorghum plants grown in both natural soils. For P levels, a reduction was observed for most species grown in the natural soils, except for maize and soybeans in the LVAd, which showed an increase in the last treatments. These same species, when grown in the LVd, in addition to sorghum grown in the LVAd, showed both a reduction and an increase in P levels. The K levels increased in all species grown in the natural soils and sunflower and soybeans grown in the SAT, with increases in soybean occurring from the second dose of La (150 mg kg^{-1}) (Table 4).

The levels of Ca and Mg increased in most species grown in the three soils, except for sorghum (Table 3) grown in the LVAd, for which reductions were observed mainly in the dose of 614 mg La kg^{-1} of dry soil. In general, S contents were reduced in all species grown in the three soils, except for sorghum (Table 3) and sunflower (Table 5) grown in SAT and soybean (Table 4) grown in the LVd, for which significant increases were also observed in the treatments with 614, 1573 and 983 mg La kg^{-1} of dry soil, respectively.

Micronutrient contents generally increased with increasing doses of La. Exceptions include reductions observed for Cu and Fe in sorghum and sunflower plants grown in the natural soils (Tables 3 and 5). For Cu levels, increases were observed for maize (Table 2) and soybean (Table 4), respectively grown in the LVAd and the LVd. As for Fe contents, increases were observed for maize and soybean in the LVAd and soybean in the LVd. The levels of Mn increased in all species grown in the LVAd and also in maize, sorghum, and soybean grown in the LVd. The Zn contents increased in maize and soybean plants from LVAd and LVd, respectively.

The levels of La in the shoot of the plants of maize (Table 2), soybean (Table 4), and sunflower (Table 5) increased gradually with the increase of La concentrations applied for the three soils. For maize, it was not possible to present the levels of La for plants grown in the SAT because the measured concentrations showed wide variation, which also occurred in the other soils, but in specific La doses (983 and 1573 mg kg^{-1} in LVAd and LVd, respectively). This wide variation was also observed for soybean grown in the SAT and LVd for La doses, 384 e 983 mg kg^{-1}, respectively.

Table 2. Nutritional composition of the shoot of maize plants grown in tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) with increasing concentration of La.

Soil	**Nutrient/ La**	**La concentration (mg kg^{-1} dry soil)**						
		0	**150**	**240**	**384**	**614**	**983**	**1573**
SAT	**N**[1]	12.33±0.09	11.74±0.17	11.52±0.37	10.15±0.13*	12.14±0.50	13.61±0.50	14.11±0.49**
	P[1]	3.38±0.12	2.82±0.16*	2.61±0.09*	1.98±0.02*	1.90±0.16*	2.12±0.22*	1.37±0.02*
	K[1]	35.25±0.63	32.15±0.84	29.83±1.93	32.15±1.59	30.10+0.66	34.52±1.53	35.36±2.49
	Ca[1]	1.15±0.05	1.08±0.01	1.43±0.07	1.51±0.03	1.56±0.11**	1.72±0.11**	1.80±0.17**
	Mg[1]	0.87±0.03	0.89±0.03	1.06±0.02*	1.11±0.02**	1.28±0.04**	1.26±0.01**	1.19±0.05**
	S[1]	0.80±0.02	0.73±0.02	0.86±0.03	0.79±0.02	0.87±0.02	0.87±0.02	0.72±0.03
	Cu[2]	2.63±0.03	2.84±0.05	2.73±0.06	2.56±0.04	2.82±0.13	2.80±0.05	1.93±0.18*
	Fe[2]	103.13±2.46	64.56±3.41*	63.22±0.95*	68.91±3.57*	61.27±2.39*	77.37±6.91*	71.52±3.40*
	Mn[2]	57.12±1.19	56.12±1.22	69.78±0.72**	74.53±3.04**	80.63±1.88**	93.85±2.06**	82.13±3.45**
	Zn[2]	17.65±0.65	17.93±1.22	21.01±0.42**	19.47±0.78	23.18±0.12**	21.18±0.26	23.32±2.33**
LVAd	**N**[1]	28.13±1.65	27.55±1.36	28.97±0.62	30.60±0.75	46.11±0.97**	52.98±3.68**	-
	P[1]	1.50±0.06	1.48±0.10	1.46±0.02	1.30±0.03	1.83±0.09**	1.59±0.04	-
	K[1]	11.81±0.57	12.58±0.77	13.23±0.50	15.89±0.18**	23.83±1.0**	26.03±0.58**	-
	Ca[1]	4.18±0.13	6.30±0.24**	7.24±0.25**	8.63±0.18**	7.22±0.13**	6.45±0.50**	-
	Mg[1]	2.88±0.09	4.25±0.15**	4.56±0.17**	5.16±0.09**	4.66±0.11**	3.43±0.11**	-
	S[1]	3.55±0.16	2.50±0.12*	2.24±0.09*	2.32±0.04*	2.84±0.06*	2.09±0.02*	-
	Cu[2]	4.60±0.27	5.44±0.28	5.95±0.05**	6.21±0.37**	8.13±0.16**	7.12±0.75**	-
	Fe[2]	98.16±1.66	104.76±0.54**	103.42±1.91**	115.61±3.61**	116.79±2.32**	106.45±5.42	-
	Mn[2]	179.02±10.03	316.80±14.43**	384.55±10.94**	443.94±3.43**	352.57±1.26**	305.30±22.86**	-
	Zn[2]	41.05±3.72	55.46±1.36**	56.77±2.79**	65.40±4.34**	96.21±4.26**	60.25±6.22**	-
	La[2]	<LOQ	5.93±0.35**	10.58±0.70**	20.63±1.43**	47.98±1.47**	-----	-
LVd	**N**[1]	46.30±1.39	47.36±1.71	46.86±2.25	45.99±1.77	62.70±9.91**	53.03±2.42	43.80±1.92
	P[1]	1.25±0.03	1.24±0.03	1.14±0.01	1.17±0.01	1.38±0.07**	0.98±0.03*	1.01±0.02*
	K[1]	16.07±0.35	18.65±0.54	22.80±0.67**	24.64±0.96**	33.44±0.74**	21.88±0.13**	21.34±1.76**
	Ca[1]	2.83±0.09	3.36±0.09	2.87±0.17	2.52±0.25	2.40±0.27	8.59±0.77**	8.09±0.05**
	Mg[1]	1.78±0.05	2.54±0.04	2.17±0.11	2.06±0.22	2.10±0.26	8.01±0.41**	7.73±0.19**
	S[1]	2.40±0.05	2.15±0.07	1.93±0.06*	1.94±0.04*	1.90±0.05*	1.91±0.13*	1.86±0.08*

Cu[2]	8.50±0.10	8.69±0.21	8.44±0.29	7.94±0.17	9.70±1.0	7.27±0.64	8.12±0.73
Fe[2]	195.31±32.81	155.01±9.18	197.0±8.27	153.20±8.31	176.82±23.19	139.70±27.49	244.77±30.44
Mn[2]	302.36±8.85	470.40±6.08**	429.75±18.39**	412.03±22.39**	379.75±25.40**	223.49±34.94*	217.19±5.63*
Zn[2]	49.20±3.48	56.37±1.16	53.93±2.69	50.48±0.97	46.13±1.80	39.65±1.99*	36.79±2.19*
La[2]	<LOQ	32.63±2.43**	54.23±1.38**	52.30±4.74**	52.60±2.14**	52.87±6.12**	-----

Asterisks indicate significant differences ($p<0.05$) according to Dunnett's test, considering the lower (*) and higher (**) means than the control; the values between brackets represent the mean ± standard error (n = 4); [1] it is expressed as mg g^{-1} shoot dry weight; [2] it is expressed as µg g^{-1} shoot dry weight; LOQ: limit of quantification; the values for La content in maize plants grown in SAT could not be shown because they presented wide variation. ----- : the values for La content could not be shown because they presented wide variation.

Table 3. Nutritional composition of shoot of sorghum plants grown in tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) with increasing concentration of La.

Soil	**Nutrient/ La**	**La concentration (mg kg^{-1} dry soil)**						
		0	**150**	**240**	**384**	**614**	**983**	**1573**
SAT	**N[1]**	19.13±0.34	19.58±0.24	23.32±0.92**	19.20±0.20	26.80±1.10**	24.53±0.48**	23.05±3.05
	P[1]	5.45±0.05	5.04±0.19	5.02±0.10	3.61±0.20*	3.68±0.28*	3.01±0.24*	1.59±0.08*
	K[1]	62.17±2.37	60.56±0.95	61.40±0.62	49.37±0.93*	54.25±0.63*	52.36±2.14*	41.34±2.44*
	Ca[1]	1.07±0.07	1.06±0.01	1.49±0.10**	1.49±0.07**	1.80±0.01**	1.87±0.05**	2.57±0.07**
	Mg[1]	1.45±0.02	1.52±0.04	1.71±0.05	1.51±0.07	1.76±0.09**	1.84±0.09**	1.79±0.11**
	S[1]	1.06±0.01	1.10±0.02	1.19±0.03	1.11±0.02	1.33±0.07**	1.30±0.06**	1.45±1.10**
	Cu[2]	6.42±0.29	5.97±0.22	6.26±0.22	5.60±0.19	6.41±0.56	5.88±0.49	4.97±0.27
	Fe[2]	75.25±5.40	79.13±7.98	74.41±1.31	73.83±5.0	74.23±1.68	80.18±8.20	64.40±1.75
	Mn[2]	124.48±4.73	120.46±2.26	137.85±6.11	124.42±4.64	131.91±9.24	116.76±8.22	111.45±2.97
	Zn[2]	62.67±1.21	60.65±2.73	67.22±2.36	58.83±2.46	66.55±4.43	61.97±0.64	69.07±3.21
LVAd	**N[1]**	37.62±1.0	32.33±0.65*	32.63±0.20*	32.0±0.81*	36.57±3.14	31.0±2.10*	31.15±1.65*
	P[1]	2.11±0.03	2.11±0.09	1.75±0.02*	1.56±0.09*	3.69±0.04**	-	-
	K[1]	20.98±0.80	21.43±0.74	19.25±1.15	19.92±0.95	31.37±3.29**	-	-
	Ca[1]	4.75±0.03	7.30±0.06**	7.48±0.05**	6.25±0.02**	4.29±0.04*	-	-
	Mg[1]	3.74±0.08	3.83±0.05	3.14±0.06*	2.05±0.08*	3.58±0.15	-	-
	S[1]	3.19±0.01	2.13±0.02*	2.02±0.03*	1.93±0.03*	2.67±0.02*	-	-
	Cu[2]	8.71±0.22	8.96±0.17	7.84±0.23*	8.40±0.27	5.09±0.18*	-	-

	Fe[2]	96.57±4.52	96.66±4.42	90.09±4.96	85.42±0.83	74.66±12.97*	-	-
	Mn[2]	233.41±3.98	314.54±7.68**	300.99±3.62**	239.52±5.75	179.19±14.95*	-	-
	Zn[2]	125.23±6.87	138.13±5.98	111.75±6.26	113.70±6.84	76.84±0.90*	-	-
LVd	**N[1]**	34.88±0.97	29.60±0.36*	28.75±0.46*	28.88±0.61*	32.93±2.05	31.82±0.75	24.10±1.78*
	P[1]	1.65±0.03	1.73±0.08	1.43±0.03	1.39±0.03	1.34±0.07*	1.06±0.06*	1.13±0.09*
	K[1]	18.40±0.66	17.58±0.60	17.66±0.70	16.88±0.51	26.71±2.07**	19.33±1.77	18.71±0.55
	Ca[1]	6.57±0.13	8.22±0.16**	8.13±0.10**	7.86±0.29**	7.36±0.42	7.29±0.20	7.20±0.06
	Mg[1]	6.80±0.17	7.47±0.19	6.65±0.20	6.65±0.61	4.12±0.38*	4.43±0.55*	4.44±0.09*
	S[1]	1.93±0.01	1.70±0.01*	1.61±0.02*	1.54±0.01*	1.58±0.03*	1.52±0.03*	1.33±0.04*
	Cu[2]	7.64±0.25	7.94±0.23	6.88±0.18	8.31±0.01	7.63±0.20	7.68±0.43	5.94±0.08*
	Fe[2]	93.92±10.69	93.10±2.24	118.19±13.20	75.0±7.87	109.89±5.65	82.16±4.59	117.0±25.34
	Mn[2]	101.30±3.18	143.07±2.80**	150.94±4.58**	148.99±12.39**	158.87±12.98**	160.20±5.22**	150.62±4.40**
	Zn[2]	66.81±4.01	57.57±2.93	62.29±4.88	52.10±5.27	63.81±4.76	57.39±5.06	53.51±3.51

Asterisks indicate significant differences ($p<0.05$) according to Dunnett's test, considering the lower (*) and higher (**) means than the control; the values between brackets represent the mean ± standard error (n = 4); [1] it is expressed as mg g^{-1} shoot dry weight; [2] it is expressed as μg g^{-1} shoot dry weight; LOQ: limit of quantification.

Table 4. Nutritional composition of shoot of soybean plants grown in tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) with increasing concentration of La.

Soil	**Nutrient/ La**	**La concentration (mg kg^{-1} dry soil)**						
		0	**150**	**240**	**384**	**614**	**983**	**1573**
SAT	**N[1]**	25.65±0.28	27.69±0.24	32.95±1.01**	27.13±0.75	42.53±2.48**	40.73±2.41**	-
	P[1]	4.02±0.15	3.66±0.25	3.29±0.05*	4.07±0.09	2.57±0.23*	2.57±0.23*	2.28±0.03*
	K[1]	39.19±1.01	42.95±0.20**	43.67±0.43**	41.17±0.50**	46.34±0.25**	46.48±0.56**	27.66±1.11*
	Ca[1]	7.21±0.13	7.66±0.35	8.43±0.10**	7.23±0.17	9.30±0.17**	9.30±0.17**	6.81±0.25
	Mg[1]	4.11±0.14	4.41±0.16	4.90±0.05**	4.37±0.02	5.09±0.10**	5.01±0.08**	4.37±0.06
	S[1]	2.66±0.13	2.23±0.11*	2.18±0.02*	2.13±0.05*	2.20±0.06*	1.94±0.04*	1.51±0.08*
	Cu[2]	2.21±0.07	2.48±0.08	2.89±0.06**	2.18±0.03	3.17±0.15**	3.81±0.20**	2.87±0.31**
	Fe[2]	83.82±2.95	98.77±0.75	107.45±1.67**	89.75±3.92	113.08±4.73**	114.41±7.32**	102.19±8.65**
	Mn[2]	222.83±3.80	260.89±7.26	327.78±0.13**	245.94±8.59	411.05±13.06**	461.85±4.24**	316.91±14.44**
	Zn[2]	62.59±1.69	63.66±2.46	67.31±0.79	64.17±0.59	73.59±1.92**	73.44±2.91	60.32±0.82

	La[2]	1.29±0.07	8.97±0.34**	18.0±0.47**	-----	22.81±1.13**	33.92±1.47**	53.97±0.13**
LVAd	N[1]	64.91±0.67	65.44±0.75	66.79±0.42	60.03±0.78*	62.87±0.71*	-	-
	P[1]	1.55±0.05	1.41±0.06	1.52±0.05	1.55±0.13	1.72±0.08	2.24±0.05**	-
	K[1]	19.67±0.42	20.46±0.23	23.80±0.55**	26.06±0.22**	27.99±0.62**	32.74±1.04**	28.15±1.10**
	Ca[1]	6.07±0.17	7.22±0.14**	8.04±0.23**	8.04±0.38**	9.58±0.37**	10.53±0.23**	-
	Mg[1]	3.63±0.09	3.80±0.15	4.28±0.06**	3.83±0.10	4.29±0.11**	4.65±0.10**	-
	S[1]	2.31±0.10	1.77±0.08*	1.74±0.01*	1.67±0.05*	1.87±0.03	2.09±0.10	2.09±0.64
	Cu[2]	3.87±0.13	1.50±0.08*	1.59±0.19*	1.17±0.11*	1.05±0.18*	-	-
	Fe[2]	101.23±4.52	92.14±3.79	104.20±5.12	101.08±3.07	123.22±5.09	140.20±1.11**	114.70±22.55
	Mn[2]	111.70±2.54	158.87±3.09**	190.12±2.20**	203.75±4.82**	230.98±8.20**	254.68±8.26**	-
	Zn[2]	55.13±1.30	53.50±1.44	52.41±1.94	57.22±2.50	59.25±1.85	75.31±1.88**	43.90±10.57
	La[2]	2.45±0.63	27.38±4.05**	25.37±1.52**	28.83±4.26**	61.19±7.37**	179.91±9.81**	-
LVd	N[1]	63.77±0.99	70.84±1.17**	66.68±0.10**	67.99±0.45**	64.92±0.19**	65.71±0.71**	-
	P[1]	1.15±0.03	1.17±0.03	1.01±0.03*	1.09±0.02	1.43±0.01**	1.42±0.01**	1.81±0.15**
	K[1]	15.02±0.06	15.81±0.56	15.96±0.61	17.40±0.36**	17.28±0.32**	22.63±0.32**	22.22±0.68**
	Ca[1]	4.20±0.18	5.99±0.23	4.86±0.39	5.21±0.39	5.29±0.18	19.67±0.64**	8.36±1.09**
	Mg[1]	3.07±0.07	4.27±0.07**	3.83±0.03**	3.77±0.21**	3.98±0.09**	2.96±0.12	4.69±0.45**
	S[1]	1.47±0.01	1.50±0.03	1.31±0.03*	1.29±0.04*	1.18±0.04*	1.74±0.04**	1.16±0.02*
	Cu[2]	3.22±0.08	3.94±0.21**	3.61±0.14	3.34±0.17	3.09±0.05	4.47±0.11**	3.48±0.40
	Fe[2]	100.86±3.56	129.08±8.08**	109.42±5.68	128.99±4.81**	113.04±5.29	147.32±6.14**	-
	Mn[2]	182.25±1.65	264.07±14.93**	216.95±12.01	248.88±12.90**	250.86±2.60**	253.13±4.27**	232.66±2.46**
	Zn[2]	50.35±1.02	46.14±1.37	44.68±0.55	43.54±2.99	41.32±1.78*	66.54±2.99**	38.62±7.07*
	La[2]	1.09±0.21	28.06±3.49**	38.49±1.16**	34.45±1.21**	30.60±2.23**	-----	154.01±7.36**

Asterisks indicate significant differences ($p<0.05$) according to Dunnett's test, considering the lower (*) and higher (**) means than the control; the values between brackets represent the mean ± standard error (n = 4); [1] it is expressed as mg g^{-1} shoot dry weight; [2] it is expressed as µg g^{-1} shoot dry weight; LOQ: limit of quantification; ----- : the values for La content could not be shown because they presented wide variation.

Table 5. Nutritional composition of shoot of sunflower plants grown in tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) with increasing concentration of La.

Soil	**Nutrient/ La**	**La concentration (mg kg^{-1} dry soil)**						
		0	**150**	**240**	**384**	**614**	**983**	**1573**

SAT	**N**[1]	24.89±0.93	26.23±1.23	25.58±1.08	27.66±1.27	26.81±0.85	26.86±2.03	38.27±0.55*
	P[1]	4.10±0.09	3.83±0.01*	3.20±0.05*	2.90±0.07*	2.55±0.10*	2.45±0.04*	2.74±0.04*
	K[1]	47.14±0.30	50.0±0.57	48.20±0.87	54.67±1.02**	60.30±0.52**	59.98±0.56**	62.71±1.09**
	Ca[1]	6.71±0.14	7.18±0.13	7.46±0.19	9.93±0.06**	11.89±0.05**	12.28±0.52**	15.88±0.59**
	Mg[1]	2.54±0.09	2.77±0.09	3.01±0.10**	4.40±0.07**	5.64±0.08**	5.12±0.12**	6.37±0.09**
	S[1]	2.12±0.02	2.21±0.06	1.92±0.04*	2.16±0.05	2.14±0.07	1.92±0.02*	2.57±0.05**
	Cu[2]	4.99±0.08	6.61±0.13**	5.05±0.23	5.45±0.32	5.98±0.40	4.87±0.30	6.35±0.36**
	Fe[2]	87.50±1.60	99.67±4.38	72.42±0.84*	78.31±3.42	87.53±5.18	79.25±2.0	89.21±1.25
	Mn[2]	233.86±10.48	322.97±29.59	280.38±6.93	460.58±34.03**	497.19±29.32**	615.50±59.41**	702.59±51.16**
	Zn[2]	82.20±2.09	89.59±2.10	90.24±1.26	110.58±5.09**	120.14±3.71**	117.77±2.47**	113.37±3.33**
	La[2]	2.49±0.15	8.0±0.15**	9.28±0.88**	14.21±0.11**	19.54±1.41**	21.04±1.05**	40.01±3.34**
LVAd	**N**[1]	-	-	-	-	-	-	-
	P[1]	2.62±0.11	2.41±0.11	2.40±0.17	1.74±0.08*	2.07±0.07*	-	-
	K[1]	39.45±1.0	40.20±0.62	41.64±1.68	51.41±1.57**	67.59±2.84**	-	-
	Ca[1]	9.25±0.23	10.27±0.16	12.89±0.50**	15.48±0.47**	17.39±0.72**	-	-
	Mg[1]	3.14±0.06	3.48±0.07**	4.12±0.02**	4.72±0.08**	4.36±0.13**	-	-
	S[1]	5.02±0.11	4.29±0.11*	4.21±0.13*	4.13±0.08*	3.88±0.10*	-	-
	Cu[2]	15.14±0.48	13.04±0.93	14.04±1.15	7.15±0.86*	2.73±0.66*	-	-
	Fe[2]	176.54±14.57	144.04±6.47	144.36±17.50	70.22±5.54*	60.48±11.49*	-	-
	Mn[2]	351.83±11.11	465.76±6.15**	515.24±28.33**	477.39±32.38**	234.30±5.93*	-	-
	Zn[2]	184.48±2.01	194.10±5.20	186.33±5.48	101.0±1.10*	58.28±2.23*	-	-
	La[2]	2.05±0.32	33.24±2.23**	40.68±0.54**	66.92±4.91**	123.35±8.04**	-	-
LVd	**N**[1]	-	-	-	-	-	-	-
	P[1]	1.29±0.01	1.05±0.02*	1.31±0.02	1.05±0.01*	-	-	-
	K[1]	43.62±0.54	52.71±0.46**	51.06±1.51**	45.57±0.31**	-	-	-
	Ca[1]	4.10±0.15	4.65±0.11**	5.51±0.08**	6.37±0.19**	-	-	-
	Mg[1]	1.98±0.08	2.58±0.14**	2.31±0.11**	2.77±0.08**	-	-	-
	S[1]	4.51±0.04	3.65±0.17*	3.94±0.04*	3.41±0.13*	-	-	-
	Cu[2]	2.89±0.50	2.01±0.53	1.94±0.60	3.48±0.79	-	-	-
	Fe[2]	83.79±4.29	28.19±4.41*	76.23±8.42	18.24±1.35*	-	-	-
	Mn[2]	174.40±14.66	100.07±16.14*	167.16±23.55	81.58±8.22*	-	-	-

Zn[2]	55.95±1.83	44.80±4.21*	47.27±2.10*	12.27±2.0*	-	-	-
La[2]	2.87±0.32	46.73±3.33**	28.78±2.05**	64.55±3.14**	-	-	-

Asterisks indicate significant differences ($p<0.05$) according to Dunnett's test, considering the lower (*) and higher (**) means than the control; the values between brackets represent the mean ± standard error (n = 4); [1] it is expressed as mg g^{-1} shoot dry weight; [2] it is expressed as µg g^{-1} shoot dry weight; LOQ: limit of quantification; ----- : the values for La content could not be shown because they presented wide variation.

4. Discussion

Rare earth elements influence ionic fluxes in plants in different ways, thus interfering with nutrient content, as shown for La in the present study. When analyzing the results individually, we evidenced the existence of a species-specific response to La, with different effects of the element on the levels of nutrients in plants (Ramírez-Olvera et al., 2018). Furthermore, we verified the influence of soil physicochemical properties on the bioavailability of nutrients and La. Although nutrient contents have not been evaluated in some cases for the last doses of La (from 614 mg kg^{-1}) applied to the natural soils, the nutritional balance was probably also affected, especially in eudicotyledons, since there was a significant reduction in plant growth (Table S1).

Although variations have occurred, in general, in one or more doses of La, the levels of N, K, Ca, Mg, Mn, and Zn increased, whereas those of P, S, Cu, and Fe decreased. Similar results were found by De Oliveira et al. (2015) when evaluating soybean plants submitted to increasing concentrations of La in nutrient solutions (0, 5, 10, 20, 40, 80, and 160 µM). The authors reported an increment in K, P, Ca, Mg, and Mn, reductions in Cu and Fe, as well as an increase in Zn in intermediate doses and a reduction in high doses.

Complex phenomena are involved in the influence of REE on nutrient absorption (Hu et al., 2004). Brioschi et al. (2013) found evidence that the uptake of REE by the roots is strongly linked to the absorption of Fe and Al. The systematic association with an essential element suggests that the REE are circumstantially absorbed during the absorption of Fe, and the mechanism seems to be the same as that of this micronutrient (Brioschi et al., 2013). Thus, the reduction in Fe levels may be the result of competition with La, since, according to Brioschi et al. (2013), the proportion of REE/Fe in the roots is higher than in the adjacent soil, indicating a preference for the absorption of REE. Moreover, the levels of La in the plants' shoot gradually increased upon increasing the doses of La applied to the soil. Thus the preference for La absorption may have occurred. The reduction in Fe content affects the chlorophyll biosynthesis since this micronutrient controls the rate of formation of aminolevulinic acid, a precursor to that biomolecule synthesis (Cakmak et al., 2010). Despite this, the reductions observed for the levels of Fe in the present study were not so severe, since there were only slight reductions in the relative levels of chlorophyll (Table S2).

Much of La absorbed by the roots is efficiently filtered by the Casparian strip, being retained in the root cell wall (Liu et al., 2013). Thus, higher levels of La are found in the roots, and the sap flow carries a small amount to the shoot (Brioschi et al., 2013). Within the sap, REE levels are also related to Fe and mainly to Mg, K, and P, indicating that the transport of REE is related to the mechanism that controls the general flow of nutrients (Brioschi et al., 2013).

The ionic radius of La is similar to that of Ca (Hu et al., 2004) and, therefore, these elements have similar modes of action in plants. As a result, there may be competition between them for the same locations, and La can replace Ca. Thus, La can interfere with Ca absorption and also enter the cells through the Ca channels in the membrane. According to Tyler et al. (2004), REE can replace Ca^{2+} or interact with this in various physiological functions. Moreover, Ce, like La, can block Ca channels, as Pošćić; Schat; Marchiol (2017) reported reductions in Ca levels even under low concentrations of Ce.

In contrast, in the present study, Ca concentrations increased in the shoot of all species grown in the three soils. As the levels of La have also increased, there may have been a more significant structural substitution of Ca for La. This substitution may have occurred in the middle lamella of the cell walls or even inside the cells, in crystals calcium oxalate, which are a source of Ca (De Oliveira et al., 2015; Liu et al., 2020). Furthermore, an increase in Ca levels may indicate an imbalance in hormonal regulation (auxin and CMI1 protein) (Hazak et al., 2019). Application of REE has been related to an increase in the endogenous auxin content (IAA) by stimulating the synthesis of the tryptophan and inhibiting the activity of the enzyme that degrades IAA (Luo et al., 2008).

The P content reduction observed for most of the evaluated species may have occurred due to the bond of La with P in the soil or the roots, as reported by Ce in a study conducted by Pošćić; Schat; Marchiol (2017). The authors reported that Ce may have precipitated with P ($CePO_4$) in the roots, not only in the apoplast but also inside the cells. Phosphorus plays an essential role in plant metabolism, mainly because it is fundamental in phosphorylation reactions for ATP production. Thus, changes in the contents of this macronutrient can result in extensive disturbances in plants' metabolic and physiological processes. As a result, growth and development can be significantly affected. Despite this, the reduction in P levels in the natural soils was less intense than in SAT, despite having occurred a significant reduction in the shoot dry matter under high concentrations of La in the natural soils. Thus, the reduction in growth in these plants is probably related to other factors. In this context, one can consider the levels of Zn, which reduced in most species cultivated in the natural soils. Zinc has an essential influence on the elongation of internodes, as it participates in auxin metabolism.

The levels of N increased for most species grown in the three soils, which can be explained by the influence that La has on macronutrient metabolism. Cao et al. (2007) in a study with soybean, reported that the pretreatment with La increased the activity of the enzymes nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH) and decreased the accumulation of nitrate and ammonium, indicating that the addition of La promotes an efficient transformation of inorganic N into proteins (Pang et al., 2002). Nitrogen is an essential component of amino acids, proteins, nucleotides, nucleic acids, chlorophylls, and coenzymes. The relative chlorophyll content has not been shown a very sensitive variable, except for sorghum, which presented a reduction in N and Mg levels. As the Mg contents also increased for most species, in general, the reductions in growth may not be a result of the Mg and N deficiency.

For maize and sorghum plants grown in the natural soils, reductions in photosynthesis were observed (Table S3). In the case of sorghum, this can be explained by reductions in N, Mg, and relative chlorophyll content. Also, Cu levels decreased in this species, which may have affected the functionality of plastocyanin, an essential protein involved in the transport of electron during the photochemical phase of photosynthesis (Cakmak et al., 2010). However, for maize, there were no reductions, such as those presented for sorghum plants, which indicates that the decreases in photosynthesis were probably the result of some effect on the biochemical phase. As reported by Sun et al. (2016), the excess of La (III) decreased the activities of the enzymes of the chloroplast ATP synthase and Rubisco, as well as reduced the ability to regenerate RuBP carboxylase.

For most species, there was an increase in the levels of K and Mn, which indicates a synergistic relationship between these elements and La. Liu et al. (2013) and De Oliveira et al. (2015) reported that La could cause accumulation of K and Mn in rice and soybean plants, respectively, grown in nutrient solutions. Besides, as reported by Brioschi et al. (2013), the levels of REE in the xylem sap correlate with the K levels. Liu et al. (2020) reported in a study with REE hyperaccumulator fern *Dicranopteris linearis,* that when in excess, REE are transported by evaporation and co-compartmentalized with Mn in necrotic lesions and the epidermis in immobile forms (Si-coprecipitate).

As previously discussed, most of the nutrients in the shoot of species grown in the SAT showed an increase in some dose(s) of La applied. For this soil, the occurrence of the phenomenon known as hormesis was observed, which is characterized by beneficial effects in low doses and harmful effects in high doses (Agathokleous et al., 2019). This may have occurred due to the soil's chemical properties, mainly pH, which contributed to less La bioavailability and, therefore, greater absorption of some nutrients. In general, doses 983 and 1573 mg La kg^{-1} resulted in harmful effects for the species evaluated, although soybean and sunflower also showed some cases of nutrient content reductions under 614 mg La kg^{-1}. The maize and sorghum plants showed slight increases in shoot dry matter, at doses 384 and 614 mg La kg^{-1}, respectively. Magnesium and Mn levels of maize at the same doses have also increased. In the case of sorghum, at the dose of 614 mg La kg^{-1}, increases in N, Mg, and S levels were also observed.

Moreover, increases in the photosynthesis were observed for maize in treatments with 983 and 1573 mg La kg^{-1}, doses for which increases in Mg levels were also observed. Besides, a significant increase in the relative chlorophyll content and the N content was observed at doses 983 and 1573 mg La kg^{-1}, respectively. Sunflower also showed an increase in the relative chlorophyll content, but this occurred at a dose of 150 mg La kg^{-1} for which an increase in Cu content was observed. These increases found for maize under concentrations 983 and 1573 mg La kg^{-1} indicate that for this species in the SAT, such concentrations would be stimulating.

Other studies have also shown that the application of La resulted in hormetic effects on plant growth. De Oliveira et al. (2015) reported that low doses of La in solution (5 and 10 µM) stimulated photosynthesis and total chlorophyll content and caused a high incidence of binucleated cells and, consequently increases in soybean biomass. On the other hand, under high doses (40, 80, and 160 µM), plant growth was reduced. Liu et al. (2013) observed improvement of the growth of rice roots in doses 0.05 and 0.1 mmol L^{-1} of La^{3+} and inhibition in doses 1.0 and 1.5 mmol L^{-1}. The authors reported that the hormetic effects of La^{3+} might be related to the absorption of some nutrients such as K, Ca, and Mo.

Given the results that we found here, it is evident how the physicochemical properties of soils influence the effects of La on plants and how these present different responses to the element. Doses (generally from 614 mg La kg^{-1}), which for plants grown in the natural soils were harmful, for maize grown in SAT were beneficial. The hormetic effects depend on the substrate's pH levels, in addition to the number of doses below the NOEC (last concentration at which there was no effect compared with the control) and the time intervals (Agathokleous et al., 2019).

The different responses to La can be explained mainly by the differences in bioavailable La concentrations between soils. In SAT, the bioavailable levels were probably much lower than those of natural soils, mainly due to their higher values of pH, CEC, and organic matter. The results of La levels reinforce this statement because the values found for plants grown in SAT were lower than those of plants grown in the natural soils. Therefore, the bioavailable concentrations referring to total concentrations for which hormetic effects have been observed (between 384 and 1573 mg La kg^{-1}) should be much lower. Thus, for future studies, aiming to determine La concentrations involved with hormetic effects in the natural soils, we suggest that the La treatments should be determined based on bioavailable concentrations.

5. Conclusion

In general, in one or more doses of La, the levels of N, K, Ca, Mg, Mn, Zn increased, and the levels of P, S, Cu, and Fe decreased in the shoot of the plants. However, specificities occurred depending on the species and the soils. In general, for plants grown in SAT, most nutrient contents increased or did not present significant differences compared with controls. Moreover, cases of hormetic effects have been observed in this soil, mainly for maize, with stimulating effects between doses 384 and 1573 mg La kg^{-1}. On the other hand, for the plants of the natural soils, the reductions in the nutrient contents were more pronounced, and the La contents were higher than for plants in the SAT. These results evince the influence of physicochemical differences in soils on the effects of La on plants, determining, in part, if they will be stimulating or harmful.

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6. References

Aide, M., Ladonin, D., Vitová, M. et al., 2019. Lanthanide Soil Chemistry and Its Importance in Understanding Soil Pathways: Mobility, Plant Uptake, and Soil Health, in: Awwad, N.S., Mubarak, A.T. (Eds.), Lanthanides. IntechOpen, London. http://dx.doi.org/10.5772/intechopen.79238

Agathokleous, E., Kitao, M., Calabrese, E.J., 2019. Hormetic dose responses induced by lanthanum in plants. Environ. Pollut. 244, 332–341. https://doi.org/10.1016/j.envpol.2018.10.007

Brioschi, L., Steinmann, M., Lucot, E., Pierret, M.C., Stille, P., Prunier, J., Badot, P.M., 2013. Transfer of rare earth elements (REE) from natural soil to plant systems: Implications for the environmental availability of anthropogenic REE. Plant Soil 366, 143–163. https://doi.org/10.1007/s11104-012-1407-0

Cakmak, I., Pfeiffer, W.H., McClafferty, B., 2010. Biofortification of durum wheat with zinc and iron. Cereal Chem. 87, 10–20.

Cao, R., Huang, X. hua, Zhou, Q., Cheng, X. ying, 2007. Effects of lanthanum(III) on nitrogen metabolism of soybean seedlings under elevated UV-B radiation. J. Environ. Sci. 19, 1361–1366. https://doi.org/10.1016/S1001-0742(07)60222-8

de Oliveira, C., Ramos, S.J., Siqueira, J.O., Faquin, V., de Castro, E.M., Amaral, D.C., Techio, V.H., Coelho, L.C., e Silva, P.H.P., Schnug, E., Guilherme, L.R.G., 2015. Bioaccumulation and effects of lanthanum on growth and mitotic index in soybean plants. Ecotoxicol. Environ. Saf. 122, 136–144. https://doi.org/10.1016/j.ecoenv.2015.07.020

de Sá Paye, H., de Mello, J.W.V., de Magalhães Mascarenhas, G.R.L., Gasparon, M., 2014. Distribution and fractionation of the rare earth elements in Brazilian soils. J. Geochemical Explor. 161, 27–41. https://doi.org/10.1016/j.gexplo.2015.09.003

Dinali, G.S., Root, R.A., Amistadi, M.K., Chorover, J., Lopes, G., Guilherme, L.R.G. 2019. Rare earth elements (REY) sorption on soils of contrasting mineralogy and texture. Environ. Int. 128, 279–291.

El-Ramady, H.R.H., 2010. Ecotoxicology of rare earth elements: ecotoxicology of rare earth elements within soil and plant environments, VDM Verlag Dr. Muller Aktiengsellsellshaft & Co. KG, Saarbrücken.

Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, 1997. Manual de métodos de análises de solo, second ed. Ministério da Agricultura e do Abastecimento, Rio de Janeiro Available online at. https://www.agencia.cnptia.embrapa.br/Repositorio/Manual+de+Metodos_000fzvhotqk02wx5ok0q43a0ram31wtr.pdf (Verified on 20 Feb 2019).

García-Jiménez, A., Gómez-Merino, F.C., Tejeda-Sartorius, O., Trejo-Téllez, L.I., 2017. Lanthanum affects bell pepper seedling quality depending on the genotype and time of exposure by differentially modifying plant height, stem diameter and concentrations of chlorophylls, sugars, amino acids, and proteins. Front. Plant Sci. 8, 1–14. https://doi.org/10.3389/fpls.2017.00308

Hazak, O., Mamon, E., Lavy, M., Sternberg, H., Behera, S., Schmitz-Thom, I., Bloch, D., Dementiev, O., Gutman, I., Danziger, T., Schwarz, N., Abuzeineh, A., Mockaitis, K., Estelle, M., Hirsch, J.A., Kudla, J., Yalovsky, S., 2019. A novel Ca^{2+}-binding protein that can rapidly transduce auxin responses during root growth, PLoS Biology. https://doi.org/10.1371/journal.pbio.3000085

Hu, Z., Richter, H., Sparovek, G., Schnug, E., 2004. Physiological and Biochemical Effects of Rare Earth Elements on Plants and Their Agricultural Significance: A Review. J. Plant Nutr. 27, 183–220. https://doi.org/10.1081/PLN-120027555

Huang, J.W.W., Grunes, D.L., Kochian, L.V., 1994. Voltage-dependent Ca^{2+} influx into right-side-out plasma-membrane vesicles isolated from wheat roots – characterization of a putative Ca^{2+} channel. Proc. Natl. Acad. Sci. U.S.A. 91, 3473–3477.

Lian, H., Qin, C., Zhang, C., Yan, M., Li, H., Zhang, S., 2019. Foliar-applied lanthanum chloride increases growth and phosphorus acquisition in phosphorus-limited adzuki bean seedlings. Plant Soil 442, 385–399. https://doi.org/10.1007/s11104-019-04197-5

Liu, D., Wang, X., Zhang, X., Gao, Z., 2013. Effects of lanthanum on growth and accumulation in roots of rice seedlings. Plant, Soil Environ. 59, 196–200. https://doi.org/10.17221/760/2012-pse

Liu, W.S., Van Der Ent, A., Erskine, P.D., Morel, J.L., Echevarria, G., Spiers, K.M., Montargès-Pelletier, E., Qiu, R.L., Tang, Y.T., 2020. Spatially Resolved Localization of Lanthanum and Cerium in the Rare Earth Element Hyperaccumulator Fern *Dicranopteris linearis* from China. Environ. Sci. Technol. 54, 2287–2294. https://doi.org/10.1021/acs.est.9b05728

Luo, J., Zhang, J., Wang, Y., 2008. Changes in endogenous hormone levels and redox status during enhanced adventitious rooting by rare earth element neodymium of *Dendrobium densiflorum* shoot cuttings. J. Rare Earths 26, 869–874. https://doi.org/10.1016/S1002-0721(09)60023-5

Malavolta, E., Vitti, G. C., Oliveira, S. A., 1997. Avaliação do estado nutricional das plantas: princípios e aplicações, Potafos, Piracicaba.

Malavolta, E., 1980. Elementos da nutrição mineral de plantas, Agronômica Ceres, Piracicaba.

Organisation for Economic Co-operation and Development – OECD, 2006. Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing https://doi.org/10.1787/9789264070066-en.

Pang, X., Li. D.C., Peng, A., 2002. Application of rare-earth elements in the agriculture of China and its environmental behavior in soil. Environ. Sci. Pollut. Res. 9, 143–148.

Paoli, L., Fiorini, E., Munzi, S., Sorbo, S., Basile, A., Loppi, S., 2014. Uptake and acute toxicity of cerium in the lichen Xanthoria parietina. Ecotoxicol. Environ. Saf. 104, 379–385. https://doi.org/10.1016/j.ecoenv.2014.02.028

Pošćić, F., Schat, H., Marchiol, L., 2017. Cerium negatively impacts the nutritional status in rapeseed. Sci. Total Environ. 593–594, 735–744. https://doi.org/10.1016/j.scitotenv.2017.03.215

Ramírez-Olvera, S.M., Trejo-Téllez, L.I., García-Morales, S., Pérez-Sato, J.A., Gómez-Merino, F.C., 2018. Cerium enhances germination and shoot growth, and alters mineral nutrient concentration in rice. PLoS One 13, 1–19. https://doi.org/10.1371/journal.pone.0194691

Ramos, S.J., Dinali, G.S., Oliveira, C., Martins, G.C., Moreira, C.G., Siqueira, J.O., Guilherme, L.R.G., 2016. Rare Earth Elements in the Soil Environment. Curr. Pollut. Reports 2, 28–50. https://doi.org/10.1007/s40726-016-0026-4

Smith Stegen, K., 2015. Heavy rare earths, permanent magnets, and renewable energies: An imminent crisis. Energy Policy 79, 1–8. https://doi.org/10.1016/j.enpol.2014.12.015

Sun, J., Hu, H., Li, Y., Wang, L., Zhou, Q., Huang, X., 2016. Effects and mechanism of acid rain on plant chloroplast ATPase. Environ. Sci. Pollut. R. 23, 18296–18306. https://doi.org/10.1007/s11356-016-7016-3.

Teodoro, J.C., Ribeiro, I.C.A., De Oliveira, C., Martins, G.C., Guilherme, L.R.G., 2020a. Lanthanum phytotoxicity: an approach to assess ecological risk in tropical soils. Ecotoxicol. Environ. Saf., Submitted.

Turra, C., Fernandes, E.A.N., Bacchi, M.A., Energia, C. De, Paulo, U.D.S., 2011. Turra et al_2011_REE in fertiliser 3, 86–92.

Tyler, G., 2004. Rare earth elements in soil and plant systems - A review. Plant Soil 267, 191–206. https://doi.org/10.1007/s11104-005-4888-2

United States Environmental Protection Agency – USEPA, 2007. Method 3051A (SW- 846): microwave assisted acid digestion of sediments, sludges, and oils, revision 1. Washington, DC. Available online at. https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf (Verified on 20 Feb 2019).

Zhang, C., Li, Q., Zhang, M., Zhang, N., Li, M., 2013. Effects of rare earth elements on growth and metabolism of medicinal plants. Acta Pharm. Sin. B 3, 20–24. https://doi.org/10.1016/j.apsb.2012.12.005

Supplementary material

Table S1. Shoot dry matter (g pot^{-1}) of plant species grown in tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) with increasing doses of La (data taken from Teodoro et al., 2020a - submitted).

Specie	Soil	0	150	240	384	614	983	1573
		--------mg La kg^{-1} dry soil--------						
Maize	**SAT**	3.58±0.11	3.79±0.09	4.0±0.04	4.20±0.15**	3.84±0.10	3.47±0.12	3.77±0.19
	LVAd	2.14±0.11	2.28±0.05	2.03±0.04	1.87±0.07	1.09±0.01*	0.61±0.16*	0.10±0.03*
	LVd	1.14±0.06	0.98±0.07	0.92±0.04	0.85±0.06*	0.59±0.05*	0.16±0.06*	0.28±0.09*
Sorghum	**SAT**	1.95±0.03	1.74±0.05	1.63±0.05	2.20±0.04	2.48±0.11**	1.94±0.11	0.96±0.13*
	LVAd	1.13±0.05	1.23±0.06	1.28±0.07	1.14±0.07	1.09±0.04	0.19±0.09*	0.25±0.08*
	LVd	1.24±0.06	1.39±0.07	1.40±0.01	1.31±0.03	1.05±0.18	0.91±0.15	0.91±0.10
Soybean	**SAT**	2.14±0.06	2.05±0.04	1.93±0.03*	2.14±0.04	1.62±0.04*	1.23±0.08*	0.36±0.01*
	LVAdD	0.85±0.02	0.92±0.03	0.81±0.05	0.78±0.03	0.66±0.05*	0.40±0.04*	0.14±0.03*
	LVd	1.10±0.07	0.78±0.02*	0.86±0.03*	0.82±0.03*	0.72±0.03*	0.78±0.02*	0.24±0.04*
Sunflower	**SAT**	1.77±0.03	1.65±0.03	1.64±0.03	1.45±0.08*	1.32±0.02*	1.02±0.05*	0.68±0.03*
	LVAd	0.75±0.22	0.58±0.03*	0.55±0.01*	0.34±0.02*	0.21±0.01*	-	-
	LVd	0.26±0.01	0.13±0.02*	0.18±0.03*	0.07±0.01*	0.06±0.01*	-	-

Asterisks indicate significant differences ($p<0.05$) according to Dunnett's test, considering the lower (*) and higher (**) means than the control (0 mg La kg^{-1} dry soil); ± standard error of the mean, n=4.

Table S2. Relative chlorophyll content **(**AtLEAF readings) of plant species grown in tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) with increasing doses of La (data taken from Teodoro et al., 2020a - submitted).

Specie	Soil	0	150	240	384	614	983	1573
		--------mg La kg^{-1} dry soil--------						
Maize	**SAT**	44.33±0.17	43.92±0.33	43.53±0.39	42.30±0.22	45.21±1.08	46.80±0.63**	41.94±0.68
	LVAd	52.66±0.78	50.78±0.62	50.27±0.35*	46.59±0.41*	40.39±0.83*	42.36±0.73*	36.25±0.47*
	LVd							
Sorghum	**SAT**	40.83±0.55	43.06±1.02	43.94±1.02	43.87±0.67	39.19±1.14	38.58±1.17	37.40±1.41
	LVAd	42.11±1.02	40.78±0.75	43.51±0.67	42.35±0.68	43.39±0.86	38.97±0.60*	40.73±0.48
	LVd	41.65±0.32	41.59±0.16	42.12±0.24	41.13±0.62	40.74±0.64	37.28±0.92*	38.73±0.56*

Soybean	**SAT**	42.47±0.45	41.93±0.57	41.48±0.12*	40.47±0.25*	38.92±0.48*	38.49±0.34*	-
	LVAdD	50.45±0.61	50.88±0.10	51.14±0.28	49.50±0.87	46.82±0.90*	43.78±0.78*	-
	LVd	52.13±0.63	51.52±0.10	51.90±0.31	51.26±0.38	50.21±0.35*	45.66±0.29*	-
Sunflower	**SAT**	45.29±0.39	48.59±0.79**	46.40±0.38	44.76±1.17	41.72±0.33*	42.35±0.77*	39.13±0.85*
	LVAd	48.58±0.80	47.75±0.82	46.66±0.63	45.98±0.58*	42.66±0.50*	-	-
	LVd	46.68±0.60	43.65±0.79*	45.70±0.92	44.85±1.20	-	-	-

Asterisks indicate significant differences ($p<0.05$) according to Dunnett's test, considering the lower (*) and higher (**) means than the control (0 mg La kg^{-1} dry soil); ± standard error of the mean, n=4.

Table S3. Photosynthesis (µmol m^{-2} s^{-1}) of plant species grown in tropical artificial soil (SAT), Dystrophic Red-Yellow Oxisol (LVAd) and Dystrophic Red Oxisol (LVd) with increasing doses of La (data taken from Teodoro et al., 2020a - submitted).

Specie	**Soil**	**0**	**150**	**240**	**384**	**614**	**983**	**1573**
		---mg La kg^{-1} dry soil---						
Maize	**SAT**	14.76±0.43	15.15±0.19	14.05±0.18	12.90±0.96	17.14±1.07	18.27±1.18**	18.64±1.26**
	LVA	26.48±0.91	24.75±0.46	24.14±0.92	21.65±0.65*	12.73±0.60*	5.16±1.18*	2.35±0.21*
	LVd	35.53±3.20	24.03±0.80*	22.06±2.21*	17.99±1.85*	22.90±2.85*	17.38±0.56*	15.26±0.84*
Sorghum	**SAT**	24.44±2.31	22.44±0.96	20.34±0.95	19.93±2.30	23.55±1.75	24.89±1.26	14.93±2.36*
	LVA	25.01±2.52	27.50±1.25	25.03±2.13	18.82±2.21	24.31±3.74	6.65±1.86*	2.57±1.91*
	LVd	24.69±1.62	26.62±2.90	25.21±0.15	20.97±3.04	23.93±2.94	14.70±0.83*	13.28±0.70*

Asterisks indicate significant differences ($p<0.05$) according to Dunnett's test, considering the lower (*) and higher (**) means than the control (0 mg La kg^{-1} dry soil); ± standard error of the mean, n= 4.