UNIVERSIDADE FEDERAL DE LAVRAS

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ECOTOXICOLOGICAL RISK OF CERIUM FOR TROPICAL SOILS

LAVRAS-MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do Solo e Nutrição de Plantas, para a obtenção do título de Doutor.

Prof. Luiz Roberto Guimarães Guilherme, Ph.D.
Orientador

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RESUMO

O cério (Ce) está presente na indústria moderna e na composição de fertilizantes fosfatados. O descarte de materiais de alta tecnologia e a aplicação intensiva de fertilizantes fosfatados, como ocorre em solos tropicais – solos de áreas agrícolas brasileiras – podem alterar o *status* natural de Ce no ambiente solo. Assim, entender as consequências ambientais da entrada de Ce em agroecossistemas tropicais é importante para avaliar o potencial risco ecológico desse elemento e garantir a segurança ambiental. Os objetivos deste estudo foram avaliar o risco ecotoxicológico para plantas que crescem em solos tropicais contaminados com Ce, bem como criar um banco de dados para apoiar futuras legislações que regulem os limites desse elemento em solos brasileiros e, possivelmente em outros solos tropicais. Além disso, este estudo avaliou os efeitos do Ce sobre os processos fisiológicos das plantas, bem como sugerir variáveis fisiológicas para avaliar os riscos ecológicos para este elemento. Oito espécies de plantas (milho, sorgo, arroz, trigo, soja, girassol, rabanete e feijão) foram expostas a um gradiente de concentração de Ce em dois solos tropicais típicos (Latossolo e Cambissolo), e um solo artificial. Os resultados mostraram que entre as variáveis de crescimento e germinação avaliados, a fitotoxicidade do Ce foi mais pronunciada na matéria seca da parte aérea do que na porcentagem de germinação e no índice de velocidade de germinação, independente das espécies ou solos estudados. A sensibilidade das plantas é específica da espécie. Propriedades do solo, como pH, capacidade de troca de cátions e carbono orgânico, podem ter influenciado a severidade da fitotoxicidade do Ce. Por isso, Ce foi mais tóxico para as plantas no Latossolo do que nos demais solos (Cambissolo e solo artificial). Nossos resultados de avaliação de risco (concentração perigosa, HC_5 = 281,6 mg Ce kg^{-1}) apoiam a ideia que a entrada não intencional de Ce através de fertilizantes fosfatados não representa um risco para os solos de agroecossistemas brasileiros. Entre as variáveis fisiológicas avaliadas, a fitotoxicidade do Ce foi mais pronunciada no índice SPAD do que na taxa fotossintética, taxa de transpiração e condutância estomática. A avaliação de risco com variáveis fisiológicas suportam a ideia que o índice SPAD pode ser uma variável usada em estudos de risco ecológico para Ce, por ser muito sensível e fácil de medir.

Palavras-chave: Elementos terras raras. Fertilizante fosfatado Fitotoxicidade. Ecotoxicologia. Segurança ambiental.

ABSTRACT

Cerium (Ce) is present in modern industry and in the composition of phosphate fertilizers. The discharge of high technology materials and intensive application of phosphate fertilizer, as it happens in tropical soils - e.g., soils of Brazilian farming areas - may change the natural status of Ce in the soil environment. Thus, understanding the environmental consequences of Ce imput in tropical agroecosystems is important to evaluate the potential ecological risk of this element and ensuring environmental safety. The aims of this study were to evaluate the ecotoxicological risk to plants growing in tropical soils contaminated with Ce, as well as to create a database to support future legislation regulating the limits of this element in Brazilian and conceivably other tropical soils. In addition, this study evaluated the effects of Ce on physiological processes of plants, as well as suggested plant physiological variables for assessing ecological risks for this element. Eight crop species (corn, sorghum, rice, wheat, soybeans, sunflower, radish, and beans) were exposed to a Ce concentration gradient in two typical tropical soils (Oxisol and Inceptsol), and an artificial soil. Our findings showed that among the growth and germination endpoints measured, Ce phytotoxicity was more pronounced on shoot dry matter than on percent germination and germination speed index, irrespectively of the soils and crop species evaluated. Sensitivity of plants is species specific. Soil properties such as pH, cation exchange capacity, and organic carbon may have influenced the severity of Ce phytotoxicity. Because of that, Ce was more toxic to plants in the Oxisol than the other soils tested (Inceptsol and artificial soil). Our risk assessment results (hazardous concentration, HC_5 = 281.6 mg Ce kg^{-1}) support the idea that unintentional Ce input through P fertilizers does not pose a risk to soils of Brazilian agroecosystems. Among the physiological variables measured, Ce phytotoxicity was more pronounced on SPAD index than on photosynthetic rate, transpiration rate, and stomatal conductance. Our risk assessment results with physiological variables support the idea that SPAD index may be a variable used in ecological risk studies for Ce, because it is both very sensitive and easy to measure.

Keywords: Rare earth elements. Phosphate fertilizer. Phytotoxicity. Ecotoxicology. Environmental safety.

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO

Os elementos terras raras (ETR) constituem um grupo de 17 elementos com propriedades físicas e químicas semelhantes. Dentre esses elementos, 15 pertencem ao grupo dos lantanídeos, e a esses, incluem-se os elementos escândio e ítrio (IUPAC, 2005). São tipicamente macios, maleáveis, dúcteis, além de reativos (JORJANI; SHAHBAZI, 2012). Esses elementos são aplicados em diversos segmentos industriais de alta tecnologia, como militar, nuclear, automotivo, energia renovável e em setores eletrônicos (ALONSO et al., 2012; BINNEMANS et al., 2013; DUTTA et al., 2016). Além disso, desde a década de 1980 na China, os ETR são utilizados como fertilizantes (Fertilizante ETR) na agricultura para incrementar o rendimento e a qualidade das culturas (HU et al., 2004; TYLER, 2004; WANG et al., 2008).

No Brasil não há relatos de aplicação direta de ETR, através de fertilizantes enriquecidos com ETR na agricultura, como ocorre na agricultura chinesa. No entanto, os insumos agrícolas derivados de rochas fosfáticas, como o fertilizante fosfatado mineral e o fosfogesso, podem conter níveis significativos de ETR em sua composição (OTERO et al., 2005; RAMOS et al., 2016b; TODOROVSKY et al., 1997; TURRA et al., 2011; WAHEED et al., 2011). Em vista disso, esses insumos aplicados na agricultura podem carrear, indiretamente, ETR e serem considerados uma fonte difusa desses elementos para o ambiente solo.

O solo tropical tipicamente encontrado em grande parte do território no Brasil apresenta baixa fertilidade natural e alta fixação de P (LOPES;

GUILHERME, 2016). Assim, intensiva aplicação de fertilizante fosfatado é realizada regularmente para fornecer as necessidades nutricionais para as culturas (ROY et al., 2016; WITHERS et al., 2018). Com base nisso, além da expansão e intensificação da agricultura brasileira nas últimas 5-6 décadas, o uso total anual de fertilizante mineral fosfatado aumentou de uma média de 0,04 Tg em 1960 para 2,2 Tg em 2016, e espera-se alcançar 4,6 Tg em 2050 (WITHERS et al., 2018). Esta alta taxa de fertilizantes fosfatados regularmente suplementados ao solo pode resultar em aumentos significativos dos níveis desses ETR no ambiente. Além disso, como apenas cerca de 1% dos ETR são reciclados de produtos finais (JOWITT et al., 2018), os materiais eletrônicos descartados inadequadamente em aterros também podem resultar em aumentos significativos dos níveis de ETR em solos dessas áreas. Por estas razões, os ETR foram considerados como “poluentes emergentes” (PAGANO et al., 2015).

O cério é o ETR mais abundante e o 25º elemento de maior ocorrência na crosta terrestre (MIGASZEWSKI; GAŁUSZKA, 2014), destacando-se dentre outros ETR em fertilizantes minerais fosfatados (RAMOS et al., 2016b; TURRA et al., 2011). Há relatos no Brasil de que os níveis de Ce na camada de 0 a 0,4 m aumentaram até 62% em uma área onde as atividades agrícolas - incluindo a fertilização do solo - eram constantes há pelo menos 20 anos com soja e trigo (NEVES et al., 2018). Além disso, há estudos que relatam o Ce causando toxicidade fisiológica em batata doce exposta a 20-80 mg Ce L^{-1} (JIANG et al., 2017) e colza exposta a 0,28-8,96 mg Ce L^{-1} (POŠĆIĆ et al., 2017), através de solução nutritiva. Independentemente disso, a compreensão do papel biológico do Ce e de outros ETR ainda está em seus estágios iniciais (SKOVRAN; MARTINEZ-GOMEZ, 2015). A literatura apresenta poucas informações sobre os testes de fitotoxicidade de Ce, principalmente testes realizados em solos tropicais, relatando sua toxicidade nos processos fisiológicos das culturas agrícolas. Neste sentido, estudos de avaliação de risco ecológico são necessários para entender melhor as consequências ambientais de enriquecer solos com este elemento e recomendar valores seguros para Ce no ambiente do solo, particularmente para solos de agroecossistemas tropicais. Do mesmo modo, esses estudos são necessários para entender os efeitos do Ce nos processos fisiológicos das culturas cultivadas em solos que são propensos a receber altas aplicações involuntárias de ETR, através da adubação fosfatada.

O Brasil se destaca no setor agrícola mundial, sendo um dos maiores produtores e fornecedores de alimentos e produtos agrícolas (OECD/FAO, 2015). No entanto, nenhum estudo avaliando o efeito do Ce nos processos fisiológicos das culturas crescidas em solos tropicais, bem como estudo sugerindo valores orientadores que poderiam ser usados para regular os níveis de Ce para solos brasileiros, como ocorre para muitos outros elementos-traço, foram conduzidos até o momento. Neste sentido, o objetivo deste estudo foi avaliar a fitotoxicidade do Ce por meio de ensaios de avaliação de risco ecológico com culturas e solos típicos brasileiros, bem como criar um banco de dados para subsidiar e orientar futuras legislações que regulamentam os limites desse elemento em solos tropicais.

2. REFERENCIAL TEÓRICO

2.1. Elementos terras raras

Os elementos terras raras (ETR) são, de acordo com a classificação da União Internacional de Química Pura e Aplicada (International Union of Pure and Applied Chemistry - IUPAC), um grupo de 17 elementos químicos. Dentre esses elementos, 15 pertencem ao grupo dos lantanídeos com número atômico entre Z=57 e Z=71: 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). A eles, se juntam o escândio (Sc, Z=21) e o ítrio (Y, Z=39) (IUPAC, 2005). Os ETR tem características físico-químicas muito semelhantes. Esta semelhança é originária da natureza de suas configurações eletrônicas, direcionando a um estado de oxidação "+3" particularmente estável e uma leve, mas constante, redução do raio iônico, com o aumento do número atômico, o que é denominado de "contração lantanídica" (MIGASZEWSKI; GAŁUSZKA, 2014). O elemento Ce pode apresentar estado de oxidação "+2" e o Eu apresentar estado de oxidação "+4" (DOŁĘGOWSKA; MIGASZEWSKI, 2013; MIGASZEWSKI; GAŁUSZKA, 2014).

No século XIX, os ETR foram aplicados pela primeira vez nas mantas incandescentes de lampiões, com a criação da iluminação a gás (FRANÇA, 2012). Com o desenvolvimento tecnológico, as propriedades dos ETR tornaram-se mais conhecidas. Atualmente, sabe-se que os ETR são elementos macios, maleáveis, dúcteis e considerados ótimos condutores elétricos, o que permite a sua aplicação em vários segmentos industriais de alta tecnologia, como o automotivo, nuclear, petrolífero, eletrônicos, bélico, metalúrgico e de energia renovável (JORJANI; SHAHBAZI, 2012; ALONSO et al., 2012; CHAKHMOURADIAN; WALL, 2012; MASSARI; RUBERTI, 2013). Os ETR também podem ser aplicados em estudos de processos pedogenéticos, como traçadores em estudos geoquímicos (KIMOTO et al., 2006; LAVEUF et al., 2008; LAVEUF; CORNU, 2009; WANG et al., 2011). Além disso, os ETR são aplicados na agricultura chinesa, como fertilizante agrícola (HU et al., 2004).

A etimologia do termo “terras raras” vem de longa data. A denominação genérica “Terra” foi dada, inicialmente, aos óxidos da maioria dos elementos metálicos. Assim, como os elementos “Terras Raras” foram, inicialmente, isolados na forma de óxidos, receberam essa denominação. O termo "rara" foi dada em função desses elementos não ocorrerem naturalmente na forma metálica, mas sim associados a minerais, além de serem de difícil separação dos outros, devido às semelhanças nas propriedades físico-químicas (MOLDOVEANU; PAPANGELAKIS, 2012). Diante disso, o termo “Terras Raras” é usado até os dias atuais.

A distribuição natural dos ETR mostra regularidade e segue a regra de Oddo-Harkins, onde os elementos com número atômico par (Ce, Nd, Sm, Gd, Dy, Er e Yb) são mais abundantes que os elementos com número atômico ímpar (La, Pr, Eu, Tb, Ho, Tm e Lu), além dos seus teores diminuírem com o aumento da massa atômica (KABATA-PENDIAS, 2011). A abundância dos ETR na crosta terrestre é relevante. Em contraste com a sua nomenclatura, os elementos terras raras não são necessariamente raros, pois são encontrados em quase todas as formações rochosas. Dos lantanídeos encontrados em menores concentrações, o Lu e o Tm são mais abundantes na crosta terrestre que o cádmio (Cd) e o selênio (Se) (TYLER, 2004). O elemento Pm é o único artificial, ou seja, não ocorre naturalmente na crosta terrestre (KABATA-PENDIAS, 2011; PANG et al., 2002).

2.2. Elementos terras raras no solo

Os ETR ocorrem em mais de 270 minerais distribuídos em todos os continentes, podendo estes serem primários e/ou secundários e estarem presente em diversas concentrações (CHAKHMOURADIAN; WALL, 2012; JORDENS et al., 2013). Apesar disto, 95% de todos os recursos de ETR do mundo estão concentrados em apenas três minerais, a bastnasita, a monazita e o xenotímio (GUPTA; KRISHNAMURTHY, 2005). Normalmente, quando um mineral contém ETR, esse pode conter todos os ETR, mas em diferentes proporções e com predominância de Ce e La (SHA; CHAPPELL, 1999; GUPTA; KRISHNAMURTHY, 2005).

As concentrações naturais de ETR encontrados nos solos são basicamente dependentes do material de origem, enquanto o fracionamento desses elementos individuais depende das características do solo que são, parcialmente, influenciadas pelo processo pedogênico (PAYE et al., 2016). Além disso, os níveis de ETR nos solos são influenciados, dentre outros fatores, pelo grau de intemperismo, potencial redox e clima. No Brasil, solos desenvolvidos a partir de rochas ígneas alcalinas apresentam maior teor médio de ETR, seguido por rochas sedimentares, rochas metamórficas, rochas ígneas básicas e rochas ígneas ácidas (PAYE et al., 2016). As concentrações totais desses elementos na camada superior do solo, geralmente, são muitas vezes menores que no material de origem, uma vez que os processos de formação do solo causam perdas de ETR por lixiviação (TYLER, 2004). Em geral, as concentrações de ETR (mg kg^{-1}) em solos mundiais são, em média: La = 27; Ce = 56,7; Pr = 7,0; Nd = 26; Sm = 4,6; Eu = 1,4; Gd = 3,9; Tb = 0,63; Dy = 3,6; Ho = 0,72; Er = 2,2; Tm = 0,37; Yb = 2,6; Lu = 0,37; Sc = 11,7; e Y = 12 (KABATA-PENDIAS, 2011).

De maneira geral, os valores de ETR em áreas com alterações antrópicas são devidos, principalmente, à aplicação de insumos agrícolas (OTERO et al. 2005; TURRA et al. 2011; WAHEED et al. 2011; ZHANG; SHAN, 2001). Entretanto, fatores ambientais como chuva, neve e transporte eólico podem contribuir para estes teores, principalmente, em áreas próximas a grandes centros industriais (SMIDT et al., 2011).

A biodisponibilidade, toxicidade ou a deficiência de qualquer elemento no ambiente, depende da sua disponibilidade, da própria característica do elemento e da capacidade do solo em liberá-lo a partir da fase mineral (BACKES et al., 1995). A disponibilidade e a absorção dos ETR pelas plantas podem ser influenciadas por atributos físico-químicos do solo, como pH, capacidade de troca de cátions (CTC) e o conteúdo de carbono orgânico no solo. Em geral, os maiores valores de CTC induzem à adsorção e, consequentemente, diminuem a disponibilidade desses elementos no solo. Uma correlação negativa entre os teores de Ce (III) em solução e a CTC de diferentes solos chineses foi relatada por Li et al. (2001). O pH do solo é tido como um dos fatores mais importantes para controlar a disponibilidade dos ETR para as plantas. O baixo pH do solo é uma condição favorável à dessorção de ETR da matriz mineral para a solução do solo, aumentando assim a disponibilidade desses elementos às plantas (CAO et al., 2001; DIATLOFF et al., 1996; LOELL et al., 2011). Estudos reportaram maiores concentrações de ETR disponíveis para as plantas, quando cultivadas em solos ácidos (CHENG et al. 2015; FANG et al., 2007; LOELL et al., 2011; PAYE et al., 2016; THOMAS et al. 2014). A matriz orgânica também pode ser outro fator determinante para os processos geoquímicos e bioquímicos do solo sendo importante para o comportamento dos ETR. Isso está relacionado aos grupos funcionais orgânicos carregados negativamente, resultando em alta capacidade de adsorção de elementos catiônicos, como os ETR. Por meio de um estudo de dessorção de ETR em solos, Wen et al. (2002) observaram que as taxas de adsorção foram maiores em solos com maior teor de matéria orgânica e maior de pH.

2.3. Elementos terras raras em insumos agrícolas

Em 1986, foi registrado na China o primeiro fertilizante comercial enriquecido com ETR e denominado de “Changle”, o qual continha em sua composição La_2O_3, CeO_2, Pr_6O_{11} e Nd_2O_3 com seus respectivos teores 25 a 28%, 49 a 51%, 5 a 6% e 15 a 17% (HU et al., 2004). No Brasil, não há relatos de aplicação intencional de fertilizantes enriquecidos com ETR na agricultura. No entanto, os fertilizantes fosfatados e fosfogesso podem apresentar teores significativos de ETR em sua composição e, assim, serem considerados como fonte difusa desses elementos, uma vez que são aplicados indiretamente ao solo (OTERO et al., 2005; RAMOS et al., 2016a; TODOROVSKY et al., 1997; TURRA et al., 2011; Waheed et al., 2011). Através de um levantamento na literatura, Ramos et al., (2016a) reportaram teores de ETR presentes na rocha fosfatada e em seus subprodutos, como fertilizantes fosfatados, fosfogesso e ácido fosfórico para diversos países (Tabela 1).

Tabela 1. Conteúdos mundiais de ETR nos principais fertilizantes fosfatados.

Produto	Concentração (mg kg^{-1})						País
	La	Ce	Nd	Sm	Eu	Tb	
Superfosfato Simples	18,4	40,2	-	2,0	0,4	0,3	Paquistão
NPK (08:23:18)	90	129	-	12	3,0	1,5	Paquistão
Superfosfato	18	8,5	-		0,3	-	Egito
Superfosfato	17	35	23,5	-	-	-	Bulgária
NPK (12:12:17)	500	600	181	33,2	9,9	3	Espanha
NPK (4:14:08)	534	1181	571	77	17,1	4,5	Brasil
Superfosfato simples	674	1499	770	122	32,5	6,5	Brasil
Termofosfato	755	1575	748	105	24,5	8,3	Brasil
Fosfogesso	1484	3015	970	150	37	6	Brasil
Fosfato monoamônico	177	449	234	43	13,6	8,7	Brasil
Superfosfato triplo	727	1332	556	89	29	13,7	Brasil
Fertilizante ETR	15400	24100	1100	2000	200	25,8	China

Fonte: Adaptado de Ramos et al., (2016a).

Como visto, os fertilizantes fosfatos e o fosfogesso utilizados na agricultura são importantes fontes difusas de ETR para os solos. O consumo de fertilizantes fosfatados pela agricultura mundial é elevado, principalmente em países de clima tropical. No Brasil, sobretudo no Cerrado, há baixa disponibilidade de fósforo às plantas, devido à forte interação deste elemento com o solo pelo processo de fixação (LOPES; GUILHERME, 2016). Assim, intensiva aplicação de fertilizante fosfatado é realizada regularmente para fornecer as necessidades nutricionais para as culturas (ROY et al., 2016; WITHERS et al., 2018). Neste sentido, devido a expansão e intensificação da agricultura brasileira nos últimos 60 anos, o uso total anual de fertilizante mineral fosfatado aumentou de uma média de 0,04 Tg em 1960 para 2,2 Tg em 2016, podendo alcançar 4,6 Tg em 2050 (WITHERS et al., 2018). Essa alta taxa de aplicação de fertilizantes fosfatados, bem como o descarte inapropriado de materiais tecnológicos que contêm ETR podem resultar em aumento dos teores naturais desses elementos nos solos dessas áreas. Dessa forma, estudos ecotoxicológicos com ETR são necessários para esclarecer os possíveis efeitos desses elementos no ambiente solo e plantas.

2.4. Elementos terras raras em plantas

As concentrações dos ETR no ambiente podem influenciar as concentrações desses elementos nas plantas. Os modos de aplicação e as concentrações de cada ETR no ambiente, como o ambiente solo podem influenciar a absorção, translocação e distribuição nas plantas (HU et al., 2004). As plantas possuem mecanismos que regulam a redistribuição de ETR, principalmente os relacionados à presença de barreiras apoplásticas nas raízes, quais são os primeiros obstáculos enfrentados por esses elementos nos vasos condutores do xilema. Geralmente, a distribuição dos ETR em plantas é crescente na seguinte sequência: flor, grãos, fruto < caule < folha < raiz (HU et al., 2004). As folhas das plantas também podem absorver ETR. Nessa situação, as barreiras apoplásticas continuam funcionando como obstáculos à translocação desses elementos para os diferentes órgãos vegetais. Porém, a distribuição desses elementos segue a ordem: semente < fruto < flor < raiz < caule < folha (BRIOSCHI et al., 2012). Dentro das plantas, a maioria dos ETR ficam ligados às paredes celulares (LIU et al., 2012; LIU et al., 2013). No entanto, alguns podem atravessar a membrana celular, acumulando em organelas e em forma de cristais, como "oxalato de ETR" (OLIVEIRA et al., 2015), inclusive em tecidos corticais das raízes, evitando também a translocação para a parte aérea da planta.

Na literatura, há relatos controversos quanto aos efeitos dos ETR nas plantas. Os relatos que sustentam os efeitos benéficos desses elementos nas plantas, quando disponíveis em concentrações ideais estão relacionadas ao aumento da eficiência do uso da água (BABULA et al., 2015), melhoria dos processos fotossintético (GIRALDO et al., 2014; WU, et al., 2014), estímulo à maior atividade do sistema antioxidante enzimático (WU et al., 2014; ZHANG ET AL., 2014), além de ao crescimento e aumento da produtividade das culturas (GUO et al., 2013; SHTANGEEVA, 2014; VILELA et al., 2018).

Por outro lado, há relatos na literatura afirmando efeitos deletérios nas plantas, provocados por elevadas concentrações de ETR no meio de cultivo. Os ETR, quando disponíveis em elevadas concentrações, podem acumular nas células vegetais, causando danos estruturais, perturbação no equilíbrio nutricional (POŠĆIĆ et al., 2017), diminuir a taxa fotossintética e o crescimento das plantas (JIANG et al., 2017), até mesmo provocar a morte celular (XU et al., 2017). Os ETR disponíveis em altas concentrações no solo também são capazes de afetar negativamente o crescimento de diferentes espécies de plantas, como Asclepias syriaca L., Desmodium canadense (L.) DC, Panicum virgatum L, Raphanus sativus L., and Solanum lycopersicum L.) (CARPENTER et al., 2015; THOMAS et al., 2014).

2.5 Cério

O cério (Ce) (Z = 58) é o ETR mais abundante e o 25º elemento mais abundante em massa na crosta terrestre (MIGASZEWSKI; GAŁUSZKA, 2014). Em solos mundiais, este elemento apresenta concentrações variando entre 13 mg kg^{-1} a 273 mg kg^{-1} (RAMOS et al., 2016a). Além disso, o Ce se destaca entre os demais ETR presentes nos fertilizantes minerais fosfatados (Ramos et al., 2016b; TURRA et al., 2011), sendo o elemento de maior capacidade de alterar seus níveis naturais nos solos, quando há aplicação desse tipo de insumo agrícola. Há relatos no Brasil dos níveis de Ce na camada de 0 a 0,4 m terem aumentados até 62% em uma área onde as atividades agrícolas - incluindo a fertilização do solo - eram constantes há pelo menos 20 anos com soja e trigo (NEVES et al., 2018).

Com relação aos efeitos do Ce em organismos vivos, há estudos que relatam o Ce causando toxicidade fisiológica em batata-doce exposta a 20-80 mg Ce L^{-1} (JIANG et al., 2017) e em colza exposta a 0,28-8,96 mg Ce L^{-1} (POŠĆIĆ et al., 2017), através de solução nutritiva. O cério também reduziu o rendimento de rábano quando as folhas foram tratadas com 80-300 µM de Ce (III) (WANG et al., 2017). Além disso, THOMAS et al. (2014) também observaram maior toxicidade do Ce para diferentes espécies de plantas quando cultivadas em solos com pH mais baixo (4,08) em comparação com solos com pH mais alto (6,74).

Pouco se sabe a respeito dos efeitos do Ce no ambiente solo, incluindo efeitos sobre plantas, animais e seres humanos. As leis brasileiras vigentes não mostram valores orientadores estabelecidos que regulamentam os teores desse elemento para os solos do Brasil, como ocorre para os elementos-traço, de acordo com a Resolução 420 do CONAMA (2009). Estes valores orientadores têm como objetivo fornecer parâmetros comparativos que auxiliam na gestão e monitoramento da qualidade do solo e suas possíveis alterações. Especificamente, o valor de prevenção, refere-se ao teor limite de determinada substância no solo, que não afete a sua funcionalidade. Ou seja, é o teor acima do qual, as principais funções do solo podem ser comprometidas, necessitando, portanto, de monitoramento (CONAMA, 2009).

O Brasil é um dos maiores produtores agrícolas mundiais, sendo responsável por fornecer alimentos não somente para o mercado interno, mas também para outras regiões do mundo (OECD/FAO, 2015). Para que este papel continue a ser exercido de maneira correta, é importante desenvolver estudos com base em avaliação de risco ambiental, a fim de se propor, futuramente, valores orientadores de Ce em solos, além de obter informações quanto aos efeitos deste elemento sobre as plantas. Com isso, o Brasil terá a garantia da sustentabilidade dos sistemas de produção agropecuários e a proteção da qualidade do solo.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1: Ecological risk assessment of cerium for tropical agroecosystems

Artigo elaborado de acordo com as normas do periódico *Chemosphere* (versão publicada).

Ecological risk assessment of cerium for tropical agroecosystems

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Abstract

Cerium (Ce) is present in high technology materials and in mineral P fertilizers and the use and discharge of such resources may change the natural status of Ce in the soil environment. Brazilian soils in farming areas are significantly exposed to increased levels of unintentionally-added Ce through intensive input of phosphate fertilizers. The aims of this study were to evaluate the ecotoxicological risk to plants growing in tropical soils contaminated with Ce, as well as to create a database to support future legislation regulating the limits of this element in Brazilian and conceivably other tropical soils. Eight crop species (corn, sorghum, rice, wheat, soybeans, sunflower, radish, and beans) were exposed to a Ce concentration gradient in two typical tropical soils (Oxisol and Inceptsol), and an artificial soil. Our findings showed that among the endpoints measured, Ce phytotoxicity was more pronounced on shoot dry matter than on percent germination and germination speed index. Sensitivity of plants is species specific and our data showed that sunflower and radish exposed to Ce were the most sensitive crop species. Soil properties such as pH, cation exchange capacity, and organic carbon may have influenced the severity of Ce phytotoxicity. Because of that, the Oxisol contaminated with this element caused higher phytotoxicity than the other soils tested. Our risk assessment results (hazardous concentration, HC_5 = 281.6 mg Ce kg^{-1}) support the idea that unintentional Ce input through P fertilizers does not pose a risk to soils of Brazilian agroecosystems.

Keywords: Cerium; rare earth elements; phytotoxicity; environmental safety; phosphate fertilizer.

Highlights

- Sensitivity of plants exposed to Ce is species specific
- Soil properties are influential in determining the severity of Ce phytotoxicity
- Ce in Brazilian tropical soils is not extremely hazardous to crop plants
- Ce indirectly applied to soil by P fertilizers does not pose a risk to agroecosystems

1. Introduction

Rare earth elements (REEs) comprise a group of 17 metals with similar physical and chemical properties and include 15 elements belonging to the lanthanide group, plus scandium and yttrium (IUPAC, 2005). REEs are applied in several industrial segments of high technology, such as military, nuclear, automotive, renewable energy and electronic sectors (Alonso et al., 2012; Binnemans et al., 2013; Dutta et al., 2016). In addition, since the 1980s in China, REEs have been widely applied as fertilizers in agriculture to improve crop yield and quality (Hu et al., 2004; Tyler, 2004; Wang et al., 2008). Since the soil environment is a primary destination of all products and by-products containing REEs (Ramos et al., 2016b), this scenario suggests that these elements are increasing in such ecosystems.

In Brazilian agriculture, there is no report of direct application of REEs through REEs fertilizers, as in China. However, mineral P fertilizers may contain significant levels of REEs in their composition and can be considered as a diffuse source of these elements to the soil environment (Otero et al., 2005; Ramos et al., 2016b; Todorovsky et al., 1997; Turra et al., 2011; Waheed et al., 2011). The typical tropical soil found in much of the Brazilian territory has low fertility and high P fixation (Lopes and Guilherme, 2016), and in order to provide nutritional requirements for crops an intensive application of mineral P fertilizer is regularly performed (Roy et al., 2016; Withers et al., 2018). For this reason, and also because of the extraordinary expansion and intensification of agriculture observed in Brazil in the last 50-60 years, total annual P fertilizer use increased from an average of 0.04 Tg in 1960 to 2.2 Tg in 2016, and is expected to reach up to 4.6 Tg in 2050 (Withers et al., 2018). This high rate of P fertilizers regularly supplemented to the soil may result in significant increases in involuntary REEs additions to soils, increasing the levels of these elements in the environment. Moreover, REEs are increasingly being used in consumer electronics and inappropriate discharge of electronic materials in landfills may also result in significant increases of REE levels in soils of these areas. For these reasons, REEs have been considered "emerging pollutants” (Pagano et al., 2015).

Cerium (Ce) stands out among other REEs in mineral P fertilizers, because it is present in higher concentrations than all others (Ramos et al., 2016a; Turra et al., 2011). Cerium is also the REE with the highest concentrations reported for soils worldwide, i.e., from 13 mg kg^{-1} to 273 mg kg^{-1} (Ramos et al., 2016b). In fact, Ce levels within the 0- to 0.4-m soil layer have been reported to have increased by up to 62% in an area where agricultural activities - including soil fertilization - have been constant for at least 20 years with soybeans and wheat in Brazil (Neves et al., 2018). Furthermore, Ce caused deleterious effects when sweet potato (Jiang et al., 2017) and rapeseed (Pošćić et al., 2017) were exposed to 20-80 mg Ce L^{-1} and 0.28-8.96 mg Ce L^{-1}, respectively, in nutrient solution. Cerium also reduced the yield of horseradish when leaves were treated with 80-300 µM Ce (III) (Wang et al., 2017). Besides Ce, relevant studies addressing the physiological effects of other REEs (e.g., lanthanum) have been recently published in the literature (de Oliveira et al., 2015; Wang et al., 2014; Wang et al., 2016; Zhang et al., 2017; Zhang et al., 2018). Irrespectively of that, the understanding of the biological role of Ce and other REEs is still in its early stages (Skovran and Martinez-Gomez, 2015) and there is limited information in the literature on Ce phytotoxicity tests conducted in tropical soils. Thus, ecological risk assessment studies are necessary to better understand the environmental consequences of enriching soils with this element and to recommend safe values for Ce in the soil environment, particularly for soils of tropical agroecosystems, which are prone to receive high involuntary applications of REEs through P fertilization.

Brazil is one of the world's largest producers and suppliers of food and agricultural products (OECD/FAO, 2015), and because of this is considered a global farm (Tollefson, 2010). However, no studies suggesting guiding values that could be used to regulate Ce levels for Brazilian soils, as occurs for many other trace metals, have been conducted so far. The aim of this study was to evaluate Ce phytotoxicity through risk assessment assays with crop plants and natural Brazilian soils, as well as to create a database to support and guide future legislation regulating the limits of this element in tropical soils. For this purpose, eight crop plants were exposed to a Ce concentration gradient in two typical tropical soils (Oxisol and Inceptsol) found in much of the Brazilian territory, and an artificial soil (standard artificial tropical soil - ATS).

2. Materials and methods

The phytotoxicity experiment conducted here assessed cerium effects on seedling emergence and plant growth in contaminated soils, and followed the OECD 208 guidelines (OECD, 2006). These guidelines recommend the use of an artificial substrate, which we used for comparison with the natural soils from Brazil.

2.1 Plant species

Eight crop plant species were tested in this experiment, as follows: four monocotyledonous - *Zea mays* L. (corn), *Sorghum bicolor* L. (sorghum), *Oryza sativa* L. (rice), *Triticum aestivum* L. (wheat) -, and four eudicotyledonous - *Glycine max* L. (soybeans), *Helianthus annuus* L. (sunflower), *Raphanus sativus* L. (radish), and *Phaseolus vulgaris* L. (beans). These crop plants are all recommended in the OECD guidelines and were selected based on their commercial importance.

2.2 Soils

The artificial tropical soil (ATS) was composed of coconut fiber (10%), kaolinite clay (20%), and fine sand (70%), by dry weight. Coconut fiber is commonly used as a substrate for horticultural purposes as a soilless potting medium and is a component easily found in tropical countries such as Brazil. Besides this, two different types of typical Brazilian tropical soils were used in all assays: an Oxisol collected in Itumirim (21°17’08’’ S, 44°47’43’’ W) and an Inceptsol collected in Lavras (21°13’46’’ S, 44°59’17’’ W), both municipalities located in the State of Minas Gerais, Brazil. Both tropical soils were collected under minimally disturbed tropical semi deciduous forest. The Inceptsol and the Oxisol were the selected soil types based on their representativeness in the Brazilian territory, and because they are commonly found under native vegetation or any anthropic activity, including agriculture.

2.3 Soils preparation and analysis

The soil samples were air dried, homogenized, and sifted with two mm sieves. Chemical and physical characterization of soils were performed according to the methodologies described by the Brazilian Agricultural Research Corporation (EMBRAPA, 2011). Total organic carbon (TOC) contents in soils were determined by the dry combustion method using the Elementar analyzer model Vario TOC Cube. Soil characterization is shown on Table 1. Maximum water holding capacity (WHC) was measured according to the procedures described by ISO 11274 (ISO, 1998).

Table 1. Physical and chemical properties of tested soils.

Soil	pH_{H_2O}	P	K	Ca	Mg	Al	CEC	TOC	Sand	Silt	Clay
		mg dm^{-3}	mg dm^{-3}	cmol$_c$ dm^{-3}	cmol$_c$ dm^{-3}	cmol$_c$ dm^{-3}	cmol$_c$ dm^{-3}	%	%	%	%
Oxisol	5.20	0.88	39.42	0.14	0.10	0.20	1.73	0.80	68	4	28
Inceptsol	6.10	1.14	39.42	2.26	0.13	0.03	4.08	1.09	20	48	32
ATS	5.60	4.89	192.71	1.44	0.44	0.16	3.63	4.00	-	-	-

CEC = Cation exchange capacity; TOC = Total organic carbon

The soils were fertilized three days before starting each assay by application of 300, 200, 150, 75, 15, 50, 0.5, 1.5, 5.0, 0.1, and 5 mg kg^{-1} of N, P, K, Ca, Mg, S, B, Cu, Fe, Mo, and Zn, respectively, by nutritive solution (Malavolta, 1980).

To validate the experimental concentrations as well as the soil contamination procedure, analysis of soil samples tested for the presence of Ce were performed from three replicates of each nominal concentration tested in the wheat test. In order to determine total Ce concentrations, samples of controls and treated soils were initially oven-dried at 60 °C until reaching constant weight, grounded in a agate mortar, and sifted with 150-μm nylon sieves. After that, aliquots of 0.5 g of each sample were taken to digestion according to the USEPA 3051A method (USEPA, 2007). Each aliquot was digested with 5 mL of concentrated nitric acid (HNO_3) in PTFE Teflon® tubes (CEM Corporation, Matthews, NC, USA) using a microwave (MARS-5®, CEM), with a temperature set at 175 °C and at a controlled pressure of 0.76 MPa for 15 min. After digestion, extracts were cooled down at room temperature and then filtered on a filter paper. At the same time of filtration, 5 mL of deionized water were added in the final volume of the extract. After filtration, the extracts were transferred into smaller vials (20 mL) and submitted to analysis. Cerium concentrations were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) – Spectro Blue (Germany). An analytical blank and a standard reference material (Calcareous Soil ERM-CC690®, Institute for Reference Materials and Measurements IRMM, Geel, Belgium) were added throughout the analytical series to substantiate the accuracy of the analytical results obtained. The percent recovery of Ce concentration of tested soils was calculated as: (mean Ce concentration determined – mean Ce concentration determined in control soil)/(nominal concentration).

2.4 Experimental procedure

Cerium concentrations used in the assays followed a geometric progression of 1.7, as follow: 0 (control), 50, 85, 144.5, 245.7, 417.6, 709.9, 1206.9, and 2051.7 mg Ce kg^{-1}. For each Ce concentration, five replicates were used. Cerium chloride heptahydrate ($CeCl_3 \cdot 7H_2O$) from Sigma-Aldrich (CAS Number: 18618-55-8) was the source of cerium used for the preparation of the contaminating solutions of Ce. For each Ce concentration tested, a contaminant solution was prepared separately.

A plastic pot containing 500 g of contaminated soil (Ø120 mm x 78 mm height for the Oxisol and the Inceptsol; Ø120 mm x 110 mm height for the artificial tropical soil, which occupies more volume) consisted of an experimental unit. To contaminate the soil of each experimental unit, contaminant solutions of each expected Ce concentration were pipetted into the plastic bag with 500 g of dry soil. Once contaminated, the soil was homogenized and transferred to a plastic pot. After that, all contaminated soil pots were left to settle for 12 hours before sowing. In each experimental unit a crop species was planted using ten seeds (corn, sorghum, soybeans, sunflower, and beans) or fifteen seeds (rice, wheat, and radish). After sowing, soil humidity was kept at 60-70% of the maximum water holding capacity with deionized water and, after 14 days top-dressing fertilization was performed using 20% of the fertilizer used at the beginning of each test. The experiment consisted of one assay for each plant species, totaling eight assays. Crop species were exposed to Ce from the time seeds were sowed until the end of each assay. The assay ended 21 days after the emergence of ≥ 50% of seedlings in zero concentration treatment (control) for each species. The experiment was carried out under greenhouse conditions with an average temperature of 26±3 °C and natural light, during the Summer season.

Germination (%G) was determined using total seedling emergence counted immediately on the first day of the assay. Germination speed index (GSI) was determined based on the following formula: GSI = ($E_1/D_1 + E_2/D_2 + E_3/D_3 + ... + Ni/Di$), where E_i is the number of new seedlings emerged and D_i is the number of days after sowing the seeds (Maguire, 1962). Germination and GSI were not assessed in beans. At the end of the assay, aboveground parts were harvested and placed in an oven at approximately 70 °C to obtain shoots dry matter (SDM) separately for each experimental unit.

In order to investigate possible partial effects of chlorine (Cl) on plant growth, which was added as accompanying ion of Ce, a test was conducted for each crop, except beans, using calcium chloride ($CaCl_2$). In this test, four Cl concentrations (316.96, 538.82, 915.7, 1557.27 mg Cl kg^{-1}) equivalent to those applied at the four highest concentrations of Ce (245.7, 417.6, 709.9, 1206.9 and 2051.7 mg Ce kg^{-1}) were used (Figure S1 - supplementary material).

2.5 Statistical analysis

Germination data were analyzed using one-way ANOVA followed by the Dunnet test ($p < 0.05$) in order to determine the no observed effect concentrations (NOEC) and the lowest observed effect concentrations (LOEC) when compared to the control treatment. For SDM and GSI, the effective concentration (ECx) of Ce resulting in 25% (EC_{25}) or 50% (EC_{50}) inhibition were derived by concentration-response curves as described by Cedergreen et al. (2005). EC_{50} and concentration-response curves for Ce effect on SDM are presented at Figure S2 - supplementary material.

Risk analysis was developed using the EC_{50} values from SDM for all plants grown on the Oxisol and the Inceptsol, since this endpoint was more susceptible to the deleterious effect of Ce. The derivation of the species sensitivity distribution (SSD) curve (Posthuma et al., 2002) was performed in order to estimate the hazardous concentration (HC_5), which is the Ce concentration that would cause risk to 5% or less of all plant species tested. To assess how the inclusion of data from plants grown on the artificial soil influences the derivation of HC_5, the same analysis was conducted using all the three soils tested (Figure S3 - supplementary material). In addition, the derivation of HC_5 for different soils separately was performed to assess difference of results between each natural soil (Oxisol and Inceptsol) and the artificial soil (Figure S4, S5 and S6 - supplementary material).

To assess the contribution of Cl for the observed effects on plant growth, one-way ANOVA, followed by the Dunnet test ($p<0.05$) were conducted with the SDM data comparing the CI experiment with and without Ce added. All statistical analysis were performed using R (R Development Core Team, 2017).

3 Results

3.1 Ce concentration in soils

The average recovery of Ce in the certified reference sample (ERM®-CC690-calcareous soil; Ce = 49.1 mg kg^{-1}) was 92%, indicating a reliable accuracy for the analytical methodology used for Ce analysis.

Table 2 shows the average of Ce content determined in the control and treated soils. Information concerning the percent recovery of Ce compared to nominal concentrations is reported in Table S1 - supplementary material. In general, recoveries of Ce from each concentration were high (>70% recovery) and ranged from 76% to 121%, except for the nominal concentrations: 50 mg Ce kg^{-1} in the Oxisol and in the artificial soil; 245.7 mg Ce kg^{-1} in the Inceptsol; and 709.9 mg Ce kg^{-1} in the ATS. The control treatment presented considerable level of Ce concentrations, especially in the Inceptsol, which was 1.5 times higher than the Ce concentration observed in the Oxisol, and practically three times higher than the Ce concentration observed in the ATS. As expected, the concentrations studied were above the expected nominal concentrations because of natural Ce concentrations in soils.

Table 2. Mean with standard error (n=5) of cerium (Ce) concentrations (mg Ce kg^{-1} dry soil) in control and treated soils (Oxisol, Inceptsol and artificial tropical soil (ATS) for each expected nominal concentration.

Nominal concentration	Mean determined concentration		
	Oxisol	Inceptsol	ATS
0 – Control	77.37 ± 1.59	116.13 ± 4.74	40.74 ± 4.82
50	109.18 ±12.84	160.49 ± 7.40	108.49 ± 6.58
85	157.46 ± 5.29	182.87 ± 11.25	111.72 ± 1.08
144.5	224.97 ± 14.88	225.69 ± 5.66	192.38 ± 12.26
245.7	272.00 ± 12.60	279.35 ± 20.58	296.44 ± 5.04
417.6	440.92 ± 2.04	507.99 ± 13.65	428.69 ± 50.52
709.9	766.16 ± 52.98	734.36 ± 39.9	996.14 ± 77.35
1,206.9	1,478.92 ± 58.94	1,363.13 ± 32.05	1,413.45 ± 71.70
2,051.7	2,301.13 ± 110.60	2,046.67 ± 74.47	2,521.10 ± 71.39

3.2 Effects on germination

The exposure to Ce decreased %G. However, the LOEC values were found only among the three highest concentrations studied (709.9, 1206.9 and 2051.7 mg Ce kg^{-1}), except for corn grown in the Inceptsol (144.5 mg Ce kg^{-1}) and in the ATS (144.5 mg Ce kg^{-1}), as well as for rice grown in the ATS (245.7 mg Ce kg^{-1}) (Table 3 and Table S3 - supplementary material). On the other hand, Ce did not cause negative effect on %G of sorghum grown in the ATS. In addition, LOEC and NOEC values from %G showed this endpoint to be less sensitive than GSI (Table S2 and Table S4 - supplementary material).

The EC_{25} and EC_{50} values for GSI varied depending on soil types and plant species (Table 4). The Oxisol generally presented the lowest EC values, followed by the Inceptsol and the ATS. Cerium contamination caused more severe negative effects on sunflower and radish GSI than in any other plant species. Differently, rice was the most tolerant plant species to Ce. In fact, most plants grown in the ATS were not sensitive enough to Ce to be able to derive EC values using the GSI endpoint.

Table 3. No observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) estimated for seed germination percentage (%G) exposed to soils (Oxisol, Inceptsol and artificial tropical soil (ATS)) contaminated with cerium (Ce).

Plant Species	Soil	NOEC (mg Ce kg^{-1})	LOEC (mg Ce kg^{-1})	*p*-value[b]
Corn	Oxisol	417.6	709.9	<0.01
	Inceptsol	85.5	144.5	0.02
	ATS	85.5	144.5	<0.01
Sorghum	Oxisol	709.9	1,206.9	<0.01
	Inceptsol	709.9	1,206.9	<0.01
	ATS	None[a]	None[a]	-
Rice	Oxisol	709.9	1,206.9	<0.01
	Inceptsol	709.9	1,206.9	<0.01
	ATS	144.5	245.7	0.03
Wheat	Oxisol	417.6	709.9	<0.01
	Inceptsol	1,206.9	2,051.7	<0.01
	ATS	1,206.9	2,051.7	<0.01
Soybeans	Oxisol	417.6	709.9	<0.01
	Inceptsol	709.9	1,206.9	<0.01
	ATS	1,206.9	2,051.7	<0.01
Sunflower	Oxisol	417.6	709.9	<0.01
	Inceptsol	417.6	709.9	<0.01

	ATS	417.6	709.9	0.04
Radish	Oxisol	417.6	709.9	<0.01
	Inceptsol	709.9	1,206.9	<0.01
	ATS	1,206.9	2,051.7	<0.01

[a] No Ce doses caused statistically significant difference, compared to the control treatment.

[b] *p*-value related to the LOEC.

Table 4. Effect concentration (EC) values estimated from concentration-response curves of eight plant species cultivated on three different soils (Oxisol, Inceptsol and artificial tropical soil (ATS)) contaminated with cerium (Ce). EC_{25} and EC_{50} (mg Ce kg^{-1}) values are shown together with the 95% confidence intervals (within brackets) representing concentrations resulting in 25% and 50% reduction respectively in the shoot dry matter (SDM) and germination speed index (GSI) as compared to control treatment.

Species	Endpoint	Soil	EC_{50}	EC_{25}
Corn	SDM	Oxisol	585.8 (548.3 – 623.2)	448.1 (387.2 – 509.0)
		Inceptsol	1,009.8 (837.5 –1,182.0)	456.4 (236.3 – 676.4)
		ATS	1,190.2 (1,088.1 – 1,292.2)	806.3 (620.3 – 992.3)
	GSI	Oxisol	1,139.9 (1,004.5 – 1,275.3)	677.0 (545.8 – 808.2)
		Inceptsol	VE	1,536.3 (1,155.9 – 1,916.8)
		ATS	N/E	N/E
Sorghum	SDM	Oxisol	347.8 (318.3 – 377.3)	249.1 (213.4 – 284.8)
		Inceptsol	945.3 (843.1 – 1,047.6)	699.2 (584.9 – 814.1)
		ATS	1,262.9 (1,121.9 – 1,403.9)	925.9 (769.1 – 1,082.8)
	GSI	Oxisol	1,010.2 (902.1 – 1,118.3)	729.3 (603.9 – 854.6)
		Inceptsol	VE	1,554.7 (1,207.6 – 1901.8)
		ATS	VE	VE
Rice	SDM	Oxisol	691.8 (628.0 – 755.0)	518.8 (430.2 – 607.3)
		Inceptsol	1,325.2 (1,092.4 – 1,557.9)	717.3 (456.2 – 978.2)
		ATS	2,184.0 (1,849.5 – 2,518.6)	1,475.8 (1,065.0 – 1,885.9)

	GSI	Oxisol	VE	1,693.4 (1,477.8 – 1,909.0)
		Inceptsol	N/E	N/E
		ATS	N/E	N/E
Wheat	SDM	Oxisol	350.6 (315.0 – 383.3)	231.6 (192.5 – 270.7)
		Inceptsol	897.1 (791.1 – 1,003.0)	616.6 (490.4 – 742.7)
		ATS	1,137.2 (1,064.4 – 1,210.1)	826.9 (754.1 – 899.8)
	GSI	Oxisol	923.7 (882.5 – 964.8)	685.8 (639.1 – 732.5)
		Inceptsol	VE	1,758.8 (1,612.6 – 1,904.9)
		ATS	VE	VE
Soybeans	SDM	Oxisol	511.8 (474.2 – 549.3)	370.4 (323.9 – 416.8)
		Inceptsol	949.1 (842.0 – 1,056.1)	616.7 (490.8 – 742.6)
		ATS	1,304.8 (1,213.4 – 1,396.2)	944.6 (828.8 – 1,060.5)
	GSI	Oxisol	964.9 (879.0 – 1,050.9)	758.8 (654.2 – 863.3)
		Inceptsol	VE	1,791.7 (1,549.6 – 2,033.7)
		ATS	N/E	N/E
Sunflower	SDM	Oxisol	303.7 (274.3 – 333.2)	213.0 (183.9 – 242.2)
		Inceptsol	466.3 (412.3 – 520.2)	245.5 (179.4 – 311.7)
		ATS	997.3 (942.0 – 1,052.5)	807.2 (732.9 – 881.6)
	GSI	Oxisol	659.9 (619.6 – 700.2)	572.2 (493.8 – 650.5)
		Inceptsol	1,043.6 (908.8 – 1,178.5)	728.6 (577.6 – 879.6)
		ATS	N/E	N/E
Radish	SDM	Oxisol	331.1 (301.2 – 362.9)	262.8 (226.1 – 299.5)
		Inceptsol	548.6 (482.3 – 614.8)	374.7 (295.7 – 453.7)

		ATS	1,146.3 (993.9 – 1,298.7)	799.8 (591.8 – 1,007.8)
	GSI	Oxisol	856.9 (811.5 – 902.2)	719.3 (674.1 – 764.4)
		Inceptsol	1,336.2 (1,203.2 – 1,469.2)	1,067.3 (894.1 – 1,240.4)
		ATS	N/E	N/E
Beans	SDM	Oxisol	589.4 (544.4 – 634.4)	489.1 (425.9 – 552.3)
		Inceptsol	1,232.1 (1,028.0 – 1,436.3)	777.5 (626.8 – 928.3)
		ATS	1,524.8 (1,330.8 – 1,718.9)	956.4 (764.2 – 1,148.6)

N/E = the effect of Ce could not be estimated.

Value exceeded (VE) = the predicted value exceeded the range of concentrations evaluated in the experiment.

3.3 Effects on shoot dry matter

The LOEC and NOEC values from SDM (Table S5 - supplementary material) showed this endpoint to be more sensitive than %G (Table 3 and Table S3 - supplementary material) and GSI (Table S2 and S4 - supplementary material). The EC values for the SDM were usually much lower than those found for GSI, and they also varied among crop species and soil types (Table 4). Of the eight plant species studied, EC_{25} results showed that radish, wheat, and especially sunflower were highly sensitive to Ce, when planted in the Oxisol and the Inceptsol. However, the EC_{25} values of the plants grown in the ATS were more similar among them. When considering the EC_{50} values for sunflower indicated a higher sensitivity to Ce for this species in all soil types. The EC_{50} values for radish, wheat, and sorghum were only slightly higher than for sunflower in all soils. In contrast, rice showed higher EC_{50} values, regardless of the soil type, and was the most tolerant species for Ce. In general, the EC_{50} and EC_{25} values were smaller in the Oxisol than in the Inceptsol and much smaller than those from the ATS. These EC_{50} values ranged from 303.74 mg Ce kg^{-1} to 691.83 mg Ce kg^{-1}, 466.25 mg Ce kg^{-1} to 1325.18 mg Ce kg^{-1} and 997.26 mg Ce kg^{-1} to 1524.81 mg Ce kg^{-1} when the plants were grown on the Oxisol, the Inceptsol and the ATS, respectively.

3.4 Influence of chloride

The highest concentrations of calcium chloride tested tended to decrease plant growth, especially the concentrations corresponding to 1206.9 and 2051.7 mg Ce kg^{-1} (Figure S1 - supplementary material). Those effects, however, were generally smaller when compared to the observed effects of $CeCl_3$ at equivalent concentrations.

3.5 Risk assessment

The SSD curves and the HC_5 value derived for Ce based on data from the Oxisol and the inceptisol are shown in Figure 1. The HC_5 estimated through the SSD curves was 281.6 mg Ce kg^{-1} dry soil. When the ATS was included in the analysis, the HC_5 estimated was 329.2 mg Ce kg^{-1} (Figure S3 - supplementary material). In addition, the HC_5 estimated for each soil separately was 262.1 mg Ce kg^{-1} in the Oxisol, 478.5 mg Ce kg^{-1} in the Inceptsol and 878.7 mg Ce kg^{-1} in the ATS (Figure S4, S5 and S6, respectively- supplementary material).

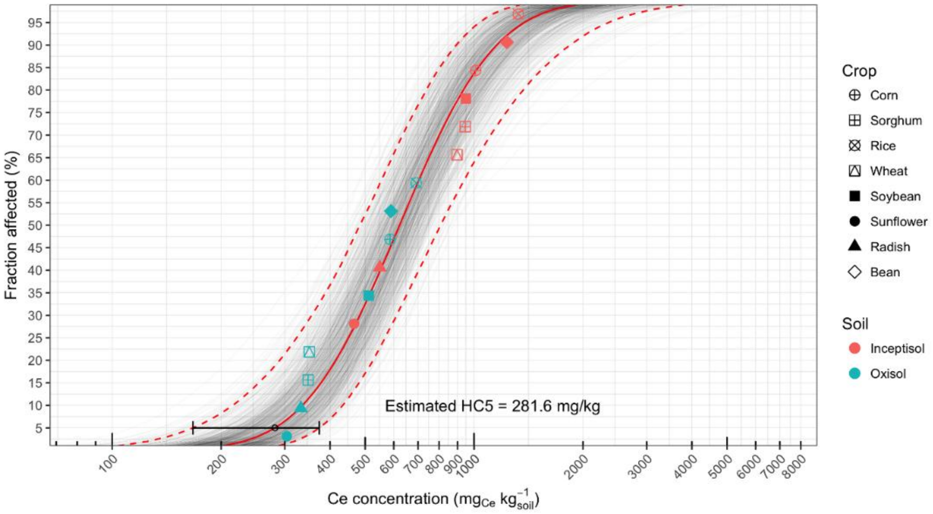

Figure 1. Species sensitivity distribution (SSD) and hazardous concentration that would be protecting at least 5% of species (HC_5) estimated from EC_{50} (concentrations resulting in 50% reduction) values using shoot dry matter endpoint for eight plant species cultivated on Oxisol and Inceptsol contaminated with cerium (Ce).

4 Discussion

4.1 Cerium effect on plants

The effect of trace elements, such as Ce, is variable according to each plant species, because of their specific anatomical and metabolic characteristics, which generates different tolerance mechanisms amongst them (Assad et al., 2017; Bonanno, 2013; Feleafel and Mirdad, 2013; Hameed et al., 2016; Memon and Schröder, 2009; Rizwan et al., 2018; Yang et al., 2017). In addition, plants (e.g., wheat and rice) belonging to same family (i.e., Poaceae) and possessing the same metabolism (C3 metabolism), presented different sensitivities to trace elements (Kim et al., 2018), such as Ce (Shtangeeva, 2014). This indicates that the relation between structure and physiology, which is peculiar to each plant, may result in more specific characteristics with respect to its mechanisms of tolerance to a certain element (Kim et al., 2018, Rizwan et al., 2018; Yang et al., 2017), such as Ce (Shtangeeva, 2014).

The SDM was the most sensitive endpoint measured in this study. Similar results were reported by Thomas et al. (2014), using plants (common milkweed (*Asclepias syriaca* L.), showy ticktrefoil (*Desmodium canadense* (L.) DC.), switchgrass (*Panicum virgatum* L.), radish (*Raphanus sativus* L.) and tomato (*Solanum lycopersicum* L.)) tested with Ce. Another study with other REEs (praseodimium, neodymium and samarium) also demonstrated that SDM was the most sensitive endpoint (Carpenter et al., 2015) for assessing phytotoxicity.

Information in the literature on the mechanism of action of Ce in plants is still incomplete. However, it is quite possible that the deleterious effects observed in this study may have occurred due to the accumulation of Ce in cells, causing structural damage and disturbance in the nutritional balance, as reported elsewhere (Xue et al., 2012; Pošćić et al., 2017). Other studies stated that Ce may decrease photosynthesis and growth (Jiang et al., 2017), as well as cause cell death (Xu et al., 2017).

Hormesis effects on the tested plant species were not observed. However, other studies have reported positive effects of Ce in low concentrations, stating that Ce has the ability to prevent the inhibition of the synthesis of photosynthetic pigments and to improve photosynthesis (Zhou et al., 2011; Yin et al., 2009), as well as to induce growth (Ma et al., 2014; Shyam and Aery, 2012) and increase crop yields (Vilela et al., 2018; Hu et al., 2004). Probably, the wide range between the concentrations tested and the short experimental period (21 days) were not sufficient to demonstrate positive Ce response in the analyzed endpoints, if there is such a positive effect.

Finally, the chloride applied in soils as an accompanying ion for Ce may have caused part of the observed negative effects on plants, since the calcium chloride test showed negative effects on plants at the highest tested concentrations (Figure S1 - supplementary material). Therefore, especially when the application of $CeCl_3$ resulted in small negative effects (e.g., 10% decrease or less) on plant growth, it is difficult to distinguish whether that effect was due to Ce or to Cl. However, at higher concentrations of $CeCl_3$, the reductions in plant growth were clearly due to Ce (Table 4 and Table S2). Excess chloride can induce severe physiological dysfunctions, impairing nutrient uptake, protein biosynthesis, and plant photosynthesis (Geilfus, 2018). In this way, the EC values may reflect an overestimation of Ce phytotoxicity, due to the partial contribution of Cl, especially at the concentrations causing smaller reductions in plant growth, and these values should be taken as conservative estimates.

4.2 Ce concentration in background soils

Ce natural concentrations in the Oxisol and mainly in the Inceptsol were higher than the average abundance of 64 mg Ce kg^{-1} in the Upper Continental Crust (UCC) (Taylor and McLennan, 1995) and of 56.7 mg Ce kg^{-1} estimated in soils worldwide (Kabata-Pendias and Pendias, 2011). However, Ce concentrations found in this study are closer to average concentration of 87.12 mg kg^{-1} (range: 0.22 – 418.75 mg Ce kg^{-1}) and 82 mg Ce kg^{-1} (range: 15 – 286 mg Ce kg^{-1}) presented for Brazilian soils by Sá Paye et al. (2014), as well as those found by Ramos et al. (2016b), respectively. According to Sá Paye et al. (2014), the total content of Ce and other REE naturally found in soils basically depends on the parent material. Brazil occupies a large territory in South America, characterized by a diversity of soil types formed from different parent material. Thus, natural content of Ce in Brazilian soils exhibits a wide variability and may be higher, such as those in this study, or lower than the average content estimated in UCC and soils worldwide.

4.3 Soil properties and Ce phytotoxicity

Bioavailability and uptake of Ce and other REEs by plants can be influenced by soil physical-chemical attributes, such as pH, cation exchange capacity (CEC), and soil organic carbon content. In general, lower CEC induces desorption and, consequently, increases Ce availability in soil. In the present study, the CEC in the Oxisol was 2.4- and 2.1-fold lower than those found for the Inceptsol and the ATS, respectively. Thus, the low CEC in the Oxisol can be one of the attributes explaining the higher Ce phytotoxicity observed for this soil in this study. In the same way, Li et al. (2001) observed that the CEC of typical Chinese soils was negatively correlated with Ce desorption.

Low soil pH is a favorable condition to desorption of Ce and other REEs from the soil mineral matrix to soil solution, thus increasing the availability of these elements to plants (Cao et al., 2001; Diatloff et al., 1996; Loell et al., 2011). In the current study, the Oxisol presented slightly lower pH values than the other soils; however, since pH is a logarithmic value, this small difference can be considered quite significant which may have contributed to higher Ce availability and, consequently, a more severe phytotoxicity in this soil. In another study, Cheng et al. (2015) reported that low pH (test ranging from 3.75 to 8.23) and CEC (5.08 – 35.8 $cmol_c$ kg^{-1}) values found in different types of orchard soils from a typical Chinese area of orange planting induced higher uptake of Ce and other REEs by the orange trees. Furthermore, Thomas et al. (2014) also observed higher toxicity of Ce to different plant species when grown in soils with lower pH (4.08) compared to soils with higher pH (6.74).

The organic matrix also contributes to soil geochemical and biochemical processes and is important for the behavior of the REEs. This is due to negatively charged organic functional groups that increase soil CEC, especially when it comes to Brazilian soils. According to Souza et al. (2007), these soils (e.g., Oxisols) have a predominance of low-activity clay minerals and oxides of Al and Fe, which are an abundant source of positive charges. The total organic carbon (TOC) content in the ATS was 5.0 and 3.6 times higher than the TOC content in the Oxisol and the Inceptsol, respectively. This higher TOC content in the ATS may have decreased the availability of Ce to plants and, subsequently, alleviated Ce phytotoxicity. Similar results were observed by Wen et al. (2002), where soils with higher organic matter content were generally related to the high amount of Ce adsorption to soils. In contrast, a previous study have shown that soil organic carbon content did not result in significant effects on the bioavailability of soil REEs (Cheng et al., 2015).

4.4 Risk assessment

A few risk assessment studies can be found in the literature for Ce and other REEs. A study with lanthanum showed a value of HC_5 of 49 mg kg^{-1} dry soil; however it was derived using EC_{10} data from soil invertebrates, bacteria and plants (Li et al., 2018). Although both elements - La and Ce - are REEs and have similar physical and chemical characteristics, the results were not similar, probably because these authors used lower values (EC_{10}) and different organisms to derive the HC_5.

The HC_5 estimated for the ATS separately was much higher than for both natural soils. In addition, there was a HC_5 overestimation when plant data grown in the ATS were included in the risk analysis, which would justify the exclusion of ATS for estimating the HC_5, and as a result, improving our confidence in assessing the impacts of Ce on plants. Despite the conservatism used in this risk assessment, there is a great difference of Ce concentrations found between the natural contents reported for this element in Brazilian tropical soils - 82 mg Ce kg^{-1} soil (Ramos et al., 2016b) and 87.13 mg Ce kg^{-1} soil (Sá Paye et al., 2014) - and the HC_5 (281.6 mg Ce kg^{-1}) found in this study, which is the safety limit value estimated for our studied soils.

As mentioned above, Brazilian tropical soils/agroecosystems generally require intensive application of inorganic phosphate fertilizer to obtain adequate crop yields. The Brazilian average annual rate of P mineral fertilizer applied to crops is 25 kg P ha^{-1} yr^{-1} (Withers et al., 2018). In addition, the Ce levels in widely used P fertilizers such as single superphosphates (SSP) can be within a range of 40.2 to 3,934 mg Ce kg^{-1} (Turra et al., 2011; Ramos et al., 2016a; Ramos et al., 2016b). Considering the application of an average rate of 25 kg P ha^{-1} yr^{-1}, which is equivalent to 57.25 kg P_2O_5 ha^{-1} yr^{-1}, a typical SSP fertilizer with 18% P_2O_5 and a high Ce content (1,866 mg Ce kg^{-1} - Ramos et al., 2016a) would provide an addition of 593.5 g Ce ha^{-1} yr^{-1}. Taking into account the cultivated soil layer of 0 - 0.2 m, this amount of Ce is equivalent to an input of 0.3 mg Ce kg^{-1} soil annually. In another scenario typically found in the Brazilian Cerrado, considering a double-crop system (soybeans followed by corn) during the same agricultural year, the recommendations of phosphate fertilization can reach up to 100 kg P_2O_5 ha^{-1} for soybeans cultivation (Sousa et al., 2004) and 60 kg P_2O_5 ha^{-1} for corn cultivation (Raij et al., 1997). In this scenario, an average rate of 1.66 kg Ce ha^{-1} could be added every year, which is equivalent to 0.83 mg Ce kg^{-1} soil in the cultivated soil layer (0 - 0.2 m).

The estimated values of average Ce rates added in Brazilian agroecosystems may be lower when using phosphate fertilizers with lower Ce content or a higher P_2O_5 concentration. In addition, most Brazilian Cerrado soils are naturally acidic (Lopes and Guilherme 2016), and, when these are under agriculture, usually liming with calcium carbonate or magnesium carbonate is performed in order to neutralize pH and increase CEC, as well as to improve soil fertility (Sánchez and Salinas, 1981; Lopes and Guilherme, 2016). In this sense, the intensity of Ce phytotoxicity is minimized and as such, it is reasonable to expect that Brazilian agriculture systems with intensive P fertilizer applications cannot be considered as a risk to soil environment with respect to their Ce inputs.

5 Conclusion

This study showed that the sensitivity of plants exposed to Ce was species specific, and that sunflower and radish were the most sensitive species. Tropical soils, especially the Oxisol, have greater potential to induce Ce phytotoxicity in plants than the ATS, which can have consequences in toxicity tests where an artificial substrate is recommended in guidelines such as OECD (OECD, 2006). The TOC, pH, and the CEC of each soil appear to be essential properties in determining the severity of Ce phytotoxicity.

Our risk assessment results (HC_5 = 281.6 mg Ce kg^{-1}) support the idea of Ce not being extremely hazardous to terrestrial plants. However, additional studies are needed to investigate a larger number of plant species, including wild noncrop species, under soils with different properties in order to obtain more robust results about the impacts of Ce contamination on crop growth and the natural vegetation. Furthermore, periodic monitoring should be carried out in areas that receive an intense application of mineral P fertilizer or landfill sites with improperly discarded electronic waste. Finally, this study provided essential reference data values that can be used in ecotoxicological screening for Ce in tropical soils.

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Supplementary material

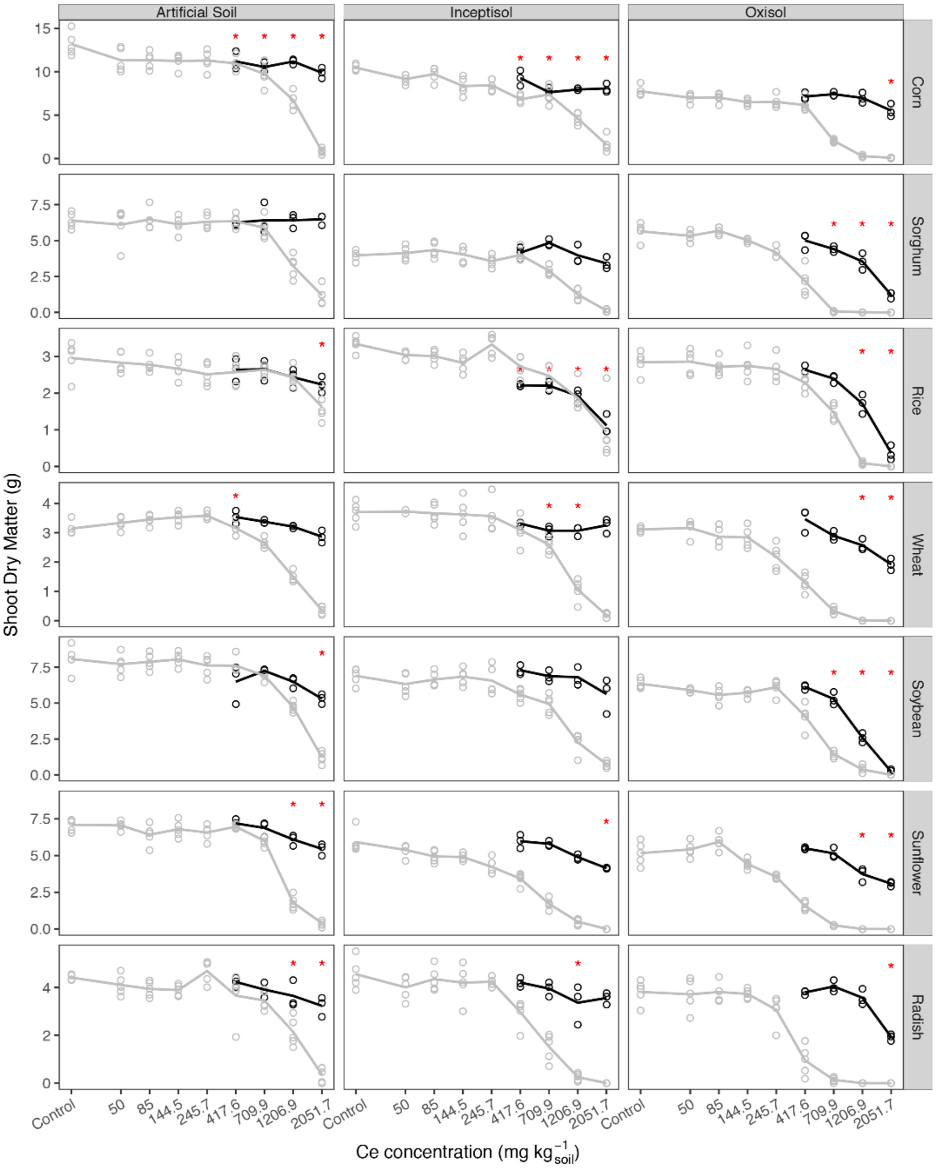
Artificial Soil
Inceptisol
Oxisol
Corn
Sorghum
Rice
Wheat
Soybean
Sunflower
Radish
Shoot Dry Matter (g)
Ce concentration (mg kg$_{soil}^{-1}$)
Control
50
85
144.5
245.7
417.6
709.9
1206.9
2051.7
0
5
10
15
0.0
2.5
5.0
7.5
0
1
2
3
4
*

Figure S1. Effects of chloride (Cl) on shoot dry matter (SDM) of plants grown on soils (Oxisol, Inceptsol and artificial soil (ATS)) contaminated with four Cl concentrations (316.96, 538.82, 915.7, 1557.27 mg Cl kg^{-1}), equivalent to those applied at the four highest concentrations of cerium (245.7, 417.6, 709.9, 1206.9 and 2051.7 mg Ce kg^{-1}). SDM for plants tested in chloride without Ce added are represented by dark black lines.

* Concentration that presented statistically significant difference compared to the control treatment (Dunnet test; $p < 0.05$).

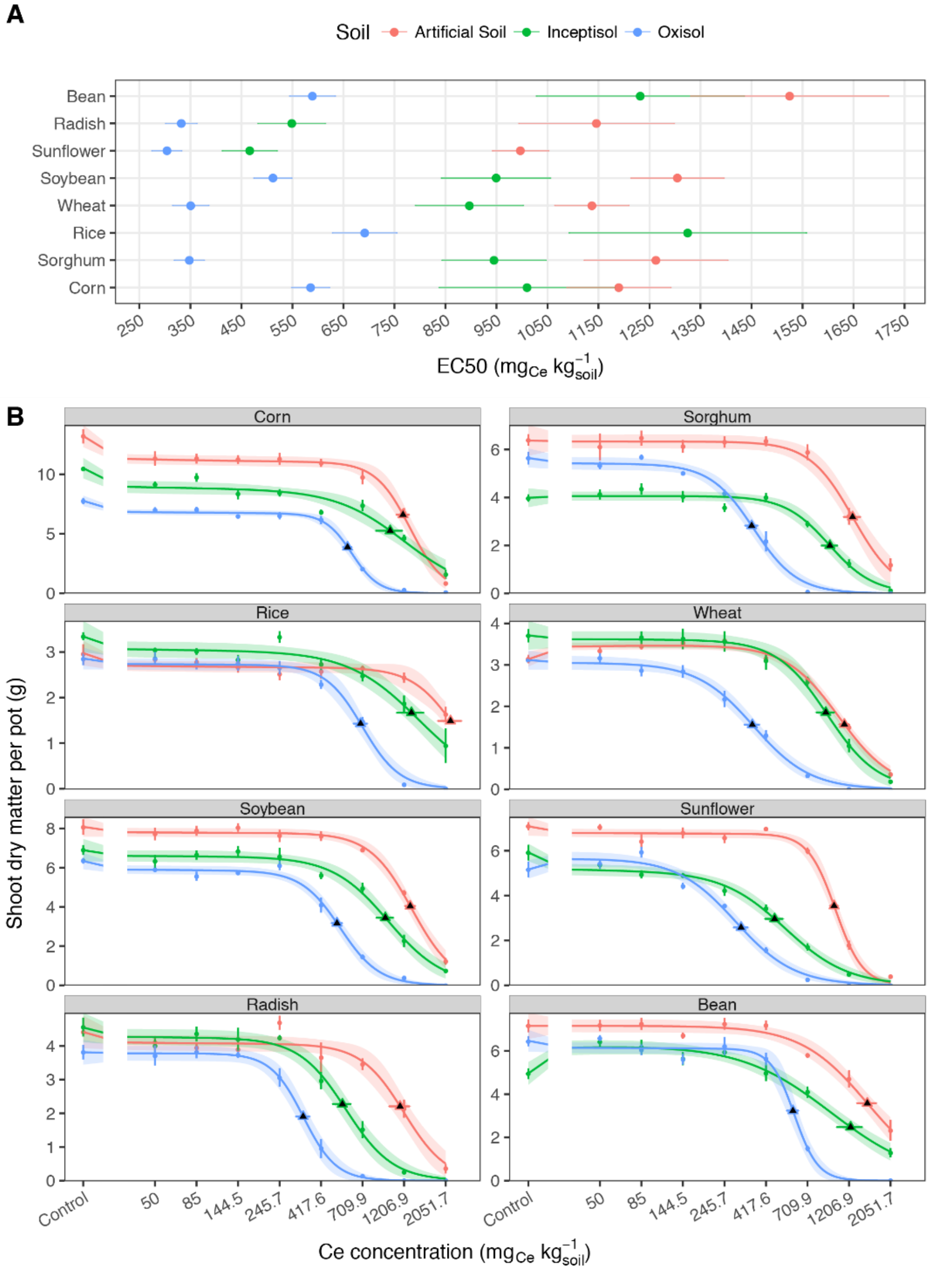
A
Soil
Artificial Soil
Inceptisol
Oxisol
Bean
Radish
Sunflower
Soybean
Wheat
Rice
Sorghum
Corn
250
350
450
550
650
750
850
950
1050
1150
1250
1350
1450
1550
1650
1750
EC50 ($mg_{Ce}\ kg^{-1}_{soil}$)
B
Corn
Sorghum
Rice
Wheat
Soybean
Sunflower
Radish
Bean
Shoot dry matter per pot (g)
Control
50
85
144.5
245.7
417.6
709.9
1206.9
2051.7
Ce concentration ($mg_{Ce}\ kg^{-1}_{soil}$)

Figure S2. Concentration−response models for the cerium (Ce effect on eight crops grown in three soil types. A) For each combination of crop (y−axis) and soil (indicated by color according to the legend at the top), the estimated EC_{50} (x−axis) (concentrations resulting in 50% reduction of the measured variable) is represented by the dots, and the 95% confidence interval is represented by the horizontal bars. B) Representation of concentration−response models for each combination of crops (panels) and soil (indicated by color according to the legend at the top), with fitted values indicated by solid lines, 95% confidence interval indicated by shaded regions, and means for each treatment represented by dots, and their standard errors (n = 4) indicated by vertical bars. The x−axis, indicating the Ce doses, is in log (base 2) scale. The estimated EC_{50} and their confidence intervals are represented by the black triangles and their horizontal error bars, respectively.

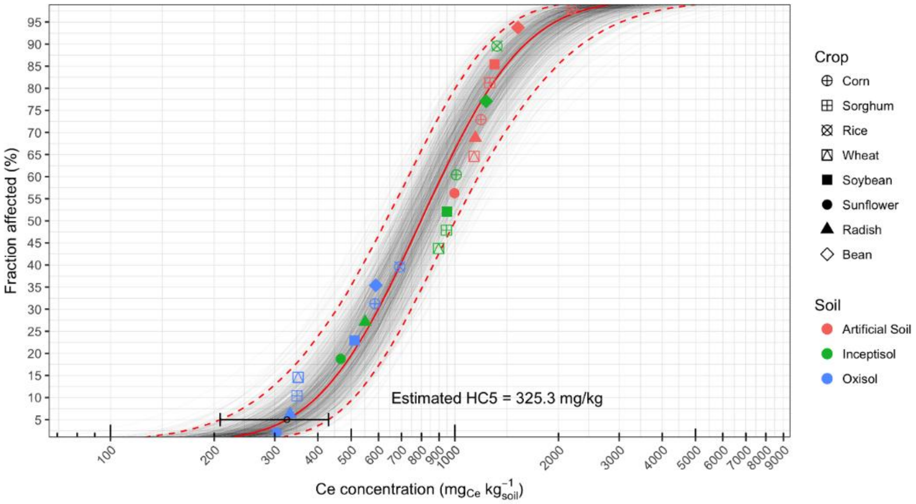

Figure S3. Species sensitivity distribution (SSD) and hazardous concentration that would be protecting at least 5% of species (HC_5) estimated from EC_{50} (concentrations resulting in 50% reduction) values of shoot dry matter (SDM) endpoint for eight plant species cultivated on Oxisol, Inceptsol and artificial soil (ATS) contaminated with cerium (Ce).

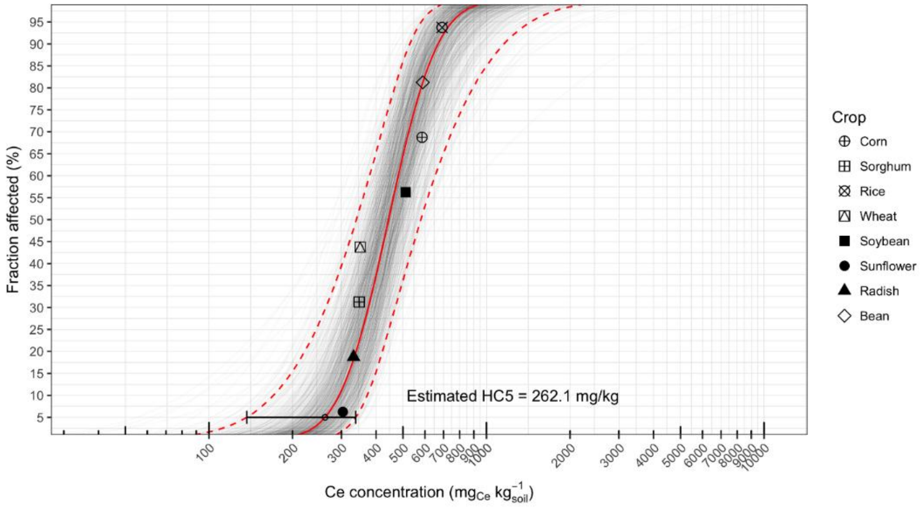

Figure S4. Species sensitivity distribution (SSD) and hazardous concentration that would be protecting at least 5% of species (HC_5) estimated from EC_{50} (concentrations resulting in 50% reduction) values of shoot dry matter endpoint for eight plant species cultivated on Oxisol contaminated with cerium (Ce).

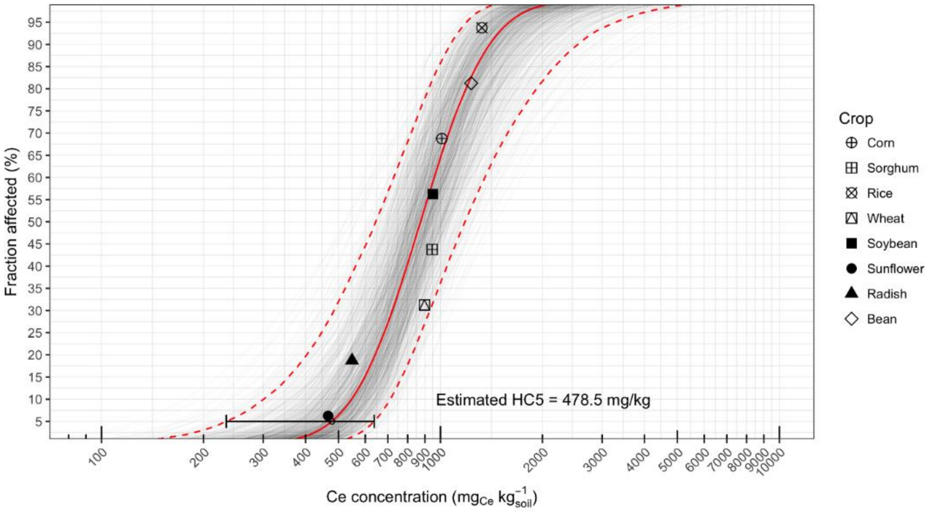

Figure S5. Species sensitivity distribution (SSD) and hazardous concentration that would be protecting at least 5% of species (HC_5) estimated from EC_{50} (concentrations resulting in 50% reduction) values of shoot dry matter endpoint for eight plant species cultivated on Inceptsol contaminated with cerium (Ce).

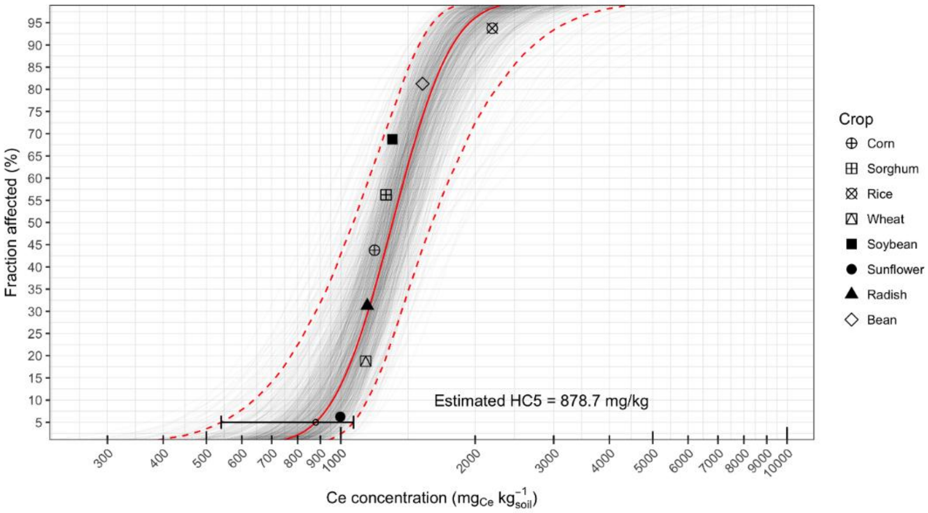

Figure S6. Species sensitivity distribution (SSD) and hazardous concentration that would be protecting at least 5% of species (HC_5) estimated from EC_{50} (concentrations resulting in 50% reduction) values of shoot dry matter endpoint for eight plant species cultivated on artificial tropical soil (ATS) contaminated with cerium (Ce).

Table S1. Recovery of cerium (Ce) on treated soils (Oxisol, Inceptsol and artificial tropical soil (ATS)) as compared to the expected nominal concentration.

Nominal concentration	% recovery*		
	Oxisol	Inceptsol	ATS
0 - Control	-	-	-
50	64	89	135
85	94	79	84
144.5	102	76	105
245.7	79	66	104
417.6	87	94	93
709.9	97	87	135
1,206.9	116	103	114
2,051.7	108	94	121

* Percent recovery was based on measured Ce concentrations minus Ce concentration in control soil divided by nominal concentration

Table S2. No observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) estimated for shoot dry matter (SDM) and germination speed index (GSI) of plant species cultivated on soils (Oxisol, Inceptsol and artificial tropical soil (ATS)) contaminated with cerium.

Plant Species	Endpoint	Soil	NOEC (mg kg^{-1})	LOEC (mg kg^{-1})	*p*-value[b]
Corn	SDM	Oxisol	85.0	144.5	< 0.01
		Inceptsol	85.0	144.5	< 0.01
		ATS	0.0	50.0	0.04
	GSI	Oxisol	417.6	709.9	< 0.01
		Inceptsol	245.7	417.6	< 0.01
		ATS	1,206.9	2,051.7	< 0.01
Sorghum	SDM	Oxisol	144.5	245.7	< 0.01
		Inceptsol	417.6	709.9	< 0.01
		ATS	709.9	1,206.9	< 0.01
	GSI	Oxisol	417.6	709.9	0.01
		Inceptsol	1,206.9	2,051.7	< 0.01
		ATS	None[a]	None[a]	-
Rice	SDM	Oxisol	245.7	417.6	0.01
		Inceptsol	0.0	50.0	0.03
		ATS	1,206.9	2,051.7	< 0.01
	GSI	Oxisol	1,206.9	2,051.7	< 0.01
		Inceptsol	None[a]	None[a]	-
		ATS	144.5	245.7	< 0.01
Wheat	SDM	Oxisol	144.5	245.7	< 0.01
		Inceptsol	417.6	709.9	< 0.01
		ATS	85.0	144.5	0.01
	GSI	Oxisol	417.6	709.9	< 0.01
		Inceptsol	1,206.9	2,051.7	< 0.01
		ATS	1,206.9	2,051.7	0.01
Soybeans	SDM	Oxisol	50.0	85.0	0.04
		Inceptsol	245.7	417.6	0.01

		ATS	417.6	709.9	0.01
	GSI	Oxisol	417.6	709.9	< 0.01
		Inceptsol	1,206.9	2,051.7	< 0.01
		ATS	None[a]	None[a]	-
Sunflower	SDM	Oxisol	144.5	245.7	< 0.01
		Inceptsol	144.5	245.7	< 0.01
		ATS	417.6	709.9	< 0.01
	GSI	Oxisol	417.6	709.9	< 0.01
		Inceptsol	417.6	709.9	< 0.01
		ATS	1,206.9	2,051.7	< 0.01
Radish	SDM	Oxisol	245.7	417.6	< 0.01
		Inceptsol	245.7	417.6	< 0.01
		ATS	50.0	85.0	0.02
	GSI	Oxisol	417.6	709.9	< 0.01
		Inceptsol	709.9	1,206.9	< 0.01
		ATS	1,206.9	2,051.7	< 0.01
Beans	SDM	Oxisol	417.6	709.9	< 0.01
		Inceptsol	709.9	1,206.9	< 0.01
		ATS	417.6	709.9	0.01

[a] No concentration caused statistically significant difference, compared to the control treatment.

[b] *p*-value related to the LOEC.

Table S3. Mean and standard error of seed germination percentage per pot of plant species cultivated on soils (Oxisol, Inceptsol and artificial tropical soil (ATS)) contaminated with cerium (Ce).

Plant	Soil	Ce Concentration (mg kg^{-1})								
		0 - Control	50	85	144.5	245.7	417.6	709.9	1206.9	2051.7
Corn	Oxisol	62.00±3.74	56.00±5.10	48.00±3.74	52.00±3.74	44.00±5.10	36.00±5.10	16.00±2.45***	6.00±4.00***	0.00***
	Inceptsol	68.00±3.74	60.00±3.16	56.00±5.10	50.00±4.47*	50.00±4.47*	40.00±3.16***	38.00±2.00***	24.00±2.45***	14.00±2.45***
	ATS	76.00±2.45	60.00±4.47	66.00±5.10	42.00±3.74**	48.00±8.60*	40.00±5.48**	50.00±4.47	50.00±3.16	22.00±7.35***
Soybeans	Oxisol	60.00±3.16	66.00±2.45	58.00±5.83	56.00±10.30	62.00±5.83	40.00±6.32	16.00±7.48***	8.00±2.00***	0.00***
	Inceptsol	56.00±5.10	50.00±7.07	64.00±10.30	72.00±5.83	68.00±2.00	58.00±9.70	52.00±4.90	28.00±5.83**	16.00±4.00***
	ATS	56.00±4.00	60.00±6.32	62.00±2.00	52.00±10.20	40.00±5.48	62.00±6.63	62.00±9.70	56.00±8.72	30.00±6.32*

Sorghum	Oxisol	82.00±3.74	82.00±4.90	82.00±4.90	62.00±3.74	72.00±8.00	74.00±5.10	50.00±4.47	6.00±6.00***	0.00***
	Inceptsol	80.00±3.16	78.00±4.90	70.00±4.47	80.00±4.47	76.00±4.00	68.00±3.74	70.00±8.94	48.00±5.83**	0.00***
	ATS	66.00±4.00	60.00±12.25	80.00±7.75	76.00±5.10	76.00±5.10	72.00±6.63	72.00±8.00	62.00±6.63	52.00±8.00
Sunflower	Oxisol	80.00±5.48	80.00±5.48	76.00±6.00	78.00±7.35	68.00±5.83	68.00±3.74	0.00***	0.00***	0.00***
	Inceptsol	88.00±3.74	86.00±5.10	84.00±4.00	84.00±2.45	72.00±8.00	90.00±5.48	46.00±5.10**	8.00±4.90***	4.00±2.45***
	ATS	94.00±4.00	88.00±3.74	88.00±3.74	90.00±3.16	82.00±6.63	88.00±3.74	72.00±7.35*	68.00±5.83**	40.00±5.48***
Radish	Oxisol	77.33±5.42	73.33±2.11	82.67±4.00	88.00±3.89	78.67±3.89	76.00±1.63	46.67±2.11**	6.67±3.65***	0.00***
	Inceptsol	76.00±4.52	81.33±3.27	72.00±4.90	74.67±6.80	68.00±6.46	78.67±1.33	56.00±4.99	38.67±6.46***	2.67±1.63***

	ATS	82.67±3.40	84.00±2.67	85.33±4.90	73.33±7.89	76.00±4.52	78.67±3.27	81.33±4.90	73.33±5.96	34.67±9.75***
Rice	Oxisol	78.67±4.42	81.33±5.73	89.33±6.18	78.67±2.49	81.33±3.89	82.67±6.86	86.67±3.65	38.67±5.33***	2.67±2.67***
	Inceptsol	77.33±4.99	78.67±3.27	76.00±3.40	77.33±4.99	80.00±3.65	72.00±4.42	66.67±4.22	41.33±7.42**	30.67±12.75***
	ATS	89.33±4.52	84.00±4.52	85.33±2.49	73.33±2.98	69.33±4.52*	69.33±2.67*	81.33±5.33	82.67±4.52	72.00±9.29
Wheat	Oxisol	98.67±1.33	96.00±2.67	97.33±2.67	98.67±1.33	94.67±3.89	90.67±3.40	49.33±4.99***	12.00±3.89***	0.00***
	Inceptsol	94.67±1.33	94.67±1.33	98.67±1.33	100.00±0.00	96.00±2.67	94.67±3.27	97.33±1.63	74.67±6.11	29.33±10.02***
	ATS	90.67±4.00	96.00±1.63	93.33±5.16	97.33±1.63	97.33±1.63	97.33±1.63	97.33±1.63	86.67±2.11	60.00±6.32***

* significant at p < 0.05; ** significant at p < 0.01; *** significant at p < 0.001 compared to the control treatment according to Dunnet test.

Table S4. Mean and standard error of germination speed index per pot of plant species cultivated on soils (Oxisol, Inceptsol and artificial tropical soil (ATS)) contaminated with cerium (Ce).

Plant	Soil	Ce Concentration (mg kg^{-1})								
		0 - Control	50	85	144.5	245.7	417.6	709.9	1206.9	2051.7
Corn	Oxisol	2.09±0.08	2.08±0.15	1.99±0.07	2.10±0.05	2.02±0.05	1.91±0.05	1.48±0.05***	0.98±0.09***	0.48±0.08***
	Inceptsol	2.28±0.03	2.15±0.05	2.15±0.07	2.09±0.06	2.15±0.03	1.96±0.05**	2.01±0.03**	1.80±0.10***	1.57±0.03***
	ATS	2.31±0.06	2.13±0.07	2.08±0.17	1.90±0.12	2.06±0.09	1.93±0.08	2.01±0.09	2.01±0.08	1.41±0.22***
Soybeans	Oxisol	1.69±0.05	1.67±0.02	1.62±0.12	1.73±0.09	1.80±0.04	1.53±0.08	1.37±0.04***	0.45±0.12***	0.00***
	Inceptsol	1.72±0.05	1.68±0.06	1.81±0.05	1.86±0.03	1.76±0.05	1.72±0.06	1.77±0.04	1.58±0.08	1.13±0.11***
	ATS	1.54±0.08	1.57±0.14	1.68±0.04	1.58±0.09	1.48±0.06	1.66±0.08	1.62±0.07	1.68±0.06	1.44±0.04

Sorghum	Oxisol	2.18±0.10	2.26±0.10	2.31±0.05	2.09±0.04	2.21±0.10	2.01±0.12	1.64±0.13*	0.89±0.12***	0.00***
	Inceptsol	2.11±0.04	2.13±0.10	2.20±0.07	2.23±0.12	2.12±0.08	1.99±0.08	2.13±0.15	1.80±0.10	1.25±0.19***
	ATS	1.96±0.12	1.81±0.24	2.19±0.12	2.18±0.05	2.10±0.06	2.12±0.14	2.07±0.17	1.90±0.10	1.48±0.10
Sunflower	Oxisol	1.42±0.09	1.49±0.07	1.50±0.06	1.40±0.06	1.40±0.05	1.40±0.05	0.52±0.03***	0.00***	0.00***
	Inceptsol	1.52±0.06	1.48±0.06	1.44±0.06	1.48±0.05	1.39±0.09	1.57±0.05	1.06±0.11**	0.63±0.13***	0.13±0.04***
	ATS	1.60±0.04	1.56±0.04	1.56±0.05	1.60±0.03	1.52±0.04	1.52±0.05	1.36±0.07	1.37±0.09	1.15±0.10***
Radish	Oxisol	2.62±0.06	2.69±0.05	2.61±0.09	2.80±0.10	2.65±0.09	2.64±0.07	2.01±0.09***	0.29±0.12***	0.00***
	Inceptsol	2.48±0.16	2.50±0.12	2.41±0.13	2.42±0.12	2.30±0.15	2.55±0.08	2.19±0.18	1.58±0.21***	0.21±0.06***

	ATS	2.65±0.09	2.74±0.09	2.83±0.05	2.58±0.17	2.61±0.10	2.60±0.06	2.77±0.08	2.63±0.09	1.26±0.36**
Rice	Oxisol	2.06±0.13	2.09±0.14	2.30±0.14	2.16±0.05	2.29±0.06	2.32±0.08	2.27±0.06	1.97±0.10	1.16±0.07***
	Inceptsol	2.06±0.10	2.10±0.08	2.02±0.08	2.04±0.11	2.23±0.04	2.10±0.06	2.17±0.07	2.15±0.03	1.91±0.12
	ATS	2.28±0.12	2.21±0.09	2.26±0.08	2.02±0.09	1.84±0.12*	2.04±0.05	2.10±0.09	2.23±0.10	2.24±0.07
Wheat	Oxisol	2.98±0.02	2.97±0.03	2.93±0.07	2.96±0.04	2.94±0.04	2.88±0.05	2.10±0.09***	0.89±0.08***	0.00***
	Inceptsol	2.93±0.03	2.96±0.02	2.98±0.02	2.99±0.01	2.88±0.08	2.89±0.07	2.94±0.05	2.70±0.09	1.85±0.15***
	ATS	2.82±0.06	2.95±0.04	2.87±0.09	2.94±0.04	2.91±0.06	2.93±0.04	2.95±0.03	2.90±0.03	2.31±0.14*

* significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$ compared to the control treatment according to Dunnet test.

Table S5. Mean and standard error of shoot dry matter (g per pot) of plant species cultivated on soils (Oxisol, Inceptsol and artificial tropical soil (ATS)) contaminated with cerium (Ce).

Species	Soil	Ce Concentration (mg kg^{-1})								
		0 - Control	50	85	144.5	245.7	417.6	709.9	1206.9	2051.7
Corn	Oxisol	7.75±0.26	7.00±0.23	7.03±0.24	6.48±0.16**	6.53±0.31*	6.16±0.34**	2.05±0.08***	0.28±0.05***	0.08±0.02***
	Inceptsol	10.47±0.17	9.15±0.24	9.73±0.36	8.35±0.45***	8.44±0.31**	6.82±0.23***	7.39±0.47***	4.62±0.29***	1.60±0.40***
	ATS	13.19±0.59	11.32±0.61*	11.33±0.42*	11.24±0.37*	11.29±0.50*	10.97±0.34*	9.74±0.59***	6.73±0.43***	0.86±0.14***
Soybeans	Oxisol	6.35±0.14	5.91±0.07	5.57±0.24*	5.73±0.12*	6.11±0.24	4.09±0.38***	1.45±0.11***	0.37±0.12***	0.00***
	Inceptsol	6.90±0.25	6.33±0.33	6.64±0.24	6.85±0.25	6.57±0.44	5.60±0.19*	4.94±0.29***	2.27±0.32***	0.74±0.09***
	ATS	8.08±0.40	7.72±0.32	7.88±0.25	8.05±0.21	7.63±0.31	7.61±0.25	6.92±0.12*	4.71±0.14***	1.22±0.18***

Sorghum	Oxisol	5.64±0.26	5.18±0.16	5.49±0.12	5.07±0.07	3.85±0.15***	2.18±0.42***	0.08±0.02***	0.02±0.00***	0.00***
	Inceptsol	4.09±0.15	4.08±0.21	4.04±0.23	3.51±0.24	3.36±0.18	3.66±0.19	2.62±0.14**	0.88±0.18***	0.04±0.05***
	ATS	6.05±0.24	6.77±0.56	5.95±0.32	6.81±0.26	5.84±0.24	6.48±0.21	5.29±0.33	4.17±0.35***	1.21±0.28***
Sunflower	Oxisol	5.17±0.35	5.42±0.22	5.93±0.23	4.43±0.14	3.55±0.06***	1.57±0.13***	0.25±0.02***	0.00***	0.00***
	Inceptsol	5.92±0.35	5.38±0.19	4.95±0.18	4.90±0.11	4.23±0.24**	3.43±0.18***	1.71±0.16***	0.50±0.09***	0.00***
	ATS	7.09±0.18	7.06±0.13	6.42±0.31	6.80±0.24	6.57±0.23	6.97±0.07	6.00±0.15**	1.79±0.21***	0.40±0.09***
Radish	Oxisol	3.82±0.22	3.72±0.30	3.81±0.18	3.74±0.09	3.06±0.28	0.95±0.28***	0.14±0.05***	0.00***	0.00***
	Inceptsol	4.56±0.28	4.01±0.22	4.36±0.22	4.20±0.34	4.24±0.08	2.97±0.25**	1.51±0.25***	0.25±0.07***	0.00***

	ATS	4.42±0.05	4.11±0.19	3.94±0.14*	3.89±0.10***	4.69±0.21	3.66±0.44	3.46±0.17***	2.14±0.26***	0.36±0.14***
Rice	Oxisol	2.85±0.14	2.86±0.14	2.72±0.10	2.75±0.16	2.66±0.15	2.29±0.10*	1.47±0.10***	0.09±0.02***	0.00***
	Inceptsol	3.34±0.09	3.04±0.05*	3.01±0.07*	2.83±0.11**	3.33±0.13	2.73±0.12**	2.48±0.12***	1.87±0.18***	0.94±0.38***
	ATS	2.96±0.21	2.83±0.12	2.78±0.09	2.66±0.12	2.52±0.13	2.58±0.16	2.64±0.06	2.44±0.11	1.63±0.17***
Wheat	Oxisol	3.11±0.04	3.16±0.12	2.87±0.15	2.84±0.15	2.18±0.19***	1.30±0.13***	0.33±0.05***	0.01±0.00***	0.00***
	Inceptsol	3.70±0.16	3.72±0.04	3.66±0.14	3.62±0.25	3.56±0.25	3.09±0.21	2.59±0.13***	1.06±0.16***	0.19±0.04***
	ATS	3.14±0.10	3.34±0.10	3.45±0.08	3.53±0.09	3.58±0.06	3.15±0.09	2.64±0.07**	1.49±0.08***	0.36±0.06***
Beans	Oxisol	6.45±0.24	6.59±0.14	6.06±0.24	5.62±0.25	6.22±0.42	5.63±0.29	1.49±0.13***	0.38±0.06***	0.00***

	Inceptsol	4.95±0.25	6.42±0.23	6.17±0.34	5.64±0.30	5.94±0.10	4.97±0.37	4.10±0.27	2.50±0.16***	1.29±0.21***
	ATS	7.17±0.30	7.18±0.26	7.25±0.28	6.71±0.15	7.26±0.27	7.19±0.22	5.79±0.11*	4.71±0.40***	2.33±0.48***

* significant at p < 0.05; ** significant at p < 0.01; *** significant at p < 0.001 compared to the control treatment according to Dunnet test.

ARTIGO 2 – Ecophysiological crop responses to cerium in tropical soils

Artigo elaborado de acordo com as normas do periódico *Chemosphere* (versão submetida).

Ecophysiological crop responses to cerium in tropical agroecosystems

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Abstract

Cerium (Ce) is present in phosphate fertilizers and in high technology materials. The discharge and use of these materials - e.g., intensive application of phosphate fertilizer, as it happens in soils of Brazilian farming areas - may change the natural status of Ce in the soil environment. Thus, understanding the environmental consequences of Ce contamination in tropical agroecosystems is important for ensuring environmental safety. The aims of this study were to evaluate the effects of Ce on gas exchange and SPAD index through toxicity tests with crop plants in tropical Brazilian soils as well as to suggest plant physiological endpoints for assessing ecological risks for this element. Seven crop species (corn, sorghum, rice, wheat, soybeans, sunflower, and radish) were exposed to a concentration gradient of Ce in two typical tropical soils (Oxisol and Inceptsol). Cerium was more toxic to plants in the Oxisol than in the Inceptsol. Positive effects were observed on some physiological endpoints at the lowest Ce concentrations, indicating that this element can function as a bioestimulant for plants at ideal concentrations in the soil. Among the physiological endpoints measured, Ce phytotoxicity was more pronounced on SPAD index than on photosynthetic rate, transpiration rate, and stomatal conductance. Our risk assessment results support the idea that SPAD index may be an endpoint used in ecological risk studies for Ce, because it is both very sensitive and easy to measure.

Keywords: Rare earth elements; biostimulant; physiological endpoints; environmental safety; ecotoxicology.

Highlights

- Cerium disturbs plant physiology more strongly in the Oxisol than in the Inceptsol
- Cerium has a hormesis effect behaviour on some physiological crop endpoints
- SPAD index could be used as an endpoint in ecological risk assessments for Cerium

1 Introduction

Rare earth elements (REEs) are a group of 17 chemical elements in the periodic table with similar physicochemical properties. This group of elements is formed by the lanthanides, plus scandium and yttrium (IUPAC, 2005). The REEs are considered malleable, soft, ductile, and reactive (Jorjani and Shahbazi, 2012), which are properties that enable their use in a broad range of advanced industrial segments, such as metallurgy, military, automotive, nuclear, electronic and environmental (Alonso et al., 2012; Chakhmouradian and Wall, 2012; Haxel et al., 2002).

In Chinese agriculture, REEs have been applied as fertilizers to improve crop yields and quality since the 1980s (Hu et al., 2004; Ramos et al., 2016b; Tyler, 2004). Unlike China, in Brazil there is no report of deliberate application of REEs in agriculture through REE-carrying fertilizers. The typical tropical soil, as found in much of Brazil has high P fixation and low fertility (Lopes and Guilherme, 2016). Thus, an intensive application of mineral phosphate fertilizer is performed regularly to provide nutritional requirements for crop plants (Roy et al., 2016), resulting in an average consumption of 2.2 Tg in 2016, which can reach up to 4.6 Tg in 2050, since the Brazilian agriculture is expanding and intensifying (Withers et al., 2018). These P fertilizers may have significant concentrations of REEs, which may be considered as a diffuse source of these elements to the soil (Otero et al., 2005; Ramos et al., 2016a; Ramos et al., 2016b; Todorovsky et al., 1997; Turra et al., 2011). In this sense, in countries that are big consumers and apply high rates of P fertilizers regularly to the soil, such as Brazil, there may be a significant unintentional input of REEs to agricultural soils, increasing the levels of these elements in the environment. In addition, because only around 1% of the REEs are recycled from end-products (Jowitt et al., 2018), the inappropriately discarded electronic materials in landfills may also result in significant increases of REEs levels in soils. For these reasons, REEs are considered “emerging pollutants” (Pagano et al., 2015).

Cerium is the most abundant REE and the 25th most abundant element by mass in earth’s crust (Migaszewski and Gałuszka, 2014), and stands out among other REEs in mineral phosphate fertilizers (Ramos et al., 2016a; Turra et al., 2011). There is a report in Brazil that Ce levels within the 0 to 0.4 m layer increased by up to 62% in an agricultural area cropped for at least 20 years with soybean and wheat (Neves et al., 2018). Cerium caused physiological toxicity in sweet potato exposed to 20-80 mg Ce L^{-1} (Jiang et al., 2017) and rapeseed exposed to 0.28-8.96 mg Ce L^{-1} (Pošćić et al., 2017), through nutrient solution. Although a few studies have addressed Ce ecotoxicity, particularly in nutrient solutions, the understanding of the biological role of Ce and other REEs is still in its early stages (Skovran and Martinez-Gomez, 2015). In addition, there is limited information in the literature on Ce toxicity tests, especially tests conducted in tropical soils, reporting toxicity of this element on physiological processes of crop plants. In view of that, ecotoxicity studies are necessary to better understand the environmental consequences of enriching soils with Ce, especially soils of tropical agroecosystems, which are prone to receive high involuntary applications of REEs through phosphate fertilization. These studies may also provide relevant data concerning physiological endpoints that could be more appropriate to derive and recommend safe values for this element in the soil environment.

The aim of this study was to evaluate Ce effects on gas exchange and SPAD index of crop plants through ecotoxicity tests with natural Brazilian soils, as well as to suggest plant physiological endpoints for ecological risk assessments of this element. For this purpose, seven crop plants were exposed to a Ce concentration gradient in two typical tropical soils (Oxisol and Inceptsol) found in much of the Brazilian territory. Since Brazil is a leading producer and exporter of food and agricultural products in the world (OECD/FAO, 2015), with this study we hope to generate relevant information for evaluating risks and ecophysiological effects of Ce in crop plants and also to suggest supplementary and expedite protocols to regulate the levels of this element in Brazilian soils.

2 Materials and methods

The cerium effect on crop plants physiology grown in contaminated soils with this REE was assessed by a phytotoxicity experiment, which was based on the guideline OECD 208 (OECD, 2006). Comprehensive information concerning this experiment is published elsewhere (Moreira et al., 2019).

2.1 Test plants and soils

Seven crop plant species were selected based on their commercial importance. Three eudicotyledonous: radish (*Raphanus sativus*), sunflower (*Helianthus annuus*), soybean (*Glycine max*), and four monocotyledonous: rice (*Oryza sativa*), wheat (*Triticum aestivum*), corn (*Zea mays*) and sorghum (*Sorghum bicolor*).

Two soils were used in this experiment as follows: an Inceptsol collected in the municipality of Lavras, Minas Gerais State, Brazil (21°13’46’’ E, 44°59’10’’ N) and an Oxisol collected in the municipality of Itumirim, Minas Gerais State, Brazil (21°17’08’’ E, 44°47’43’’ N), both tropical soils. The two tropical soils were collected under native vegetation (reference areas).

The physical and chemical properties of the soils (Table S1 - supplementary material) were determined according to standard procedures described by Embrapa (EMBRAPA, 2011). The maximum water holding capacity (WHC) was determined according to the procedures defined by ISO 11274 (ISO, 1998) and the total organic carbon (TOC) contents by the dry combustion method through the Elementar analyzer model Vario TOC Cube.

The soil samples were previously air dried, homogenized and sieved (2 mm mesh). Three days before the beginning of each assay, the soil samples were fertilized by applying 300 mg N, 200 mg P, 150 mg K, 75 mg Ca, 15 mg Mg, 50 mg S, 0.5 mg B, 1.5 mg Cu, 5.0 mg Fe, 0.1 mg Mo, and 5 mg Zn kg^{-1} soil, according to Malavolta (1980).

2.2 Cerium analysis

The analysis of Ce on soil samples (three replicates of each nominal dose) tested in the wheat assay was performed to validate the experimental concentrations and the soil contamination procedure. In order to determine total Ce concentrations, samples of controls and treated soils were initially oven-dried at 60°C until reaching constant weight, grounded in a agate mortar, and sifted with 150-μm nylon sieves. After that, aliquots of 0.5 g of each sample were taken to digestion according to the USEPA 3051A method (USEPA, 2007). Each aliquot was digested with 5 mL of concentrated nitric acid (HNO_3) in PTFE Teflon® tubes (CEM Corporation, Matthews, NC, USA) using a microwave (MARS-5®, CEM), with a temperature set at 175 °C and at a controlled pressure of 0.76 MPa for 15 min. After digestion, extracts were cooled down at room temperature and then filtered on a filter paper. At the same time of filtration, 5 mL of deionized water were added in the final volume of the extract. After filtration, the extracts were transferred into smaller vials (20 mL) and submitted to analysis. Cerium concentrations were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) - Spectro Blue (Germany). A standard reference material (Calcareous Soil ERM-CC690®, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium) and an analytical blank were added throughout the analytical series in order to verify the accuracy of the analytical results. The % recovery of Ce concentration of the tested soils was calculated as follows: (mean Ce concentration analyzed - mean Ce concentration analyzed in control soil) / (nominal dose concentration).

2.3 Experimental procedure

The Ce concentrations used in the experiment were 0 (control), 50, 85, 144.5, 245.7, 417.6, 709.9, 1206.9 and 2051.7 mg Ce kg^{-1} of soil, following the geometric progression of 1.7. The chosen doses were applied through solutions containing Ce as a cerium chloride heptahydrate ($CeCl_3 \cdot 7H_2O$) pure salt, from

Sigma-Aldrich (CAS Number: 18618-55-8). For each Ce concentration tested, a contaminant solution was prepared separately. Five replicates were used for each dose.

A plastic pot (Ø120 mm x 78 mm height) containing 500 g of soil consisted of an experimental unit. To contaminate the soil of each experimental unit, contaminant solutions of each expected Ce concentration were pipetted into a plastic bag with 500 g of dry soil. Once contaminated, the soil was homogenized and transferred to a plastic pot. After that, all contaminated soil pots were left to settle for 12 hours before sowing. In each experimental unit a crop species was planted using ten seeds (corn, sorghum, soybean, and sunflower) or fifteen seeds (rice, wheat, and radish) and soil humidity was kept at 60-70% of the maximum water holding capacity with deionized water. Fourteen days after sowing, the top-dressing fertilization was performed using 20% of the fertilizer used at the beginning of each Crop species were exposed to Ce from the time seeds were sowed until the end of each assay. The assay of each species ended 21 days counted after the emergence of ≥ 50% of the seedlings in the control treatment. The whole experiment was carried out at greenhouse conditions with natural light and average temperature of 26 ± 3°C.

The SPAD Index (SI) was analyzed using a portable chlorophyll meter (SPAD-502, Konica Minolta Co., Japan). Photosynthetic rate (*A*), transpiration rate (*E*), and stomatal conductance (g_s) on the crop species were analyzed using an Infrared Gas-exchange Analyzer, IRGA (Li-6400, Li-Cor, Lincoln, NE, USA) with its chamber programmed for a photosynthetically active photon flux density at 1000 µmol m^{-2} s^{-1}. All physiological endpoints were analyzed on a fully expanded leaf of a plant (randomly chosen) from each experimental unit, 20 days after seedlings emergence, between 9 and 11 a.m.

2.4 Data analyses

Photosynthetic rate, transpiration rate, stomatal conductance, and SPAD index data were analyzed using one-way ANOVA followed by the Dunnet test ($p < 0.05$), for calculating the no-observed-effect-concentrations (NOEC) and the lowest-observed-effect-concentrations (LOEC). Risk assessment was performed using the LOEC values from SPAD index because this endpoint was more susceptible to the negative effect of Ce. LOEC and NOEC values from photosynthetic rate, transpiration rate, and stomatal conductance are presented in Tables S4, S5, and S6 - supplementary material. The species sensitivity distribution (SSD) curve (Posthuma et al., 2002) was derived in order to estimate the hazardous concentration HC_5, which is the Ce concentration that would cause risk to 5% or less of crop plant species tested. Since the biomass is an endpoint recommended to be analyzed in toxicity tests according to guidelines such as OECD (OECD, 2006), the HC_5 was also estimated for comparison purposes, using the LOEC values from shoots dry matter (SDM) (Figure S1 and Table S7 - supplementary Material) analyzed on the respective plants grown in the Oxisol and the Inceptsol, according to Moreira et al. (2019). All statistical analyses were conducted using R (R Development Core Team, 2017).

3 Results

3.1 Cerium content recovery in soil

The mean determined Ce concentration in the standard reference material (ERM®-CC690-calcareous soil; Ce = 49.1 mg kg^{-1}) was 45.18 ± 0.29 mg kg^{-1} (n = 4). Thus, the measured recovery of Ce (mean recovery = 92%) reveals reliable analytical data accuracy for Ce analysis.

The averages of Ce concentration in treated and control soils are given in Table S2- supplementary material. Table S3- supplementary material shows the percent recovery of Ce compared with the nominal concentration. Recoveries of Ce from each concentration ranged from 76% to 121% (average = 100 ± 30%), except for nominal concentrations 50 mg kg^{-1} in the Oxisol and 245.7 mg kg^{-1} in the Inceptsol, which were lower than 70%. Both soils presented a high level of natural Ce concentrations, being higher than the average abundance of 64 mg Ce kg^{-1} in the Upper Continental Crust (UCC) (Taylor and McLennan, 1995); yet, closer to average concentration of 87.12 mg Ce kg^{-1} to and 82 mg Ce kg^{-1} reported by Ramos et al. (2016b) in Brazilian soils. Thus, not surprisingly, Ce concentrations in treated soils were above the expected nominal concentrations because of natural Ce concentrations in soils.

3.2 Photosynthetic rate

The photosynthetic rate of all crops significantly decreased at some Ce concentrations when compared with the control, and varied depending on the soil type and plant (Figure 1). In general, the Oxisol required lower Ce concentrations to cause more severe negative effects on plants than the Inceptsol. Among the crops grown in the Oxisol, soybean and sunflower were more sensitive, being negatively affected from concentrations of 144.5 and 245.7 mg Ce kg^{-1}, respectively, and rice was the most tolerant species for Ce, requiring a concentration of 1206.9 mg Ce kg^{-1} to decrease its photosynthetic rate. On the other hand, among the crops grown in the Inceptsol, rice was more sensitive, requiring a Ce concentration of 417.6 mg kg^{-1}, and soybean, radish, sorghum, and wheat were the most tolerant species for Ce to decrease its the photosynthetic rate. Positive effects on photosynthetic rate were also observed in plants grown on the lowest Ce concentrations. There was an increase of 62%, 25%, and 28% in the photosynthetic rate of sorghum, rice and sunflower, respectively, cultivated on Oxisol, and 21% in the photosynthetic rate of wheat cultivated on Inceptsol.

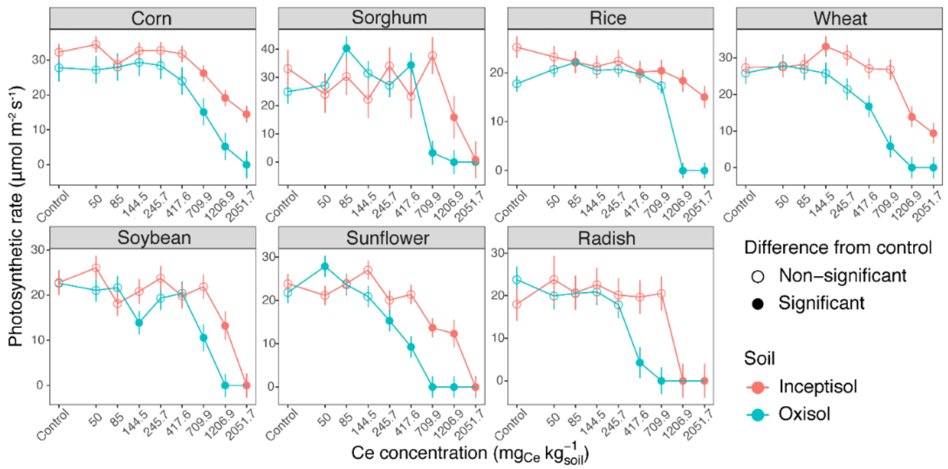

Figure 1. Photosynthetic rate (µmol m^{-2} s^{-1}) of plant species growing in two tropical soils (Oxisol and Inceptsol) contaminated with cerium (Ce). The dots represent the means (n = 4) and the vertical bars represent the standard error. The closed dots indicate statistically significant difference from the control treatment (Dunnet test; $p < 0.05$).

3.3 Transpiration rate

The transpiration rate of the plants exposed to Ce also decreased significantly in relation to the control, varying according to the species and soil types (Figure 2). Plants grown in the Oxisol were more sensitive to Ce concentrations, showing decreased transpiration rates at lower Ce concentrations than the respective plants when grown in the Inceptsol. Sunflower and radish growing in the Oxisol were the most sensitive for Ce with respect to transpiration rate. In contrast, rice growing in the Inceptsol was the most tolerant species, and Ce concentrations tested were not sufficient to decrease their transpiration rate. Positive effects were observed under low Ce concentration, i.e., an increase of 57%, 85%, and 47% in the transpiration rate of corn, soybean and radish, respectively, cultivated on Inceptsol, and of 80%, 57%, and 31% in the transpiration rate of sorghum, rice and sunflower, respectively, grown on Oxisol.

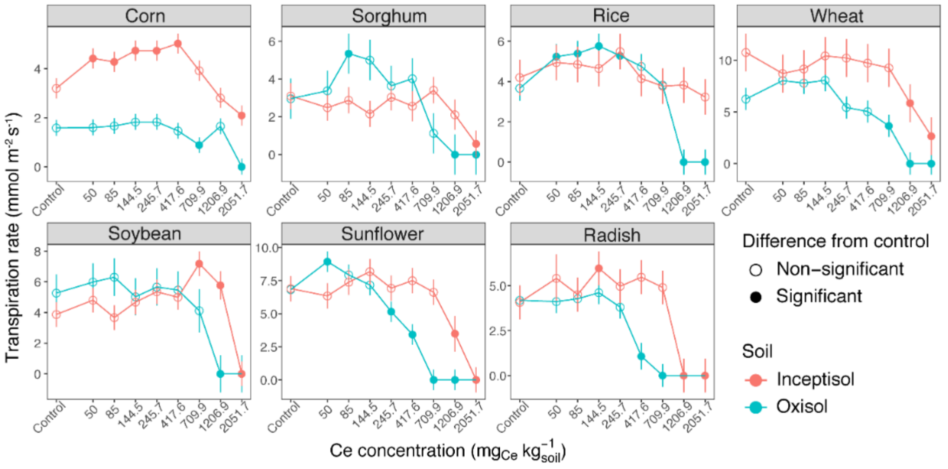

Figure 2. Transpiration rate (mmol m^{-2} s^{-1}) of plant species growing in two tropical soils (Oxisol and Inceptsol) contaminated with cerium (Ce). The dots represent the means (n = 4) and the vertical bars represent the standard error. The closed dots indicate statistically significant difference from the control treatment (Dunnet test; $p < 0.05$).

3.4 Stomatal conductance

The stomatal conductance of the plants also decreased in some Ce concentrations significantly in relation to the control and varied according to the plant species and soil types (Figure 3). Sunflower, radish, and wheat grown in the Oxisol were the most sensitive species with negative effects of Ce on the stomatal conductance starting at 417.6 mg Ce kg^{-1}. The plants grown in the Inceptsol were more tolerant, with stomatal conductance decreasing only from the concentrations of 1206.9 or 2051.7 mg Ce kg^{-1}. In addition, the Ce concentrations tested caused no negative effect on stomatal conductance of rice. There was a positive effect of Ce on stomatal conductance, increasing 46% and 87% for corn and soybean, respectively, grown in the Inceptsol, and 89%, 78%, and 96% for sorghum, rice, and sunflower grown in the Oxisol.

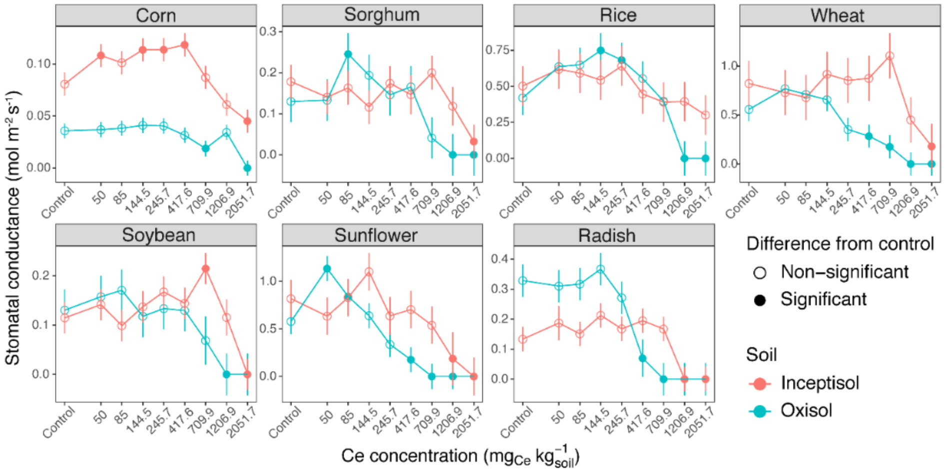

Figure 3. Stomatal conductance (mol m^{-2} s^{-1}) of plant species growing in two tropical soils (Oxisol and Inceptsol) contaminated with cerium (Ce). The dots represent the means (n = 4) and the vertical bars represent the standard error. The closed dots indicate statistically significant difference from the control treatment (Dunnet test; $p < 0.05$).

3.5 SPAD index

The negative effects of increasing Ce concentrations on SPAD index varied among plants and soil types (Figure 4). Both soils showed a low Ce concentration required to decrease the SPAD index of plants but in the Oxisol the highest Ce concentrations affected more severely this endpoint than in the Inceptsol. Plants grown in the Oxisol required Ce concentrations ≤ 417 mg kg^{-1}, except for rice, which needed 1206 mg kg^{-1} to decrease its SPAD index. Differently, plants grown in the Inceptsol required concentrations ≤ 709.9 mg Ce kg^{-1}, except for soybean and rice, which needed 1206 mg Ce kg^{-1} to decrease their respective SPAD indexes.

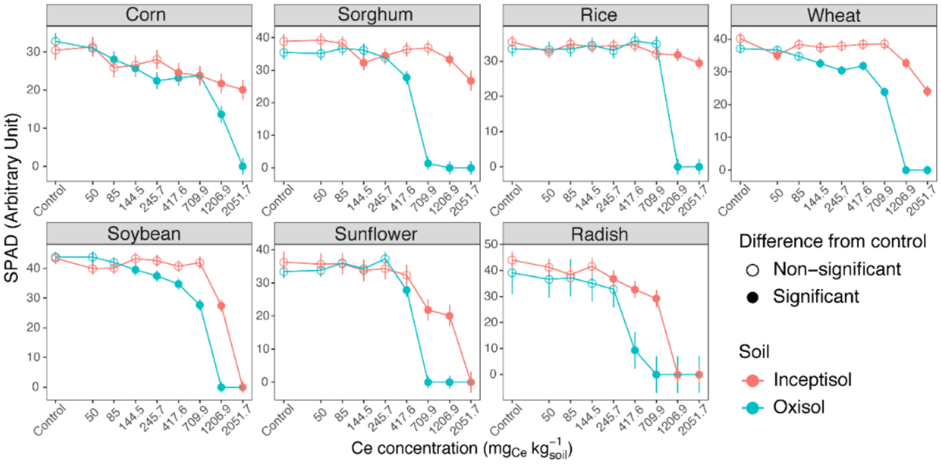

Figure 4. SPAD index of plant species growing in two tropical soils (Oxisol and Inceptsol) contaminated with cerium (Ce). The dots represent the means (n = 4) and the vertical bars represent the standard error. The closed dots indicate statistically significant difference from the control treatment (Dunnet test; $p < 0.05$).

3.6 Risk assessment

The NOEC and LOEC values for the SPAD index (Table 1) were usually much lower than those found for others physiological endpoints analyzed (Table S4, S5 and S6 - supplementary material), showing this endpoint to be more sensitive. Figure 5 shows the species sensitivity distribution (SSD) curves and the hazardous concentration (HC$_5$) value derived for Ce based on data from the Inceptsol and the Oxisol. The HC$_5$ estimated based on SPAD index was 60.4 mg Ce kg^{-1} dry soil. The HC$_5$ value estimated from LOEC values of SDM was 128.6 mg Ce kg^{-1} dry soil (Figure S1 - supplementary Material).

Table 1. No-observed-effect-concentrations (NOEC) and lowest-observed-effect-concentrations (LOEC) estimated for SPAD index of plants exposed to soils (Oxisol and Inceptsol) contaminated with cerium (Ce).

Plant Species	Soil	NOEC (mg Ce kg^{-1})	LOEC (mg Ce kg^{-1})	*p*-value[b]
Corn	Oxisol	50.0	85.0	*
	Inceptsol	245.7	417.6	*
Sorghum	Oxisol	245.7	417.6	***
	Inceptsol	85.0	144.5	**
Rice	Oxisol	709.9	1,206.9	***
	Inceptsol	709.9	1,206.9	*
Wheat	Oxisol	85.0	144.5	***
	Inceptsol	0.0	50.0	**
Soybean	Oxisol	85.0	144.5	*
	Inceptsol	709.9	1,206.9	***
Sunflower	Oxisol	245.7	417.6	**
	Inceptsol	417.6	709.9	***
Radish	Oxisol	245.7	417.6	***
	Inceptsol	144.5	245.7	*

[b] *p*-value related to the LOEC; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$ compared with the control treatment.

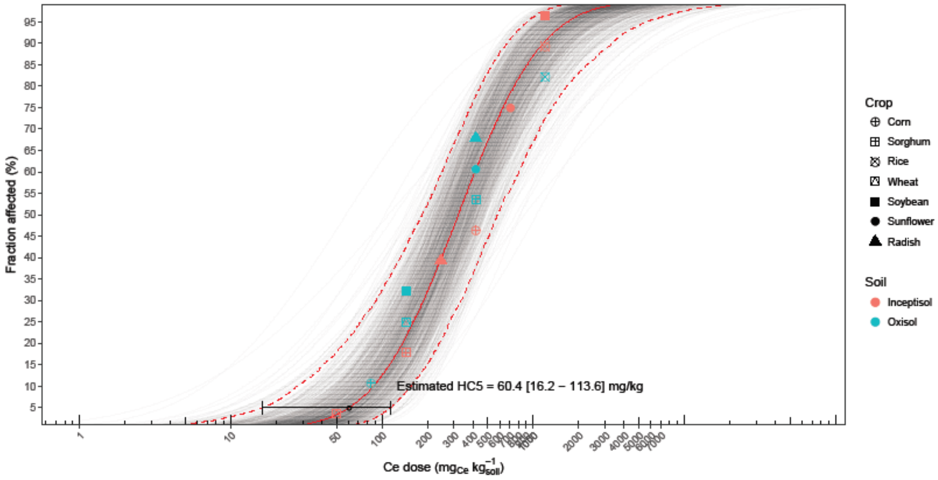

Figure 5. Species sensitivity distribution (SSD) and hazardous concentration that would be protecting at least 5% of species (HC_5) estimated from LOEC (lowest-observed-effect-concentrations) values using the SPAD index endpoint (SI) for seven plant species cultivated on two soils (Oxisol and Inceptsol) contaminated with cerium (Ce).

4 Discussion

Soil physical-chemical properties, such as pH, cation exchange capacity (CEC), and total organic carbon content (TOC) can influence the bioavailability and uptake of Ce and other REEs, increasing the possibility of plant phytotoxicity and death. In general, lower CEC, pH, and TOC are favorable conditions to increase Ce availability in soil. In the present study, the pH, CEC, and TOC in the Oxisol were lower than those found for the Inceptsol. Thus, the lower values of these attributes in the Oxisol may have contributed to higher Ce bioavailability and, consequently, to the more severe phytotoxicity observed in this soil.

Thus, in the Oxisol, lower Ce concentrations caused more severe negative effects, mainly in soybean and sunflower, which are eudicotyledoneous species.

On the other hand, rice - a monocotyledonous species -, was the most tolerant crop plant tested. This fact can be explained by the greater thickness of apoplastic barriers found more often in monocotyledonous than in eudicotyledoneous (Krishnamurthy et al., 2011). This prevents Ce to reach the shoots of monocotyledonous plants, as is the case of rice, protecting the photosynthetic apparatus, and making it more tolerant to Ce. Apoplastic barriers have an important role in protection against various stresses (Enstone et al., 2003), such as the protection of internal tissues and shoot tissues against the effect of toxic elements (Lux et al., 2004). Oliveira et al. (2015) reported a low La translocation from roots to shoots, probably related with the roots retaining a greater amount of this element via thickening of the apoplastic barriers in soybean.

However, the action mechanism of Ce on plants is still poorly understood, with limited information available in the literature, yet most of these few studies show contradictory results. In addition, there is limited information available about Ce effects on plant physiology in tests conducted in soils, especially tropical soils. Cerium in soil is absorbed through the roots, transported to the shoots, and finally accumulates in edible tissues (Jiang et al., 2017), and may cause different effects on crop plants, depending on its concentration in the soil, as shown in our results, as well as on specific Ce tolerance characteristics of each species. Although Ce has neither been characterized as an essential element for life nor as a toxic element in the environment, our findings revealed that crops grown on soils containing Ce, especially at the highest Ce concentrations, may suffer phytotoxic effects, damaging their photosynthetic capacity and decreasing the SPAD index, which represents the leaf chlorophyll content. Since Ce can inhibit photosystem II and damage the chloroplast ultrastructure (Xu et al., 2017), the decrease in photosynthetic rate and SPAD index of plants may be associated with these reasons. In addition, the leaf chlorophyll content is an important factor determining the photosynthetic potential (Gitelson et al., 2003), and the decrease of the SPAD index, probably related to damages in the chloroplast ultrastructure or in the chlorophyll biosynthesis process, may have influenced the negative effects of Ce on the photosynthetic ability of plants. Moreover, previous toxicological investigations have suggested that Ce may accumulate in cells, causing structural damage (Pošćić et al., 2017; Wang et al., 2017), disturbances in the nutritional balance, (Guo et al., 2007; Hu et al., 2002; Pošćić et al., 2017), inhibition of cell division, increase the frequency of aberrant cells (Kotelnikova et al., 2018) and cell death (Xu et al., 2017), which could also be associated with the negative effects observed in the current study.

Our results of toxicity on the gas exchange also coincide with the findings reported by Jiang et al. (2017), who observed inhibition of photosynthesis and reduction of chlorophyll content of sweet potato when grown in nutrient solution with high Ce concentration of 20-80 mg L^{-1} and 80 mg L^{-1}, respectively. In the same way, the studies of Xu et al. (2017) and Zicari et al. (2018) reported a decrease of photosynthetic pigment (carotenoid, chlorophyll *a* and *b*) content in *Spirodela polyrhiza* L. and *Lemna minor* L. Fronds, respectively, after exposure to Ce through nutrient solution.

In general, at the lower Ce concentrations, we observed the absence of significant negative effects or the occurrence of the significant positive effects on the endpoints studied. Therefore, the results of this study revealed that in some situations this element, at ideal concentrations, could function as a biostimulant in plants. This dose-response phenomenon is known as “hormesis effect” (Agathokleous et al., 2018; Calabrese and Blain, 2009). Shyam and Aery (2012) reported an increase of 46% in the chlorophyll content of cowpea plants (*Vigna unguiculata* (L.) Walp.) exposed to 17,841 μM of Ce, compared with the control treatment. However, at higher doses this element caused negative effects. Xue et al. (2012) also reported an increase in chlorophyll content of *Arabidopsis thaliana* seedlings exposed to 0.5 mol Ce L^{-1}, whereas at higher Ce concentrations at the culture medium, chlorophyll content of seedlings decreased. The observed positive effects of Ce in the photosynthetic rate of sorghum, rice, wheat, and sunflower exposed at doses of 85, 85, 144.5, and 50 mg Ce kg^{-1}, respectively, agrees with data presented by Fashui et al. (2002), who reported an increase of 31.57% in the photosynthetic rate of spinach when exposed to 5 µg Ce mL^{-1}. Other reports of positive effects of Ce at optimal concentrations are also available (Hu et al., 2004; Ma et al., 2014; Shyam and Aery, 2012; Vilela et al., 2018).

The observed increase of the photosynthetic rate at low Ce concentrations may be due to an increase in the electronic flux in the photochemical phase of photosynthesis, resulting in more reducing power and energy, and potentiating the photosynthetic rate in the presence of Ce, as observed by Oliveira et al. (2019) (submitted) and Wang et al. (2011), in rice, Giraldo et al. (2014), in *Spinacia oleraceae*, and Zhou et al. (2011), in maize. A hypothesis explaining the beneficial effects of REE such as Ce in plant metabolism include a positive effect on biomass production in lower concentrations, by induction of a higher photosynthetic rate, due to an increase in electron transport rate in the photochemical phase of photosynthesis (Ramos et al., 2016b). However, at higher concentrations, Ce is prejudicial to chloroplast structure, negatively affecting the photosynthesis process, due membrane disruption (Shen et al., 2014).

Stomatal conductance is a component of the photosynthetic rate, therefore, an increase or decrease in this endpoint will contribute with the increase or decrease in photosynthetic rate. Oliveira et al. (2019) (submitted) observed an increase in photosynthetic rate of rice exposed to Ce, due to the increase in stomatal conductance and increase in SPAD index. Beside this, in general, the transpiration rate is directly related with stomatal conductance, indicating that whenever a higher transpiration rate occurs, there is also a greater stomatal conductance. This showed that higher stomatal conductance promotes an increased entrance of CO_2, thus intensifying the photosynthetic process.

It should be pointed out that the toxic effects of Ce on gas exchange and SPAD index observed in our study can be partially related to chloride, which was applied in soils as an accompanying ion for Ce. In order to investigate possible partial effects of this anion (Cl^-) on plant growth, Moreira et al. (2019) conducted a test with all crops exposing them to four Cl concentrations (316.96, 538.82, 915.7, 1557.27 mg Cl kg^{-1}), in the form of calcium chloride ($CaCl_2$), equivalent to those applied at the four highest concentrations of Ce (245.7, 417.6, 709.9, 1206.9 and 2051.7 mg Ce kg^{-1}). These authors reported that the highest concentrations of calcium chloride reduced crops growth, especially at the concentrations corresponding to 1206.9 and 2051.7 mg Ce kg^{-1}. However, those effects were less intense when compared with the observed effects of $CeCl_2$ at equivalent concentrations. Nutrient uptake, protein biosynthesis, and plant photosynthesis may be impaired by excess chlorine (Geilfus, 2018). In this way, our results, especially those related to higher Ce concentrations tested, may reflect an overestimation of Ce phytotoxicity in gas exchanges and SPAD index, and its values should be taken as conservative estimates.

There are few studies available in the literature on risk assessment for Ce and other REEs. A study with lanthanum reported a HC_5 value of 49 mg kg^{-1} dry soil, which was derived from EC_{10} (effective concentration of Ce resulting in 10% inhibition) data from soil invertebrates, bacteria, and plants (Li et al., 2018). Although the endpoints to derive the HC_5 were different, the result cited was close to that found in this study (HC_5 = 60.4 mg Ce kg^{-1} dry soil), probably because both elements - Ce and La - are REEs and have similar physical and chemical characteristics.

Our risk assessment results showed that the SPAD index was the most sensitive to Ce among the endpoints studied. In addition, the HC_5 from SDM was twice higher than the HC_5 from SPAD index, showing that this physiological endpoint is also more sensitive to Ce than the growth endpoint, already recommended in guidelines such as OECD (OECD, 2006) in toxicity tests. Phytotoxic effects of a contaminant are first expressed in the physiological processes and, only if not mitigated, they translate on negative effects on plant growth. SPAD index is an important physiological endpoint, which together with gas exchanges and other physiological characteristics, such as chloroplast development and leaf nitrogen content, make up the photosynthetic rate, and consequently are essential for the status of plants health. In this sense, our study suggests the use of SPAD index as an additional endpoint for Ce toxicity tests. Other physiological endpoints could also be considered, e.g., the chlorophyll content, which can be determined spectrophotometrically in a laboratory (Arnon, 1949; Lichtenthaler and Wellburn, 1983). However, this method requires adequate infrastructure, the use of organic solvents and is destructive, as well as relatively expensive and time-consuming. On the other hand, the SPAD index is a fast, accurate, and non-destructive way to estimate leaf chlorophyll concentrations, measured by a SPAD-502 portable meter, which is used extensively in both research and agricultural applications, with a range of different plant species (Ling et al., 2011). In view of this, we understand that Ce toxicity tests could include as an endpoint the SPAD index measured by a portable SPAD meter, because it is practical, fast and not expensive. In addition, *in situ* monitoring of plants in areas favorable to the increase of Ce concentration or areas already contaminated with high Ce concentration in the soil, especially in areas of Oxisols, a typical tropical soil, may be carried out periodically and, if necessary, immediate decision-making may be taken.

5 Conclusion

Our study showed that physiological endpoints are more sensitive than growth-related endpoints for evaluating the ecotoxicological risks of high Ce concentrations in tropical soils. Nevertheless, lower concentrations of this element may result in positive effects (hormesis phenomenon).

Plants growing in the Oxisol were more sensitive than in the Inceptsol, probably due to different soil properties, especially pH, CEC, and TOC. The sensitivity of crops exposed to Ce shows to be peculiar to each plant, being the most sensitive species: wheat, growing in the Inceptsol and corn, growing in the Oxisol.

The risk assessment results support the idea that the SPAD index may be an endpoint used in ecological risk studies for Ce, because of its high sensitivity and practical handling. It could be used together with other endpoints recommended in guidelines such as OECD (OECD, 2006).

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Supplementary material

Table S1. Physical and chemical properties of tested soils.

Soil	pH_{H_2O}	P	K	Ca	Mg	Al	CEC	TOC	Sand	Silt	Clay
		mg dm^{-3}	mg dm^{-3}	$cmol_c$ dm^{-3}	$cmol_c$ dm^{-3}	$cmol_c$ dm^{-3}	$cmol_c$ dm^{-3}	%	%	%	%
Oxisol	5.20	0.88	39.42	0.14	0.10	0.20	1.73	0.80	68	4	28
Inceptsol	6.10	1.14	39.42	2.26	0.13	0.03	4.08	1.09	20	48	32

CEC = Cation Exchange Capacity; TOC = Total Organic Carbon

Table S2. Mean with standard error of cerium (Ce) concentrations (mg Ce kg^{-1} dry soil) in control and treated soils (Oxisol and Inceptsol) for each expected nominal concentration*.

Nominal concentration	Mean determined concentration	
	Oxisol	Inceptsol
0 - Control	77.37 ± 1.59	116.13 ± 4.74
50	109.18 ±12.84	160.49 ± 7.40
85	157.46 ± 5.29	182.87 ± 11.25
144.5	224.97 ± 14.88	225.69 ± 5.66
245.7	272.00 ± 12.60	279.35 ± 20.58
417.6	440.92 ± 2.04	507.99 ± 13.65
709.9	766.16 ± 52.98	734.36 ± 39.94
1,206.9	1,478.92 ± 58.94	1,363.13 ± 32.05
2,051.7	2,301.13 ± 110.60	2,046.67 ± 74.47

*After Moreira et al. (2019)

Table S3. Recovery of cerium (Ce) on treated soils (Oxisol and Inceptsol) as compared with the expected nominal concentration.

Nominal concentration	% recovery*	
	Oxisol	Inceptsol
0 - Control	-	-
50	64	89
85	94	79
144.5	102	76
245.7	79	66
417.6	87	94
709.9	97	87
1,206.9	116	103
2,051.7	108	94

*Percent recovery was based on measured Ce concentrations minus Ce concentration in control soil divided by nominal concentration. Data from Moreria et al. (2019).

Table S4. No-observed-effect-concentrations (NOEC) and lowest-observed-effect-concentrations (LOEC) estimated for photosynthetic rate of plants exposed to soils (Oxisol and Inceptsol) contaminated with cerium (Ce).

Plant Species	Soil	NOEC (mg Ce kg^{-1})	LOEC (mg Ce kg^{-1})	*p*-value[a]
Corn	Oxisol	417.6	709.9	***
	Inceptsol	417.6	709.9	**
Sorghum	Oxisol	417.6	709.9	***
	Inceptsol	709.9	1,206.9	*
Rice	Oxisol	709.9	1,206.9	***
	Inceptsol	245.7	417.6	*
Wheat	Oxisol	245.7	417.6	***
	Inceptsol	709.9	1,206.9	***
Soybean	Oxisol	85.0	144.5	***
	Inceptsol	709.9	1,206.9	***
Sunflower	Oxisol	144.5	245.7	*
	Inceptsol	417.6	709.9	***
Radish	Oxisol	245.7	417.6	***
	Inceptsol	709.9	1,206.9	***

[a] *p*-value related to the LOEC; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table S5. No-observed-effect-concentrations (NOEC) and lowest-observed-effect-concentrations (LOEC) estimated for transpiration rate of plants exposed to soils (Oxisol and Inceptsol) contaminated with cerium (Ce).

Plant Species	Soil	NOEC (mg Ce kg^{-1})	LOEC (mg Ce kg^{-1})	*p*-value[a]
Corn	Oxisol	417.6	709.9	*
	Inceptsol	1,206.9	2,051.7	**
Sorghum	Oxisol	709.9	1,206.9	**
	Inceptsol	1,206.9	2,051.7	***
Rice	Oxisol	709.9	1,206.9	***
	Inceptsol	None[b]	None[b]	-
Wheat	Oxisol	417.6	709.9	**
	Inceptsol	709.9	1,206.9	**
Soybean	Oxisol	709.9	1,206.9	***
	Inceptsol	1,206.9	2,051.7	***
Sunflower	Oxisol	144.5	245.7	*
	Inceptsol	709.9	1,206.9	**
Radish	Oxisol	245.7	417.6	***
	Inceptsol	709.9	1,206.9	***

[a] *p*-value related to the LOEC; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

[b] No Ce doses caused statistically significant difference, compared with the control treatment.

Table S6. No-observed-effect-concentrations (NOEC) and lowest-observed-effect-concentrations (LOEC) estimated for stomatal conductance of plants exposed to soils (Oxisol and Inceptsol) contaminated with cerium (Ce).

Plant Species	Soil	NOEC (mg Ce kg^{-1})	LOEC (mg Ce kg^{-1})	*p*-value[a]
Corn	Oxisol	50.0	709.9	*
	Inceptsol	1,206.9	2,051.7	***
Sorghum	Oxisol	709.9	1,206.9	**

	Inceptsol	1,206.9	2,051.7	***
Rice	Oxisol	709.9	1,206.9	***
	Inceptsol	None[b]	None[b]	-
Wheat	Oxisol	245.7	417.6	*
	Inceptsol	1,206.9	2,051.7	**
Soybean	Oxisol	709.9	1,206.9	***
	Inceptsol	1,206.9	2,051.7	***
Sunflower	Oxisol	245.7	417.6	**
	Inceptsol	709.9	1,206.9	**
Radish	Oxisol	245.7	417.6	***
	Inceptsol	709.9	1,206.9	***

[a] *p*-value related to the LOEC; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

[b] No Ce doses caused statistically significant difference, compared with the control treatment.

Table S7. No-observed-effect-concentrations (NOEC) and lowest-observed-effect-concentrations (LOEC) estimated for shoot dry matter (SDM) of plants exposed to soils (Oxisol and Inceptsol) contaminated with cerium (Ce).

Plant Species	Soil	NOEC (mg kg^{-1})	LOEC (mg kg^{-1})	*p*-value[b]
Corn	Oxisol	85.0	144.5	< 0.01
	Inceptsol	85.0	144.5	< 0.01
Sorghum	Oxisol	144.5	245.7	< 0.01
	Inceptsol	417.6	709.9	< 0.01
Rice	Oxisol	245.7	417.6	0.01
	Inceptsol	0.0	50.0	0.03
Wheat	Oxisol	144.5	245.7	< 0.01
	Inceptsol	417.6	709.9	< 0.01

Soybeans	Oxisol	50.0	85.0	0.04
	Inceptsol	245.7	417.6	0.01
Sunflower	Oxisol	144.5	245.7	< 0.01
	Inceptsol	144.5	245.7	< 0.01
Radish	Oxisol	245.7	417.6	< 0.01
	Inceptsol	245.7	417.6	< 0.01

*Source Moreira et al. (2019).

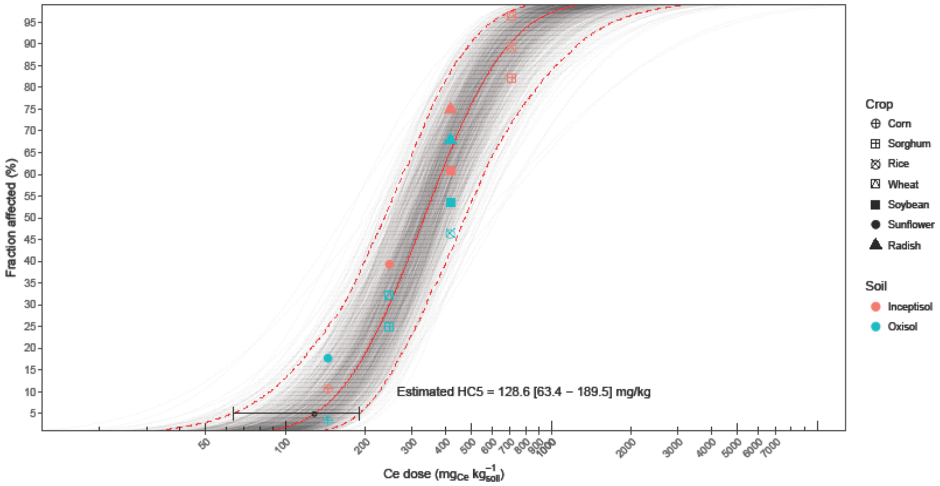

Figure S1. Species sensitivity distribution (SSD) and hazardous concentration that would be protecting at least 5% of species (HC_5) estimated from LOEC (lowest-observed-effect-concentrations) values using shoots dry matter (SDM) parameter for seven plant species cultivated on two soils (Oxisol and Inceptsol) contaminated with cerium (Ce).