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Avaliação do cogumelo A*garicus bisporus* como um agente protetor contra os efeitos tóxicos do chumbo na exposição materna

Evaluation of the mushroom *Agaricus bisporus* as a protective agent against the toxic effects of lead in maternal exposure

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RESUMO

Chumbo (Pb) é um contaminante ambiental nocivo capaz de impactar vários tecidos dentro do corpo humano. O uso de recursos naturais, como cogumelos medicinais, mostra-se promissor para diminuir os efeitos nocivos do Pb. Com isso em mente, nossa investigação centrou-se no potencial do Agaricus Bisporus, um tipo de cogumelo, para neutralizar as consequências tóxicas da exposição ao Pb por meio de experimentos envolvendo ratos e nematoides. Em nossos ensaios, submetemos os animais a doses combinadas de Agaricus Bisporus (Ab) por e Pb. Esta dupla exposição foi explorada em dois modelos in vivo separados: ratas grávidas e C. elegans. No caso de ratas grávidas, as ratas Wistar foram divididas em quatro grupos distintos (n = 5/grupo): Grupo I - Controle; Grupo II – Ab 100 mg/kg; Grupo III – Pb 100 mg/L; Grupo IV – Ab+Pb - 100 mg/kg + 100 mg/L. Este regime de exposição persistiu até o 19º dia de gestação, quando as ratas prenhes foram eutanasiadas. Avaliamos uma série de resultados, incluindo ganho de peso, toxicocinética, características hematológicas, marcadores bioquímicos, indicadores de estresse oxidativo, bem como capacidade reprodutiva e desenvolvimento embriofetal. Notavelmente, a coadministração desses cogumelos desempenhou um papel significativo no alívio dos impactos nocivos da exposição ao Pb e na promoção da recuperação. Notavelmente, Agaricus Bisporus exibiu propriedades antioxidantes, levando a melhorias nos parâmetros de estresse oxidativo. Além disso, o cogumelo exibiu uma capacidade parcial de reverter os danos à morfologia fetal e aos atributos ósseos. Em nossa investigação envolvendo C. elegans, fizemos observações dignas de nota. A co-exposição ao Pb juntamente com 100 mg/mL de Agaricus Bisporus demonstrou redução na mortalidade embrionária e larval. Essa combinação também levou ao aumento do tamanho e da motilidade da ninhada em comparação com os nematóides expostos apenas ao Pb. Além disso, observamos a mitigação da transmissão de toxicidade reprodutiva dos pais do nematoide para seus descendentes. Com base nessas descobertas, propomos que os atributos antioxidantes de Agaricus Bisporus têm o potencial de mitigar as consequências desfavoráveis da toxicidade reprodutiva induzida por Pb. Consequentemente, este antioxidante natural pode servir como uma contramedida valiosa contra o impacto prejudicial do Pb na saúde reprodutiva.

Palavras-Chave: *Agaricus bisporus*. *C. elegans*. Chumbo. Intoxicação. Toxicologia da reprodução.

ABSTRACT

Lead (Pb) is a harmful environmental contaminant capable of impacting various tissues within the human body. The use of natural resources such as medicinal mushrooms shows promise in lessening the harmful effects of Pb. With this in mind, our investigation focused on the potential of Agaricus Bisporus, a type of mushroom, to counteract the toxic consequences of Pb exposure by through experiments involving rats and nematode. In our assays, we submitted the animals to combined doses of Agaricus Bisporus (Ab) por and Pb. This double exposure was explored in two separate in vivo models: pregnant rats and C. elegans. In the case of pregnant rats, Wistar rats were divided into four distinct groups (n = 5/group): Group I - Control; Group II – Ab 100 mg/kg; Group III – Pb 100 mg/L; Group IV – Ab+Pb - 100 mg/kg + 100 mg/L. This exposure regimen persisted until the 19th day of gestation, when the pregnant rats were euthanized. We evaluated a range of outcomes, including weight gain, toxicokinetics, haematological characteristics, biochemical markers, oxidative stress indicators, as well as reproductive capacity and embryofetal development. Notably, co-administration of these mushrooms played a significant role in alleviating the harmful impacts of exposure to Pb and promote recovery. Notably, Agaricus Bisporus exhibited antioxidant properties, leading to improvements in oxidative stress parameters. Furthermore, the mushroom exhibited a partial ability to reverse damage to fetal morphology and bone attributes. In our investigation involving C. elegans, we made noteworthy observations. Co-exposure to Pb together with 100 mg/mL of Agaricus Bisporus demonstrated a reduction in embryonic and larval mortality. This combination also led to increased clutch size and motility compared to nematodes exposed to Pb alone. In addition, we observed mitigation of transmission of reproductive toxicity from nematode parents to their offspring. Based on these findings, we propose that the antioxidant attributes of Agaricus Bisporus have the potential to mitigate the unfavorable consequences of Pb-induced reproductive toxicity. Consequently, this natural antioxidant may serve as a valuable countermeasure against the detrimental impact of Pb on reproductive health.

Key Words: *Agaricus bisporus.C. elegans.* Lead. Intoxication. Reproductive toxicity.

LISTA DE ABREVIATURAS

Ab – *Agaricus bisporus*

ALT - Alanina aminotranferase

AST - Aspartato aminotranferase

CAT - Catalase

CEUA - Comissão de ética no uso de animais

DTNB - 5-5-ditio-bis-2-ácido nitrobenzóico

EAG - Equivalente de ácido gálico

EDTA - Ácido etilenodiamino tetra-acético

EROS – Espécie reativa de oxigênio

GR - Glutationa redutase

GPx - Glutationa peroxidase

GSH - Glutationa reduzida

Hb - Hemoglobina

HCl - Ácido clorídrico

H_2O_2 - Peróxido de hidrogênio

H_3PO_4 - Ácido fosfórico

HTC – Hematócrito

IAL –Instituto Adolf Lutz

KCl - Cloreto de potássio

KOH - Hidróxido de potássio

LAPETOX - Laboratório de Pesquisa Toxicológica

LDL - Lipoproteína de baixa densidade

MDA - Malonaldeído

mM - Milimolar

NaOH - Hidróxido de sódio

nm - Nanômetro

OH - Radical hidroxila

OMS - Organização Mundial da Saúde

O_2 – Oxigênio

Pb - Chumbo

PLT - Plaquetas

RBC – Red Blood Cells (Contagem total de eritrócitos)

rpm - rotação por minuto

TBA - Ácido tiobarbitúrico

TBARS - Substâncias reativas ao ácido tiobarbitúrico

TCA - Ácido tricloroacético

TFK - Tampão fosfato de potássio

UNISO - Universidade de Sorocaba

USP - Universidade de São Paulo

WBC – White Blood Cells (Contagem total de Leucócitos)

SUMÁRIO

1 INTRODUÇÃO

Os metais são elementos naturais encontrados em toda a crosta terrestre. Entretanto, alguns desses elementos não tem funcionalidade/essencialidade para o organismo, e assim tem potencial de toxicidade humano ou ambiental (ZORODDU et al.,2019). Sabe-se que a contaminação pelos metais e compostos metálicos alteram funções de alguns órgãos e sistemas como o sistema hematopoiético, fígado, rins e sistema nervoso central (FU; XI, 2020). A contaminação humana por metais ocorre principalmente através da ingestão de água e alimentos contaminados, por via dérmica e por meio da inalação (ar atmosférico). Os indivíduos mais suscetíveis são, principalmente as crianças, idosos e gestantes (CAUSSY et al., 2003; AL OSMAN; YANG; MASSEY, 2019).

Os elementos químicos classificados como essenciais (cobre, ferro, manganês e zinco, por exemplo) têm funções fisiológicas no corpo, agindo como cofatores enzimáticos ou como grupos funcionais de proteínas. Esses elementos são indispensáveis para o normal funcionamento do organismo (ZORODDU et al., 2019). Contrariamente, elementos químicos classificados como não essenciais (chumbo, mercúrio e cádmio) não desemprenham nenhum papel na fisiologia humana e são considerados tóxicos, pois alteram a função de tecidos, causam inibição enzimática, e geram espécies reativas de oxigênio (EROs), e, consequentemente, o estresse oxidativo (FU, XI, 20119; AMADI et al., 2019; WANI; ARA; USMANI, 2015).

De acordo com Ress; Fuller (2020) a preocupação com a saúde pública associada à contaminação ambiental por chumbo (Pb) tem aumentado. A contaminação pode estar diretamente relacionada ao descarte irregular de resíduos deste metal pesado por fábricas e mineração. Já foi provado que o Pb pode permanecer no solo por muitos anos, mesmo após o fechamento da principal fonte de contaminação, devido a sua estabilidade química. Na população, em geral, a absorção oral do Pb é a via mais comum, e adultos podem absorver no máximo 10% do Pb ingerido, contrastando com as crianças (entre 2 meses e 6 anos de idade), que podem absorver até 50% da quantidade ingerida (WANI; ARA; USMANI, 2015). Quanto aos efeitos tóxicos, em adultos os danos cardiovasculares são os mais comuns, enquanto as crianças estão suscetíveis a danos neurológicos e dificuldades de aprendizagem (CDC, 2007; FREITAS et al., 2007). Outro efeito tóxico bem conhecido é a anemia, de leve a moderada em adultos, e severa em crianças, que ocorre pela inibição da síntese do grupo heme que é uma molécula complexa que desempenha papel crucial em vários processos biológicos, particularmente no transporte e armazenamento de oxigênio dentro do corpo sendo um composto contendo ferro encontrado na hemoglobina, proteína responsável pelo transporte de

oxigênio nas células vermelhas do sangue, e na mioglobina, proteína que armazena e libera oxigênio nas células musculares. A molécula heme consiste em uma estrutura de anel de porfirina com um átomo de ferro em seu centro desta forma íon de ferro podem se ligar ao oxigênio, permitindo o transporte e liberação eficiente de oxigênio nos tecidos debvido a isso na presença de oxigênio, a molécula heme torna-se oxigenada, dando à hemoglobina e à mioglobina sua cor vermelha característica. (WOOD; SPERLING, 2019). Do sangue, o Pb é distribuído a todos os tecidos (especialmente tecidos moles como os rins e fígado), inclusive ao feto. Posteriormente, cerca de 90% do Pb é depositado nos ossos (MINOZZO et al., 2008).

Os antioxidantes presentes no organismo humano são os principais mecanismos de defesa contra os efeitos tóxicos de Pb (BOTTARI et al., 2020). No entanto, este mecanismo de defesa pode não ser suficiente, sendo necessária a administração de agente quelante. A principal função desses agentes quelantes é a quelação (sequestro) do metal tóxico, formando preferencialmente uma espécie menos tóxica que é excretada (KIM; KIM; KUMAR, 2019). Alguns dos quelantes sintéticos utilizados para eliminar o Pb do organismo como EDTACaNa2 (ácido etilenodiamino- tetracético cálcico dissódico), BAL (Dimercaprol) e DMSA (ácido dimercaptosuccínico). Porém, algumas reações adversas já foram relatadas durante o tratamento, como desconforto gastrointestinal, reações cutâneas, elevação das enzimas hepáticas, além da quelação de metais essenciais (ANDERSEN, 2004; AMADI et al., 2019).

Assim, o desenvolvimento de novos quelantes, mais seguros, adequados para administração oral em longo prazo é essencial. O cogumelo *Agaricus bisporus*, popularmente conhecido como champignon, possui antioxidantes e, especialmente, contém quitina, um polissacarídeo constituído por um polímero de cadeia longa de N-acetilglicosamina, precursor direto da quitosana. Segundo Abuja; Albertini (2001), essas substâncias presentes no *Agaricus bisporus* são capazes de neutralizar os efeitos tóxicos do Pb, sequestrando o metal livre. A quitina e a quitosana possuem boas propriedades quelantes de Pb e do cádmio, além da função de adsorção, já comprovada em tratamento de água para redução de metais pesados (BORNET; TEISSEDRE, 2008; MENK et al., 2019; CASTANHO et al., 2021).

Dada a problemática do Pb e a possível solução com o uso de cogumelo comestível, essa tese foi construída na forma de artigos científicos. Primeiramente, tem-se a sessão de revisão da literatura e objetivos gerais do estudo. Em seguida, a tese está dividida em capítulos (três no total), sendo que cada capítulo é um artigo autônomo. Assim, as seções de materiais e métodos, resultados e discussão estão incorporados em cada um dos artigos. A formatação de cada artigo segue as normas das revistas escolhidas. O primeiro artigo, “Influence of *Agaricus bisporus* mushroom on Pb toxicokinetic in pregnant rats” foi aceito em fevereiro de 2023; o

segundo manuscrito, “Protective effect of *Agaricus bisporus* mushroom against maternal and fetal damage induced by lead administration during pregnancy, in rats.” Foi aceito em junho de 2023; e o terceiro manuscrito, “Protective efficacy of *Agaricus bisporus* against lead-induced toxicity in *Caenorhabditis elegans*” foi submetido em maio de 2023.

2 REVISÃO DA LITERATURA

2.1 Chumbo: usos e fontes de exposição

O chumbo (Pb) é um elemento químico de número atômico 82, massa atômica 207,2 g e pertencente ao grupo 14 da tabela periódica. Tem coloração branca-azulada, tornando-se acinzentado quando exposto ao ar, sendo geralmente encontrado na forma dos minerais galena (PbS), anglesita ($PbSO_4$) e cerusita ($PbCO_3$) (CARR, 2004; HAYNES, 2014). O Pb caracteriza-se por ser um metal pesado, tóxico e maleável de ocorrência natural onipresente, sendo um dos primeiros metais descobertos (WIWANITKIT; SUWANSAKSRI, 2006). O Pb existe nas formas orgânica e inorgânica, ambas tóxicas, sendo o Pb orgânico mais tóxico que o inorgânico por ser mais rapidamente absorvido; pode ser ainda encontrado em dois estados de oxidação Pb (+2) e Pb (+4) (KING et al, 2014).

No ambiente, o íon estável do Pb é encontrado principalmente no estado (+2). Em compostos inorgânicos e combinado com o enxofre (S), forma a galena (PbS = 86,6% de Pb e 13,4% de S), um dos mais abundantes minérios, do qual é extraído quase todo o chumbo primário. O Pb (+4) é relativamente instável, e formado apenas sob condições fortemente oxidantes. Nessas condições, forma os compostos orgânicos chumbo tetrametila $Pb(CH_3)_4$) e chumbo tetraetila ($Pb(C_2H_5)_4$), que são extremamente tóxicos (SYVERSON et al., 2019; ALKHATIB et al., 2014).

Propriedades únicas do Pb, como maleabilidade elevada, ductilidade, baixo ponto de fusão e resistência à corrosão, resultou no seu uso difundido em diferentes indústrias, como de automóveis, pintura, cerâmica, plásticos entre outros (GAGAN; GUPTA; TIWARE,2012). A importância comercial da Pb baseia-se em suas propriedades físicas, incluindo seu baixo ponto de derretimento, facilidade de fundição, alta densidade, maciez, maleabilidade, baixa resistência, facilidade de fabricação, ácido resistência, reação eletroquímica com ácido sulfúrico e estabilidade química no ar, água e solo (KING et al., 2014). Tais características levaram ao aumento múltiplo na ocorrência de Pb livre no ambiente, e sua concentração ambiental varia de local para local (NEEDLEMAN, 2004). Pelo seu amplo uso, o Pb é considerado um agente tóxico ocupacional potente e sua natureza não biodegradável é a principal razão de sua prolongada persistência no ambiente (JACOB et al., 2002).

O Pb pode ser usado na forma de metal puro ou ligado com outros metais. Estima-se que o Pb seja utilizado em mais de 200 processos industriais diferentes, incluindo mineração, fundição, fabricação de produtos, combustão de carvão e petróleo, com destaque para a

produção de baterias elétricas (LEE et al., 2016). Esta utilização não é realizada apenas em grandes empresas, com melhor controle das condições ambientais de trabalho, mas também em pequenas empresas, muitas das quais instaladas em regiões residenciais, e funcionando nem sempre de acordo com a legislação trabalhista, ambiental e de saúde (BRASIL, 2006).

Além da produção e reforma de baterias automotivas, o Pb é utilizado na indústria de plástico, na lapidação de pedras preciosas, instrução e aprendizado de tiro, reparação de radiadores de carro, reciclagem de baterias automotivas, redução de minérios ricos em ouro para obtenção deste, entre outros (MENEZES; D'SOUZA; VENKATESH, 2003; LO et al., 2012; SCHIFER et al., 2005).

A exposição da população humana ao Pb era relativamente baixa antes da revolução industrial, mas aumentou com a industrialização e mineração em larga escala. Vários países são fontes permanentes de exposição ao Pb. No Brasil, no município de Adrianópolis, no Paraná, funcionou entre 1945 e 1995 a usina Plumbum Mineração e Metalurgia Ltda, responsável pelas atividades metalúrgicas. Como consequência dessas atividades, cerca de três milhões de toneladas de rejeitos de Pb foram lançados diretamente no Rio Ribeira, sem tratamento e em 1995, as minas e a refinaria encerraram suas atividades, abandonado um importante passivo ambiental que ameaça a saúde da população (DI GIULIO et al., 2012).

No ano de 2010, durante a vigilância rotineira da meningite no estado de Zamfara na Nigéria, realizada pelos Médicos Sem Fronteiras e as autoridades locais de saúde pública identificaram crianças com sintomas de intoxicação por Pb, relacionado ao processamento de minério de ouro, sendo uma das principais fontes de rendas no local (LO et al., 2012, AJUMOBI et al., 2014). A China enfrenta um desafio de saúde pública e estabilidade social em relação ao envenenamento por Pb, especialmente em crianças. De 2009 a 2011 em várias províncias da China, foram relatados incidentes de intoxicação por Pb com impactos negativos em mais de 4.000 crianças, além de estudos documentando altos níveis de Pb nas populações trabalhadoras nas áreas de fundição e mineração de Pb, bem como nas fábricas (CHEN et al., 2012; WANG; ZHANG, 2006).

Segundo o Ministério da Saúde, no Brasil não existem registros ou estimativas confiáveis do número de indivíduos expostos ocupacional e ambientalmente ao metal. Entretanto, a literatura especializada vem apontando grupos de trabalhadores intoxicados principalmente entre os envolvidos na produção, reforma e reciclagem de baterias automotivas (BRASIL, 2006; MINOZZO et al., 2009).

A atmosfera é o principal meio de transporte ambiental do Pb, o qual se deposita em águas e solos superficiais (EPA 2006). Após a liberação na atmosfera, as partículas de Pb são

dispersas e, finalmente, removidas da atmosfera por deposição úmida ou seca (TONG; SCHIRNDING; PRAPAMONTOL, 2000).

No ar, Pb está na forma de partículas sendo removido por chuva ou assentamento gravitacional. Com base em dados do Inventário Nacional de Emissões (NEI, 2014), os seguintes setores contribuem com as maiores parcelas das emissões totais de Pb: Aeronaves, processos industriais, combustão/geração elétrica e carvão (EPA, 2016). Embora os níveis de Pb em tintas para uso interno tenham sido restritos desde a década de 1950 em muitos países, casas e móveis mais antigos ainda podem ser cobertos com tinta contendo Pb. As liberações de tintas à base de Pb são frequentemente confinadas à área nas proximidades das superfícies pintadas, e a deterioração ou remoção da tinta por lixamento ou jateamento de areia pode resultar em altas concentrações localizadas de pó Pb tanto no ar interno quanto externo (GOTTESFELD, 2015).Com relação à contaminação aquática, os setores da indústria que representam a maior parte da liberação do Pb para a água superficial são os de produtos químicos, papel, metais primários, equipamentos de transporte e utilitários elétricos (TRI15, 2017). A quantidade de Pb solúvel em águas superficiais depende do pH e da força iônica da água, podendo estar presente em revestimentos superficiais, em partículas minerais de sedimentos, ou pode ser transportado como parte de matéria orgânica suspensa ou não viva na água (ATSDR, 2007).

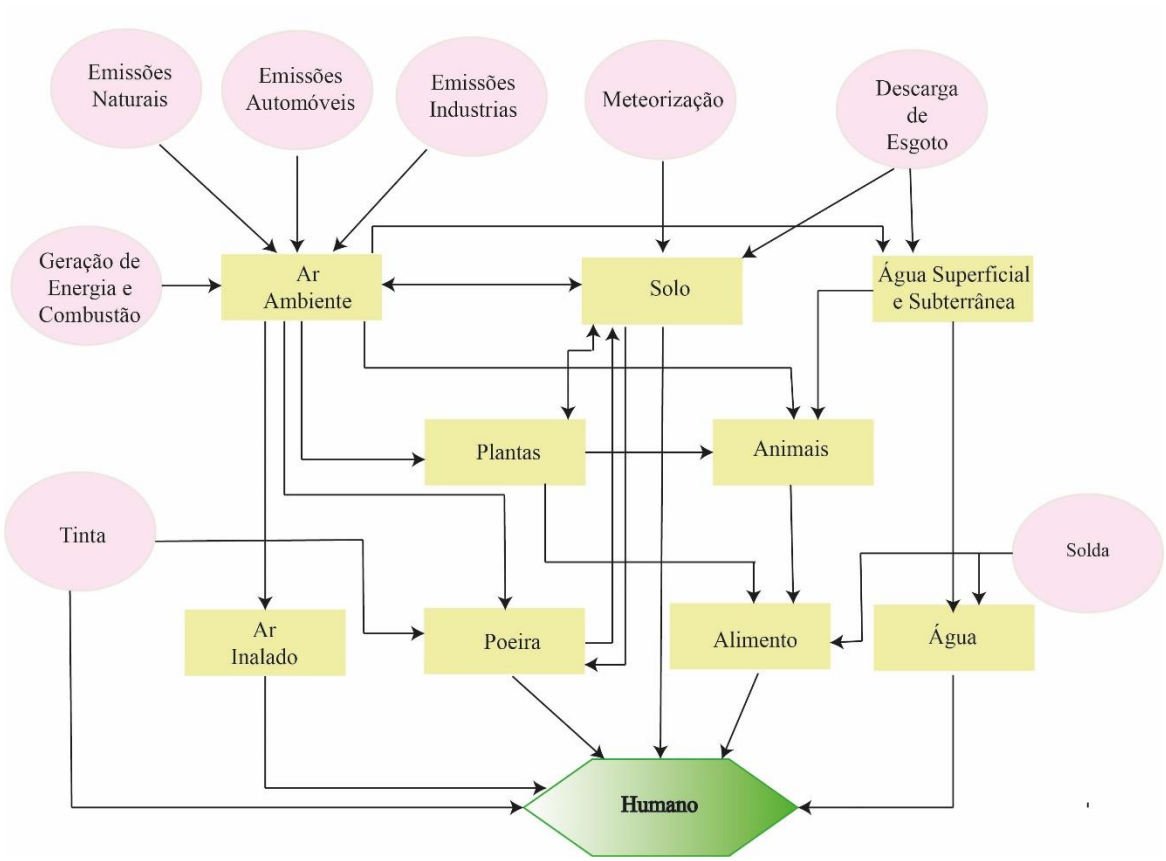
Dados divulgados pelo Environment and Climate Change, Canadá (2016), mostram que outras indústrias, como de ferro e aço, indústria de petróleo e gás e a indústria de cimento e concreto, contribuíram com 136,9 toneladas do total de Pb liberado na água, em 2014. Esta liberação inclui 134,1 toneladas de Pb que foram liberadas quando uma barragem que assegura um lago de rejeitos da mina de Mount Polley, no centro da Colúmbia Britânica, rompeu em 4 de agosto de 2014, derramando resíduos de mineração no Lago Polley e águas circundantes.

Em 2015, no Brasil a barragem de Fundão, da mineradora Samarco, uma empresa *joint venture* da companhia Vale do Rio Doce e da anglo-australiana BHP- Billiton, se rompeu causando grande impacto ambiental. Foram identificados altos níveis de contaminação por metal pesado na água de rios próximos a represa. E resultados de análises em amostras de peixes e crustáceos apresentaram grande parte das amostras com níveis de arsênio, cádmio e Pb acima da legislação (FREITAS; SILVA; MENEZES, 2016).

Já a poluição do solo com metais pesados é um problema sério do ponto de vista do meio ambiente e da saúde pública porque os metais tendem a persistir, circulando indefinidamente e se bioacumulando em toda a cadeia alimentar. O solo é considerado um dos principais depósitos, uma vez que, ao alcançá-lo, o Pb pode permanecer ali indefinidamente

(WIECZOREK et al., 2018; AFRIFA et al., 2015). É importante ressaltar ainda que os ambientes aquático, terrestre e aéreo estão em constante comunicação, e assim as rotas de exposição são interligadas, conforme Figura 1. O Pb é liberado no ar pela mineração de metais, por exemplo, ou pela água em irrigação, ou descarte irregular em rios, e se instalará em solo, sedimento, folhagens ou outras superfícies (KOWALSKA et al., 2016; BARAN et al., 2017).

Figura 1 - Rotas de exposição humana ao chumbo (Pb), considerando os ambientes aquático, aéreo e terrestre.



Fonte: Adaptado de IPCS, 1995.

2.1.1 Toxicocinética do Pb no organismo

O Pb é um elemento tóxico, sem qualquer atividade fisiológica. Ele é absorvido, distribuído, armazenado e excretado, e por não ser metabolizado - e sim complexado por macromoléculas (PAOLIELLO; CHASIN, 2001), é importante entender a cinética deste metal no organismo.

As principais vias de introdução do Pb inorgânico são a inalação de poeira contaminada e a ingestão de material contaminado, fragmentos de tinta, alimentos e água. Já as formas orgânicas de Pb podem ser absorvidas rapidamente pelos pulmões, trato gastrointestinal e pela pele (BRASIL, 2006).

A extensão e taxa de absorção gastrointestinal do Pb são influenciadas pela fisiologia do indivíduo (por exemplo, idade, jejum, estado nutricional e gravidez), características físico-químicas do metal (tamanho das partículas, solubilidade, característica orgânica ou inorgânica) e a dose de Pb ingerida (GREEN; PAIN, 2019). As crianças absorvem maior proporção do Pb (40 a 50%) de uma dose oral de Pb solúvel em água, em comparação com adultos (3-10%), possivelmente devido a combinação de diferenças fisiológicas e diferenças na dieta e nutrição (CAPITANI, 2009).

Após a absorção, o Pb é transportado pela corrente sanguínea e distribui-se da mesma forma, independentemente da rota de absorção. Cerca de 90% do Pb se encontra nos glóbulos vermelhos (90%), ligando a várias proteínas intracelulares; o restante se encontra no plasma, provavelmente ligado à albumina, globulina ou como íons livres disponíveis para o transporte aos tecidos (SMITH et al., 2002; MANTON; ROTHENBERG; MANALO, 2001). A enzima Ácido delta aminolevulínico desidratase (ALAD) é a ligante com maior afinidade pelo Pb. Assim que o Pb se liga, inibe a atividade de ALAD, deslocando o zinco, que é um mineral essencial para a atividade de ALAD (JAFFE et al., 2000).

Em seguida, o Pb é transferido do sangue para tecidos moles e para os ossos, onde se acumula. Em adultos expostos, aproximadamente 90% da carga corporal total de Pb é encontrada nos ossos, sendo um reservatório. O depósito de Pb nos ossos pode manter os níveis de Pb no sangue muito tempo após o término da exposição, e pode servir como fonte de transferência de Pb para o feto, pois durante o desenvolvimento do esqueleto fetal, ocorre maior mobilização do cálcio do osso materno (GULSON et al., 2003). Isso ocorre pois o Pb forma complexos altamente estáveis com fosfato, substituindo o cálcio na hidroxiapatita (cálcio e fosfato), que compreende a matriz cristalina primária do osso. Como resultado, o Pb se deposita no osso durante o processo normal de mineralização que ocorre durante o crescimento e remodelação óssea, e é liberado ao sangue durante o processo de resorção óssea (MEIRE et al., 2011).

A maior parte de Pb em tecidos moles está no fígado, cérebro e no córtex renal. Em contraste com o osso, que acumula Pb com exposição contínua, as concentrações em tecidos moles são relativamente constantes em adultos, refletindo uma rotatividade mais rápida de Pb no tecido mole, em relação ao osso (MARI et al., 2014).

Independente da via de exposição, o Pb absorvido e não armazenado é excretado em maior quantidade pela urina e fezes; suor, saliva, cabelo e unhas, leite materno e fluidos seminal são rotas menores de excreção (HERNÁNDEZ-OCHOA et al., 2005; SEARS et al., 2012). A meia-vida de eliminação do Pb nos três compartimentos (sangue, osso e tecidos moles) é bastante diferente, sendo estimada em 30 dias para o sangue, 40 dias para os tecidos moles e várias décadas no osso (USASTDR, 2007).

2.1.2 Efeitos do Pb na saúde

Desde o desenvolvimento do Perfil Toxicológico do Pb, os resultados de inúmeros estudos epidemiológicos têm despertado a atenção crescente aos efeitos adversos à saúde, pois esta exposição está associada à toxicidade a todos os sistemas e órgãos.

Interferências no funcionamento das membranas celulares e enzimas são resultado da toxicidade do Pb. O metal forma complexos estáveis com ligantes contendo enxofre, fósforo, nitrogênio ou oxigênio (grupamentos –SH, $–H_2PO_3$, $–NH_2$, –OH), que funcionam como doadores de elétrons. As interações bioquímicas do Pb com grupamentos sulfidril (–SH) são consideradas de grande significado toxicológico, visto que se tal alteração ocorrer em uma enzima, sua atividade pode ser alterada e resultar em efeitos tóxicos (MOREIRA; MOREIRA, 2004). Os principais efeitos tóxicos observados são os efeitos neurológicos, renais, cardiovasculares, hematológicos e gastrointestinal.

O sistema nervoso central (SNC) é o conjunto de órgãos mais sensível à contaminação por Pb. Os efeitos neurológicos são os de maior preocupação pois são observados em concentrações sanguíneas menores que 10 μg/dL em bebês e crianças, e aparece nos adultos em concentrações em torno de 40 μg/dL (KRIEG et al., 2010; PATRICK et al., 2006; RESS; FULLER; 2020). Os principais sintomas relatados em crianças são: função cognitiva reduzida, alteração do humor, déficit de aprendizagem, incluindo déficits de atenção, hiperatividade, comportamentos autistas, transtornos de conduta, neuropatia periférica e encefalopatia, tendo como principais efeitos sensorial, motores dor ,Alterações cognitivas e comportamentais e alteraçoes neurológicas (WASSERMAN et al., 2003; BELLINGER, 2008; BOUCHER et al., 2012, BRAUN et al., 2012).

Os efeitos neurológicos em adultos, associados à exposição ao Pb, incluem: diminuição da função cognitiva, abrangendo atenção, memória e aprendizado, alteração da função neuromotor e neurossensorial, incluindo diminuição do tempo de reação e velocidade de caminhada, tremor e aumento do risco de esclerose lateral amiotrófica (ELA), além de alteração

de humor e comportamento (sintomas psiquiátrico, como ansiedade, depressão e esquizofrenia) (POWER et al., 2014; FANG et al., 2010; BOUCHARD et al.,2009).

Os efeitos neurotóxicos no SNC ocorrem principalmente pois o Pb substitui o cálcio e, em menor grau, o zinco, e desencadeia inadequadamente processos dependentes da calmodulina. Por este mecanismo, o Pb promove alterações na entrada do cálcio na célula modificando a sinalização neuronal. O resultado consiste na interferência de liberação de neurotransmissores, interrompendo a função dos sistemas GABAérgicos, dopaminérgicos e colinérgicos, além de inibir os canais de N-metil D-Aspartato (NMDA) durante o período neonatal (MASON; HARP; HAN, 2014).

A toxicidade induzida pela Pb ao sistema hematológico também é muito importante e foi descoberta há muito tempo. O Pb inibe a síntese do heme, levando ao desenvolvimento de anemia microcítica e hipocrômica geralmente leve a moderada em adultos e, algumas vezes, é severa em crianças (IOLASCON et al., 2009; WOOD; SPERLING, 2019). A anemia mostra consistentemente inibição de enzimas de síntese do heme, particularmente δ-ALAD, e subsequentes reduções na hemoglobina sanguínea. Outros efeitos hematológicos observados em estudos epidemiológicos incluem alterações na função do eritrócito (diminuição das atividades de pirimidina 5'-nucleotidase e membrana Ca^{2+}/Mg^{2+}ATPase), alterações na concentração de eritropoietina sérica e diminuição da contagem de plaquetas (ERGURHAN-ILHANET al., 2008; AHAMED et al., 2006; ABAM et al., 2008). Os limites do Pb, em sangue, necessários para induzir decréscimo dos níveis de hemoglobina, em adultos e crianças, estão estimados em 50 e 40 μg/dL, respectivamente (PAOLIELLO; CHASIN, 2001).

Exposições agudas ou crônicas ao Pb podem causar ainda alteração na função renal, contribuindo para o desenvolvimento da doença renal crônica (KIM et al., 2015). A nefrotoxicidade aguda é caracterizada por disfunção dos túbulos proximais, distal e coletores manifestada por aminoacidúria, glicosúria e fosfatúria, aumento da excreção renal de sódio e redução da excreção de ácido úrico. Na nefropatia crônica, observa-se fibrose intersticial progressiva, dilatação dos túbulos, hiperplasia das células tubulares, redução da taxa de filtração glomerular, perda de capilares peritubulares, injuria tubular e presença de infiltrado inflamatório (SUN et al., 2008; RONCAL et al., 2007).

O efeito da exposição ao Pb na pressão arterial é o desfecho cardiovascular mais estudado. Inúmeros fatores afetam a pressão arterial, incluindo idade, massa corporal, etnia, tabagismo, consumo de álcool, histórico contínuo ou familiar de doenças cardiovasculares/renais, níveis de colesterol LDL e vários fatores alimentares. Além disso, a doença renal, bem como danos renais induzidos pelo Pb, pode levar a efeitos cardiovasculares,

incluindo aumento da pressão arterial (NAVAS-ACIEN et al., 2007). Os efeitos cardiovasculares do Pb, no entanto, não se limitam ao aumento da pressão arterial e da hipertensão. A exposição ao Pb também foi associada ao um aumento da incidência de desfechos cardiovasculares clínicos, como doença cardíaca coronária, acidente vascular encefálico (AVE) e doença arterial periférica, e com outras anormalidades da função cardiovascular, como hipertrofia ventricular esquerda e alterações no ritmo cardíaco (NAVAS-ACIEN et al., 2004; MENKE et al., 2006).

Os estudos epidemiológicos que avaliaram os efeitos gastrointestinais associados à exposição crônica ao Pb relataram consistentemente sintomas como cólica abdominal/dor, náusea, vômito, diarreia e/ou prisão de ventre. Este quadro é observado com concentrações de Pb entre 8,04 e 100 μg/dL. A exposição aguda ao Pb está associada a alterações gastrointestinais e paralisia intestinal (KURUVILLA et al.,2006; ROSENMAN et al., 2006).

2.1.3 Legislação

Em muitos países, o uso de Pb em partes de processos e produtos é restringido, e em alguns é proibido. No Brasil, o uso do Pb tetraetílico na gasolina foi proibido desde 1978. Em 2008, foi publicada a lei (n° 11.762) estabelecendo um limite máximo de 0,06% em peso de Pb em tintas imobiliárias e de uso infantil e escolar, vernizes e materiais similares de revestimento de superfícies, fabricadas, comercializadas e distribuídas no país. A legislação brasileira não regulamenta a utilização de Pb em tintas para outras finalidades como, por exemplo, as de uso gráfico (BRASIL, 2008).

No entanto, ainda existem países que utilizam o Pb na gasolina e em tintas usadas na fabricação de brinquedos. Apesar da legislação brasileira determinar limite sobre o Pb em tintas de brinquedos, existe a preocupação com a falta de controle na importação de brinquedos, principalmente chineses que, muitas vezes, não estão adequados aos limites estabelecidos (CAPITANI et al., 2009).

Frente a várias fontes de exposição ao Pb, alguns países estabeleceram concentrações máximas de Pb no sangue para pessoas expostas e não expostas. No Brasil, a Norma Regulamentadora NR-7, do Ministério do Trabalho, estabelece os valores de referência, para pessoas não expostas, de 40 μg/dL de sangue, e o Índice Biológico Máximo Permitido (IBMP) é de 60 μg/dL para pessoas expostas (BRASIL,1994). A *Agency for Toxic Substances and Disease Registry* (ATSDR) recomenda como segura uma concentração sanguínea de Pb inferior

a 30 μg/dL em adultos não expostos e 60 μg/dL em adultos expostos ocupacionalmente (ABADIN et al., 2007).

A Portaria nº 685 da Secretaria Nacional de Vigilância Sanitária, estabelece limites máximos de tolerância (LMT) para o Pb em alimentos, nas condições em que são consumidos. Esses valores variam de 0,05 a 2 mg/kg de alimento (BRASIL, 1998). A ingestão diária tolerável provisória para o Pb é de 3,6 μg/kg de peso corpóreo, enquanto a ingestão semanal tolerável provisória é de 25 μg/kg de peso corpóreo, ambas recomendadas pela Organização Mundial da Saúde (FAO/WHO, 1993).

Em relação a presença de Pb na água, a Portaria n.º 518 do Ministério da Saúde, estabelece valor máximo permitido (VMP) de 0,01 mg/L de Pb, garantindo padrão de potabilidade para substâncias químicas que representam risco à saúde (BRASIL, 2004).

A concentração máxima dos compostos orgânicos do Pb (tetraetila) que é utilizada como antidetonante na gasolina segue legislação específica e delimitada em alguns países e em muitos outros esta aplicação foi banida (ATSDR, 1993). No Brasil, o Pb foi banido da gasolina em 1978 e substituído por etanol. Porém, boa parte da contaminação dos solos é ainda proveniente do uso do Pb na gasolina, mesmo após anos passados (PAOLIELLO; CHASIN, 2001).

2.1.4 Tratamentos para a intoxicação por Pb

Os principais métodos disponíveis para evitar os efeitos tóxicos do Pb são baseados em sua capacidade de reduzir a carga corporal do Pb por quelação. Todos os agentes quelantes se ligam ao Pb, e aumentam sua excreção facilitando a transferência do Pb de tecidos moles para a circulação onde pode ser excretado (AMADI et al., 2019).

Várias substâncias farmacológicas estão disponíveis para terapia de quelação na intoxicação Pb. Os agentes atualmente em uso são Dimercaprol ou British anti-Lewisite (BAL), CaNa2-EDTA (ou EDTA), e 2,3-dimercaptosuccinico ácido (DMSA; Succimer®). Dosagens e protocolos de administração para esses agentes variam com a idade do paciente, nível de Pb, tipos de sintomas e gravidade (ANDERSEN, 2004).

- **Dimercaprol:** Também chamado de 2,3-dimercaptopropanol ou anti-Lewisita britânico (BAL), foi desenvolvido como um antídoto para o lewisite (um agente químico de guerra química agora baseado em arsênico obsoleto) por bioquímicos britânicos durante a Segunda Guerra Mundial. Atualmente, é usado principalmente para tratar arsênico, mercúrio, ouro, Pb antimônio e outras intoxicações por metais tóxicos (TIAN; SHI, 2017). Após a

administração do dimercaprol, o início da ação é 30 min, aumentando a excreção fecal e urinária de Pb quelatado. Porém foram associados com o dimercaprol efeitos adversos, incluindo náuseas, vômitos, hipertensão, taquicardia, dor de cabeça, ansiedade, dor abdominal e febre (ARCHER, 2008).

- **CaNa2-EDTA (ou EDTA):** EDTA funciona formando complexo de metal-quelato estável que é excretado pelo rim. Aumentando a excreção renal do Pb em 20-50 vezes. Porém inúmeros efeitos adversos foram descritos, incluindo erupção cutânea, febre, fadiga, mialgias, calafrios e arritmias cardíacas. Além do EDTA quelar minerais essenciais, principalmente o zinco (KOSNET, 2005).
- **2,3-Ácido dimercaptosuccinico (DMSA; Succimer®):** É um medicamento quelante administrado por via oral, rotulado para envenenamento por Pb. É tão eficaz quanto ao EDTA no aumento da excreção urinária do Pb. E os efeitos adversos relatados são mínimos (anorexia, náusea, vômito, e erupções cutâneas). O DMSA aumenta a excreção de zinco, mas em grau muito menor que os outros quelantes, além de ter poucos efeitos sobre cálcio, ferro, magnésio e cobre (CAO et al., 2011; SMITH; STRUPP, 2013).

2.2 Alimentos funcionais

Na década de 1950, pesquisas relacionando nutrição e doenças degenerativas ganharam espaço, especialmente na conexão entre doenças cardíacas e ingestão dietética de gordura (MELLENTIN, 2001). A demanda por alimentos saudáveis está em constante crescimento, motivada pela preocupação com a saúde e pelo aumento da expectativa de vida. Os consumidores estão cada vez mais preocupados com sua saúde e promovem cuidados extras com seu estilo de vida e com a saúde de sua dieta (SIRÓ et al., 2008).

Na década de 1980, os japoneses começaram a perceber a importância da prevenção da saúde, devido ao aumento de patologias relacionadas com o estilo de vida, além de começarem a prestar mais atenção no envelhecimento da população (OHAMA; IKEDA; MORIYAMA, 2006). E na década de 80 o termo “comida funcional” foi usado e introduzido pela primeira vez no Japão, com o desenvolvimento de um refrigerante contento fibra dietética “Fibe Mini”, lançado em 1988 (ARAI, 2002).

Assim, alguns cientistas e consultores de negócios, no início da década de 1990, começaram a estudar sobre os efeitos fisiológicos de vários alimentos e seus ingredientes e promover ativamente a nova tendência de comidas funcionais. Desta forma, o conceito “alimentos funcionais” só ganhou reconhecimento durante os anos 1990 (MELLENTIN, 2001).

Porém, em 1991, os japoneses abandonam o termo "alimento funcional", devido à proibição do governo, pois o uso da palavra alimento funcional, poderia implicar em duplo entendimento dos consumidores, que usariam o alimento como medicamento, com a expectativa de prevenção ou até mesmo cura. Por tal razão foi necessário substituir o termo. E o conceito de alimentos funcionais foi integrado aos Alimentos para Sistema de Usos de Saúde Especificados (*FOSHU*), que é uma regulamentação específica para processos de aprovação de alimentos funcionais. Ou seja, para ser integrado aos alimentos *FOSHU*, os alimentos devem ter efeito específico sobre a saúde devido a sua constituição química e não podem causar risco a saúde (BIANCO, 2008).

Apesar do conceito de alimentos funcionais estar relacionada aos japoneses, a história, desse tipo de alimento é bem mais antiga. Nos países asiáticos, há muitos anos estes alimentos funcionais são considerados como parte integrante da cultura e são tradicionalmente associados a benefícios específicos de saúde (WESTSTRATE et al., 2002).

Com o passar dos anos, o alimento funcional ganhou maior percepção de marketing, sendo usualmente conceituado e promovido no mercado a partir dos benefícios que pode oferecer à saúde (URALA; LÄHTEENMÄKI, 2007). No entanto, somente nos últimos anos os cientistas e os órgãos reguladores começaram a concordar que a "funcionalidade" dos alimentos deve ser encontrada no alimento como um todo, ao invés de seus componentes individuais (VERSCHUREN, 2007).

De fato, grande variedade de produtos alimentícios são, ou serão no futuro, caracterizados como alimento funcional, afetando variedade de funções corporais relevantes para um estado de bem-estar e saúde e/ou para a redução do risco de uma doença (ROBERFROID, 2000). Desta forma para um alimento ser considerado como funcional, além de ser excelente produto alimentício como tal, deve oferecer o efeito específico para a saúde, com funções de um ou mais alvos benéficos no corpo (MARK-HERBERT, 2004).

Nas últimas décadas, uma variedade de definições sobre alimentos funcionais foi descrita tanto na literatura científica quanto na voltada para o *marketing*. Estas definições vão desde simples declarações como: "Alimentos que, além de serem componentes da nutrição básica, proporcionam benefícios sanitários" (IFIC Foundation, 1995), e "Alimentos ou produtos alimentares comercializados com a mensagem do benefício para a saúde*"* (RIEMERSMA, 1996); ou definições mais elaboradas de acordo com Health Canada (1997), "Alimentos similares na aparência à comida convencional, que são consumidos como parte da dieta usual e tem demonstração de benefício fisiológico e/ou redução o risco de doença crônica além da nutrição básica", e como descrito por Food and Nutrition Board (1994), "Alimentos que englobam produtos potencialmente úteis, incluindo qualquer alimento modificado ou

ingrediente alimentar que pode fornecer um benefício de saúde além do tradicional nutriente que contém".

No Brasil, a legislação brasileira não define "alimentos funcionais"; define alegação de propriedades funcionais por meio da Portaria nº 398, em 30 de abril de 1999, de responsabilidade do Ministério da Saúde, segundo a Agência Nacional de Vigilância Sanitária (BRASIL, 1999), como sendo

> *Alimento ou ingrediente que alegar de propriedades funcionais e/ou de saúde, além de funções nutricionais básicas, produzir efeitos metabólicos e/ou fisiológicos benéficos à saúde, devendo ser seguro para consumo sem supervisão médica, porém no caso de uma nova propriedade funcional, há necessidade de comprovação científica da alegação de propriedades funcionais e/ou de saúde, e da segurança de uso (BRASIL,1999).*

O desenvolvimento de alimentos funcionais deve ter conhecimento científico relevante em relação a suas funções alimentares. A Alegações de Propriedade Funcional e, ou de Saúde num alimento é de caráter opcional, a empresa que desejar fazer uso da alegação deverá requerê-la junto a ANVISA e apresentar a comprovação da segurança e eficácia do alimento por meio de um Relatório Técnico Científico (BRASIL, 1999).

Há exigência que os alimentos tenham sua segurança de uso comprovada, visto que muitos desses alimentos possuem em suas formulações a adição de novos ingredientes, que possuem recomendações de consumo que quando não obedecidas podem provocar efeitos adversos para determinados grupos da população (BRASIL, 2013).

Os alimentos e ingredientes funcionais podem ser classificados de dois modos: quanto à fonte, de origem vegetal ou animal, ou quanto aos benefícios que oferecem, atuando em seis áreas do organismo: no sistema gastrointestinal; no sistema cardiovascular; no metabolismo de substratos; no crescimento, no desenvolvimento e diferenciação celular; no comportamento das funções fisiológicas e como antioxidantes (SOUZA, et al., 2003).

Desta forma o componente de um alimento funcional pode ser um macronutriente, ou um micronutriente como por exemplo (magnésio, ácidos graxos poliinsaturados, ácidos graxos monoinsaturados e diferentes grupos de fenólicos), se a sua quantidade for superior à dose diária recomendada. Pode também ser um componente alimentar que, apesar de ter algum valor

nutritivo, não é essencial (por exemplo, alguns oligossacarídeos) (LEYVA-JIMÉNEZ et al., 2019).

Segundo Roberfroid (2000), podemos considerar um 'alimento funcional' quando:

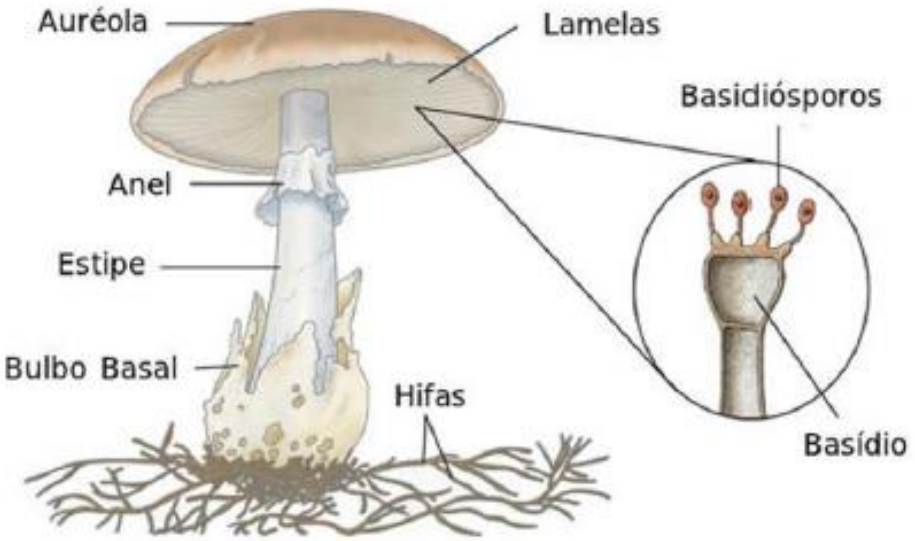
- ✓ Consumido como parte da dieta normal/usual, e composta de ocorrência natural;
- ✓ Ter um efeito positivo sobre a função de destino, podendo melhorar o bem-estar e/ou reduzir o risco de doenças, além de valor nutricional básico;
- ✓ Elimina um componente conhecido por causar um efeito prejudicial quando consumido (por exemplo, uma proteína alergênica);
- ✓ Adiciona um componente que normalmente não está presente nos alimentos e que não é necessariamente um macronutriente ou um micronutriente, mas que foi provado o seu efeito benéfico para os consumidores;
- ✓ Substitui um componente, geralmente um macronutriente (por exemplo, gorduras) que causa efeitos nocivos, por outro componente que apresenta efeitos benéficos para saúde;
- ✓ Aumenta a biodisponibilidade ou a estabilidade de um componente conhecido por produzir um efeito funcional ou reduzir o potencial risco de doença do alimento.

Desta forma, os alimentos não modificados, tais como a fruta, legumes, cereais, vegetais, cogumelos e condimentos, podem representar a forma mais simples de alimentos funcionais (ALI; RAHUT, 2019).

2.3 Cogumelos

Os cogumelos pertenciam ao reino vegetal até 1969, quando eles inauguraram um novo reino da natureza, o Reino *Fungi*. Portanto, os cogumelos são considerados fungos visíveis com frutificação macroscópica (basidiocarpos), pertencentes aos filos Ascomycota (ascomicetos) e Basidiomycota (basidiomicetos), sendo essa frutificação a estrutura de reprodução sexuada destes organismos (DAS, 2010). Morfologicamente, são classificados como *puffballs*, *stinkhorns*, *bracket* e fungos branqueados, podendo ser comestíveis, não comestíveis ou venenosos (BATES, 2006). Um esquema das estruturas básicas de um cogumelo está apresentado na Figura 2.

Figura 2 - Esquema das principais estruturas do cogumelo.



Fonte: MENEZES, 2014.

A Anvisa (Agência Nacional de Vigilância Sanitária) define cogumelos comestíveis na Resolução RDC nº 272 de 2005 como sendo:

> *Um produto obtido de espécie(s) de fungo(s) comestível(is), tradicionalmente utilizada(s) como alimento. Pode ser dessecado, inteiro, fragmentado, moído ou em conserva, submetido a processo de secagem, defumação, cocção, salga e ou fermentação e/ou outro processo tecnológico considerado seguro para a produção de alimentos (BRASIL,2005).*

Os cogumelos são considerados importantes recursos biológicos devido aos diversos benefícios ecológicos, nutricionais e medicinal (ODEYEMI et al., 2014). E por conterem lignina e celulose, são excelentes decompositores de matérias orgânicas mortas, além de colaborar com o meio ambiente na reciclagem de nutrientes no solo e na água (ADENIYI et al., 2018).

Além disso, algumas espécies são muito consumidas pelo homem por serem ricas em proteínas, fibras brutas, vitaminas e minerais. Mais ainda, algumas espécies possuem benefícios medicinais, incluindo hipocolesterolêmico, antitumoral, antimicrobiano, antiviral, antineoplásico, antimutagênico, antioxidante, hipoglicêmico, antiparasitário, anti-inflamatório

hepatoprotetor, e propriedades imunomodulatórias (RATHEE et al., 2011; PATEL et al., 2012; DURU; CAYAN, 2015).

2.3.1 Propriedade nutricional e medicinal dos cogumelos comestíveis

Os cogumelos são consumidos pelos seres humanos desde os tempos antigos, não apenas como parte da dieta normal, mas também como iguaria (devido ao seu sabor atraente e aroma). Além disso, as propriedades nutricionais e medicinais dos cogumelos são reconhecidas há muito tempo. Na última década, os cogumelos comestíveis tornaram-se cada vez mais atraentes como alimentos funcionais por seu potencial benefício à saúde humana (GUILLAMÓN et al., 2010). Os cogumelos contêm altas quantidades de carboidratos e fibras, além de concentração alta de proteína (19% a 35%, incluindo todos os aminoácidos essenciais) e têm baixo teor de gordura (SINGH et al., 2015), conforme reportado na Tabela 1.

Tabela 1- Composição química dos cogumelos em relação a outros alimentos, por 100 g de parte comestível.

Alimento	Água	Carboidrato	Proteína	Gordura	Calorias
Cogumelo*	90-98	22.4-61	19.5-50.4	0.8-2.19	28
Ovo**	75,6	1.6	13.0	8.9	143
Carne**	60.4	0.0	27.3	10.9	215
Leite**	87	4.9	3.5	3.5	65
Cenoura**	88	9.7	1.1	0.2	42

Nota: *Adaptado: LIU et al; 2019; ** TACO, 2001.

Em relação aos minerais, esses são encontrados na faixa de 800 a 40000 mg/kg para elementos como o potássio, fósforo, enxofre e magnésio, enquanto outros, como o zinco, ferro, cobre, cálcio, sódio e manganês estão entre 5 a 500 mg/kg (WANG et al; 2014). No geral, os cogumelos têm concentração muito baixa de sódio, o que é benéfico para pacientes hipertensos, e alto teor de potássio e fósforo, que é um importante aspecto ortomolecular (CHANGE; WASSER, 2012). Os cogumelos comestíveis também fornecem conteúdo nutricionalmente significativo de vitaminas (B 1, B 2, B 12, C, D e E). Além disso, os cogumelos têm um baixo índice glicêmico e alto manitol, o que é especialmente benéfico para os diabéticos (KOZARSKI et al., 2015).

Sabe-se também que os cogumelos possuem muitos compostos bioativos nutricionais, medicinais e farmacologicamente importantes, incluindo proteínas inativadoras de ribossomos, proteases, proteínas antifúngicas, polissacarídeos, polifenóis e lectinas (SINGH et al., 2015). As lectinas presentes nos cogumelos têm sido relatadas e nos últimos anos atraíram muita atenção devido as suas atividades antiproliferativas e antitumorais (JUNG et al., 2007; THAKUR et al., 2007; ZHANG et al., 2010). Os polissacarídeos, como as β-glucanas, estão presentes na parede celular. Esses compostos têm efeitos de modulação imune, e ação antineoplásica, exercendo atividade imunomoduladora com inibição do crescimento tumoral (WAN-JHEN et al., 2015; VOLMAN et al., 2010). E ainda foi relatado que muitas espécies de cogumelos comestíveis possuem atividade antioxidante (WASSER, 2010). Os compostos antioxidantes encontrados são: fenólicos, flavonóides, glicosídeos, polissacarídeos, tocoferóis, ergotioneína, carotenóides e ácido ascórbico (YU et al., 2014; KLAUS et al., 2013; CHEN et al., 2012).

2.3.2 *Agaricus bisporus* (Champignon de paris)

O cogumelo *Agaricus bisporus,* popularmente conhecido como “champignon de paris”, “cogumelo branco” ou “cogumelo paris” é um dos cogumelos comestíveis mais cultivados no mundo. Ele é classificado como basidiomicetos, e tem sua origem nas regiões de clima temperado e, por isso, necessita de temperaturas mais baixas para a indução de sua frutificação. Seus recursos foram descobertos pela primeira vez na Europa e, em seguida seu cultivo foi introduzido na américa (SUN et al., 2019).

No Brasil, esta foi a primeira espécie comercialmente cultivada. O champignon de paris foi introduzido em 1953, devido à crise avícola, quando imigrantes chineses e italianos se fixaram em Mogi das Cruzes. Desde então, o cultivo de cogumelos comestíveis no país expandiu (ELISANDRA et al., 2014). O cultivo era realizado de forma rudimentar, e no início, enfrentaram dificuldades devido ao clima e aos substratos utilizados até a adaptação (SAMPAIO; QUEIROZ, 2006).

Inicialmente, a produção do *Agaricus bisporus* era baixa e feita em condições bastante precárias, utilizando palha de arroz, esterco de cavalo e de galinha como substrato. Porém, devido à falta de concorrência, o preço era muito alto (BONONI, 2003). Com o passar dos anos e com o incremento da pesquisa, variedades de substratos foram testadas, e hoje os substratos mais utilizados são: capins secos, bagaço de cana, farinha de osso e de sangue, calcário, ureia, sulfato de potássio, farelos de soja e milho. Os compostos podem ter diferentes formulações,

desde que apresentem os elementos necessários para o desenvolvimento do fungo. Para isso devem sofrer um processo de fermentação controlada e após esse processo devem ser pasteurizados para eliminar fungos, insetos e ácaros indesejáveis (ANDRADE et al., 2008).

A importância deste cogumelo vai além de dados econômicos e nutricionais. A participação em processos biogeoquímicos em pastagens, bem como seu potencial para biorremediação de metais pesados ou outros poluentes tóxicos como fenóis, já foram relatados (GARCIA et al., 2005; KAMEDA et al., 2006; MENK et al; 2019).

Segundo Furlani; Godoy (2007), o *Agaricus biporus* possui 54 ± 7 % de carboidratos, 28 ± 7 % de proteína; 5 ± 1 % de lipídios e 20 ± 2 % de fibras. O ácido ascórbico e o fósforo foram também determinados e atingiram valores médios de 6,3 mg/100g e 113,3 mg/100g, respectivamente. Assim, ficou evidente que o *Agaricus bisporus* tem excelente valor nutricional, pois apresenta alto teor de proteínas e fibras, baixo teor de lipídeos, e componentes nutricionais nobres.

Estudos indicam que o consumo dietético do cogumelo *Agaricus bisporus* reduz fatores de risco de diabetes, sugerindo que os cogumelos contêm compostos com potenciais benefícios anti-inflamatórios e antioxidantes para a saúde que podem ocorrer ao longo do tempo em adultos predispostos ao diabetes tipo 2 (CALVO et al., 2016). E outros relatos demostram que o *Agaricus bisporus* tem atividade hipoglicêmica e hipolipidêmica (JEONG et al., 2010).

Segundo Twardowski et al. (2015), o cogumelo *Agarius bisporus* contém compostos bioativos que demonstram propriedades imunomoduladoras e anticâncer. Além de apresentarem efeito contra a sepse, possivelmente relacionado ao potencial anti-inflamatório de seu polissacarídeo (heterogalactano) (RUTHES et al., 2012).

Há mais de 200 anos, o botânico francês Henri Braconnot, graças a sua pesquisa sobre cogumelos comestíveis, descobriu um novo polissacarídeo que recebeu o nome de quitina. A quitina é um polissacarídeo natural derivado de numerosos organismos vivos e é o segundo carbono polimerizado mais abundante presente na natureza, depois da celulose. (MUZZARELLI et al., 2012). Tanto a quitina quanto a quitosana exibem várias propriedades biológicas, tais como: anticolesterolêmico, agentes de cicatrização, anticâncer, analgésico, antiácido, antiulcerante e antioxidante entre outros (ZARGAR; ASGHARI; DASHTI, 2015; DUTTA; DUTTA; TRIPATHI, 2004; LIM et al., 2003)

A atividade antioxidante da quitina, quitosana e derivados corresponde à sua capacidade de eliminação de diferentes espécies de radicais de oxigênio, como alquil, superóxido, hidroxil e DPPH (2,2-difenil-1-picril-hidrazil). O mecanismo ainda não está claro, mas deve estar

relacionado à quelação de íons metálicos livres pelos grupos hidroxila e amino polissacarídeo, o que leva à formação de um sistema estável (CASADIDIO et al., 2019.

Na ciência cosmética, a quitina e a quitosana foram investigadas como excipientes em potencial e como agentes ativos biológicos, graças a suas propriedades peculiares, como ausência de toxicidade, biocompatibilidade e biodegradabilidade. E seu uso é conhecido na aplicação industrial. No tratamento de resíduos a quitosana é investigada como quelante de metais pesados (CASADIDIO et al., 2019; Li et al., 2014).

E devido à ausência de toxicidade e sua a ação como quelante conhecida em tratamento de água, poderia ser utilizado como agente protetor, especialmente em gestantes intoxicadas pela exposição ao Pb, uma vez que os quelantes comerciais, além de apresentarem efeitos adversos, eliminam, junto com o Pb, minerais essenciais como ferro, zinco e cálcio, importantes na fase gestacional.

3 OBJETIVOS

3.1 Objetivo geral

Avaliar a capacidade protetora do cogumelo *Agaricus bisporus* contra os efeitos tóxicos do chumbo em dois modelos *in vivo:* ratos e nematoides

3.2 Objetivos específicos

- ✓ Caracterizar nutricionalmente o cogumelo *Agaricus bisporus* por meio da análise de composição centesimal, dosagens de compostos fenólicos, β-glucanas e micronutrientes;
- ✓ Caracterizar o quadro hematológicos das ratas prenhez;
- ✓ Avaliar marcadores bioquímicos e de estresse oxidativo nas ratas prenhez;
- ✓ Quantificar o Pb presentes no sangue, ossos, placenta, fígado, rim e cérebro das ratas prenhez, objetivando entender a toxicocinética;
- ✓ Avaliar os efeitos do *Agaricus bisporus* sobre o desenvolvimento intrauterino de ratas.
- ✓ Avaliar parâmetros como letalidade embrionária e letalidade larval em *c. elegans*
- ✓ Avaliar tamanhos e a motilidade dos vermes hermafroditas adultos após 72 horas de exposição, e os tamanhos de seus descendentes após 144 horas de exposição.
- ✓ Avaliar o estresse oxidativo na cepa transgênica *C. elegans* CL2166 (dvIs19 [(Paf15)gfp-4p::NLS] III) após a exposição ao Pb, por meio de mudanças na fluorescência de GFP

CAPÍTULO 1

Influence of *Agaricus bisporus* Mushroom on Pb Toxicokinetic in Pregnant Rats.

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Influence of *Agaricus bisporus* Mushroom on Pb Toxicokinetic in Pregnant Rats

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Abstract: (Pb) is a toxic metal, responsible for several damages to human health. *Agaricus bisporus* (Ab) is a mushroom with promising antioxidant properties to be used as an alternative chelator in Pb intoxication. The aim was to understand the Pb toxicokinetic and the potential of Ab as a protective agent. A total of 20 female Wistar rats were distributed into 4 groups (n = 5/group): Control (receiving water); Group Ab 100 mg/kg (gavage); Group Pb 100 mg/L in water; and Group Ab + Pb—100 mg/kg + 100 mg/L (gavage and water). Pb administration occurred daily until the 19th day of pregnancy. On day 19 of gestation, the rats were euthanized, and the blood and tissues were collected for Pb measurement, using an inductively coupled plasma mass spectrometer. The results showed that the levels of Pb in the blood, placenta, and liver of the mothers, and in the brain of the fetuses increased significantly in the Pb group. On the other hand, the combined exposure to Pb + Ab showed a significant decrease in the metal concentration in relation to the Pb group, returning to normal levels. Kidney and bone lead levels also increased significantly in the Pb group. However, in the combined exposure group, levels did not return to the control amounts; there was protection, but the Pb concentration was still significantly higher than in the control. In the brain, no significant differences were observed. In conclusion, we suggest *A. bisporus* is a natural chelator, because the co-administration of the mushroom was able to interact with Pb ions, minimizing the Pb absorption and distribution. These effects are suggested since *A. bisporus* have antioxidants and beta glucan that interact with Pb, chelating it and, thus, reducing its toxic effects.

Keywords: lead; *Agaricus bisporus*; combined exposure; toxicokinetic; pregnancy

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1. Introduction

Lead (Pb) is a non-essential element with a proven neurotoxic effect, and with a significant risk for humans exposed to it by environmental, occupational, or dietary factors [1]. Sensitivity to Pb is greater in children; therefore, pregnant women should be aware of the risk of exposure to Pb, because the metal can pass to the fetus during pregnancy through the placenta, and breastfeeding can also be another source of exposure for babies [2,3].

In the general population, gastrointestinal absorption is the most common route, and adults can absorb a maximum of 10% of ingested Pb, in contrast to children (between 2 months and 6 years of age), who can absorb up to 50% of the amount ingested. Considering the toxic effects, in adults, the most common damages are cardiovascular, hypertension, loss of appetite, hallucinations, headache, insomnia, and joint pain [4], while children are susceptible to neurological damage, delay in psychomotor development, and learning difficulties, among others [5,6]. Another well-known toxic effect is the inhibition of the body's ability to produce hemoglobin, affecting the synthesis of the heme group, by inhibiting the activity in the enzymes, δ-aminolevulinic acid dehydratase (ALA-D), coproporphyrinogen

oxidase, and ferrochelatase. Such inhibition causes mild to moderate anemia in adults and severe anemia in children [7]. From the blood, Pb is distributed to all tissues (especially soft tissues such as the kidneys and liver), including the fetus during pregnancy. Subsequently, about 90% of the Pb is deposited in bones [8,9].

The World Health Organization's 2021 update on the impact of chemicals on public health determined that, of the 2 million people who died in 2019 from exposure to known chemicals, nearly half were due to Pb exposure. Pb exposure is estimated to be responsible for 21.7 million years lost to disability and death [10]. Regarding blood Pb reference values, the Centers for Disease Control and Prevention (CDC) uses a reference value of 3.5 µg/dL to identify children with high Pb levels. The CDC estimates that approximately 800 million children worldwide have Pb in their blood [3]. In addition to affecting adults and children, the release of Pb into the environment can cause mortality and also limit population size among non-human animals, such as populations of wild animals and waterfowl species [11].

The administration of a chelating agent is the current therapy for metal intoxication [12]. EDTACaNa2 (disodium calcium ethylenediaminetetracetic acid), BAL (Dimercaprol), and DMSA (dimercaptosuccinic acid) are some of the synthetic chelating agents used to remove Pb from the body. However, some adverse reactions have already been reported during treatment, such as gastrointestinal discomfort, skin reactions, and elevation of liver enzymes, in addition to the chelation of essential metals [13,14]. In this way, alternative therapies using natural substances such as garlic, vitamin C, and vitamin E have been studied. Some findings have shown the use of different nutrients; for example, vitamins, flavonoids, and mineral elements as defenders against Pb poisoning [15–17].

Garlic, a natural substance used in food, contains several essential nutrients and antioxidants, as well as being rich in flavonoids and selenium [18]. It is recommended as an active antioxidant to combat toxicity caused by heavy metals, and the natural chelating ability of the allicin and sulfhydryl groups present in garlic makes it a strong antioxidant in the treatment of lead-induced toxicity, especially long-term toxicity [17,19]. Vitamin E acts as an antioxidant, inhibiting lipid peroxidation. Vitamin E neutralizes the oxidative stress generated by Pb, inhibiting the additional production of free radicals by chelation [17,20].

In this way, culinary and medicinal mushrooms can also be suggested as a complementary therapy. The mushroom *Agaricus bisporus* is highly nutritious [21]. This mushroom contains many bioactive compounds, including alkaloids, flavonoids, steroids, terpenes, and phenolic compounds responsible for reducing oxidative damage [22,23]. In addition, *A. bisporus* also has glucans, fibers, and these polysaccharides, especially the triple helix 1,3-1,6-β-d-glucans, which showed chelating characteristics [24], along with chitin and chitosan, which are known for their antioxidant activity, have the ability to eliminate different species of oxygen radicals, such as alkyl, superoxide, and hydroxyl. The mechanism is still unclear but must be related to the chelation of free metal ions by hydroxyl and amino polysaccharide groups, which leads to the formation of a stable system [25]. In waste treatment, chitosan is investigated as a heavy metal chelator [25,26].

In this way, we evaluated the presence of Pb in the blood and tissues of pregnant rats exposed to the metal, and in the brains of their fetuses, aiming to understand the Pb toxicokinetic in the presence of *A. bisporus* as a protective agent.

2. Materials and Methods

Fresh *A. bisporus* mushrooms were obtained commercially from a local producer. The mushrooms were sliced and dried in a ventilated oven at 38 ± 2 °C, until constant mass. The dried mushroom was ground in a mill to obtain a kind of thin flour. The mushroom flour was given to the animals every day as described below.

2.1. Experimental Design

Healthy Wistar rats, male and female, were obtained from the UNICAMP vivarium, Campinas, São Paulo state. The experiment was approved by the Committee for the

Care and Use of Experimental Animals of the University of Sorocaba, under the protocol number 175/2020. The research was developed in the experimental animal vivarium at the Laboratory of Toxicological Research-LAPETOX, University of Sorocaba. The procedures followed the ethical precepts and the animal welfare standards [27]. Rats were kept in boxes with constant cycles of light and dark cycles—12 h each—at a temperature of 21–23 °C, with free access to a standard chow diet and filtered water. The animals were kept for a week in adaptation before starting the procedures.

For the induction of pregnancy, 01 male and 03 females were mated at night in an independent box, in the proestrus and estrus phases of the estrous cycle. Day 1 of gestation occurred when sperm were found in the vaginal wash, performed in the morning after mating [28].

All pregnant rats were randomly divided into 4 groups (n = 5 per group): Group I—Control (received water); Group II—Ab 100 mg/kg (received *A. bisporus* mushroom); Group III—Pb 100 mg/L (received Pb); Group IV—Ab + Pb (100 mg/kg +100 mg/L; combined exposure). Exposure to Pb was made in drinking water, while Ab was solubilized in water and administered orally (by gavage). The administration of Pb and Ab was carried out until the 19th day.

The dose of 100 mg/kg/day of *A. bisporus* was defined based on previous studies from our team [29,30]. The Pb dose of 100 mg/L was defined in a pilot study, which found out that the dose represented values above 90 µg/dL in the blood of pregnant rats. These levels can cross the placental barrier, causing damage to the cognitive development of the fetus [31,32]. At the end of the experiment, the animals were euthanized with ketamine, xylazine, and acepromazine. Blood and tissues were collected for Pb measurement.

2.2. Determination of Pb Levels

Pb was quantified using an inductively coupled plasma mass spectrometer, and the operating conditions are reported in Table 1. Reference materials (blood and tissue) were used as a control of the quality of the analysis. Recovery of blood reference material (Seronorm Trace Elements Blood L-2, ALS Scandinavia AB, Lulea, Sweden) was (103%). Concerning tissue (CRL-ISS 12th Proficiency Test Bovine meat), the recovery was (105.48%).

Table 1. Operating conditions for ICP-MS.

ICP-MS: Operating Conditions	
Monitored isotope	208Pb
Radio frequency power	1550 W
Argon flow	15 L min^{-1}
Nebulization chamber	0.9 L min^{-1}
Collision cell	Helium (purity > 99.999%)
Nebulization chamber	Scott (double pass)
Interface	Nickel cones
Sample	0.90 mm
Skimmer	0.45 mm

For blood and tissue, the methodological limits of detection were 1.9535 µg/L and 0.0435 µg/L, respectively. The methodology of Batista and colleagues [33] with some modifications was used for the determination of Pb in blood. In metal-free tubes, 100 µL of blood was added with 4.9 mL of a solution containing 0.5% v v^{-1} of HNO_3 and 0.01% v v^{-1} of Triton X-100. The samples of blood were shaken and analyzed by ICP-MS.

Tissues were lyophilized for 48 h (lyophilizer Liotop l101, Liobras, São Carlos, SP, Brazil). After that, 35–250 mg of the dried sample was added in metal-free tubes and pre-digested for 48 h with 2 mL of sub-distilled HNO_3. The samples were then heated at 90 °C, for 4 h. After cooling, the volume was made up to 50 mL with ultrapure water for analysis by ICP-MS [34], with modifications.

2.3. Statistical Analysis

Data were reported as mean ± standard deviation (SD). The comparison of the mean values among the experimental groups were determined by one-way nonparametric ANOVA (Kruskal–Wallis test), followed by Tukey–Kramer's interval tests. Differences were considered statistically significant when $p < 0.05$. The results were analyzed using the Statistica® 8.0 and Graph Pad Prism® 5 programs.

3. Results

The Pb concentrations in the pregnant rats in the blood, bone, brain, placenta, liver, and kidney samples are shown in Figure 1 and in the Supplementary Material.

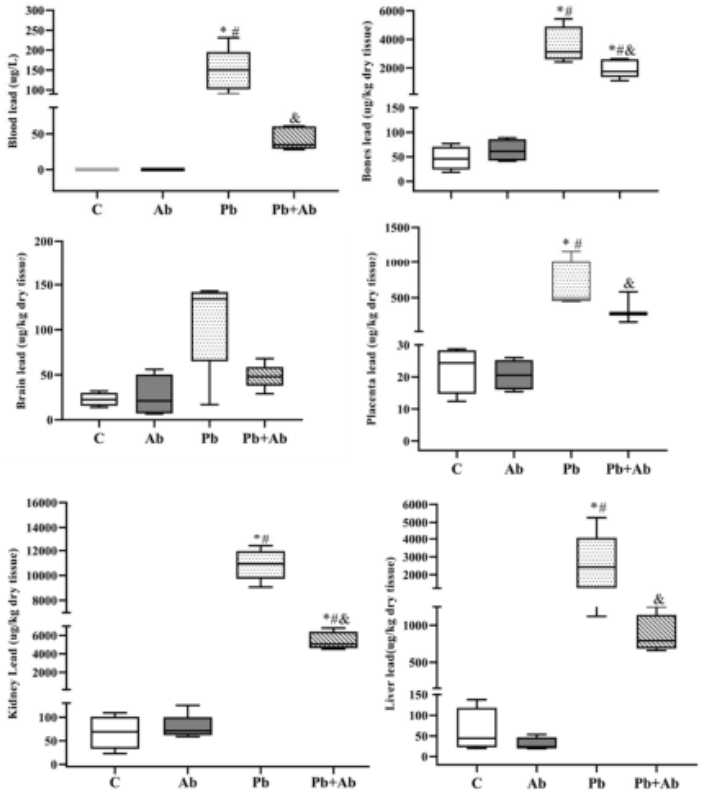

Figure 1. Lead concentrations (mean ± standard deviation) in blood and tissue samples from pregnant rats exposed to *A. bisporus* and lead, from gestation day 1 to 19. C (Control), Ab (*A. bisporus* 100 mg/kg/day), Pb (Lead 100 mg/L/day), Pb + Ab (100 mg/L/day of Pb + 100 mg/kg/day of *A. bisporus*), n = 5/group. * $p < 0.05$ in comparison to Control group, # in comparison to *A. bisporus*, and & in comparison to lead. Kruskal–Wallis test, followed by Tukey–Kramer's.

A significant increase in the Pb concentration was observed in the blood, placenta, and liver in the Pb group. On the other hand, the group that received both Pb + Ab showed a significant drop in the metal concentration in relation to the Pb group. The blood, brain, placenta, and liver did not present statistical differences in lead levels when comparing the control and Pb + Ab. Numerically, it seems to be a difference; however, the large variation within each group (see Supplementary Material) may have been responsible for the lack of significance. In the placenta, a tendency was found when comparing the Pb + Ab group to the control ($p = 0.62$). The lead concentration in the kidneys and bones also increased

significantly in the Pb group. However, in the Pb + Ab group, despite the levels did not return to the similarity of the control, the lead levels diminished compared to the Pb group.

Concerning the brain of the fetuses, the Pb levels are shown in Figure 2. A significant increase in the Pb concentration was observed in the Pb group, compared to the control. On the other hand, the Pb + Ab group showed a significant drop in the metal levels in relation to the Pb group, showing the mushroom has a chelator potential.

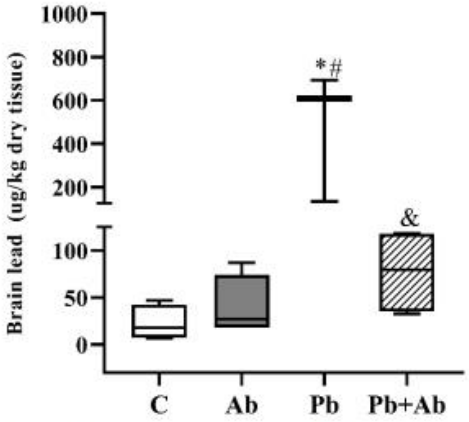

Figure 2. Lead concentrations (mean ± standard deviation) in brain samples of fetuses from female rats exposed to *A. bisporus* and lead, from gestation day 1 to 19. C (Control), Ab (*A. bisporus* 100 mg/kg/day), Pb (Lead 100 mg/L/day), Pb + Ab (100 mg/L/day of Pb + 100 mg/kg/day of *A. bisporus*), n = 5/group. * $p < 0.05$ in comparison to Control group, # in comparison to *A. bisporus*, and & in comparison to lead. One-way ANOVA, followed by the Tukey–Kramer's test.

4. Discussion

Nowadays, Pb toxicity is still a key environmental health problem causing harmful effects for children and pregnant women, but not restricted to them. Several and distinct species of wild animals are affected by Pb poisoning in the environment [11]. Pb toxic effects on pregnancy are complex, and Pb can affect the mother and fetus with harmful effects and prolonged consequences [35].

The concentrations of Pb in the blood reflect the amount that enters the body through all exposure routes through the respiratory and gastrointestinal tracts, and skin [36]. Thus, we quantified the Pb concentrations in the blood of pregnant rats to assess their level of exposure. The mean blood Pb level for the pregnant rats in the Pb group was around 140 µg/L. This value is two times greater than the maximum allowed biological index—60 µg/L—for Pb in the blood, in Brazil [37]. This finding was similar to those reported by Saleh et al. (2018) [38], with pregnant rats exposed to 160 and 320 mg Pb/kg for 20 days. Those authors found blood lead concentrations of 100 and 125 µg/dL, respectively, and these concentrations are clinically toxic.

We positively observed a decrease in the level of Pb in maternal blood with the *A. bisporus* co-exposures (around 50 µg/dL, almost three times lower than the Pb group) being below the maximum permitted biological index. Comparatively, similar data were reported by Sadeghi et al. (2021) [39], in which pregnant rats were exposed to Pb and treated with vitamin C and garlic extract. These findings corroborated with several studies and indicated that antioxidants can reduce toxic metals in the blood [40,41].

In the current study, a significant rise in the Pb level was observed in the kidney and hepatic tissues of the Pb-exposed group. This increase was in accordance with results reported by Takano et al. (2015) [42], for animals exposed to Pb for 4 weeks. The high concentrations of Pb in the maternal liver were probably caused by an altered Pb pharmacokinetics in pregnancy. Since the kidney was identified as a soft tissue target

for Pb accumulation, research has been published on the Pb concentration in animal kidneys [43,44]. One possible explanation is that the Pb distribution is due to its reabsorption. In the kidney, Pb is filtered in the glomerulus; however, most of the Pb filtered is reabsorbed by the distal tubule and collecting duct [45].

During pregnancy, the internal store of Pb can escape from the bone, due to high bone turnover, and cross the placenta by diffusion in the developing embryo [46]; in our study, we found high concentrations of bone lead in the group exposed to Pb (3621.20 ± 1249.23). However, in the Pb + Ab group (1932.40 ± 657.63), the concentrations did not return to the similarity of the control group. This finding is in agreement with that reported by Mumtaz et al. (2020) [6], who reported that bones are one of the main storage sites for Pb, with the bone matrix being the main target due to its ability to replace cations (Fe^{2+}, Ca^{2+}, and Mg^{2+} Na^{+}) in the body, altering the mineral metabolism.

The accumulation of Pb in the body of women of reproductive age can be transferred to the fetus during pregnancy through the placenta, the structure responsible for fetal nutrition, allowing the passage of Pb to the fetus, a fact that increases the risk of pregnancy complications and appearance of diseases after birth [47]. Thus, concentrations of Pb in the placenta can be good biomarkers of fetal exposure to Pb [48]. In our study, we found high Pb concentrations in the placenta of the group exposed to Pb (around 652 µg/L). On the other hand, *A. bisporus* co-exposure decreased the Pb concentration by half (about 340 µg/L), suggesting that the co-administration of the mushroom *A. bisporus* was beneficial.

In fetuses, the blood–brain barrier, because it is not fully developed, becomes the gateway to toxic products. For this reason, studies suggest that Pb can enter the brain more easily, becoming more harmful to newborns than adults [49,50]. In our study, an increase in the Pb concentrations in the brain of the fetuses in the group exposed to Pb (478.33 ± 301.21) was found, unlike in the Pb + Ab group where we observed a decrease in the metal (77.61 ± 46.42), returning to normal concentrations. In a study carried out by Antonio-Garcia and Masso-Gonzalez (2008) [51], rats were exposed to 300 mg Pb/L, a higher dose than in our study, and to a natural antioxidant, during pregnancy and lactation. The results were similar to ours, with an increase in Pb levels in the brain of the fetuses, showing an alteration in the antioxidant defense systems, as well as a reduction in the Pb concentrations in the groups exposed to antioxidants such as vitamin C. Thus, we can hypothesize that the exogenous supplementation of antioxidant agents, such as the mushroom *A. bisporus*, may be an alternative therapy for Pb toxicity due to its ability to cross the blood–brain barrier and exert its inhibitory effects on the brain. In studies carried out by Pachauri et al. (2008) [52] and Flora et al. (2003) [53], both highlighted the importance of antioxidant supplementation in metal poisoning, as supplementation can be beneficial to increase metal mobilization. In addition to the mushroom having antioxidants, it has important amounts of beta glucan, a soluble fiber that is directly related to the Pb metabolism. According to Visweswar et al. (2008) [54], three cyclic beta glucans interact with Pb, chelating it and, thus, reducing its toxic effects.

5. Conclusions

In this study, we observed that Pb has a rapid distribution for both the mother and fetus. Therefore, we conclude that there is a link between the Pb concentrations in the blood and tissues of the mothers and in the pups' brain, demonstrating Pb transfer across the placenta. In addition, co-administration of the mushroom *A. bisporus* was able to interact with Pb ions. These results highlight the potential use of *A. bisporus* as neuroprotective agents from Pb-induced harmful effects.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph20043114/s1, Table S1: Lead levels in different tissues.

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CAPÍTULO 2

Protective effect of *Agaricus bisporus* mushroom against maternal and fetal damage induced by lead administration during pregnancy in rats.

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Protective effect of *Agaricus bisporus* mushroom against maternal and fetal damage induced by lead administration during pregnancy in rats

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Abstract

Introduction: Lead (Pb) is a toxic pollutant, which can affect different tissues of the human body. The use of natural elements, as medicinal mushroom can reduce the toxic effects of Pb.

Objective: We evaluated, through preclinical tests, the oral co exposures to mushroom *Agaricus bisporus* (Ab) by gavage and Pb in drinking water, and the capability of Ab be a protective agent for both pregnant rats and their fetuses.

Methods: Female Wistar rats were divided into four groups (n = 5/group): Group I—Control; Group II—Ab 100 mg/kg; Group III—Pb 100 mg/L; Group IV—Ab + Pb −100 mg/kg +100 mg/L. Exposure was performed until the 19th day of gestation. On the 20th day, pregnant rats were euthanized, and the outcomes evaluated were weight gain; hematological profile; biochemical markers; oxidative stress markers; reproductive capacity; and embryo fetal development.

Results: The characterization of mushrooms reveals them to be a valuable source of nutrients. However, Pb ingestion resulted in reduced weight gain and negative impacts on hematological and biochemical parameters. Fortunately, co administration of mushrooms helped to mitigate these negative effects and promote recovery. The mushroom also showed antioxidant activity, improving parameters of oxidative stress. In addition, Ab partially recovered the damage in fetal morphology and bone parameters.

Conclusion: Our findings indicated that the co administration of Ab improved the toxicity caused by Pb, and the mushroom could be used as a natural alternative as a protective/chelator agent.

KEYWORDS

Agaricus bisporus, embriotoxicity, fetotoxicity, lead, maternal toxicity, pregnancy

1 | INTRODUCTION

Mushrooms are higher fungi, belonging to the Ascomycetes and Basidiomycetes classes and they are characterized by having a heterotrophic mode of nutrition. Among the popular and most commonly cultivated mushrooms in the world belonging to the phylum Basidiomycota, we have *Agaricus bisporus* (Ab; Suwannarach et al., 2022).

Agaricus bisporus is a species of edible mushroom widely cultivated and used in cooking around the world. It is known since antiquity for its nutritional qualities and beneficial effects on health, due to its high-quality protein content, high fiber content, vitamins, minerals, lectins, and also bioactive compounds, such as protein–polysaccharide complexes, β-glucans, and polyphenols (Cebin et al., 2018). Furthermore, from a functional point of view, mushrooms are low in fat, and from a culinary perception, Ab is known for its meaty texture and earthy flavor (Ramos et al., 2019).

Different studies have shown that Ab have significant medicinal properties, such as antitumor, antidiabetic, immunostimulatory, antioxidant, and antiviral effects due to the presence of those bioactive compounds (Amara & El-Baky, 2023; Goyal et al., 2006; Ramos et al., 2019). Natural substances like mushrooms, garlic, or isolated ones as vitamin E (α-tocopherol), and ascorbic acid (vitamin C) have been used since ancient times for the prevention and treatment of various pathologies (Chopra & Dhingra, 2021; Stojkovic et al., 2019).

Garlic, which is used as both a food and medicinal herb contains essential nutrients and antioxidants such as sulfur compounds, selenium, and flavonoids (Martins et al., 2016), and is available in various forms such as pills, capsules, and liquid. Studies have demonstrated that taking garlic as a dietary supplement enhances antioxidant capacity, reduces oxidant levels in the blood, and promotes the excretion of lead and other heavy metals from the body (Askari et al., 2021; Moosavian et al., 2020).

Vitamins E and C are vitamins that serve several organic functions, acting as antioxidants to prevent lipid peroxidation by disrupting the free radical chain reaction (Traber & Stevens, 2011). They work by deactivating reactive oxygen species (ROS) through scavenging the free radical chain reaction, inhibiting further ROS production through chelation, and keeping the Pb ion in a redox state. Therefore, they can be used as complementary or still prophylactically to gain the protective effects against potential heavy metal poisoning, with a reduced chance of adverse side effects (Lindequist et al., 2005).

Metals are natural elements found throughout the Earth's crust, characterized by their metallic luster, good electrical and thermal conductivity, among other properties. However, some of these elements do not have functionality/essentiality for the organism and, therefore, have potential for human or environmental toxicity (Zoroddu et al., 2019). Lead (Pb) is a toxic pollutant, widely used in industries in the manufacture and recycling of batteries, being the noncomplied way of disposing waste the main cause of Pb contamination (Bottari et al., 2020).

The human body can be affected by Pb in various tissues, and prolonged exposure to it has been associated with damage to the liver and kidneys, as well as an increased risk of cardiovascular disease, neurological disorders, anemia, osteoporosis, and several types of cancer (Bottari et al., 2020; Fu & Xi, 2020). Inhalation, skin absorption, and ingestion of contaminated food or water are the common routes of Pb intoxication, and the most susceptible individuals are mainly children, the elderly, and pregnant women (Al Osman et al., 2019).

The antioxidants present in the human body are valuable defense mechanisms against the toxic effects of Pb (Bottari et al., 2020). However, this defense mechanism may not be sufficient, requiring the administration of adequate and more effective drug treatment. Chelators are chemical compounds that can bind to metal ions and form stable, complex molecules. They can remove excess metal ions from the body (Andersen, 2004). Antioxidants are among the complementary therapies that can be used to treat metal intoxication. Nam and colleagues discovered that gintonin, a novel substance derived from ginseng, effectively lowered blood Pb levels in both maternal rats and their offspring additionally. Gintonin provided protection against Pb-induced damage to the cerebellum in developing rats. Based on these findings, the authors suggested that gintonin could be a promising preventive agent for Pb poisoning (Nam et al., 2020).

Considering mushrooms, according to Visweswar et al. (2018) three cyclic beta glucans from *Bradyrhizobium japonicum* can interact with Pb by chelating it. Additionally, edible mushrooms residues demonstrated adsorb lead and copper from contaminated water, probably due to the chitin and chitosan contents in mushroom (Castanho et al., 2021). In this way, we designed a novel experimental study in female rats investigating the biochemical role of *A. bisporus* in the protection against maternal and fetal damage induced by the Pb exposure during pregnancy.

2 | MATERIALS AND METHODS

2.1 | Obtaining and characterization of the mushroom *A. bisporus*

The fresh mushrooms Ab were sliced and dried in a ventilated oven at 38°C ± 2°C until constant mass. The dried mushrooms were later ground in a mill into powder.

To verify the centesimal composition, the mushroom powder was analyzed in triplicate. The quantification of moisture, ash, lipids, and proteins was made following adapted methods described by the Instituto Adolfo Lutz Institute (2008). Carbohydrate content was calculated by

the difference of moisture, ash, lipids, and proteins. Glucans were determined by the enzymatic and chemical hydrolysis, in triplicate, following the commercial kit Mushroom and Yeast Beta-Glucan—Megazyme®.

The determination of the phenolic compounds followed the method adapted from Folin–Ciocalteu (Scalbert et al., 1986), with modifications made by Ramirez-Anguiano et al. (2007). The assay was performed in triplicate, and the reading was made in a spectrophotometer (MultiSpec-1501, SHIMADZU®), at 760 nm. Results were expressed in mg of gallic acid equivalent (GAE) per gram of sample on a dry basis (mg EAG/g).

Essential and nonessential elements from Ab were determined in triplicate, by x-ray fluorescence, using a compact EDXRF apparatus from Malvern Panalytical® (Santo Amaro, SP, Brazil), composed of a silicon detector (Silicon Drift Diode) with area of 25 mm^2 and thickness of 500 μm, protected by a beryllium window of 12.5 μm.

2.2 | Experimental design

The study and the experiments were approved by the Committee for the Care and Use of Experimental Animals of the University of Sorocaba, under protocol number 175/2020. Healthy male and female Wistar rats were obtained from the animal house at UNICAMP, Campinas, state of São Paulo, and they were maintained in the animal house at LAPETOX, University of Sorocaba, in accordance with animal welfare standards (Percie du Sert et al., 2020). During the study, animals were kept in microenvironment isolation cages (Alesco®), under standard temperature (22°C–24°C), humidity (55% ± 5%), and lighting (12:12 hr, light:dark). They were fed a standard chow diet ad libitum and they had free access to filtered water.

For mating, one male was housed up with three females, for an overnight period. Gestation was confirmed by positive identification of spermatozoa in a vaginal lavage smear, by microscopic observations (Biological Microscope, Model Axio Lab.A1, ZEISS®), and it was designated as the first day of gestation (Gerenutti et al., 2006).

Our team established the dose of 100 mg/kg/day of Ab by referring to our earlier research (Laurino et al., 2019; Spim et al., 2021), in which different doses of edible and medicinal mushrooms showed safety and efficacy (Grotto et al., 2016). This dose was chosen because as it demonstrated beneficial effects without any associated toxicity. Considering an adult weighing 70 kg, we have a daily intake of 7 g, which could be consumed by food or supplemented. Here, the Ab powder was solubilized in water and administered by gavage. The Pb solution was prepared, every 2 days to minimize the Pb precipitates, by dissolving lead acetate in filtered water, and a dose of 100 mg/L was used based on a pilot study that demonstrated levels above 90 μg/dL of lead in the blood of pregnant rats. These levels can cross the placental barrier, causing damage of the fetus (Caetano et al., 2023). The administration of Pb and Ab was carried out at the first day of gestation until the 19th day.

We chose to administer the Pb in the water first because it reduces the stress and discomfort that animals may experience when subjected to repeated gavage procedures. Second, along with the mushroom, administering Pb through gavage may lead to inconsistent results due to variations in the mixing of the substance and the chelating properties of the mushroom. By administering Pb through drinking water, the animals can consume the substance at their own pace, and this condition mimics real-life exposure scenarios, where humans are typically exposed to Pb through contaminated water sources. Thus, the results obtained from the study can better reflect the effects of chronic Pb exposure in humans.

The pregnant rats were kept in individual cages, and they were randomly divided into four groups ($n = 5$ per group):

1. Group I—Control, receiving water;
2. Group II—Ab 100 mg/kg, receiving Ab daily, by gavage;
3. Group III—Pb 100 mg/L, receiving Pb in drinking water;
4. Group IV—Ab (100 mg/kg) by gavage + Pb (100 mg/L) in drinking water.

Animals were weighed and observed throughout gestation for some morbidity daily. On the 20th day of gestation, the animals were euthanized with ketamine (100 mg/kg), xylazine (20 mg/kg), and acepromazine (3 mg/kg), intraperitoneally. Maternal blood was collected by hepatic venipuncture and transferred to two tubes: tube 1, containing anticoagulant ethylenediaminetetraacetic acid, for hematological and redox parameters, and tube 2, without anticoagulant, for biochemical evaluation. The cesarean section procedure was performed with a longitudinal incision in the linea alba to expose the uterus and ovaries.

Following the euthanasia of the females, an evaluation of their reproductive capacity was conducted. The number of fetuses, implantations, and visible resorptions in the uterine cavities were checked. Additionally, the ovaries were weighed, and the corpus luteal were manually removed with a scalpel and counted. The fetuses were then removed from the gestational sacs, counted, weighed, and sacrificed under halothane. The

reproductive capacity was evaluated based on the percentage of preimplantation loss, postimplantation loss, offspring vitality, and fetus weight. The fetal groups analyzed were Control ($n = 54$), Group Ab ($n = 51$), group Pb ($n = 17$), and group Pb + Ab ($n = 48$).

The rate of preimplantation (PreI) loss was calculated using the formula:

$$\text{PreI loss} = \left(\frac{\text{Cl} - \text{Imp}}{\text{Cl}}\right) \times 100$$

The rate of postimplantation (PostI) loss was calculated using the formula:

$$\text{PostI loss} = \left(\frac{\text{Imp} - \text{Lf}}{\text{Imp}}\right) \times 100$$

Offspring's survival was determined according to the formula:

$$\text{Survival} = \left(\frac{\text{Lf}}{\text{Ft}}\right) \times 100$$

where Cl is the number of corpus luteum, Imp is the number of implantations, Lf refers to live fetuses, and Ft is the total number of fetuses.

2.2.1 | Maternal hematological and biochemical parameters

With regard to hematological parameters, hemoglobin, hematocrit, white blood cells (WBC), red blood cells (RBC), and number of platelets were analyzed in total blood of the rats, using an automatic equipment Hematology XS 1000i WAS, Roche®.

The biochemical assay was performed in the serum of the rats, using the automatic equipment Cobas C111, Roche® and commercial kits. In serum from maternal rats, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, calcium, and alkaline phosphatase were analyzed.

2.2.2 | Redox outcomes

Total thiols were quantified by the concentration of reduced glutathione (GSH), based on the Ellman method (Ellman, 1959). For this, 150 μL of total blood was mix by vortexing in 100 μL of 10% (m/v) Triton X-100 and 100 μL of 30% (v/v) trichloroacetic acid. Then, the sample was centrifuged at 4000 rpm for 10 min. 900 μL of 1 M potassium phosphate buffer (PPB), 50 μL of supernatant, and 50 μL of 10 mM 5,5′-dithio-bis (2-nitrobenzoic acid) were mixed gently, forming a yellow complex, read at 412 nm. A calibration curve, with different concentrations of GSH standard, was used to calculate GSH concentrations in blood. The results were expressed in mM/mL blood.

The GSH peroxidase (GPx) activity in whole blood was determined based on the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH); 10 μL of whole blood was diluted in 390 μL of 100 mmol/L PPB. A 20 μL aliquot was added to 880 μL of a solution containing GSH, GSH reductase, NADPH, sodium azide, and 100 μL of H_2O_2. The GPx activity was monitored for 2 min at 340 nm, according to Paglia and Valentine (1967). The decrease in absorbance was proportional to the NADPH consumption by GPx, expressed mmol of NADPH per gram of Hemoglobin, per minute, using Hemoglobin concentration (in g/L).

Catalase (CAT) activity follows the method of Aebi (Aebi, 1984), based on the decomposition of H_2O_2, monitored at 240 nm. For this, the whole blood was diluted in 50 mM PPB. An aliquot of 20 μL was added to 1910 μL of the same PPB and 70 μL of H_2O_2. CAT activity was monitored for three minutes, and the activity was expressed using a constant of variation (κ), related to Hb, per minute (κ/gHg/min).

Thiobarbituric acid reactive substances (TBARS) were used as a biomarker of lipid peroxidation (Ohkawa et al., 1979). For this, 150 μL of plasma aliquots were mixed with 50 μL of 12% (m/v) sodium hydroxide, 50 μL Milli-Q ultrapure water, and incubated at 60°C for 30 min. After that, aliquots of 250 μL of 6% (v/v) phosphoric acid, 250 μL of 0.8% (m/v) thiobarbituric acid (TBA) and 100 μL of 10% (m/v) sodium dodecyl sulfate were added to the samples, which were maintained at 90°C for 1 h. Lipid peroxides reacted with TBA in an acidic medium to form a pink compound and were read in a spectrophotometer at 532 nm. A calibration curve with different concentrations of malondialdehyde standard (the main product from lipid peroxidation) was used to calculate TBARS concentrations in plasma.

2.2.3 | Fetal morphological assessment

Fetuses were separated from the placentas and weighed. The fetuses were euthanized with halothane (vat saturation). Body measurements were made in the dimensions: anterior–posterior of the skull, lateral–lateral of the skull, anterior–posterior of the thorax, lateral–lateral of the thorax, craniocaudal, and tail. Dimensions were measured in mm.

For the skeletal study, our protocol followed the previously described procedures, evaluating only the

skeleton of the fetus (Charest et al., 2022; Zin et al., 2022). Fetuses were placed in ethanol 85% for 1 week. After that, the viscera were removed. The cleaned skeletons were placed in ethanol 100% for 2 weeks and then stained in a solution with Alizarin red S plus and Alcian blue for 48 h. The skeletons were then transferred to a 2% KOH solution for 24 h; in sequence, they were transferred to a glycerin +1% KOH solution for 1 week. The double-stained skeletons were stored in a 50% glycerin solution.

2.3 | Statistical analysis

Data are expressed as mean ± standard deviation. The normality was assessed, and the results were identified as nonparametric. Thus, Kruskal–Wallis test was applied, followed by Duncan test, to verify differences among treatment protocols. Moreover, a nonparametric two-way analysis of variance. Value of $p < .05$ was used to verify the influence of time and treatments on weight gain. Value of $p < .05$ were considered significant. Litter was used as a comparison unit. The results were analyzed using the Statistica® 8.0 and Graph Pad Prism® 8 programs.

3 | RESULTS

3.1 | Ab characterization

The nutritional composition of Ab per 100 g of dry mushroom is as follows: 30.1 ± 2.2 g of protein, 41.7 ± 1.2 g of carbohydrates, 2.5 ± 0.2 g of lipids, 8.9 ± 3.3 g of total solids, and 11.1 ± 0.2 g of ash. The 100 g of dry mushroom also contains 14.35 ± 0.7 g of beta-glucans and 2.98 ± 0.2 mg of GAE.

Table 1 presents the micronutrients and toxic metals concentrations in the powder of Ab.

It is important to observe the high content of micronutrient, particularly calcium, magnesium, and phosphorus. Moreover, the nonessential elements are present in very low concentration, demonstrating safety and quality control in the mushroom cultivation process.

3.2 | Maternal parameters

Figure 1 displays the changes in body weight gain, and it shows that on the 20th day, the group exposed to Pb had a decrease in weight gain compared with the control group.

Table 2 presents the reproductive capacity data. A notable increase in pre and postimplantation losses was observed in the Pb group compared with the Control group. In contrast, the group coexposed to Pb and Ab showed improvement compared with the Pb group. Furthermore, there was a concerning trend of reduced offspring vitality in the Pb group when compared with the control group ($p = .09$). These observations can be attributed to the fact that the Pb group had only two litters in total. The Pb group experienced a significantly higher percentage of preimplantation and postimplantation losses. However, the administration of Ab alongside Pb provided protection against these losses.

TABLE 1 Essential and nonessential elements identified and quantified in the powder of the mushroom *Agaricus bisporus*.

Chemical elements	Concentration (μg/g ± SD)
Calcium	354.4 ± 1.7
Potassium	115.4 ± 0.1
Sodium	0.0 ± 0.0
Magnesium	2904.8 ± 259.7
Manganese	11.0 ± 1.0
Iron	93.8 ± 10.3
Phosphorus	9492.7 ± 81.3
Cobalt	0.0 ± 0.0
Copper	59.2 ± 1.1
Zinc	73.3 ± 1.3
Lead	0.9 ± 0.1
Mercury	0.1 ± 0.2

Note: Nonessential elements are lead and mercury.

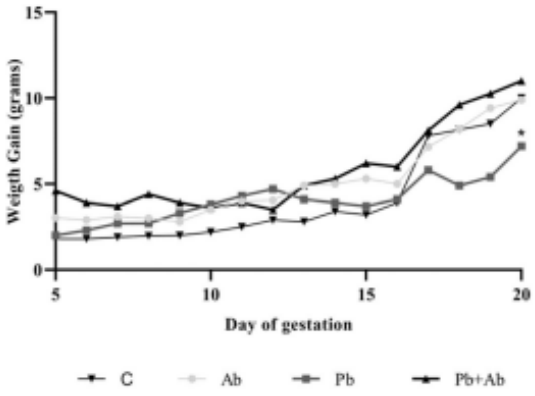

FIGURE 1 Weight gain of pregnant rats in the Control (C), *Agaricus bisporus* (Ab), Lead (Pb), and Pb + Ab groups. Data are presented as the mean ± SD ($n = 5$ per group). *$p < .05$ compared with C, using nonparametric two-way analysis of variance, followed by Duncan test.

Parameter	Groups C	Ab	Pb	Pb + Ab
Offspring vitality (%)	93.3 ± 9.4	92.5 ± 11.6	58.6 ± 53.5	84.6 ± 10.9
Preimplantation loss (%)	0.0 ± 0.0	6.5 ± 8.3	54.0 ± 38.8[a,b]	6.2 ± 9.1[c]
Postimplantation loss (%)	6.7 ± 9.4	7.5 ± 11.6	41.4 ± 53.5[a,b]	11.2 ± 10.9[c]
Fetus weight (g)	2.3 ± 0.1	2.3 ± 0.2	2.4 ± 0.2	2.4 ± 0.3
Number of litters	5	5	2	5
Number of fetuses	54	51	17	48

Note: Data are presented as the mean ± SD or percentage (%), $n = 5$ rats/group.
[a] $p < .05$ compared with C.
[b] $p < .05$ compared with Ab.
[c] $p < .05$ compared with Pb, using Kruskal–Wallis followed by Duncan test.

TABLE 2 The reproductive capacity in the Control (C), *Agaricus bisporus* (Ab), Lead (Pb), and Pb + Ab groups.

TABLE 3 Hematological parameters of pregnant rats in the Control (C), *Agaricus bisporus* (Ab), Lead (Pb), and Pb + Ab groups.

Parameters	Groups C	Ab	Pb	Pb + Ab
White blood cells (10^3/μL)	4.99 ± 1.02	6.71 ± 1.66	4.8 ± 1.2	6.091 ± 1.72
Red blood cells (10^6/μL)	5.47 ± 0.44	5.90 ± 0.59	6.5 ± 0.6[a]	5.41 ± 0.48
Hemoglobin (g/dL)	11.12 ± 1.04	11.60 ± 1.04	9.5 ± 0.7[a,b]	10.22 ± 0.88
Hematocrit (%)	33.40 ± 1.98	37.76 ± 3.78[a]	38.7 ± 3.9[a]	33.60 ± 2.89[c]
Platelets (10^3/μL)	740.60 ± 63.18	750.00 ± 92.31	765.4 ± 25.1	762.60 ± 49.30

Note: Data are presented as the mean ± SD ($n = 5$/group).
[a] $p < .05$ compared with C;
[b] $p < .05$ compared with Ab.
[c] $p < .05$ compared with Pb, using Kruskal–Wallis followed by Duncan test.

The hematological indices are given in Table 3. A statistical increase in RBC combined with a reduction in hemoglobin were observed in Pb exposed rats in relation to control and Ab; in addition, hematocrit percentage increased in the Ab and Pb groups compared with control and decreased in the Pb + Ab group in relation to the Pb group. Although the statistical differences being present, when we evaluate the levels found compared with the reference values in the literature, we recognize that the values are still within the expected range for the species (Delwatta et al., 2018).

Figure 2 shows the biochemical parameters, considering hepatic, renal, and osseous profiles. An increase in ALT levels was observed in all groups in relation to control, and in the Pb group compared with Ab. Although the statistical differences, when we compare the levels found to the reference values (Delwatta et al., 2018), we recognize they are still within the expected range for the species.

AST levels remained similar in all groups, and ALP increased in the Pb and Pb + Ab groups compared with control. Renal function, represented by urea and creatinine, stayed unaltered comparing the groups. Calcium levels enhanced in Pb + Ab in relation in relation to all groups.

The results of oxidative stress are shown in Figure 3. Regarding catalase and GSH-Px enzymes, there were an increase in their activities when rats were exposed to Pb; however, the coadministration with Ab returned the enzymes activities to values similar to control. With regard to GSH, a reduction in its levels was detected in both Pb and Pb + Ab groups compared with control. On the other hand, there was an increase in GSH levels in Pb + Ab compared with the Pb group, suggesting a protective effect of the mushroom under the Pb oxidative effect. In TBARS biomarker, an increase in its levels was observed in the Pb group, and the mushroom reversed this peroxidation, since Pb + Ab was equal to the control, and significantly different from the Pb group.

3.3 | Fetal morphological assessment

Table 4 presents the fetal morphometric measurements. In terms of external abnormalities, the Pb + Ab group had a significant decrease in the anteroposterior portion

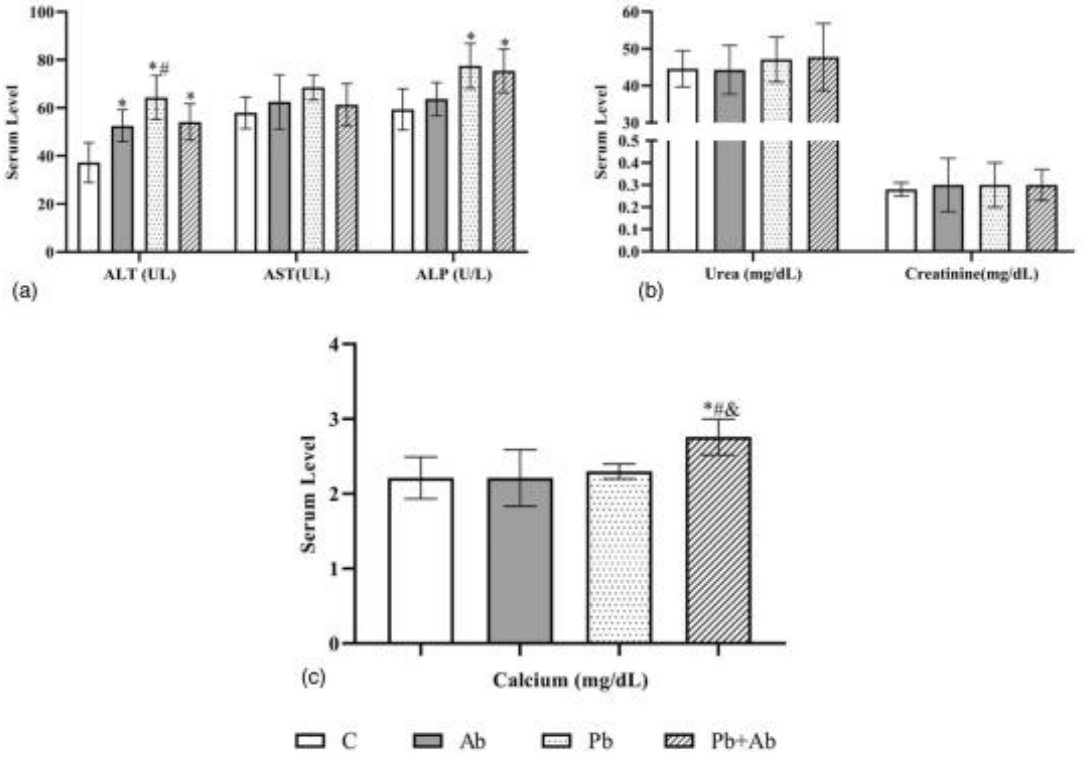

FIGURE 2 Biochemical parameters of pregnant rats in the Control (C), *Agaricus bisporus* (Ab), Lead (Pb), and Pb + Ab groups. In (a), hepatic biomarkers represented by ALT (alanine aminotransferase), AST (aspartate aminotransferase), and ALP (alkaline phosphatase); in (b), renal biomarkers; in (c) Calcium levels. Data are presented as mean ± SD ($n = 5$/group). $^{*}p < .05$ compared with C; $^{\#}p < .05$ compared with Ab; $^{\&}p < .05$ compared with Pb, using Kruskal–Wallis followed by Duncan test.

of the cranium measurement compared with the control and Pb groups. However, there was a significant increase in all groups in the laterolateral portion of the thorax measurement when compared with control. In the anteroposterior section of the thorax, both Ab and Pb + Ab showed a significant increase compared with control. There was also a tendency towards an increase in the length of anteroposterior section of the thorax in the Pb group compared with control ($p = .088$). No differences were observed in the other parameters.

The examination of fetal morphology showed no malformations in the cranial and vertebral regions across the control, Ab, and Ab + Pb groups. Considering the sternum, this measurement was analyzed by the number of ossification points presented which, normally, are six, depending on the age of the fetuses. The Ab and Pb groups showed a decrease in the number of ossifications compared with the control. In contrast, the Pb + Ab group exhibited an increased number of ossification points compared with the Ab and Pb groups.

4 | DISCUSSION

Edible mushrooms are known to have good quality proteins, high fiber content, large amounts of vitamins and minerals, lectins, and also bioactive compounds such as polysaccharides, β-glucans and polyphenols (Amara & El-Baky, 2023).

In our study, the centesimal composition of Ab showed this mushroom is a good food option due to its high protein content, about 30%, being higher than the protein content noticed by Goyal et al. (2006); (24.43% ± 0.10%). Furthermore, our mushroom had 14.35 g of beta-glucans/100 g of dry mushroom, similar that found by Cebin et al. (2018), 16.37 g of beta-glucans/100 g of dry mushroom. The main polysaccharides present in mushrooms are the beta-glucans, and they are directly related to the reduction of metabolic diseases and biomarkers related to immune function (Aoe, 2021). Concerning phenolic compounds, their concentrations corroborate other studies (Noha et al., 2018). Phenolics

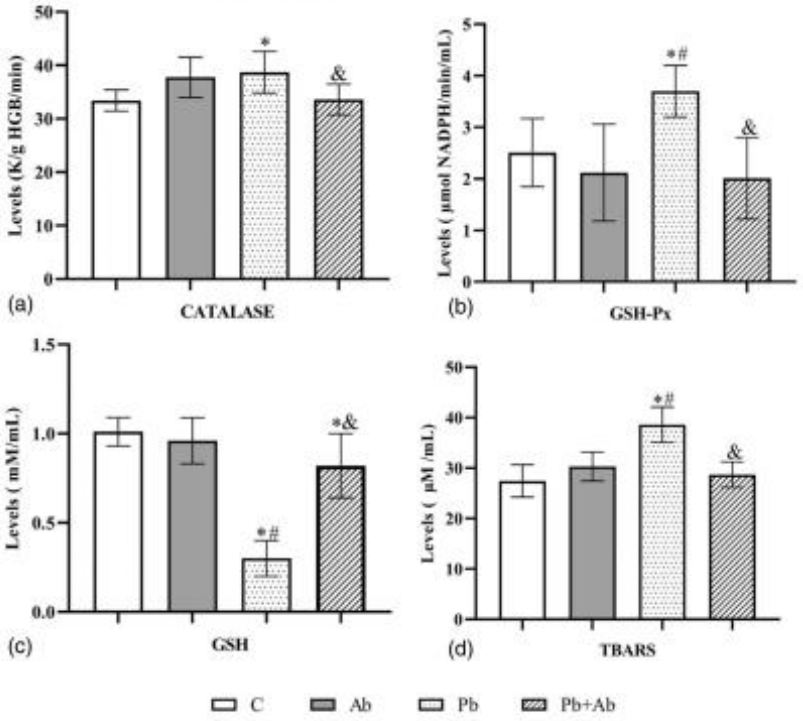

FIGURE 3 Redox status assessed in the Control (C), *Agaricus bisporus* (Ab), Lead (Pb), and Pb + Ab groups through Catalase activity (a), glutathione peroxidase (GSH-Px) activity (b), glutathione (GSH) levels (C), and thiobarbituric acid reactive substances (TBARS) levels (d). Data are presented as mean ± SD ($n = 5$/group). *$p < .05$ compared with C; #$p < .05$ compared with Ab; &$p < .05$ compared with Pb, using Kruskal–Wallis followed by Duncan test.

TABLE 4 Mean ± SD length (mm) of sections of the head and body and the incidence (%) of morphological anomalies of fetuses of maternal rats from Control (C), *Agaricus bisporus* (Ab), Lead (Pb), and Pb + Ab groups.

	Groups			
Parameter	**C**	**Ab**	**Pb**	**Pb + ab**
Number of litters	5	5	2	5
Number of fetuses	54	51	17	48
External measurements				
Cranium anteroposterior	14.10 ± 0.60	13.97 ± 0.65	14.13 ± 0.65	13.70 ± 0.89[a,b]
Cranium laterolateral	8.70 ± 0.47	8.54 ± 0.40	8.64 ± 0.55	8.48 ± 0.61
Thorax laterolateral	8.52 ± 0.53	9.46 ± 0.96[a]	9.07 ± 0.79[a]	9.12 ± 0.72[a]
Thorax anteroposterior	8.74 ± 0.65	9.77 ± 1.09[a]	9.11 ± 0.56	9.73 ± 0.75[a]
Cranium-caudal	30.06 ± 1.97	30.05 ± 1.73	30.34 ± 1.34	29.69 ± 1.58
Tail	11.91 ± 0.84	11.77 ± 0.65	11.62 ± 1.20	11.73 ± 1.06
Skeletal malformations				
Incompletely ossified skull, *n* (%)	0	0	8	0
Incompletely ossified vertebrae, *n* (%)	0	0	8	0
Sternebrae (number)	3.1 ± 1,1	2.8 ± 1.1[a]	3.0 ± 1.6[a]	3.9 ± 0.5[b,c]
Incompletely ossified ribs, *n* (%), *n* (%)	15	19	100	55
Short rib	11	12	58	33
Discontinuous ribs, *n* (%)	3	5	52	5.5

[a]$p < .05$ compared with C.
[b]$p < .05$ compared with Pb, using Kruskal–Wallis followed by Duncan test.
[c]$p < .05$ compared with Ab.

are well-known antioxidant compounds capable of reducing oxidative stress.

Mushrooms, in general, are characterized by a significant accumulation of macro and trace elements in their composition. In short, Ab highlighted as an important source of Ca, K, Mg, Cu, Fe, and Zn. Similar results were reported by Siwulski et al. (2020), who also analyzed the multielement composition of Ab, demonstrating that the mushroom is an elemental source of essential minerals. Nonessential and toxic elements such as Pb and Hg are associated with high risk to human health (Gulson et al., 2016). Our analysis revealed that the Ab contained trace amounts of toxic metals. The origin of the toxic metals may have been from the substrate where the mushroom was cultivated, (e.g., Bach et al., 2018). However, the levels detected were negligible and did not have any significant impact on our results, except for ALT enzyme; this biomarker isolated increased in the Ab group.

Research suggests that individuals who are at risk of being exposed to toxic metals should consume a diet that is rich in antioxidants, vitamins, iron, zinc, selenium, and other essential nutrients (Martins et al., 2016; Mumtaz et al., 2020). Nutritional supplements are a better option than chelation therapy as they have fewer side effects and are cost-effective (Askari et al., 2021). This is particularly important for the billions of individuals worldwide who are unknowingly exposed to contaminated metals on a regular basis (Monachese et al., 2012). In this way, nutraceuticals as mushrooms could be taken prophylactically to gain the protective effects against potential toxic metals exposure. In 2009, researchers administered 250 mg tablets containing 96% dry mushroom powder to participants three times daily for 16 weeks. The study found that these individuals demonstrated enhancements on the cognitive function scale, and the mushroom did not exhibit any toxicity (Mori et al., 2009).

Discussing the effects of Pb exposure and Ab coadministration on maternal rats, data revealed a remarkable decrease in body weight gain in the Pb-exposed group compared with the control animals. This finding was also reported by Saleh et al. (2018), that mentioned significant decrease in body weight gain in pregnant rats treated with oral Pb acetate (160 and 320 mg/kg). Weight loss is directly related to pre- and postimplantation fetus losses caused by Pb. In addition, Pb exposure can lead to malnutrition or reduced food intake by disrupting the mechanism of food satiety in the digestive tract (Li et al., 2021).

One of the targets of Pb toxicity is the hemopoietic system (Abdel-Moneim et al., 2015), and our results showed that there was anemia, with a reduction in the hemoglobin count in animals intoxicated by Pb. We suggest two mechanisms to the Pb-induced anemia: (i) erythropoiesis of erythrocyte precursor cells, due to the reduction of circulating RBC in the bone marrow; (ii) reduced heme synthesis, since Pb is a known inhibitor of δ-aminolevulinic acid dehydratase (ALAD), which is a vital key enzyme contributing to heme synthesis (Andjelkovic et al., 2019). These results are consistent with previous studies conducted by Adham et al. (2011) and Abdel-Moneim et al. (2015).

The hematological indices in the group coexposed to Ab showed improvement like the control values; with regard to WBCs, they tended to increase in number in the Ab group ($p = .08$), showing the immunomodulatory capacity of the mushrooms, which may be due to the ability of antioxidants present in the mushroom to chelate metal ions and reduce Pb toxicity (Cherrak et al., 2016).

The liver is the main storage site and one of the targets in heavy metal poisoning (Abdelrazek et al., 2022). Our findings are similar to those reported by Abdelrazek et al. (2022); in their study, they administered Pb acetate at a dosage of 50 mg/kg/day for 6 weeks, in rats. The study showed a significant increase in ALT and ALP levels after Pb exposure, corroborating our data. On the other hand, the administration of Ab also ran to increased levels of ALT individually, compared with the control group. It is possible that the presence of small amount of Pb and Hg in the mushroom may explain this finding.

Urea and creatinine are the main excretory products of kidney. Significant elevation of serum urea and creatinine is indicative of chronic kidney damage or disease (Missoun et al., 2010). In our study, we did not observe significant changes in these parameters. Results contrary to our study were reported by Abdelrazek et al. (2022) with increased creatinine and urea in the group exposed to Pb acetate, unlike the group treated with Betaine Glycine, a natural compound, reduced urea and serum creatinine, showing natural products can reduce the toxic effects caused by Pb.

By replacing bivalent cations like calcium, Pb can interfere with numerous vital biological functions (Almaimani et al., 2019). In our study, we did not observe any difference in calcium levels in the group exposed to Pb. In contrast, in the Pb + Ab group, we observed an increase in relation to all groups. Our hypothesis suggests that Pb may displace calcium in bones, making calcium available in the bloodstream. This, combined with the calcium source from the mushroom, may provide a beneficial effect. This aligns with studies reporting the positive effects of calcium supplementation against Pb toxicity (Basha & Reddy, 2015).

Exposure to Pb has known injuries as it promotes the induction of oxidative damage, increasing oxidative stress

(Hassan et al., 2019). Catalase is the main antioxidant enzyme that has heme as its prosthetic group, being responsible for catalyzing the degradation reaction of hydrogen peroxide, an element that is toxic to the cells of living beings (Antonio-García & Massó-Gonzalez, 2008). The endogenous antioxidant enzyme GPx catalyzes the reduction of both H_2O_2 and fatty acid hydroperoxides. Its activity depends on reduced GSH, which is oxidized to oxidized GSH (El-Tantawy, 2015).

Catalase and GSH-Px activities, here, were significantly increased in animals exposed only to Pb, indicating that Pb is contributing to oxidative stress. These data corroborated the results presented by Antonio-García and Massó-Gonzalez (2008), in rats exposed to 300 mg/L of Pb during pregnancy and lactation, and Al-Megrin et al. (2019), in male rats exposed to 20 mg/kg, ip, daily. They showed a strong increase in catalase and GSH-Px. However, this effect was significantly attenuated in the Pb + Ab group, therefore the activities of the enzymes were significantly reduced in relation to the Pb group, remaining similar to the control.

Composed of cysteine, glycine, and glutamic acid, GSH is a tripeptide. It can act enzymatically as a cofactor or coenzyme, detoxifying ROS via the GPx enzyme, or it can act nonenzymatically through the interactions of ROS with sulfhydryl groups (SH; Adebisi et al., 2022). A marker for lipid peroxidation and oxidative stress is TBARs (Javorac et al., 2020).

The results of this study showed a significant decline in the level of GSH and an increase in lipid peroxidation in rats exposed only to Pb in relation to control. These findings are in agreement with Saber et al. (2022), which study reported oxidative stress Pb induced. These outcomes may be a result of Pb's high affinity for the SH groups from GSH, resulting in oxidative stress condition (Manoj Kumar et al., 2017).

In contrast, coexposition to Pb and Ab increased GSH concentration and reduced TBARs compared with the Pb group, returning to normal control levels. This result suggests the ability of the mushroom to improve oxidative stress by scavenging free radicals produced by Pb poisoning. The use of exogenous antioxidants increases the total amount of endogenous antioxidants in the body and reduces the likelihood of inducing oxidative damage (Olcha et al., 2022; Winiarska-Mieczan et al., 2021). This improvement in oxidative stress parameters may be related to the presence of beta-glucan in the mushroom Ab, since, according to a study carried out by Tatli Seven et al. (2021). Regarding the protective role of beta-glucan in the toxicity induced by Pb acetate in rats, it demonstrated improvement in parameters of oxidative stress, with increased antioxidant activity in groups exposed to beta-glucan.

The reproductive consequences of Pb intoxication have already been investigated with different species, with different doses, and in different gestational periods (Aglan et al., 2021; Saleh et al., 2018), corroborating the results reported in this study. The increased rates of pre- and postimplantation losses, and the decreased fetal vitality in the Pb group are clearly related to Pb toxicity. The Pb has the ability to cross the placenta, consequently directly exposing the fetus, causing acute harmful effects such as intrauterine deaths, spontaneous abortions, and/or low birth weight (Amadi & Igweze, 2017; Baranowska-Bosiacka et al., 2011).

Our findings corroborated the high incidence of fetal mortality reported in a study with pregnant rats receiving 250 mL/L of Pb (Saleh et al., 2018). The fetal mortality is reflected by the increase in resorptions and stillbirths in maternal rats treated with Pb, together with a high percentage of postimplantation loss and a significant decline in the number of live fetuses compared with control maternal rats. Despite we did not found differences in fetus body weight, the birth weight is an important parameter in toxicology, as it allows evaluating the effect of substances administered during pregnancy on the development of the fetus (Adham et al., 2011). Other authors administered Pb in doses lower than ours (8, 16, and 24 mg/L), in female rats in the period prior to pregnancy, during and until weaning the pups. In their results, the authors found no significant difference in the body mass of the offspring, similar to our findings (Baranowska-Bosiacka et al., 2011).

Regarding the safety of medicinal mushrooms, *Lentinula edodes*, which is also an edible mushroom, was evaluated during the gestational period and, like Ab, induced decreases in the morphological measurements of fetuses (Frizo et al., 2014). Our results are still similar to those of Viroel and collaborators (Viroel et al., 2022), who found an increase in some parameters of fetal measurements after using the *Ganoderma lucidum* mushroom in pregnant rats. Considering the increase in thoracic measurements, all groups exposed to Pb and Ab showed this alteration.

Contemplating fetal bone analysis, we found malformations and variations in the skull, vertebrae, sternum, and ribs in the group exposed to Pb. The detrimental Pb effects on bone formation and mineralization were previously described in a study with female rats (Monir et al., 2010). In contrast, the Ab and Pb + Ab groups presented preserved and intact skull and vertebrae parameters.

The number of sternal ossifications points present in fetus were counted, which, normally, is six. The Pb and Ab groups showed a decrease in the number of ossifications compared with the control. This discrepancy

between the values of the Ab and Pb groups in relation to the control indicates a delay in bone formation in these groups; however, it cannot be said that there is no ossification, because it could still occur in the future. The coexposition with the mushroom reversed the delays in the sternum ossification induced by the metal or the mushroom, when administered alone.

The ribs were examined for any abnormalities, which were defined as short and discontinuous (Monir et al., 2010). Short rib refers to a shorter than normal ribs and discontinuous rib is a rib that has interruptions or gaps along its length. Abnormalities in the ribs were identified across all groups, but the Pb group exhibited abnormalities in 100% of the fetuses analyzed. Our findings are consistent with those of Aglan et al. (2021), who reported skeletal variations and malformations, including incomplete and absent ossifications in the offspring of female rats exposed to Pb at a concentration of 250 mg/L.

Regarding the bone alterations found in fetuses exposed to mushroom, we hypothesize these changes may have been caused by a deficiency of some nutrient. Since mushrooms can chelate toxic metals because they have compounds able to bind to chemical elements, such as chitin and chitosan (Visweswar et al., 2018), These compounds have the potential to bind with essential minerals, such as calcium, magnesium, manganese, and iron, all of which are bivalent cations. A similar finding was reported with the mushroom *L. edodes*, which was responsible to decreased levels of copper, iron, manganese, and cobalt in the liver of healthy male rats (Grotto et al., 2015). In contrast, the Pb + Ab group exhibited an increased number of ossification points compared with the Ab and Pb groups. This increase may be attributed to the antioxidant properties of the mushroom, which could potentially reduce fetal damage. About the ribs, in all groups some type of alteration was observed, highlighting the Pb group, which had malformations in 100% of the analyzed fetuses.

5 | CONCLUSION

There is no known safe level for Pb exposure or time to exposure. Here we stated that even just exposure during pregnancy, in female rats, was able to induce deleterious effects in both mother and fetus. On the other hand, our findings indicated the Ab improved hematological parameters, increased antioxidant activity by scavenging free radicals, partially remedied the damage in fetal morphological and bone parameters and did not affect body weight of the fetus in mushroom-treated groups. Based on these results, the coadministration of the mushroom Ab, due to the presence of phenolics and glucans in its composition, which could play a significant role in reducing the toxic effect of Pb.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data and publication materials are available from the corresponding author upon a reasonable request.

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CAPÍTULO 3

Protective efficacy of *Agaricus bisporus* against lead-induced toxicity in *Caenorhabditis elegans*.

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Protective efficacy of *Agaricus bisporus* against lead-induced toxicity in *Caenorhabditis elegans*

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Abstract

Caenorhabditis elegans is a nematode found worldwide, and due to its resemblance to mammalian systems and expression of about 40% of human disease-related genes, it serves as a suitable model for researching the toxic impacts of lead (Pb). Considering that *Agaricus bisporus*, an edible mushroom, has demonstrated antioxidant properties, our investigation focused on examining whether *Agaricus bisporus* could protect *C. elegans* from the toxic effects of Pb. A dose-response study was run involving Pb and *Agaricus bisporus*, to the chosen of the doses. After, a co-exposure study using *C. elegans* strains N2 and CL2166 was developed, considering the groups: Control, Pb, *Agaricus bisporus* and Pb+*Agaricus bisporus*. Our findings demonstrated that the co-exposition Pb + 100 mg/mL *Agaricus bisporus* reduced embryonic and larval lethality as well as increased brood size and motility in comparison to nematodes exposed only to Pb. Based on our observations, we have noticed a transfer of reproductive toxicity from nematode parents to their offspring. Thus, we suggest that the antioxidant properties of *Agaricus bisporus* could potentially mitigate the adverse effects of Pb-induced reproductive toxicity. Consequently, this natural antioxidant could potentially serve for neutralizing the detrimental effect of Pb on reproductive health.

Keywords: Alternative animal models. *Agaricus bisporus*. *C. elegans*. Lead.

Abbreviations: Ab, *Agaricus bisporus,* C, Control, Lead.

1 INTRODUÇÃO

Lead is a chemical element with the symbol Pb deemed a heavy metal, widely used throughout human history due to its unique physical and chemical properties [1]. Considering its widespread use, Pb can be found in many environments, including soil, water, and air [2]. However, it is known to be a toxic substance that can cause a variety of health problems in humans and other animals [3]. The mechanisms by which Pb causes toxicity are not fully understood, but it is known that Pb can interfere with many cellular processes, including energy production, protein synthesis, and DNA repair, also disrupting the function of enzymes and other proteins, leading to oxidative stress and inflammation [4].

Pb toxicity can affect multiple organs and systems in the body, including the nervous, cardiovascular, urinary and reproductive systems [5]. In children, Pb exposure can cause developmental delays, learning disabilities, and behavioral problems. In adults, Pb exposure can cause high blood pressure, kidney damage, and reproductive problems [6].

Caenorhabditis elegans, or simply *C. elegans*, is a small, transparent, and easy-to-grow organism that has been extensively studied due to its simple anatomy, short lifespan, and well-characterized genetics [7]. It is often used as a research model in several areas of biology, including genetics, developmental biology, neuroscience, cellular and molecular biology, aging, and toxicology [8]. One of the main advantages of *C. elegans* as a research model is the ease of cultivation in the laboratory, in addition to the ability to produce large numbers of progenies in a short period of time [9]. In toxicological research, *C. elegans* is often used as a model to study the toxic effects of various agents, including environmental chemicals, medications, and toxic substances [10].

Agaricus bisporus, also known as the Paris mushroom, is a species of mushroom widely grown and consumed around the world [11]. In addition to its use in cooking, this mushroom has also been studied for its medicinal effects, as anti-inflammatory and antioxidant [12,13]. That being said, some studies have suggested that certain compounds found in mushrooms, such as polysaccharides and beta-glucans, may have the potential to reduce toxic effects on the body caused Heavy metals, Environmental pollutants, Chemical toxins and Oxidative stress [14].

Some estudies have focused on exploring the antioxidant properties of mushrooms and their potential impact on enhancing reproductive health. Research on *Ganoderma lucidum* (reishi mushroom) has indicated that its antioxidant compounds could potentially enhance sperm quality and provide protection against reproductive toxicity caused by specific environmental factors or toxins [15]. Furthermore, other mushroom species such as *Macrolepiota procera* (parasol mushroom), *morchella esculenta, Agaricus blazei* and *shiitake* mushroom have also been investigated for their potential benefits on reproductive health [16,17,18]. However, further research is necessary to establish the precise mechanisms involved in these effects.

In this way, we developed this study using C. *elegans* to investigate whether supplementation with this mushroom is capable of protecting the nematodes from reprotoxicity-induced by Pb.

2 MATERIAL AND METHODS

2.1 Characterization of the mushroom *Agaricus bisporus*

Fresh *Agaricus bisporus* mushrooms were obtained commercially from a local producer, were sliced and dried in a ventilated oven at 38 ± 2 °C, until constant mass. The dried mushroom was ground in a mill. To verify the antioxidant capacity, the mushroom powder was analyzed in triplicate, following the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method (1995) [19].

The fingerprint of *Agaricus bisporus* (resuspended in water - 100 mg/mL) was run using High Performance Liquid Chromatography (HPLC), on a Shimadzu-model Class-VP-HPLC instrument. The significance of this method becomes evident as the chemical fingerprinting technique, backed by esteemed organizations like the World Health Organization (WHO), the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and others, plays a crucial role in providing a comprehensive chemical description of plants and food products [20].

The chromatographical methodology followed Kim et al. (2008) [21]: 20 µL was the injection volume, in a c18 column (250 mm × 4.60 mm, 5 µm particle size; Luna, Phemomenex). The mobile phase was distilled water with 0.1% glacial acetic acid (solvent A) and acetonitrile with 0.1% glacial acetic acid (solvent B), and chromatographic analysis was performed in a gradient system. The gradient was 92% A (0 minute); 90% A (0-2 minutes);

70% A (2-27 minutes); 10% A (27-50 minutes; 0% A (50-51 minutes); 0% A (51-60 minutes); 92% A (60-63 minutes). Run time was 60 minutes, using a flow rate of 1 mL/minute. The absorbance of each sample solution was measured at 280 nm.

2.2 *C. elegans* strains and experimental design

C. elegans strains were cultured according to Brenner (1974) [22], at 20 °C on nematode growth medium (NGM) plates with cholesterol The following strains were used in the study: N2 (wild type) and CL2166 (*dvIs19 [(Paf15)gfp-4p::NLS]III*). All strains were maintained at 20 °C on NGM agar plates seeded with *E. coli* OP50.

To define the appropriate doses of Pb and *Agaricus bisporus* for administration in *C. elegans*, a pilot dose-response study was conducted. This preliminary study involved testing three different concentrations of Pb and *Agaricus bisporus*. The dose-response study considered three Pb levels: 10, 100 and 1,000 mM. The dose of 100 mM was chosen based on a previous study of Pb in *C. elegans* [23], and the other doses were chosen considering a concentration 10 times higher and 10 times lower. The dose-response test enabled us to choose an appropriate Pb dose for the co-exposure study.

The analysis of different doses of *Agaricus bisporus* in *C. elegans* enabled the identification of the mushroom safety profile, which was of utmost importance for the subsequent study. We based the choice of mushroom dose on the safe and effective dose in rats (100 mg/mL) [24], and the other doses were chosen also considering the concentration 10 and 100 times lower (10 and 1 mg/mL).

After determining the doses suitable for *C. elegans*, the co-exposure study was run. The experimental groups were Control; Pb (with se selected dose); *Agaricus bisporus* (with the selected dose) and Pb + *Agaricus bisporus*.

Exposure was carried out by placing synchronized L1 stage worms synchronized by sodium hypochlorite treatment as in Stiernagle (2006) [25] onto plates seeded *with Escherichia coli* strain OP50 (OD600 = 24) and treatment: vehicle (H_20); Pb; *Agaricus bisporus* and Pb + *Agaricus bisporus*. Plates were then incubated for ∼72 hours at 20 °C until worms reached the adult stage. At least two independent biological replicates for each condition/assay were carried out.

2.3 Scoring embryonic lethality, larval lethality, and brood size

The experiment consisted in transferring exposed N2 hermaphrodites (n=10) individually to NGM plates to observe their embryonic lethality, larval lethality, and brood size. This process was repeated for four consecutive days, with the worms being transferred to fresh NGM plates every 24 hours. We calculated the total number of fertilized eggs laid, the percentage of fertilized eggs that hatched and of larvae that reached adulthood.

2.4 Assessing worm length and motility

After exposure, n2 worms were transferred to a slide containing 10 µl of m9 buffer and levamisole hydrochloride (VETRANAL®, analytical standard) at two different times, that is, hermaphrodites exposed to pb for 72 hours (P0), and their descendents for 144 hours (F1). we then photographed the worms under a microscope and measured their length using imagej software.

It is important to verify the health of the descendants of *C. elegans* because hamaphrodites can transfer toxic compounds or altered gene expression patterns to their offspring through the process of maternal transfer. the toxic substances or altered gene expression can be transmitted via the eggs, germline, or maternal factors present in the reproductive process. To conduct the motility assay, nematodes were recorded under a microscope camera for approximately two minutes. Using a mechanical click counter (CTL-DIFD-08KP; LW Scientific, Lawrenceville, USA) and a stopwatch, we recorded their thrashes for one minute, as shown, following a period of adaptation of 30 seconds.

2.5 Glutathione s-transferase expression

For this evaluation, we used a reporter strain carrying GFP expressed under control of *gst-4* promoter (CL2166) as a readout of the increased oxidative stress state Detienne et al. (2016) [26], associated with Pb exposure. CL2166 exposed worms were examined utilizing a Cytation V (Multi-Modal, Biotek Cytation 5). The fluorescence intensities of the worms and background were quantified using ImageJ software as reported in Hornos Carneiro et al. (2020) [27].

2.6 Statistical analysis

Data are expressed as mean ± standard deviation. follow-up test Duncan was performed to verify differences among treatment protocols. Values of $p<0.05$ were considered significant. The results were analyzed using the Statistica® 8.0 and Graph Pad Prism® 8 programs.

3 RESULTS

The application of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) methodology showed that the mushroom exhibited 50% antioxidant activity within 15 minutes of the test. Moreover, the mushroom maintained its antioxidant activity at approximately 48% over a period of 30, 45, and 60 minutes.

Figure 1 illustrates the presence of phenolic compounds. As reported in the chromatographic analysis of phenolic standards, by Kim et al. (2008) [16], the following compounds were identified at retention times of 5.5, 6.1, 9.2, 16.8, and 37.8 min, respectively: gallic acid, pyrogallol, protocatechuic acid, naringin, and naringenin. These phenolic compounds are the active ones commonly found in the fruiting bodies of *Agaricus bisporus.*

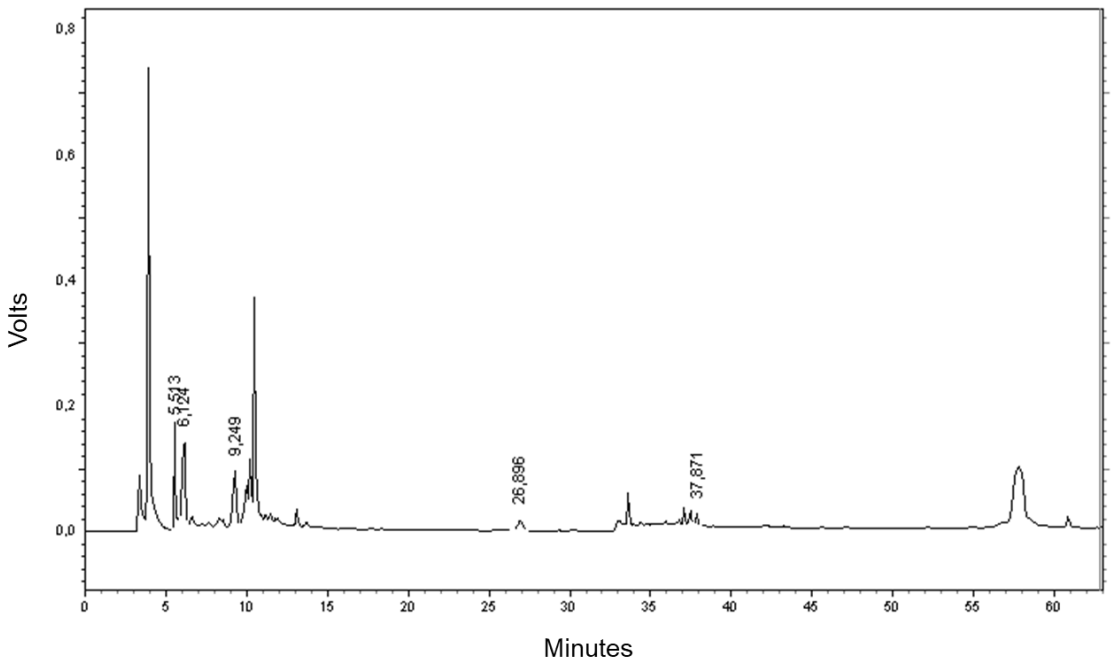


Figure 1 - Chromatogram of phenolic compounds from of *Agaricus bisporus*.

The dose-response study yielded valuable insights regarding the toxic effects of Pb in *C. elegans*. The results helped identify the threshold at which Pb toxicity becomes significant, while also avoiding excessively high doses that could have overwhelming effects on developmental parameters. Figure 2 reports the clutch size, embryonic and larval lethality in the three different doses of Pb. The mean of eggs laid by worms did not differ comparing the three Pb doses. Embryonic lethality was significantly higher in all doses comparing to control, and larval lethality was significantly higher in 100 and 1,000 µM Pb. With these findings, we chosen the dose of 100 µM, considering the existing literature [23] and also considering that the high larval lethality in 1,000 µM, compared to 100 µM, could interfere negatively in the co-exposure study. Thus, the chosen dose can induce observable toxic effects without compromising the overall health and development of the organisms.

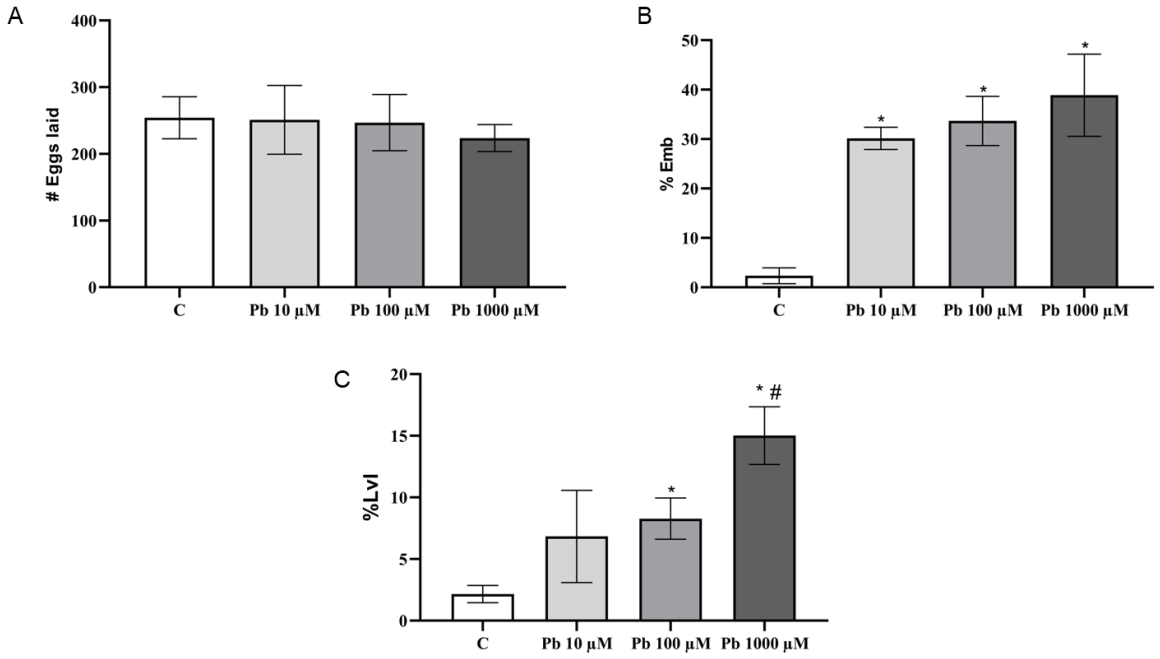


Figure 2 - Embryonic lethality (Emb), larval lethality (Lvl), and brood size following exposure of hermaphrodites to different doses of Pb. (A), Mean of eggs laid by worms exposed to the indicated conditions, (B) Emb and (C) Lvl. Data are presented as mean ± SD of 09-10 hermaphrodites per group. *p≤0.05 compared to C; #p≤0.05 compared to Pb 100 µM. Control (C), Lead (Pb).

Figure 3 presents the impact of different doses of *Agaricus bisporus* on clutch size, embryonic lethality and larval lethality. Compared to the control group, the results revealed no significant differences in litter size and larval lethality across the various doses. Moreover, there was a significative decrease in embryonic lethality at 100 mg/mL, suggesting that the use of

this mushroom is safe for *C. elegans*. Based on these findings and considering previous *in vivo* study [24], we considered the dose of 100 mg/mL for the co-exposure study. This chosen dose is expected to have a positive effect on the reproductive development of *C. elegans*, helping to reduce the damage caused by Pb exposure.

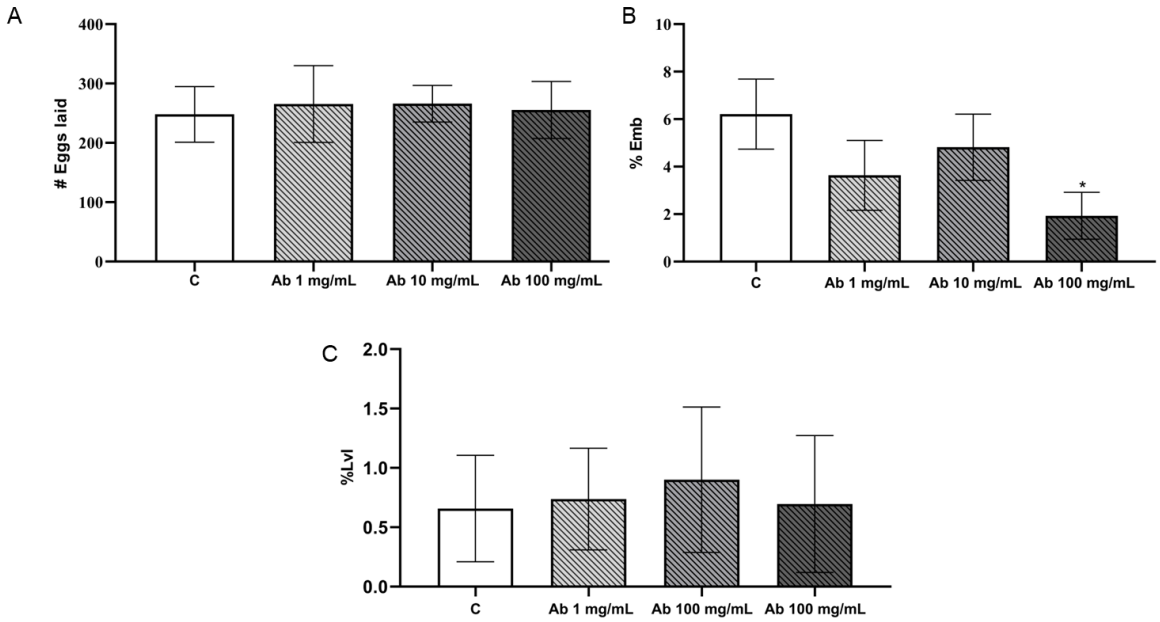


Figure 3 - Embryonic lethality (Emb), larval lethality (Lvl), and brood size following exposure of hermaphrodites to different doses of Ab. (A), Mean of eggs laid by worms exposed to the indicated conditions, (B) Emb and (C) Lvl. Data are presented as mean ± SD of 09-10 hermaphrodites per group. *p≤0.05 compared to C. Control (C), *Agaricus bisporus* (Ab).

The results of the clutch size, embryonic and larval lethality of the co-exposure study of Pb and *Agaricus bisporus* are shown in figure 4. In contrast to the control group, exposure to Pb in *C. elegans* led to reduced brood size and elevated rates of embryonic and larval lethality. However, the administration of *Agaricus bisporus* alone or in combination with Pb was found to improve these fertility outcomes in the experimental groups.

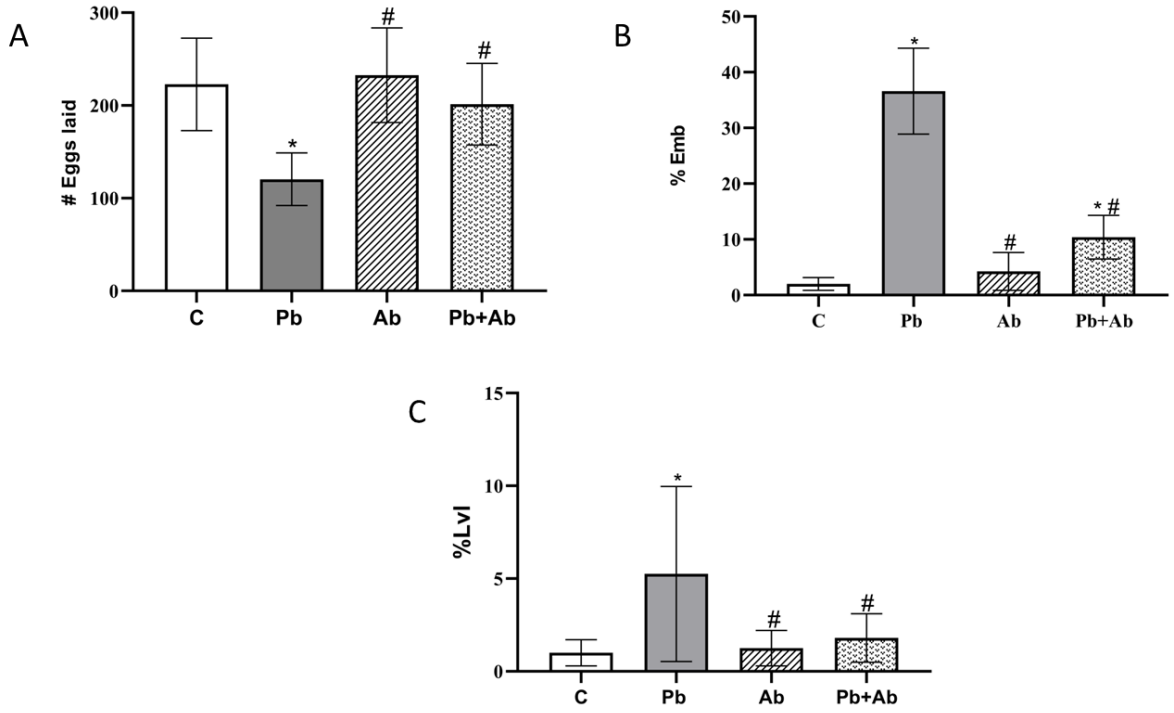
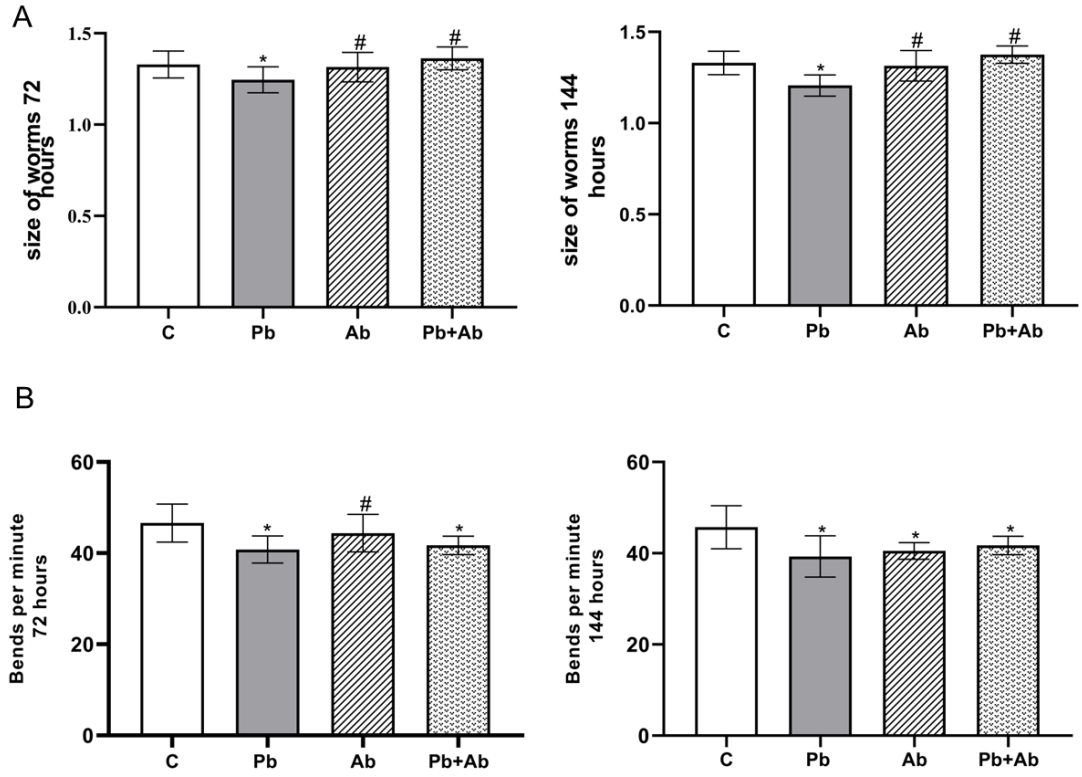


Figure 4 - Embryonic lethality (Emb), larval lethality (Lvl), and brood size following exposure of hermaphrodites to Pb and/or Ab.(A), Mean of eggs laid by worms exposed to the indicated conditions, (B) Emb and (C) Lvl. Data are presented as mean ± SD of 09-10 hermaphrodites per group. *p≤0.05 compared to C; #p≤0.05 compared to Pb. Control (C), Lead 100 µM (Pb), *Agaricus bisporus* 100 mg/mL (Ab).

Regarding the length of the worms the co-exposure, a significant decrease was observed both in Pb-exposed hermaphrodites as well as in their progeny (F1) when compared to control (figure 5, A). Conversely, the groups exposed to Pb + *Agaricus bisporus* exhibited length an improvement in this parameter.

We noticed a decrease in the worm motility in hermaphrodites exposed to Pb since L1 for 72h as well as for their progeny when compared to the control group (figure 5, B). In contrast to what was observed for length, co-treatment with Ab was not able to counteract this neurotoxicity phenotype induced by Pb. Moreover, we did not observe beneficial effects of Ab on F1´s motility, either alone or as a co-treatment with Pb.



225

226 **Figure 5 -** Lenght (A) and Motility (B) of hermaphrodites Exposed to Pb and *Agaricus bisporus* from L1 for 72
227 hours and their progeny (F1). Data are presented as mean ± SD **(**n = 20/group) *p≤0.05 compared to C; #p≤0.05
228 compared to Pb. Control (C), Lead 100 µM (Pb), *Agaricus bisporus* 100 mg/mL (Ab).

229

230 The outcomes of GFP fluorescence the co-exposure in strain CL2166 are illustrated in
231 Figure 6. We noticed a significant increase in GFP signal in the worms exposed to Pb compared
232 to control. Moreover, worms supplemented with *Agaricus bisporus* and exposed to Pb (Pb +
233 Ab) showed significantly less GST-4 expression than those exposed to only Pb. Also, a decrease
234 in GFP intensity was observed in the nematodes exposed only to *Agaricus bisporus* when
235 compared to the Pb group.

236

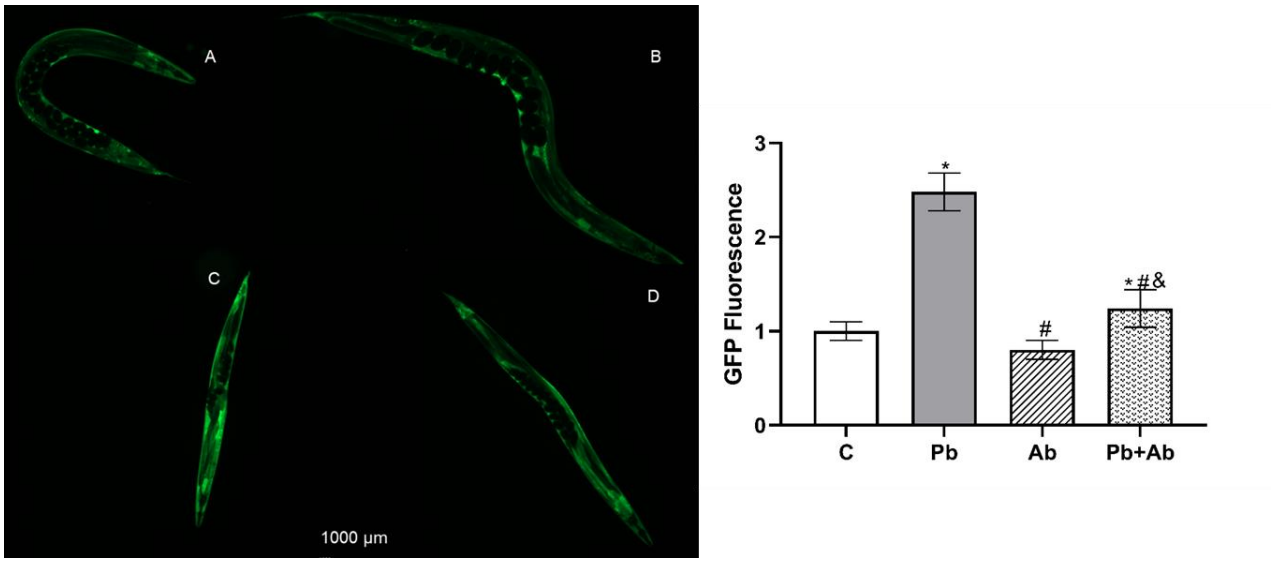


Figure 6 - Fold change of GFP fluorescence showing GST-4 expression indicating that co-treatment with Ab ameliorates the elevated signal in worms exposed to Pb. Data are presented as mean ± SD (n = 20/group). *p≤0.05 compared to C; #p≤0.05 compared to Pb; & p≤0.05 compared to Ab. Control (C), Lead 100 µM (Pb), *Agaricus bisporus* 100 mg/mL (Ab).

4 DISCUSSION

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is a commonly used tool to measure the antioxidant activity of various compounds, including natural products such as mushrooms [28,29]. Mushrooms are known to possess various beneficial bioactive compounds that contribute to their antioxidant activity, which can be measured using the DPPH assay [30]. For instance, according to a study conducted by Kim et al. in 2008 [21], the DPPH radical activity of certain edible mushrooms was observed to be 57% in *Agaricus bisporus*, 49% in Lentinus edodes, and 51% in Pleurotus ostreatus. The presence of gallic acid, pyrogallol, protocatechuic acid, naringin, naringenin, ferulic acid, myricetin, quercetin, and total phenolic compounds was found to be positively associated with the antioxidant activity observed in the study.

In the context of the DPPH assay, a higher percentage of inhibition indicates stronger antioxidant activity, whereas a lower percentage indicates weaker antioxidant activity [31]. In our study, *Agaricus bisporus* demonstrated a 50% inhibition of DPPH, indicating a moderate level of antioxidant activity.

The dose-response study is essential for determining the point at which a toxic substance begins to produce harmful effects and establishing a safe dosage [32]. By investigating different doses, we were able to observe and evaluate the occurrence and intensity of toxic reactions

caused by Pb, as well as assess the safety and protective properties of *Agaricus bisporus* in *C. elegans*. These findings yielded valuable insights that guided the selection of suitable doses for the co-exposure study: 100 µM for Pb and 100 mg/mL for *Agaricus bisporus*.

Pb is a naturally occurring heavy metal in the earth's crust that is not essential to human biology. Its neurotoxicity is complex and involves the generation of oxidative stress, membrane disruptions, dysregulation of cell signaling, and impaired neurotransmission [33]. The simplicity of the *C. elegans* model has made it a popular choice for studying heavy metals toxicity [34,35,36]. Exposure to Pb in *C. elegans* leads to a decrease in brood size and an increase in embryonic lethality [37]. Reproductive impairments have been observed in various animal species exposed to heavy metals, such as decreased quality and production of both sperm and oocytes, as well as increased embryonic lethality and recurrence of miscarriages in humans [38,39,40].

In order to investigate the potential impact of Pb on the reproductive ability of *C. elegans*, as well as the potential protective effects of *Agaricus bisporus*, we exposed the worms to a concentration of 100 µM Pb combined to 100 mg/mL *Agaricus bisporus*. Considering the results, we noticed that worms exposed to Pb had a significant decrease in the average number of eggs laid (resulting in a 54% reduction, a significant sterility), as well as an increase in both embryonic lethality (37%) and larval lethality (5.25%) when compared to the control group. Collectively, these results suggest Pb negatively affects the reproductive abilities of *C. elegans* and may result in errors during chromosome segregation [41].

These results were consistent with those reported by Guo, Yang, and Wang (2009) [23], in their research on *C. elegans* nematodes exposed to the same Pb concentration. They reported that exposure to Pb reduced brood size, indicating Pb-induced reproductive toxicity on germ cells. On the other hand, we observed a beneficial effect in the groups co-exposed to *Agaricus bisporus* (Pb+Ab). Given that *Agaricus bisporus* exhibited antioxidant properties and Pb has the potential to impair reproduction via oxidative stress, we postulate that co-exposure to *Agaricus bisporus* could safeguard nematode embryonic development from Pb-induced oxidative damage. A similar observation was reported by Li et al. (2013) [36] in a study about the protective effect of selenium against Pb-induced toxicity in C elegans. Their findings revealed that selenium antioxidant properties may aid in reducing Pb toxicity in *C. elegans*.

Measuring the body of *C. elegans* is a sensitive way to assess toxicity, with commonly used measurements including body length, width, area, and aspect ratio. In a manner consistent with the findings of Tang et al. (2019) [42], our study revealed a reduction in the length of *C. elegans* when exposed to Pb. However, we also observed that supplementing with *Agaricus*

bisporus resulted in an improvement of this parameter in both hermaphrodites and their offspring (1.35 and 1.36 mm, respectively), comparable to the measurements observed in the control group (1.34 and 1.33 mm, respectively) (Figure 5, A). The observed enhancement in the length of *C. elegans* (hermaphrodites and their descendants) following *Agaricus bisporus* supplementation could be attributed to several potential transgenerational mechanisms. These mechanisms may include nutritional factors, epigenetic modifications, hormone regulation, and the response to stress and oxidative balance [43]. However, it is crucial to acknowledge that these mechanisms are speculative, and further in-depth investigations are necessary to fully comprehend the specific mechanisms responsible for the transgenerational effects on *C. elegans* length resulting from *Agaricus bisporus* supplementation.

The locomotion of *C. elegans* has been widely used to study its response to sensory stimuli. Behavior assays, such as body turns, head strikes, and reversal frequency, are used to investigate the neuronal circuits that control behavior [44]. In this study, the effect *of Agaricus bisporus* on Pb-induced toxicity was examined using thrashes as a parameter. The number of thrashes was significantly reduced by Pb exposure at both when P0 and F1 compared to controls (Figure 5, B). When comparing the groups co-exposed to Pb+Ab to the control group, either considering the exposed hermaphrodites or F1, a reduction in body movements was still observed.

Transgenerational inheritance, including epigenetic modifications, can contribute to the transmission of changes in movement patterns from the exposed generation (P0) to the first generation (F1). This suggests that alterations in movement behavior can be inherited across generations in a non-genetic manner, potentially involving epigenetic mechanisms [45]. Our study aligns with the findings reported by Li et al. in 2013 [36] regarding lead-induced neurotoxicity and selenium treatment. Li et al. observed a decrease in the frequency of body curves in the group exposed to 100 μM of Pb, while selenium supplementation at a dose of 0.01 μM mitigated the decline in locomotion behaviors in *C. elegans.*

In contrast to what was observed for Li et al. in 2013 [36], co-treatment with Ab was not able to counteract this neurotoxicity phenotype induced by Pb. Moreover, we did not observe beneficial effects of Ab on F1´s motility, either alone or as a co-treatment with Pb.

There is evidence to support that exposure to Pb can cause oxidative damage and increase the production of reactive oxygen species (ROS) in *C. elegans* [46]. The transgenic strain *C. elegans* CL2166 expresses green fluorescent protein (GFP) under control of the gst-4 promoter [47]. In our study, we investigated the impact of Pb and *Agaricus bisporus* exposure

on worm oxidative metabolism response. We found that Pb exposure led to an increase in fluorescence in the worms, indicating an increase in the expression of gst-4. This observation is consistent with the findings of Lu et al. (2018) [48], In their study, the researchers evaluated the toxicity of manganese, lead (Pb), and cadmium in the model organism *C. elegans*. These substances have the potential to induce the formation of reactive oxygen species (ROS) in *C. elegans*, just like Pb.

On the other hand, the groups that received *Agaricus bisporus* supplementation showed decreased levels of GST-4 expression compared to those exposed solely to Pb. This finding indicates that this mushroom supplementation could potentially alleviate the intracellular ROS levels in *C. elegans* formed due Pb exposure, and therefore protect the nematodes from Pb-induced oxidative stress.

5 CONCLUSION

Our results demonstrated that *Agaricus bisporus* has the ability to reduce the toxicity resulting from Pb exposure, potentially due to the amelioration the Pb-induced oxidative stress. This is reflected in the protective effects of Ab in embryonic and larval lethality, reduced length and motility, and GST-4 expression. In this way, our findings present novel evidence supporting the antioxidant properties of the *Agaricus bisporus* and its importance as a neutralizing agent for Pb-induced reprotoxicity.

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6 CONCLUSÃO GERAL

Atualmente, não há limite seguro estabelecido ou duração de exposição ao Pb. A exposição de ratos e nematóides ao Pb nesta pesquisa levou a consequências prejudiciais.

Nossos achados demonstram que o Pb é rapidamente distribuído tanto para a mãe quanto para o feto. No entanto, observamos que o *Agaricus bisporus* melhorou os parâmetros hematológicos, aumentou a atividade antioxidante por reduzir os radicais livres, corrigiu parcialmente os danos morfológicos e ósseos e não afetou o peso corporal fetal nos grupos tratados com o cogumelo.

Além disso, no modelo *C. elegans*, o emprego do cogumelo *Agaricus bisporus* demonstrou a capacidade de diminuir a toxicidade causada pelo estresse oxidativo induzido por Pb, juntamente com o aumento associado da letalidade embrionária e larval, redução do tamanho da ninhada e comprometimento do sistema locomotor.

Assim, os resultados desta pesquisa fornecem novas evidências que suportam as propriedades antioxidantes do mecanismo de ação de *Agaricus bisporus*.

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