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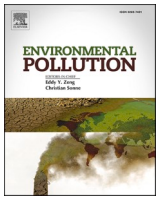

Review

The effects of heavy metal exposure on brain and gut microbiota: A systematic review of animal studies☆

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ABSTRACT

The gut-brain axis is a crucial interface between the central nervous system and the gut microbiota. Recent evidence shows that exposure to environmental contaminants, such as heavy metals, can cause dysbiosis in gut microbiota, which may affect the gut-brain communication, impacting aspects of brain function and behavior. This systematic review of the literature aims to evaluate whether deleterious effects on brain function due to heavy metal exposure could be mediated by changes in the gut microbiota profile. Animal studies involving exposure to heavy metals and a comparison with a control group that evaluated neuropsychological outcomes and/or molecular outcomes along with the analysis of microbiota composition were reviewed. The authors independently assessed studies for inclusion, extracted data and assessed risk of bias using the protocol of Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) for preclinical studies. A search in 3 databases yielded 16 eligible studies focused on lead (n = 10), cadmium (n = 1), mercury (n = 3), manganese (n = 1), and combined exposure of lead and manganese (n = 1). The animal species were rats (n = 7), mice (n = 4), zebrafish (n = 3), carp (n = 1) and fruit fly (n = 1). Heavy metals were found to adversely affect cognitive function, behavior, and neuronal morphology. Moreover, heavy metal exposure was associated with changes in the abundance of specific bacterial phyla, such as *Firmicutes* and *Proteobacteria*, which play crucial roles in gut health. In some studies, these alterations were correlated with learning and memory impairments and mood disorders. The interplay of heavy metals, gut microbiota, and brain suggests that heavy metals can induce direct brain alterations and indirect effects through the microbiota, contributing to neurotoxicity and the development of neuropsychological disorders. However, the small number of papers under review makes it difficult to draw definitive conclusions. Further research is warranted to unravel the underlying mechanisms and evaluate the translational implications for human health.

1. Introduction

Heavy metals are a group of high-density elements that are naturally present in the earth's crust in variable concentrations. However, anthropogenic activities such as mining, agriculture, combustion and industrial processes significantly contribute to their emission (Al osman et al., 2019; Ishchenko, 2019; Tchounwou et al., 2012). These metals have the capacity to persist in the ecosystem over extended periods, accumulating within the food chain and posing a significant environmental threat, particularly to groundwater sources (Tahir and Alkheraije, 2023). Heavy metal exposure has become a global public health concern, as some of them are systemic toxicants even at low concentrations in the human body (Okechukwu Ohiagu et al., 2022). Human exposure to heavy metals occurs through contaminated food, water, inhalation or skin contact (Buckley et al., 2020). The ability of these elements to cross the blood brain barrier (BBB) and, therefore, to interfere with the biological processes of the central nervous system (CNS) has been fully demonstrated (Dack et al., 2022; Grandjean and Landrigan, 2014; Gundacker et al., 2021; Killin et al., 2016; Smith et al., 2022). Notably, the toxic effects of heavy metals are particularly

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harmful for infant and children due to the underdeveloped BBB and the long period for brain development, that extends from prenatal stage until adolescence (Stiles and Jernigan, 2010), resulting in cerebral damage and neurological disorders (Hauptman and Woolf, 2017; Saunders et al., 2012). The brain can accumulate heavy metals, and this buildup is linked to cognitive aging and neurological diseases (Baj et al., 2023; Karimi et al., 2022; Madden and Merenstein, 2023; Pyatha et al., 2022). Previous studies reported that early exposure to lead (Pb) induced neurotoxicity and mental retardation in infants (Gundacker et al., 2021; Nevin, 2009). On the other hand, cadmium (Cd) exposure is associated with mental retardation, dyslexia (Wang and Du, 2013), autism spectrum disorder symptoms (Fiore et al., 2020) as well as visuomotor and externalizing behavior alterations (Viaene et al., 2000). Early exposure to mercury (Hg) has been related to children's impaired neuropsychological development in populations with high fish consumption (main source of exposure to Hg) (Bastien et al., 2022; Debes et al., 2016). However, the evidence at lower doses is controversial, which could be indicating the influence of certain influencing factors, such as, genetics, beneficial nutrients from fish or gut microbiota. Manganese (Mn) is a heavy metal essential element for human with a role in the synthesis and metabolism of neurotransmitters. However, high early exposure to Mn has been associated with cognitive, behavioral and motor deficits (Kim et al., 2022; Soetrisno and Delgado-Saborit, 2020). Additionally, a recent study reported that prenatal exposure to Hg and deficiency in Mn could enhance neurotoxicity of Pb in young children (Farías et al., 2022).

Gut microbiota participates in the regulation of numerous physiological functions, such as aiding digestion, the synthesis of proteins and amino acids, energy metabolism, modulation of the immune system, growth, neurodevelopment and behavior (Sirisinha, 2016). Experiments conducted with rats have shown that the microbiota plays a crucial role in the development of the brain during the early stages of life as well as in the neurogenesis of the adult hippocampus. It is also strongly involved in the modulation of learning, memory and behavioral responses to stress (Dinan and Cryan, 2017a). Results reported in literature proved that heavy metals could induce gut microbiota dysbiosis (Arun et al., 2021; Bist and Choudhary, 2022; Duan et al., 2020; Singh et al., 2022). This condition could be related to the development of diverse conditions, including inflammatory bowel disease (Schippa and Conte, 2014) and cancer (Choiniere and Wang, 2016). Notably, in recent years attention has been focused on the effects of heavy metals on the so-called "gut-brain axis", to highlight the close connection between the two systems. Numerous studies have shown a link between the gut microbiota and brain disorders, such as depression, autism, anxiety, Parkinson and Alzheimer's disease (Cerdó et al., 2020; Cryan et al., 2020; Dinan and Cryan, 2017b; Hsiao et al., 2013; Mangiola et al., 2016; Scheperjans, 2016; Valles-Colomer et al., 2019). Experimental models, such as germ-free animals (Luczynski et al., 2016), probiotic and antibiotic treatments (Petschow et al., 2013), have been used to evaluate the impact of the gut microbiota on the brain (Xu et al., 2021). However, there is still limited knowledge about the association between heavy metal exposure, gut microbiota changes, and neurological effects. Therefore, the aim of this work was to systematically review the evidence for the relationship between exposure to heavy metals and effects observed on the gut-brain axis. Specifically, the focus was placed on how changes on gut microbiota occurred after exposure to heavy metals could influence cognition and/or behavior as well as neurochemistry in related brain areas, thus evaluating the mediating role of the gut microbiota in the observed metal neurotoxicity.

2. Methods

We conducted a systematic review to evaluate the role of the gut microbiota on the neurotoxic effects associated to heavy metals exposure. We took in consideration neuropsychological domains ranging from changes in behavioral and cognitive aspects occurring after heavy metals exposure (i.e. neuropsychological outcomes). We also reviewed alterations such as neurotoxicity, neurochemical or morphological changes in brain areas (molecular outcomes) affected by heavy metals exposure. Therefore, only papers that included simultaneously exposure to heavy metals, neuropsychological and/or molecular effects and gut microbiome profile were assessed.

This review was conducted in compliance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA Statement, Table S1) (Page et al., 2021).

2.1. Eligibility criteria

The selection criteria for inclusion and exclusion of studies took place using the PECO strategy, which include Population, Exposure, Comparison and Outcomes. The criteria to include or exclude a study in this systematic review were summarized in Table 1. Our focus was on studies assessing the impact of heavy metals on a broad spectrum of brain-related outcomes and gut microbiota changes in animal models. We excluded studies that lacked cognitive or behavioral testing (neuropsychological outcomes), did not quantify biomarkers indicative of neurological function, or did not evaluate morphological alterations at the cellular and tissue levels in the brain (molecular outcomes). Furthermore, studies that did not examine gut microbiota changes were also excluded. Moreover, non-English articles, review articles, book chapters, conference abstracts, editorials, letters, human studies, and reports from *ex vivo* and *in vitro* studies were excluded from this systematic review.

2.2. Information sources and search strategy

This systematic review was conducted by searching in PubMed (MEDLINE), Scopus and Web of Science international databases for experimental studies conducted in animal models. Papers included investigated the role of gut microbiota on the effects of heavy metals on neuropsychological and molecular aspect of the brain. The search strategy focused on a convergence of Medical Subject Headings (MeSH) and the following entry terms: (heavy metal [MeSH Terms]) AND (microbiota OR 'gut microbiome' OR 'brain-gut axis') AND ('central nervous system' OR cognit* OR brain OR neurodevelopment* OR neuropsych* OR neurotoxicity OR neuro* OR behavior OR behaviour). The heavy metal MeSH term used in this search included the metals actinium, americium, antimony, barium, berkelium, bismuth, cadmium, californium, cesium, cesium isotopes, chromium, chromium isotopes, cobalt, copper, curium, einsteinium, fermium, francium, gallium, gallium isotopes, germanium, gold, gold isotopes, hafnium, indium, iridium, iron, iron isotopes, lawrencium, lead, manganese, mendelevium, mercury, mercury isotopes, molybdenum, neptunium, nickel, niobium, nobelium, osmium, palladium, platinum, plutonium, protactinium, radium, rhenium, rhodium, rubidium, ruthenium, and silver. The Boolean operators "AND" or "OR" were used to enhance the search

Table 1
Criteria of the research question formulation strategy and for inclusion and exclusion of studies, according to PECO parameters.

Parameter	Inclusion Criteria	Exclusion Criteria
Population	Animal studies	No animal studies; Human studies; *ex vivo* and *in vitro* studies
Exposure	Heavy metals exposure	No heavy metal exposure
Comparison	Control group	No control group
Outcomes	Assessment of neuropsychological outcomes (e.g., cognitive, motor and behavior) and/or molecular outcomes (e.g., biomarkers, neurotoxicity) Microbiota analysis.	No neuropsychological and/or molecular outcomes assessment. No microbiota analysis.

and alerts were set up in each database to keep track of new study releases. There were no limitations on publication date. In addition, the reference lists of all included articles were evaluated to identify relevant studies for inclusion in the systematic review. The search was performed periodically until November 6, 2023.

2.3. Study selection and data collection

The first screening and selection of articles was made by two independent reviewers (SP and AE) using the Rayyan online platform (rayyan.qcri.org) for systematic review. This platform allows the removal of duplicate articles and streamlines the screening process, which is later performed independently by the reviewers. Any inconsistencies were verified by discussion with the other reviewers (JMD

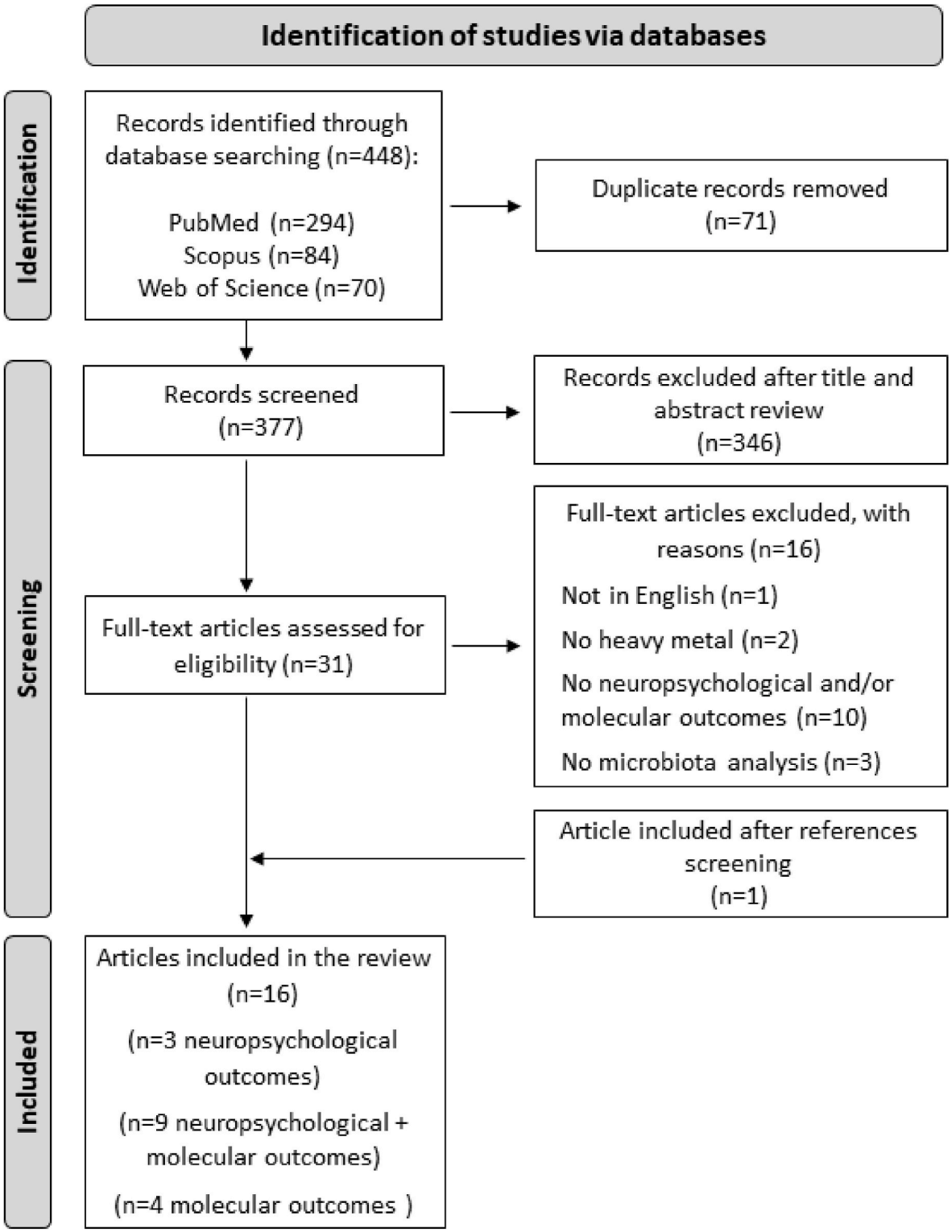

Fig. 1. Flow diagram of study selection based on the PRISMA statement.

and SL), which did not participate in the initial phase. Moreover, the literature was manually reviewed to find any additional relevant articles to be considered in the final list of papers included in this review.

2.4. Risk of bias assessment

Two independent reviewers (SP and JMD) evaluated the risk of bias and study quality in accordance with the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) protocol (Hooijmans et al., 2014). This protocol is a bias assessment tool that includes selection, performance, detection, attrition, and reporting bias. Both independent reviewers assigned an appropriate level of bias. Bias types for each included study were categorized as "high" (+), "low" (−), or "unclear" (?). The remaining authors (AE and SL) joined in the discussion of the disagreements to the risk of bias.

3. Results

3.1. Study selection

The initial screening identified 448 records through the databases searching. After removing 71 duplicates, 346 studies were excluded after reading the titles and abstracts, followed by a full-text analysis of 31 studies. Furthermore, an in-depth manual review of the literature from these 31 papers resulted in the identification of 1 additional relevant article to include in the final list. Finally, 16 articles were included in this review: 3 including neuropsychological domains (neuropsychological outcomes), 3 including biochemical markers in the brain (molecular outcomes) and 9 including neuropsychological and molecular outcomes assessment. 16 articles were excluded for the following reasons: n = 1 not in English, n = 2 no heavy metal, n = 10 no neuropsychological and/or molecular outcomes measure, n = 3 no gut microbiota measure. The detailed results of this process were illustrated in the flow diagram (Fig. 1).

3.2. Characteristics of the included studies

The studies within this review considered 4 heavy metals, Pb (n = 10), Cd (n = 1), Hg (n = 3), Mn (n = 1), and combined exposure of Pb and Mn (n = 1). Among the 16 studies selected and included in this systematic review, 3 of these investigated neuropsychological outcomes only (Chen et al., 2022; Sun et al., 2020; Xia et al., 2020) while 4 studies explored neurochemical changes (Lin et al., 2021, 2020; Wang et al., 2020; Yu et al., 2023) upon exposure to the heavy metal under study. The remaining 9 articles (X. Chen et al., 2021; Cheng et al., 2019; Hua et al., 2023; Li et al., 2022; Sun et al., 2022; Xia et al., 2023; Xiao et al., 2020; Zhang et al., 2022, 2023) considered both the effects on neuropsychological outcomes and molecular changes in the brain. Moreover, all the studies evaluated changes within the gut microbiota. All studies were conducted in China. Regarding the species of animals that have been used, n = 7 studies employed rats, n = 4 mice, n = 3 zebrafish, n = 1 carp, and n = 1 fruit fly.

3.2.1. Heavy metals exposure

The most used route of administration of heavy metals is oral administration. This method involves administering the metal through the animals' food or drinking water, or via direct gavage. Oral administration allows for a more representative exposure route, mimicking the ingestion of contaminated substances. It enables researchers to assess the direct impact of heavy metals on the gut microbiota and its associated effects. A detailed description of the animal models, route, doses and times of administration for each of the included studies is presented in Table 2.

3.2.2. Neuropsychological outcomes

Among the 3 included articles, 2 studies used Pb as a heavy metal (Chen et al., 2022; Sun et al., 2020), while 1 study exposed animals to Cd (Xia et al., 2020). To evaluate the effects on the neuropsychological domain, the studies exposed the animals to different concentrations of the heavy metal and then carried out behavioral tests. For instance, Chen et al. (2022) used the sucrose preference test, forced swim test, and

Table 2
Characteristics of heavy metal doses, animal models, administration route and timing, and microbiota samples in the selected studies.

Reference	Heavy metal and dose	Animal model	Exposure	Duration of exposure	Microbiota sample
Cheng et al. (2019)	Pb chloride, 1.34 g/L^{-1}	Mice (7-week-old male Kunming mice)	Oral (drinking water)	Chronic (8 weeks)	Feces
Lin et al. (2020)	MeHg, 10 μg/kg	Rats (4-week-old male Sprague-Dawley)	Oral	Acute (single administration)	Feces
Sun et al. (2020)	Pb acetate, 100 and 200 mg/L	Fruit flies (*Drosophila melanogaster* – wt (w^{1118}) parents and offspring)	Oral (food)	Chronic (7 days)	Intestinal tissues
Xia et al. (2020)	Cd, 1.25, 2.5 and 5 μg/L	Zebrafish (Larvae *Danio rerio*)	Waterborne	Chronic (7 days)	Feces
Xiao et al. (2020)	Pb acetate, 125 ppm	Rats (pregnant and their female offspring -Sprague -Dawley)	Oral (drinking water)	From in utero until sacrifice.	Feces
Wang et al. (2020)	Mn chloride, 200 mg/L	Rats (SPF Adult male Sprague–Dawley)	Oral (drinking water)	Chronic (5 weeks)	Feces
X. Chen et al. (2021)	Pb acetate, 300 mg/kg	Rats (SPF Adult male Sprague -Dawley)	Oral (drinking water)	Chronic (24 weeks)	Feces
Lin et al. (2021)	MeHg, 10 mg/kg; IHg, 10.8 mg/kg	Rats (4-week-old male Sprague-Dawley)	Oral	Acute (single administration)	Feces
Chen et al. (2022)	Pb acetate, 300 mg/L	Rats (SPF Adult male Sprague -Dawley)	Oral (drinking water)	Chronic (24 weeks)	Feces
Li et al. (2022)	Pb acetate, 100 mg/L	Mice (4-week-old male C557BL/6J)	Oral (drinking water)	Chronic (10 weeks)	Gut contents
Sun et al. (2022)	Pb acetate, 100 and 300 ppm	Mice (3-week-old C57BL/6J)	Oral (drinking water)	Chronic (8 weeks)	Feces
Zhang et al. (2022)	Hg chloride, 30 μg/L	Common carp (*Cyprinus carpio*)	Waterborne	Chronic (30 days)	Feces
Hua et al. (2023)	Pb acetate, 0.2%	Rats (Adult female Sprague-Dawley and offspring)	Oral (drinking water)	Chronic (for 2 weeks before the mating date until delivery)	Feces
Xia et al. (2023)	Pb acetate, 0.05 mg/L Mn chloride, 0.3 mg/L	Zebrafish (Larvae *Danio rerio*)	Waterborne	Chronic (7 days)	Intestinal tissues
Yu et al. (2023)	Pb acetate, 50 μg/L	Zebrafish (4-month-old *Danio rerio*)	Waterborne	Chronic (21 days)	Gut contents
Zhang et al. (2023)	Pb acetate, 1 g Pb^{2+}/L,	Mice (3-week-old male C557BL/6J)	Oral (drinking water)	Chronic (8 weeks)	Feces

tail suspension test to assess the depression-like behavior in rats (Chen et al., 2022). Sun et al. (2020) employed a T-maze to evaluate the climbing assay, social interaction, and associative memory test in fruit fly (Sun et al., 2020). Xia et al. (2020) conducted the locomotor activity test in zebrafish larvae (Xia et al., 2020).

3.2.3. Molecular outcomes

Out of the 4 articles included, 2 studies (Lin et al., 2021, 2020) investigated the effects of Hg exposure, 1 study (Wang et al., 2020) focused on Mn exposure, and 1 study (Yu et al., 2023) examined the effects of Pb exposure. Lin et al. (2020) and Wang et al. (2020) examined brain degeneration and necrosis, respectively, in the hippocampus of rats through histological examination. Lin et al. (2021) investigated neurochemical markers such as brain-derived neurotrophic factor (BDNF), while Wang et al. (2020) explored β-amyloid (Aβ), receptor-interacting protein kinase 3 (RIP3), and caspase-3 using Enzyme Linked Immunosorbent Assay (ELISA) and immunohistochemistry in the hippocampus. Additionally, Yu et al. (2023) studied the effects of Pb exposure in zebrafish, specifically focusing on the histological effects on Purkinje cell nuclei and analyzing the expression of *bdnf* and *trh* genes in the brain using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) (Yu et al., 2023).

3.2.4. Neuropsychological and molecular outcomes

Among the 9 included articles, 7 employed Pb (X.Chen et al., 2021; Cheng et al., 2019; Hua et al., 2023; Li et al., 2022; Sun et al., 2022; Xiao et al., 2020; Zhang et al., 2023), 1 employed Hg (Zhang et al., 2022) and 1 study exposed animals to a mix of Pb and Mn (Xia et al., 2023).

One study assessed the aversive memory and the activity of acetylcholinesterase in the brain (Cheng et al., 2019). Another study evaluated the spatial memory, and the loss of dendritic spines in the hippocampus (Xiao et al., 2020). Zhang et al., 2022) investigated the effects of Hg in spatial memory and the ferroptosis in the brain. X. Chen et al. (2021) assessed the depression-like behavior, morphometric changes and levels of neurotransmitters in the striatum. Li et al. (2022) explored the learning ability, the anxiety-like behavior and some parameters of inflammation in the hippocampus. Sun et al. (2022) studied memory, fear conditioning and expression of markers of autophagy in the hippocampus (Sun et al., 2022). Zhang et al. (2023) assessed anxiety-like and depression-like behaviors, inflammation and neurotoxicity in the hippocampus (Zhang et al., 2023). Hua et al. (2023) investigated the effects of Pb exposure on learning memory and on hippocampal structures using morphological analyses (Hua et al., 2023). Finally, Xia et al. (2023) examined the effects of Pb and Mn exposure, both alone and in combination, on the locomotor activity and on the expression levels of genes involved in the gut-brain axis (Xia et al., 2023).

3.2.5. Characterization of gut microbiota

All included studies characterized the composition of the gut microbiota in response to the exposure of heavy metals. The technique common to all studies for measuring the microbiota was 16S rRNA gene sequencing. Only two studies used shotgun metagenome sequencing (Wang et al., 2020; Zhang et al., 2023). Shotgun metagenome sequencing involves sequencing all the DNA in a microbial sample, providing comprehensive information on both taxonomic composition and functional potential of the microbiota. In contrast, 16S rRNA gene sequencing targets a specific region of the microbial DNA (such as V3 and/or V4 regions), providing information on bacterial species and their relative abundance. The present review considered the results relating to the alpha index, which represents intra-individual diversity, or the beta index, which represents overall inter-individual differences. Various indices, such as the Shannon index (Chen et al., 2022; X.Chen et al., 2021; Hua et al., 2023; Sun et al., 2022, 2020; Xia et al., 2023, 2020; Xiao et al., 2020; Yu et al., 2023; Zhang et al., 2023, 2022), Simpson index (X.Chen et al., 2021; Hua et al., 2023; Li et al., 2022; Sun et al., 2020; Xia et al., 2023; Xiao et al., 2020; Zhang et al., 2023), Chao1 index (Chen et al., 2022; X.Chen et al., 2021; Hua et al., 2023; Xia et al., 2023; Xiao et al., 2020) and ACE index (X. Chen et al., 2021; Zhang et al., 2022), are used to measure alpha diversity. Moreover, various methods, such as Principal component analysis (X. Chen et al., 2021), Principal coordinate analysis (Xia et al., 2023; Yu et al., 2023), Bray-Curtis distance (Hua et al., 2023; Sun et al., 2022, 2020; Zhang et al., 2023), Weighted UniFrac diversity (Hua et al., 2023; Xia et al., 2020), linear discriminant effect size (LEfSe) analysis (Li et al., 2022; Xia et al., 2020; Xiao et al., 2020), were used to measure beta diversity. Finally, all data were recorded using the same original taxonomic rank and spelling presented in the respective articles, to ensure consistency in reporting results.

3.3. Synthesis of results

The data from the selected studies were collected and grouped in tables using Microsoft Office Excel 2019, with the following information: first author & year of publication, heavy metal, animals, sample size & exposure, methods, and relevant results. Study characteristics and results were summarized in Tables 3–5.

3.4. Quality assessment of the included studies

To assess the risk of bias, the SYRCLE risk of bias tool (Hooijmans et al., 2014) was employed to examine and validate all potential factors that affect the quality of the papers included in this review. In general, the risk of bias remained uncertain for most of the studies examined. None of the studies provided clarification regarding the concealment of allocation or whether the animals were housed randomly during the experiments. Random sequence generation was reported in 11 papers (68,7%) (Hua et al., 2023; Lin et al., 2020; Wang et al., 2020; Xia et al., 2023; Yu et al., 2023; Zhang et al., 2022) but without disclosing the randomization method. Only one study reported a power analysis or sample size calculation justifying the number of animals per group (Xiao et al., 2020). Baseline animal characteristics, including species, strain, sex, and/or weight, were described in all studies, as well as all papers were free of selective reporting items, ensuring a low risk of bias. Information about caregiver and investigator blinding to the intervention that animals received was provided in one of the studies (Sun et al., 2020). Random outcome assessment was conducted in 3 studies (18,7%) (X.Chen et al., 2021; Xiao et al., 2020; Yu et al., 2023), whereas 5 papers (31,2%) reported blinding of outcome assessment (Cheng et al., 2019; Lin et al., 2021; Sun et al., 2020; Wang et al., 2020; Xiao et al., 2020). Finally, a high risk of bias was observed regarding incomplete outcome data in 5 studies (31,2%)(Chen et al., 2022; Hua et al., 2023; Li et al., 2022; Lin et al., 2021; Sun et al., 2020). Specifically, the numbers of animals included in the results of analyses varied and did not reflect the number of animals in the materials and methods. The summary of the risk of bias assessment is shown in Fig. 2. A detailed version of risk of bias domains and questions are reported in the Supplementary Information, List S1.

4. Discussion

This systematic review examined the evidence from preclinical studies that explored associations between gut microbiota changes and brain-related outcomes (from behavioral to neurochemical aspects) in animal models exposed to heavy metals. As far as we know, no review of the emerging evidence has currently evaluated whether the microbiota plays a role in mediating the observed effects of heavy metals exposure on the CNS, in terms of cognition, behavior, biochemical and morphological brain changes, through the gut-brain axis. Thus, a review of the literature is needed examining the relationship between brain and behavior alterations upon exposure to heavy metals and the role of the gut microbiota on these associations after it is disrupted or re-established upon exposure.

Table 3
Characteristics of neuropsychological and microbiota outcomes of included studies.

Reference	Heavy metal	Animals, sample size & exposure	Methods	Neuropsychological outcomes **(Changes observed compared with control group)**	Microbiota outcomes **(Changes observed compared with control group)**
Sun et al. (2020)	Pb	Fruit fly n = 400 4 groups: -Control groups (n = 100 male parents; n = 100 female parents) -Pb exposed group (n = 100, 100 mg/L male parents; n = 100, 200 mg/L female parents). Offspring flies fed in normal food until tested (3–7 days old).	**Behavior**: Climbing assay, social avoidance, social interaction, associate learning and short-term memory. **Microbiota**: 16sV4 rRNA sequencing	In Pb-exposed group: **Climbing assay and short-term learning and memory test for parents flies:** ↓ climbing ability, short-term memory of female and male **Climbing assay, social avoidance test and short-term learning and memory test for offspring flies:** ↓ climbing ability of male offspring from male Pb-exposed parents ↓ possibility to alter its destination correctly of male offspring from Pb-exposed parents in social avoidance test. ↓learning and memory in male and female offspring from Pb-exposed parents.	In Pb-exposed groups: **Genus level:** ↓*Lactobacillus* and *Bifidobacterium* in the Pb-exposed parents ↓α- diversity in male offspring of Pb-exposed parents. Female offspring of Pb-exposed parents showed a depletion of *Bilophila*, *Coprococcus*, *Desulfovibrio* and *Ruminococcus*. Lactobacillus and Bifidobacterium exhibited significant correlations with learning and memory, and positive correlations with social behavior. *Bilophila, Coprococcus, Desulfovibrio*, and *Ruminococcus* showed significant correlations with climbing ability.
Xia et al. (2020)	Cd	Zebrafish n = 200 4 groups: -Control group (n = 50) -Cd-exposed group (n = 50/ concentration, 1.25, 2.5 and 5 μg/L)	**Behavior**: Locomotor activity (spontaneous movements). **Microbiota**: 16sV3-V4 rRNA sequencing.	In Cd-exposed group: **Locomotor activity**: ↓average distance of movement and number of activities, in a dose-dependent manner.	In Cd-exposed group: **Phylum level:** ↑ *Proteobacteria* ↓ *Firmicutes* **Class level:** ↑*Gammaproteobacteria* ↓ *Clostridium* **Genus level:** ↑*Pseudomonas* in 5 μg/L group ↑ Pseudomonas, *Ruminococcaceae*, *Blautia*, *Bacteroides, Lactobacillus, Lachnospiraceae*, and *Phascolarctobacterium*
Chen et al. (2022)	Pb	Rat n = 45 3 groups: -Control group (n = 15) -Pb-exposed group (n = 15, 300 mg/L) -Intervention group (n = 15, 300 mg/L Pb + *Lactobacillus* and *Bifidobacterium*: 6 billion live bacteria/2 g)	**Behavior**: Sucrose preference test, forced swimming test, tail suspension test. **Microbiota**: 16sV4 rRNA sequencing	In Pb-exposed group: **Sucrose preference test:** ↓sucrose intake **Forced swimming test and tail suspension test:** ↑immobility time In intervention group: **Sucrose preference test:** ↑sucrose intake **Forced swimming test and tail suspension test:** ↓immobility time	In Pb-exposed group: **Phylum level:** ↑*Bacteroidetes* and *Proteobacteria* ↓*Firmicutes* **Family level**: ↑*Ruminococcaceae* ↑*Clostridium* ↑*s24-7 (Muribaculaceae)* ↓*Lactobacillus* ↓*Spirochaetes* ↓*Turicibacterales* **Genus level**: ↑*Clostridium difficile* ↑*Bacteroidetes* ↓*Lactobacillus* ↓*Spirobacteria* ↑Gut microbiota abundance following probiotic intervention.

Abbreviations: Cd, cadmium; Pb, lead.
↑: higher/increased; ↓: lower/decreased

4.1. Main findings

Findings from the reviewed studies revealed several changes of microbiota composition including increases and decreases at different phylogenetic levels (phylum, family, genus, order and species) in different animal models upon exposure to metals.

Specifically, the main changes are evident in the phyla *Firmicutes* and *Proteobacteria*, albeit with conflicting results. Concerning *Firmicutes*, on one hand, a significant decrease was observed in three studies involving animals exposed to Pb, resulting in increased anxiety and depression-like behaviors (Chen et al., 2022; X. Chen et al., 2021; Sun et al., 2020). On the other hand, a notable increase was found in two studies involving animals exposed to Pb, which subsequently developed memory and learning deficits (Li et al., 2022; Sun et al., 2022). Regarding *Proteobacteria*, three studies demonstrated a significant abundance reduction. This was observed both after exposure to Pb, resulting in spatial memory deficits and loss of dendritic spines in the hippocampus (Xiao et al., 2020), as well as upon exposure to Hg, with decreased levels of BDNF in the serum and brain, respectively (Lin et al., 2021, 2020). Moreover, a reduction in *Proteobacteria* was observed following the combined treatment of Pb and Mn on the developmental toxicity, resulting in decreased locomotor activity and alterations in genes related to the serotonin signaling pathway (Xia et al., 2023). In contrast, an increase in *Proteobacteria* abundance was observed following Pb exposure, associated with more pronounced depressive-like behavior (Chen et al., 2022; X. Chen et al., 2021), as well as upon exposure to Cd, leading to increased locomotor activity (Xia et al., 2020). These differences may be due to the different experimental methodologies, as well as to the doses (i.e., concentrations and times) of exposure in the different protocols.

At the genus level, in most studies included in this review, a significant decrease in the abundance of *Lactobacillus* was observed following the administration of Pb (Chen et al., 2022; X. Chen et al., 2021; Sun et al., 2020; Xiao et al., 2020; Zhang et al., 2023), Hg (Lin et al., 2021, 2020), and Mn (Wang et al., 2020). However, this decrease was restored following the administration of probiotics (Chen et al., 2022; Li et al.,

Table 4
Characteristics of molecular and microbiota outcomes of included studies.

Reference	Heavy metal	Animals, sample size & exposure	Methods	Molecular outcomes **(Changes observed compared with control group)**	Microbiota outcomes **(Changes observed compared with control group)**
Lin et al. (2020)	Hg	Rat n = 6 2 groups: - Control group (n = 3) - MeHg exposed group (n = 3, 10 μg/kg)	**Histological brain examination:** HE staining **Biochemistry:** ELISA assay **Microbiota:** 16sV3-V4 rRNA sequencing	In MeHg-exposed group: **HE staining:** no injury **ELISA:** ↑IL-1β and IL-6 in serum ↓BDNF in serum	In MeHg-exposed group: **Phylum level:** ↓ *Bacteroidetes* ↓ *Proteobacteria* ↑ *Firmicutes* **Family level:** ↓*Lactobacillaceae* ↓*Bacteroidaceae* ↓*Streptococcaceae* ↓*Sutterellaceae* ↑*Desulfovibrionaceae* ↑*Helicobacteraceae* ↑*Peptococcaceae* ↑*Rhodospirillaceae*
Lin et al. (2021)	Hg	Rat n = 15 3 groups: -Control group (n = 5) -MeHg-exposed group (n = 5, 10 mg/kg) -IHg-exposed group (n = 5, 10.8 mg/kg)	**Biochemistry:** ELISA assay **Microbiota:** 16sV3-V4 rRNA sequencing	In MeHg- and IHg-exposed groups: **ELISA:** ↓BDNF in serum and brain in MeHg-exposed group. No difference in IHg-exposed group.	In MeHg-exposed group: **Phylum level:** ↓*Bacteroidetes* ↓*Firmicutes* ↓*Proteobacteria* ↑ *Actinobacteria* ↑*Verrucomicrobia.* **Family level:** ↑*Verrucomicrobiaceae* ↑*Desulfovibrionaceae* ↑*Helicobacteraceae* ↑*Lachnospiraceae* ↓ *Rikenellaceae* ↓*Erysipelotrichaceae* ↓*Sutterellaceae* ↓*Anaeroplasmataceae* ↓*Coriobacteriacea*
Wang et al. (2020)	Mn	Rat n = 30 2 groups: -Control group (n = 15) -Mn-exposed group (n = 15, 200 mg/L)	**Histological brain examination:** HE staining **Biochemistry:** Immunohistochemistry **Microbiota:** Shotgun metagenomic sequencing	In Mn-exposed group: **HE staining:** Degeneration and necrosis of the pyramidal cell layer in the hippocampus **Immunohistochemistry**: ↑Amyloid $\beta_{1\text{-}40}$ protein ↑ Receptor-interacting serine/threonine-protein kinase 3 and caspase-3	In Mn-exposed group: **Family level:** ↓*Prevotellaceae*, ↓*Fusobacteriaceae* ↓*Lactobacillaceae* ↑*Clostridiales noname* ↑*Faecalibacterium*
Yu et al. (2023)	Pb	Zebrafish n = 100 2 groups: -Control group (n = 50) -Pb-exposed group (n = 50)	**Histological brain examination:** HE staining **Biochemistry:** qRT-PCR **Microbiota:** 16sV3-V4 rRNA sequencing	In Pb-exposed group: **HE staining:** Purkinje cell aggregation in the cerebellum **Biochemistry**: ↓*bdnf* expression ↓*trh* expression	In Pb-exposed group: Changes in relative abundance: ↑*Proteobacteria* ↓*Fusobacteriota* ↓*Actinobacteriota*

Abbreviations: BDNF, Brain-derived neurotrophic factor; ELISA, Enzyme Linked Immunosorbent Assay; HE, Hematoxylin eosin; Hg, mercury; IHg, inorganic mercury; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; MeHg, methylmercury; Mn, manganese; qRT-PCR, Quantitative Real-Time Polymerase Chain Reaction; *trh*, Thyrotropin-releasing hormone.
↑: higher/increased; ↓: lower/decreased.

2022; Xiao et al., 2020; Zhang et al., 2023).

4.1.1. Gut microbiota: a potential mediator in heavy metal effects on the CNS

The intestinal microbiota constitutes an intricate ecosystem that has a crucial impact on the host. It also contributes to maintaining the state of well-being under normal conditions. The relationship between brain and gut microbiota is described as the "brain-gut-microbiota axis" and is a bidirectional pathway, in which microbiota plays a key role in the neurophysiological processes (Sampson and Mazmanian, 2015). There is a growing interest of the influence of gut microbiota in cognitive function, anxiety, depression and autistic spectrum disorders (Góralczyk-Bińkowska et al., 2022). Following oral exposure and even before reaching the brain, heavy metals get in touch with the gut microbiota. When exposed to heavy metals, the gut microbiota undergoes changes in their composition and metabolic functions. Consequently, the gut microbiota plays a role in modifying the way that metals are absorbed and metabolized. This is achieved by acting as a protective barrier against heavy metals absorption and by influencing factors such as pH, oxidative balance, and the levels of enzymes and proteins involved in their metabolism (Duan et al., 2020; Pajarillo et al., 2021). Additionally, the interaction of heavy metals with the gut microbiota can trigger an inflammatory response. Chronic inflammation can damage the intestinal mucosa and impair its nutrient absorption function (Duan et al., 2020; Pajarillo et al., 2021; Zhong et al., 2021). Gut dysbiosis could increase the toxicity and harmful effects of heavy metals, which are related to various brain disorders, such as anxious behaviors (Bercik et al., 2011) or learning and memory deficits (Gu et al., 2022). Sun et al. (2020) found a significant correlation between *Lactobacillus* and *Bifidobacterium* abundance decrease with learning, memory and social behavior in *Drosophila melanogaster* administered with Pb. Interestingly, the study found a sexual dimorphism in which the alpha diversity was significantly decreased in the male offspring of Pb-exposed parents compared with that of the normal parents, but also, they were more vulnerable to the loss of crawling, memory, and social abilities (Sun et al., 2020). In a study by Li and colleagues (2022) they observed that *Roseburia, Lachnospiraceae NK4A136 group*, and *Eubacterium siraeum group* were positively correlated with swimming distance in the target quadrant in mice treated with Pb in Morris water maze test, while *Bacteroides* and *Alloprevotella* were negatively correlated. Furthermore,

Table 5
Characteristics of neuropsychological, molecular and microbiota outcomes of included studies.

Reference	Heavy metal	Animals, sample size & exposure	Methods	Neuropsychological outcomes (Changes observed compared with control group)	Molecular outcomes (Changes observed compared with control group)	Microbiota outcomes (Changes observed compared with control group)
Cheng et al. (2019)	Pb	Mouse n = 40 4 groups: -Control group (n = 10) -Pb-exposed group (n = 10, 1.34 g/L$^{-1)}$ - Chlorogenic acid (CGA) group (n = 10, 30 mg/kg) -Pb + CGA-exposed group (n = 10, 1.34 g/L^{-1} + 30 mg/kg)	**Behavior**: Step-down inhibitory avoidance task **Biochemistry:** measurement of AChE by reagent kit **Microbiota**: 16sV3-V4 rRNA sequencing.	In Pb-exposed group: **Step-down inhibitory avoidance task:** ↓ the latency time. CGA administration reverted this effect.	In Pb-exposed group: **AChE measure:** ↓activity of AChE in brain CGA administration reverted this effect.	In Pb-exposed group: **Genus level:** ↓ *Helicobacter* ↑*Lachnospiraceae_NK4A136_group* CGA administration reverted this effect.
Xiao et al. (2020)	Pb	Rat n = ~40 4 groups -Control group (n = 6–10) -Pb-exposed group (n = 6–10, 125 ppm) -Probiotic* group (n = 6–10, 10^{10} organism/rat) -Intervention group (n = 6–10, Pb + probiotic group) *Bifidobacterium longum BL986, Lactobacillus acidophilus LA1063, Lactobacillus fermentum LF26, Lactobacillus helveticus LH43, Lactobacillus paracasei LPC12, Lactobacillus rhamnosus LRH10, and Streptococcus thermophilus ST30	**Outcomes assessed in rat offspring.** **Behavior:** Morris water maze and spatial working memory (Y-maze) **Biochemistry:** Golgi–Cox staining **Microbiota**: 16sV4 rRNA sequencing	In Pb-exposed group: **Morris water maze:** ↑ latency to find the target platform. **Y-maze:** ↓ the alternation rates Probiotic administration reverted these effects.	In Pb-exposed group: **Histological brain examination:** ↓ dendritic spines of hippocampus Probiotic administration restored spine densities in adolescence or adulthood.	In Pb-exposed group: **Phylum level:** ↑ ratio of *Bacteroidetes* to *Firmicutes* ↓ *Proteobacteria* ↓ *Actinobacteria* In Probiotic group: **Genus level:** ↑*Helicobacter* ↑ *Bifidobacterium* ↑*Bacteroides* ↓*Anaerovibrio* ↓*Ruminococcaceae_UCG-008* ↓*Lactobacillus*
X.Chen et al. (2021)	Pb	Rat n = 30 2 groups: -Control group (n = 15) -Pb-exposed group (n = 15, 300 mg/kg)	**Behavior**: Sucrose preference test, forced swimming test, open field test, elevated plus maze test. **Histological brain examination:** -HE staining **Biochemistry:** -HPLC-ECD, ELISA, qRT-PCR and immunohistochemistry. **Microbiota**: 16sV4 rRNA sequencing.	In Pb-exposed group: **Sucrose preference test:** ↓sucrose intake **Forced swimming test:** ↑immobility time **Open field test:** ↑total travel distance ↑center residence time **Elevated plus maze test:** ↑Percentage of open arm entry ↓open arm time	In Pb-exposed group: **HE staining**: nuclear condensation in the striatum **HPLC-ECD, ELISA, qRT-PCR and Immunohistochemistry:** ↓5-HT and 5-HT3R in the striatum	In Pb exposed group: **Phylum level:** ↓*Firmicutes* ↑*Proteobacteria* **Class level:** ↑*Clostridium* ↑*Bacteroides* ↓*Bacillus* ↓*Spirulina* **Order level:** ↑*Clostridium* ↑*Bacteroides* ↑*deta-proteus* ↓*Lactobacillus* ↓*Spirulina* ↓*Turicibacterales* **Family level:** ↑*Ruminococcaceae* ↑*Clostridium* ↑*deta-proteus* ↑*S24-7* ↓*Lactobacillus* ↓*Spirulina* ↓*Turicibacterales* **Genus level:** ↑*Clostridium* ↑*Bacteroides* ↑*deta-proteus* ↓*Lactobacillus* ↓*Spirulina* ↓*Turicibacterales*
Li et al. (2022)	Pb	Mouse n = 18 3 groups:	**Behavior**: Open field test, Morris water maze	In Pb-exposed group: **Open field test:**	In Pb-exposed group: **ELISA:** ↑IL-6, IL-1β, and TNF-	In Pb-exposed group: **Phylum level:**

(*continued on next page*)

Table 5 (*continued*)

Reference	Heavy metal	Animals, sample size & exposure	Methods	Neuropsychological outcomes (Changes observed compared with control group)	Molecular outcomes (Changes observed compared with control group)	Microbiota outcomes (Changes observed compared with control group)
		-Control group (n = 6) -Pb exposed group (n = 6, 100 mg/L) -Intervention group (n = 6, 100 mg/L + *Lactobacillus plantarum WSJ-06* 10^9 CFU)	test **Biochemistry**: ELISA assay **Microbiota**: 16sV3-V4 rRNA sequencing.	↓ distance in central area ↓ time spent in central area ↑ time spent in corner area **Morris water maze test:** ↓time and distance in target quadrant ↑platform crossing times L. plantarum WSJ-06 reverted these effects.	α in hippocampus L. plantarum WSJ-06 reverted these effects.	↑*Firmicutes* ↓*Bacteroidetes* The swimming distance in the target quadrant Levels were: • Positively correlated with levels of *Roseburia*, *Lachnospiraceae_NK4A136_group*, *[Eubacterium]_siraeum_group*. • Negatively correlated with *Bacteroides*, Alloprevotella The swimming time in the target quadrant Levels were: • Positively correlated with levels of *Roseburia*, *[Eubacterium]_siraeum_group*. • Negatively correlated with *Bacteroides, Alloprevotella* In L. plantarum WSJ-06 group: **Genus level**: ↑ *[Eubacterium]_siraeum_group*, ↑*Roseburia* ↑*Lachnospiraceae_NK4A136_group* ↑*Lactobacillus* ↓*Alloprevotella* ↓*Rikenellaceae_RC9_gut_group* ↓*Parabacteroides*
Sun et al. (2022)	Pb	Mouse n = 25 3 groups: -Control group (n = 8–9) -Pb-exposed groups (n = 8–9/ concentration, 100 or 300 mg/kg)	**Behavior:** Morris water maze, Cue and Contextual Fear Conditioning **Biochemistry:** immunofluorescence **Microbiota**: 16sV3-V4 rRNA sequencing	In Pb-exposed groups: **Fear conditioning:** ↓Freezing time in context and conditioning stimulus **Morris water maze:** ↓the platform and edge frequencies ↑latency period	In Pb-exposed groups: **Immunofluorescence:** ↓ BrdU (+) cells and ↓ SOX2 (+) cells in the dentate gyrus of hippocampus ↑ markers of autophagy (Beclin 1, LC3I, and LC3II) in the dentate gyrus of hippocampus	In Pb-exposed groups: ↑*Bacteroides* ↑*Lactobacillus* ↑*Escherichia/Shigella* ↑*Clostridium XIVb* ↑*Roseburia* ↓*Alloprevotella* ↓*Parabacteroides* ↓*Saccharibacteria_genera_incertae_sedis* ↓*Prevotella* ↓*Ruminococcus* ↓*Clostridium IV* ↓*Butyricicoccus* ↓*Eubacterium* Indexes of cognitive behavior (freezing time in context and freezing time in conditioning stimulus) were significantly correlated with changes of microbiota in the gut.
Zhang et al. (2022)	Hg	Carp n = 80 2 groups: -Control group (n = 40) -Hg exposed group (n = 40, 30 μg/L)	**Behavior:** memory test in a maze. **Biochemistry:** Ferroptosis detection kits **Microbiota**: 16sV3-V4 rRNA sequencing.	In Hg-exposed group: **Memory test:** ↑ more errors ↑ time to find food in the maze.	In Hg-exposed group: **Ferroptosis:** ↑Levels of MDA and Fe^{2+} ↓Levels of ATP and GSH Ferroptosis inhibitor ferrostatin 1 reverted these alterations.	In Hg-exposed group: ↑ *Aeromonas hydrophila*. ↓ diversity of gut microbiota The levels of richness were similar to those in the control group.
Hua et al. (2023)	Pb	Rat n = ~24 4 groups: -Control group (n = 6–11) -Pb-exposed group (n = 6–11, 0.2%) -Prenatal stress (Ps) group (n = 6–11) - Pb + Ps group (n = 6–11) Prenatal stress (Ps) and Pb + Ps groups were subjected to repeated physical daily restraint stress during gestational days 15–21, 3 sessions/day	**Outcomes assessed in rat offspring.** **Behavior:** Morris water maze test **Histological brain examination:** TEM analysis and HE staining **Biochemistry:** GFAP immunohistochemistry **Microbiota:** 16sV4 rRNA sequencing	Pb + Ps group: **Morris water maze test:** ↓ time spent in the target quadrant ↓ Percentage of distance in target quadrant ↓ Numbers of crossing the platform No significantly difference between Pb and control groups.	Pb and Pb + Ps groups: **TEM analysis**: mitochondrial swelling, mitochondrial internal cristae dissolution and vacuolar degeneration in the hippocampus. **HE staining**: Hippocampal neuronal nuclei displayed abnormal density and cytoplasmic vacuolation **GFAP immunohistochemistry**: astrogliosis.	In Pb + Ps group: **Phylum level:** ↑*Firmicutes* **Class level:** ↑*Bacilli* **Order level:** ↑*Lactobacillales* **Family level:** ↑*Lactobacillaceae* **Genus level:** ↑*Helicobacter* ↑*Lactobacillus* ↑*Bifidobacterium* *Lactobacillus* and *Helicobacter* showed higher relative abundances in the Pb or Pb + Ps exposed group compared to the control or Ps group.
Xia et al. (2023)	Pb, Mn	Zebrafish n = 96 (behavior effects) n = 120	**Behavior**: Locomotor activity (spontaneous movements).	In Pb, Mn and Pb + Mn exposed groups: **Locomotor activity**:	In Mn-exposed group: ↓serotonin transporter (slc6a4a) expression	In Pb and Pb + Mn exposed groups: ↑ *Firmicutes/Bacteroidetes* ratio ↓ *Proteobacteria*

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Table 5 (*continued*)

Reference	Heavy metal	Animals, sample size & exposure	Methods	Neuropsychological outcomes (Changes observed compared with control group)	Molecular outcomes (Changes observed compared with control group)	Microbiota outcomes (Changes observed compared with control group)
		(biochemistry effects) n = 160 (microbiota effects) 4 groups: -Control group -Pb exposed group (0.05 mg/L) -Mn exposed group (0.3 mg/L) Pb + Mn exposed group (0.05 mg/L + 0.3 mg/L)	**Biochemistry:** qPCR **Microbiota**: 16sV3-V4 rRNA sequencing.	↓average distance of movement and number of activities in Pb group and Pb + Mn group. The combined exposure to Pb + Mn produces a more pronounced reduction than the effects of Pb alone.	In Pb and Pb + Mn groups: ↑transcripts for MAO ↑ ABCG and ABCC expression In Pb + Mn group: ↑ tph2 mRNA expression	The *Firmicutes/Bacteroidetes* ratio was higher in the Pb + Mn group. In Pb + Mn group: ↑ *Bacteroides* ↑ *Faecalibacterium* ↑ *Agathobacter* ↑ *Roseburia* ↑ *Campylobacter* ↑ *Fusicatenibacter* ↑ *Lachnospira* ↑ *Bifidobacterium* ↑ *Subdoligranulum* ↑ *Blautia* ↑ *Pseudocatenulatum*
Zhang et al. (2023)	Pb	Mouse n = 36 3 groups: -Control group (n = 12) -Pb exposed group (n = 12, 1 g Pb^{2+}/L) -Intervention group (n = 12, Pb + Probiotics *Lactobacillus fermentum* HNU312 (Lf312) 8Log10 CFU)	**Behavior:** Marble burying test, forced swim test **Biochemistry:** ELISA and Immunofluorescence **Microbiota:** Shotgun metagenomic sequencing.	In Pb-exposed group: **Marble burying test:** ↑ number of marbles buried. **Forced swimming test:** ↑ immobility time Probiotic administration reverted the time of immobility.	In Pb-exposed group: **ELISA:** ↑ MDA ↓ GSH ↓ activity of SOD A significant reduction on the observed oxidative damage in the Probiotic group. **Immunofluorescence:** ↑ GFAP ↑ iba-1 in the hippocampus ↓ ZO-1 in the striatum Probiotic Lf312 intervention significantly reverted these effects.	In Pb-exposed group: ↓ *Bacteroides faecichinchillae* ↓ *Faecalibaculum rodentium,* ↓ *Lachnospiraceae bacterium 284* ↓ *Lactobacillus intestinalis* ↓ *Lactobacillus johnsonii* ↓ *Lactobacillus murinus* ↓ *Lactobacillus reuteri* In Probiotic group: ↑ *Bacteroides uniformis* ↑ *Bacteroides caecimuris* ↑ *Lactobacillus fermentum* ↑ *L. reuteri* ↑ *Parabacteroides distasonis* ↑ *Parabacteroides goldsteinii*

Abbreviations: 5-HT, 5-Hydroxytryptamine (serotonin); 5-HT3R, serotonin receptor type 3; AChE, acetylcholinesterase; ABCC and ABCG, ATP-binding cassette transporters C and G; ATP, adenosine triphosphate; BrdU, 5-bromo-2′-dexoyuridine; ELISA, Enzyme Linked Immunosorbent Assay; GFAP, glial fibrillary acidic protein; GSH, glutathione; HE, Hematoxylin eosin; HPLC-ECD, High Performance Liquid Chromatography coupled to Electrochemical Detection; iba-1, ionized calcium binding adapter molecule-1; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; MAO, monoamine oxidase; MDA, Malondialdehyde; Mn, manganese; Pb, lead; qRT-PCR, Quantitative Real-Time Polymerase Chain Reaction; SOD, superoxide dismutase; SOX2(+); TEM, transmission electron microscopy; TNF-α, tumor necrosis factor α; tph2, tryptophan hydroxylase 2; ZO-1, zonula occludens-1.
↑: higher/increased; ↓: lower/decreased.

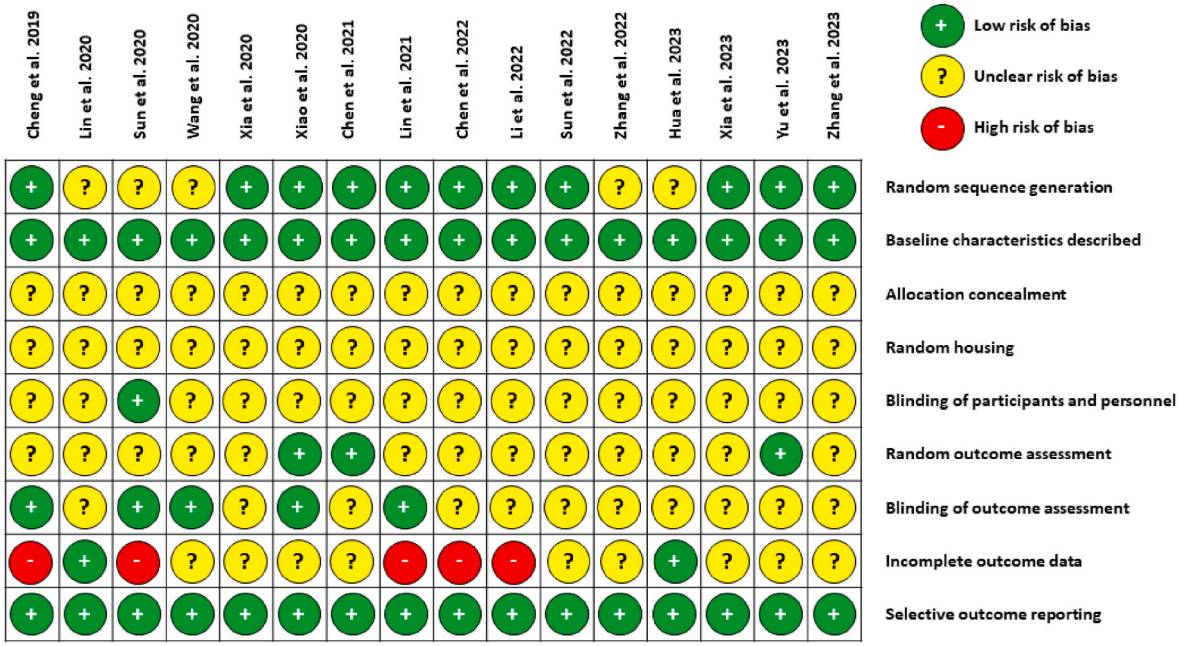

Fig. 2. Quality assessment of the included studies according to SYRCLE items.
"Positive, green circle" indicates low risk of bias; "Negative, red circle" indicates high risk of bias; and "Question mark, yellow circle" indicates an unclear risk of bias. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Roseburia and *Eubacterium siraeum group* were positively correlated with swimming time in the target quadrant, whereas *Bacteroides* showed a negative correlation (Li et al., 2022). In another study conducted by Sun et al. (2022) they showed that gut microbiota dysbiosis were significantly correlated with the serum Pb level and the freezing time in the fear conditioning paradigm, that may contribute to the cognitive deficits (Sun et al., 2022). Although the specific roles of gut microbiota in regulating brain functions are not yet fully understood, it is possible that gut microbiota plays a crucial role in behavior and cognition. Hua et al. (2023) performed mediation analyses to investigate the role of relative abundances of fecal microbiota on the relationship between prenatal Pb exposure, stress co-exposure, and learning/memory impairments in rats. The results of the mediation analyses revealed that co-exposure to Pb and stress led to spatial memory impairments by increasing the relative abundance of *Helicobacter*. Additionally, the study found that the increased relative abundances of the genus *Lactobacillus* could mitigate the neurodevelopmental impairments caused by prenatal Pb and stress co-exposure (Hua et al., 2023). Therefore, this study highlights the importance of considering the impact of heavy metals on neurodevelopment and the potential role of gut microbiota as a mediator in mitigating these effects.

The interaction of heavy metals with the gut microbiota can trigger different effects depending on the type of metal, route and duration of exposure, and host factors. Some possible effects include dysbiosis, which can be restored through probiotic administration, or alteration of metabolic activity, which may interfere with the production of beneficial substances such as short-chain metabolites (Nicholson et al., 2012).

4.1.2. Role of probiotics

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Gibson et al., 2017). There is wide literature demonstrating the effectiveness of probiotics against heavy metals toxicity, such as Cd (Tinkov et al., 2018; Zhu et al., 2021), and Pb (Zanjani et al., 2017; Zhai et al., 2019). Interestingly, probiotic administration can modify behavior, brain function and gut microbial composition in healthy male and female adults (Bagga et al., 2018). It can also improve cognitive performance in patients with major depression (Rudzki et al., 2019). Desbonnet et al. (2010) showed that the administration of a probiotic (*Bifidobacterium infantis*) reversed depressive behavior, suggesting that changes in gut bacteria were able to modify the psychiatric state of the host organism (Desbonnet et al., 2010). Two studies included in this review showed that *Lactobacillaceae* and *Bifidobacteriaceae*, which were positively associated with the ability of learning and memory in rats and fruit flies, were significantly decreased with Pb administration (Chen et al., 2022; Sun et al., 2020). Lactic acid bacteria have the ability of adsorbing metal ions (Gerbino et al., 2011), consequently, *Lactobacillus* strains could have protective effects against heavy metals (Bist and Choudhary, 2022). *Lactobacillus brevis 23017*, a specific probiotics strain with strong Hg binding abilities, was able to block oxidative stress and inflammation through MAPK and NF-κB pathways in rodents (Jiang et al., 2018). Similarly, previous studies showed that *Lactobacillus rhamnosus* (JB-1) could modulate the immune system (Karimi et al., 2009), reduce stress-induced corticosterone and anxiety- and depression-related behaviors in rodents (Bravo et al., 2011). Moreover, *Lactobacillus plantarum* significantly reduced alcohol-induced learning and memory impairment, hippocampal morphology changes, neuronal apoptosis and synaptic dysfunction in a mouse model of cognitive dysfunction (Xu et al., 2022).

In a paper included in this review, the neurotoxic effects of chronic Pb on spatial memory were antagonized by a multispecies probiotic treatment (*Bifidobacterium* and *Lactobacillus*) from prenatal period to adulthood, as well as the ratio of *Firmicutes* and *Bacteroidetes,* which was rebalanced (Xiao et al., 2020). It was found that *Lactobacillus plantarum* (WSJ-06) could restore the altered ratio between *Firmicutes* and *Bacteroidetes* occurred upon chronic Pb exposure by modulating the gut microbiota composition (Shamsipour et al., 2021). Moreover, these probiotics reduced neurodegenerative diseases, such as motor impairments in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse models of Parkinson's disease (Liao et al., 2020), and spatial learning impairment in combination with *Bifidobacterium bifidum* in a rat model of Alzheimer's disease (Shamsipour et al., 2021). In another paper, treatment with the probiotic *Lactobacillus fermentum* HNU312 (Lf312) prevented oxidative damage and inflammatory responses induced by Pb exposure. Authors also reported that treatment contributed to maintaining BBB integrity in the brain, and improving anxiety and depression-like behavior in mice (Zhang et al., 2023).

Additionally, Roy Sarkar et al. (2020) showed that gut microbiota dysbiosis induced by antibiotics may lead to neurobehavioral changes in mice. Reduced hippocampal neuronal density and reduced cognition, increased cortico-hippocampal acetylcholinesterase, and oxidative stress were also reported. However, these changes were partially reversed by probiotic treatment (Roy Sarkar et al., 2020). Probiotics can act by increasing the production of amino acid precursors, and consequently the synthesis of neurotransmitters, such as serotonin (5-Hydroxytryptamine, 5-HT) from tryptophan (Li et al., 2022). In a study included in this review, administration of Pb increased depressive-like behavior, reduced the expression of 5-HT and serotonin receptor 5-HT3R (serotonin receptor type 3, 5-HT3R) in the striatum of rats, and significantly reduced the abundance of *Lactobacillus* (Chen et al., 2022). Moreover, probiotics administration reduced the levels of pro-inflammatory cytokines (TNF-α, IL1-β) induced by chronic exposure of Pb in the hippocampus of mice (Li et al., 2022). Furthermore, Wang et al. (2020) showed that chronic administration of Mn elicited a significant increase of the expression of crucial mediators of necrosis (RIPK-3) and apoptosis (caspase-3) in the brain, caused hippocampal degeneration and necrosis and reduced gut microbiota richness, especially for *Prevotellaceae, Fusobacteriaceae* and *Lactobacillaceae* (Wang et al., 2020).

Taken together, these results suggest that probiotics could restore altered neurochemistry and behavior occurring upon heavy metal exposure.

4.1.3. Role of short-chain fatty acids (SCFAs)

The pathways through which the gut microbiota communicates with the brain are various, including the vagus nerve, the production of neurotransmitter precursors or the release of metabolites such as SCFAs (Cerdó et al., 2020). SCFAs are saturated fatty acids made from anaerobic fermentation of dietary fiber and resistant starch in the large intestine. *Bacteroidetes* and *Firmicutes* are the most abundant phyla in the intestine, with members of the *Bacteroidetes* mostly generating the SCFAs acetate and propionate, while *Firmicutes* mostly producing butyrate (Venegas et al., 2019). Members of other genus are also able to generate SCFAs, such as *Bacteroides*, *Bifidobacterium*, *Propionibacterium*, *Eubacterium, Lactobacillus, Clostridium, Roseburia* and *Prevotella* (Tran and Hasan Mohajeri, 2021). SCFAs can reach the brain by crossing the BBB via monocarboxylate transporters (Singh et al., 2022). Notably, it has been demonstrated that SCFAs have a significant role in microglial maturation and functioning (Cerdó et al., 2020), as well as in gastrointestinal disorders in patients with autism spectrum disorder (ASD) (Wang et al., 2012). In ASD children a significant decrease of SCFAs and butyrate producing bacteria (*Ruminococcaceae, Eubacterium, Lachnospiraceae* and *Erysipelotrichaceae*) has been observed (Liu et al., 2019). Moreover, exposure to heavy metals can alter the production of SCFAs. Chronic Cd exposure significantly decreased gut microbial richness and lowered abundance of SCFAs-producing bacteria in mice (He et al., 2020). In the study of Chen et al. (2022), Pb exposure resulted in depression-like behaviors, changes in gut microbiota diversity and altered levels of SCFAs in rats. In particular, the levels of fecal acetic acid, propionic acid and butyric acid were significantly decreased in the Pb-exposed group, compared with the control group. However, in this study, abundance of some SCFAs-producing bacteria belonging to the

Firmicutes phylum, such as *Clostridium* and *Ruminococcus*, significantly increased with heavy metal administration (Chen et al., 2022). This could be explained by the chronic stress of experimental animals following Pb-exposure (X. Chen et al., 2021). Moreover, with the administration of probiotic (*Bifidobacterium* combined with *Lactobacillus*) the abundance of *Spirobacteria* significantly increased, as well as fecal SCFAs alongside a reduction of depression-like behavior of the lead-exposed rats. A correlation between mood disorders and levels of SCFAs has been suggested in previous research using depression animal models (Valvassori et al., 2014). Li et al. (2022) demonstrated that administration of probiotic *L. Plantarum WSJ-06* may inhibit Pb-induced inflammation by increasing the abundance of SCFA producers, such as *Roseburia* (Li et al., 2022). Furthermore, probiotics *Lactobacillus fermentum* HNU312 (Lf312) modulated the structure and function of gut microbiota (Zhang et al., 2023)., This involved the upregulation of several metabolic pathways related to antioxidant, neurodevelopmental and neurodegenerative diseases and also promoted the release of SCFAs (Zhang et al., 2023).

The reviewed evidence highlights the importance of SCFAs produced by several gut bacteria as they are capable of modulating learning processes, behavior and cognitive function. In addition, their production could be affected by changes in gut microbiota occurred upon exposure to metals. Further investigations are needed to explore the mechanisms by which these metabolites take part in complex gut-brain interactions.

4.1.4. Role of neurotransmitters

Neurotransmitters, such a dopamine, serotonin or γ-Aminobutyric acid (GABA), are actively involved in various brain functions including movement, emotion, learning and memory (Dicks, 2022). It has been shown that bacteria can produce a range of important neurotransmitters (Strandwitz, 2018). For example, approximately 90–95% of serotonin is produced in the gastrointestinal tract (Gershon and Tack, 2007) generally from *Clostridial* and *Lactobacillus* species (Clarke et al., 2014). On the other hand, more than 50% of dopamine in the human body is synthesized in the gut (Strandwitz, 2018), mainly from *Enterococcus* and *Lactobacillus* genera (Caspani and Swann, 2019). Moreover, the major inhibitory neurotransmitter in the brain, GABA, can be produced by *Bacteroides* (Otaru et al., 2021), as well as *Parabacteroides*, *Eubacterium* and *Bifidobacterium* (Y. Chen et al., 2021) in the gut. The neurotransmitters synthesized by the gut microbiota can reach other parts of the body through the bloodstream. However, most neurotransmitters cannot cross the BBB, unlike their precursors, which instead can participate in the neurotransmitter synthesis cycle in the brain (Y. Chen et al., 2021; O'Mahony et al., 2015; Sampson and Mazmanian, 2015). It has been demonstrated that some metabolic products of bacteria are precursors of neurotransmitters, such as dopamine, serotonin or GABA (Frost et al., 2014; Lukić et al., 2022). Thus, changes in the microbiota responsible of producing these neurotransmitter precursors could affect the levels of related metabolites in the brain, thereby affecting brain function and cognition (Góralczyk-Bińkowska et al., 2022). The metabolite acetic acid (acetate), produced by carbohydrate metabolism in the colon, can reach the brain and is included into the GABA synthesis cycle (Frost et al., 2014). The probiotic *Bifidobacterium infantis* can increase plasma levels of serotonin precursor, tryptophan, thereby affecting the transmission of brain serotonin (Desbonnet et al., 2010). Production of diet-derived amino acids, such as L-tryptophan or L-tyrosine, participates in the brain synthesis of serotonin and dopamine, respectively (Caspani and Swann, 2019; Lukić et al., 2022). These studies suggest a relationship between intestinal microbial metabolism and neurotransmitter biosynthesis in the brain. In turn, the gut microbiota can transmit signals to the brain through the local nervous system (e.g., vagus nerve) (Dicks, 2022). Administration of *Lactobacillus rhamnosus*, a GABA-producing species, has been found to increase GABA levels in the brain, nevertheless these changes have not been recorded in vagotomized animals (Bravo et al., 2011). Thus, the neurotransmitter synthesis pathway in the gut could potentially have a direct (via production of metabolites) or indirect (via vagal stimulation) effect on brain chemistry and behavior.

Interestingly, exposure to environmental contaminants, such as heavy metals, can lead to changes in gut microbial composition, which consequently affect neurotransmitter function. (X. Chen et al., 2021) found a decrease of 5-HT and 5-HT3R expression in striatum and intestine of Pb-exposed rats. This effect was associated by the authors to an increase in depressive-like behavior in animals due to Pb treatment (X. Chen et al., 2021). A previous study reported that *Bifidobacteria* could modulate depressive-like behavior by increasing the concentration of 5-HT in brain regions such as prefrontal cortex, amygdala, and striatum (Savignac et al., 2016). Furthermore, it has been demonstrated that Hg disrupts neurotransmitter function by preventing the binding of neurotransmitters to receptors on the postsynaptic cell and preventing their activation (Weber et al., 2012). Lin et al. (2021) showed that the administration of MeHg reduced the levels of glutamate, dopamine and serotonin precursors, such as L-glutamate, L-tyrosine and L-tryptophan, respectively (Lin et al., 2021). This effect could be due to that MeHg specifically inhibits glutamate uptake in astrocytes (Aschner et al., 2007). Moreover, MeHg down-regulated *Bacteroides, Firmicutes* and *Proteobacteria*, and up-regulated of *Actinobacteria* and *Verrucomicrobia* at phylum level (Lin et al., 2021). Mn exposure has been shown to affect dopaminergic neurons, intestinal permeability, and gut microbiota composition, thereby contributing to the release of neurotoxic metabolites and the development of psychological disorders (Tinkov et al., 2021). It was assessed that *Lactobacillus paracasei PS23* increased dopamine in the hippocampus and prefrontal cortex of corticosterone-treated animals (Wei et al., 2019), while another research showed that treatment with *Bifidobacterium CECT 7765* decreased dopamine level in the hypothalamus of early-life stress-induced animal model (Moya-Pérez et al., 2017). Interestingly, Xia et al. (2023) recently demonstrated that the administration of Pb and Mn can induce alterations in gene expression, including genes related to the serotonin pathway. Specifically, they found that the expression of serotonin transporter (*slc6a4a*), monoamine oxidase (MAO), and tryptophan hydroxylase (*tph2*) genes were significantly altered in response to Pb and Mn combined exposure (Xia et al., 2023).

Overall, these studies suggest a potential biological mechanism by which exposure to heavy metals could induce changes in microbiota involved in the production of some neurotransmitters, which in turn could indirectly affect brain function and behavior.

4.1.5. The role and challenges of animal models in translational research

Animal models play an essential role in biomedical research, particularly for elucidating biological mechanisms of diseases influenced by the gut-brain axis (Arun et al., 2021). Among these, murine models, such as germ-free mice, are helpful for investigating the microbiota's pathogenic and therapeutic potentials. These models facilitate the exploration of host-microbiome interactions, shedding light on metabolic, immune, and neurological processes (Hou et al., 2022). Preclinical research utilizing these models has established the influence of gut microbiota on cognitive functions, behavior, and social interactions. In germ-free murine models, notable differences in gene expression within brain regions such as the prefrontal cortex, cerebellum, striatum, and hippocampus have been observed (Hoban et al., 2017; Hou et al., 2022). This highlights the gut-brain axis's role in neurodevelopment and behavior. However, the translation of these findings to human applications faces challenges due to some variations. Humans and rodents differ in the abundance of Actinobacteria and the Firmicutes/Bacteroides ratio, although the presence of shared species like Akkermansia indicates some level of comparability (Nagpal et al., 2018). Additionally, zebrafish models have proven invaluable in studying the impact of heavy metals on neurodegenerative diseases, offering insights applicable to human conditions such as Alzheimer's and Parkinson's disease. However, it is critical to consider the physiological and anatomical differences between zebrafish and humans in

these studies (Paduraru et al., 2023). Standardizing gut microbiota in preclinical studies is essential for creating accurate models that mimic human health conditions. These models are crucial for understanding how the microbiota interacts with its host and for translating research into significant health advancements (Macpherson and McCoy, 2015). Animal models are integral to bridging theoretical and practical aspects of clinical research, providing vital insights into disease mechanisms. While they are essential for translating laboratory research into clinical applications, their inability to perfectly replicate the human microbiome necessitates a balanced approach in translational research. It is important to acknowledge and address these discrepancies through careful and systematic analysis to ensure the findings are relevant and applicable to human conditions.

5. Limitations

Our systematic review, using the SYRCLE's risk of bias tool (Hooijmans et al., 2014), found several methodological limitations in the studies we examined. These limitations include unclear allocation concealment and random housing (Chen et al., 2022; X. Chen et al., 2021; Cheng et al., 2019; Hua et al., 2023; Li et al., 2022; Lin et al., 2021, 2020; Sun et al., 2022, 2020; Wang et al., 2020; Xia et al., 2023, 2020; Xiao et al., 2020; Yu et al., 2023; Zhang et al., 2022, 2023), uncertain blinding of caregivers (Chen et al., 2022; X. Chen et al., 2021; Cheng et al., 2019; Hua et al., 2023; Li et al., 2022; Lin et al., 2021, 2020; Sun et al., 2022; Wang et al., 2020; Xia et al., 2020, 2023; Xiao et al., 2020; Yu et al., 2023; Zhang et al., 2022, 2023) and outcome assessors (Chen et al., 2022; X. Chen et al., 2021; Hua et al., 2023; Li et al., 2022; Lin et al., 2020; Sun et al., 2022; Xia et al., 2023, 2020; Yu et al., 2023; Zhang et al., 2023, 2022), and a lack of transparency in reporting data (Chen et al., 2022; Cheng et al., 2019; Li et al., 2022; Lin et al., 2021; Sun et al., 2020). These issues could introduce biases in selection and performance. Allocation bias, which leads to differences in baseline characteristics between groups, can be reduced through randomization and concealed allocation. Additionally, blinding of investigators can help prevent performance bias by ensuring that their knowledge does not influence how they administer interventions, observe outcomes, or interpret results. This is crucial in maintaining the integrity of the experimental process, as unblinded researchers might consciously or unconsciously treat groups differently or interpret results in a biased manner, leading to skewed or unreliable results. To reduce detection bias, blinding of outcome assessors is also important, and various methods are available to achieve this, such as coded data analyzed by an independent researcher (Krauth et al., 2013). Procedural issues, such as small sample sizes (Lin et al., 2020), require comprehensive power analyses to minimize the risk of type II errors (Vesterinen et al., 2011). Moreover, inadequate handling of outcome data (Chen et al., 2022; X. Chen et al., 2021; Li et al., 2022; Lin et al., 2021; Sun et al., 2020) highlights the need for transparent management and reporting of missing or excluded data to maintain study integrity and reliability. Furthermore, while many studies use basic statistical analyses like ANOVA and t-tests, there is a notable gap in exploring deeper causal relationships. Few studies report correlations between heavy metal exposure and outcomes (Li et al., 2022; Sun et al., 2022, 2020; Zhang et al., 2023), but the transition from correlation to causation remains unclear. Only one study conducted a mediation analysis (Hua et al., 2023). Advanced statistical methods, such as mediation analyses and multivariate techniques like MANOVA, are valuable for understanding complex interactions. These methods provide insights into differences and underlying mechanisms, aiding in establishing more robust causal relationships. The importance of enhancing methodological rigor in animal studies, including allocation concealment, blinding, transparent reporting, and advanced statistical analysis, is emphasized for reliable clinical applicability and bridging the gap between animal research and clinical application (Krauth et al., 2013; Van Luijk et al., 2014).

Finally, it is important to acknowledge some limitations in our systematic review. Firstly, it only includes articles published in English, potentially missing out on global research. Additionally, data extraction relies solely on published reports, without further communication with authors, which could result in missing unpublished details. We chose not to include data from other approaches such as fecal microbiota transplantation to maintain focus and manage the review's scope. Another significant limitation is the variability of animal models used in the included studies. This variability, especially in terms of species, could influence gut microbiota composition and interaction with the host, thus impacting the global interpretation of findings. Lastly, a significant limitation arises from the small number and heterogeneity of the reviewed studies, complicating direct comparisons and potentially affecting the generalizability of our findings. This heterogeneity requires a cautious approach when interpreting the data. The evidence suggests that exposure to heavy metals affects both CNS outcomes and gut microbiota. However, the exact nature of these effects is unclear. It's challenging to determine if heavy metal exposure directly causes changes in gut microbiota that affect the CNS, if it first induces neurological changes influencing the gut microbiota, or if it independently affects both. Therefore, further research is required to understand their relationships.

6. Future directions

Recent investigations have significantly advanced our understanding of the impact of heavy metals on gut microbiota and brain functions. Some research, though not systematic, has reviewed the mechanisms and effects of heavy metals on the gut microbiota and the gut-brain axis, analyzing how these contaminants influence neurological health and behavior (Chiu et al., 2020; Kaur and Rawal, 2023; Rosenfeld, 2017; Singh et al., 2022). Additionally, specific research has focused mainly on the effects of single metals like Pb (Tizabi et al., 2023) and Mn (Tinkov et al., 2021), highlighting their neurotoxicity. This comprehensive body of research underscores the need for ongoing research to understand the complex relationship between heavy metal mixtures, gut microbiota, and brain function at both the behavioral and neurochemical levels, which has been the focus of this systematic review. Building on these findings, the next phase of gut-brain axis research promises rapid advancement with the introduction of novel theoretical frameworks and practical applications. Advanced techniques such as metagenomics and metabolomics are becoming central to uncovering complex molecular mechanisms (Ramírez-Acosta et al., 2021). Furthermore, precision medicine shows promise in addressing issues within the gut-brain axis by customizing treatments based on individual microbiota profiles (Sinagra et al., 2020). This approach accentuates the need for large-scale clinical studies to validate findings from preclinical models and to understand the impact of heavy metals on populations.

Special emphasis is placed on pediatric research, a sensitive field that demands special attention due to the specific vulnerability of children to heavy metal exposure (Stiles and Jernigan, 2010). The consequences of early life exposures underscore the necessity for targeted research across all developmental stages. An example could be the Europe-funded project called ATHLETE (athleteproject.eu), which exemplifies large-scale, interdisciplinary research tackling public health challenges linked to early-life environmental exposures. International collaboration plays a pivotal role in this area, providing a global perspective on the impact of heavy metals and aiding in the development of universal solutions in gut-brain research. Investigating the combined effects of heavy metals with other environmental factors opens new avenues in understanding these complex interactions.

Moreover, developing microbiota-based therapies, such as fecal microbiota transplantation or personalized probiotics, offers innovative approaches to employ the gut microbiota's protective properties against heavy metal toxicity (Antushevich, 2020; Forero-Rodríguez et al., 2022; Long-Smith et al., 2020). This expanding field holds the potential for innovative treatment strategies, addressing the specific needs and

conditions of individuals impacted by heavy metals.

In summary, the future of gut-brain pathway investigations encompasses a multifaceted approach that includes personalized treatments, innovative therapies, and international collaboration. These research directions hold the promise of significant advancements in understanding and mitigating the neurological impacts of heavy metal exposure, paving the way for more effective interventions and a deeper comprehension of the gut-brain connection.

7. Conclusions

This systematic review highlights the significant role of the gut microbiota in mediating the effects of heavy metals on the CNS. The interaction between heavy metals and the gut microbiota can lead to alterations in the diversity of the microbiota. The relationship between *Firmicutes* and *Proteobacteria*, two major bacterial phyla, is influenced by heavy metals, although the direction of that change varies between studies. Exposure to heavy metals contributes to intestinal dysbiosis, which in turn exacerbates the vulnerability of the CNS to their harmful effects. The interaction between heavy metals and the intestinal microbiota alters microbial diversity, thereby increasing the central nervous system's vulnerability to the harmful effects of heavy metals. Metal exposure is associated with an increase in gram-negative species, such as *Bacteroidetes*, and a reduction in gram-positive species, like *Lactobacillus*, leading to neuropsychological symptoms such as depression, anxiety, and deficits in learning and memory. Probiotic interventions, particularly those involving *Lactobacillus*, appear promising in mitigating these neurotoxic effects.

In conclusion, the interaction between heavy metals, gut microbiota, and the CNS suggests that heavy metals can induce direct brain alterations and indirect effects through the gut microbiota, contributing to neurotoxicity and neuropsychological disorders. Monitoring heavy metal levels and maintaining a healthy gut microbiota might be crucial in mitigating the toxic effects on the CNS. Future research should focus on unraveling the complex mechanisms underlying this interaction to develop effective intervention strategies.

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CRediT authorship contribution statement

Simona Porru: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Ana Esplugues:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Sabrina Llop:** Methodology, Writing – review & editing. **Juana María Delgado-Saborit:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.123732.

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