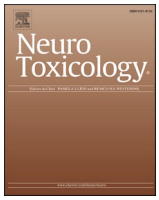
Neurotoxicology 113 (2026) 103390



Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro





Elevated fecal silver, lithium, and platinum in cognitive impairment: A pilot exploration of microbiota–metal interactions

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A R T I C L E I N F O

Keywords:
Gut microbiota
Cognitive impairment
Alzheimer's disease
Metals
Silver
Lithium
Platinum

A B S T R A C T

Background: Gut microbiota (GMB) and metal exposure have both been implicated in cognitive impairment (CI), including amnestic mild cognitive impairment (aMCI) and Alzheimer's disease (AD). However, studies integrating these areas remain scarce.
Objective: This pilot study aimed to investigate whether exposure to metals modulates the relationship between GMB composition and clinical outcomes in individuals with CI.
Methods: Stool samples were collected from aMCI (n = 12), AD (n = 18), and cognitively healthy controls (HC, n = 30). Participants were categorized into CI (n = 30) and HC (n = 30). Gut microbial diversity was assessed using shotgun sequencing, and 25 metals were quantified by inductively coupled plasma mass spectrometry (ICP-MS). Cognitive, neuropsychological, neuropsychiatric, and functional assessments were also conducted.
Results: No significant differences were observed between groups in microbial richness, alpha-diversity (Shannon index), or beta-diversity (Bray–Curtis). Likewise, microbial diversity measures were not associated with cognitive outcomes. In contrast, aMCI and AD participants exhibited significantly higher fecal concentrations of silver (Ag), lithium (Li), and platinum (Pt) compared to HC (all $p < 0.001$).
Conclusion: This multidimensional pilot study integrating microbiota profiling, metal exposure assessment, and cognitive evaluation, revealed elevated fecal excretion of Ag, Li, and Pt in participants with cognitive impairment, suggesting potential interactions between trace metals and neurodegenerative processes. While no significant differences in overall microbial diversity were observed between groups, these findings emphasize the need for larger, longitudinal investigations to elucidate the complex relationships among gut microbiota, metal homeostasis, and cognitive decline.

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https://doi.org/10.1016/j.neuro.2026.103390
Received 6 October 2025; Received in revised form 22 December 2025; Accepted 9 January 2026
Available online 10 January 2026
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1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia worldwide and represents a major public health challenge due to its increasing prevalence, socioeconomic burden, and lack of effective curative treatments (WHO, 2020). Clinically, AD is characterized by a progressive decline in cognitive functions, most notably memory, language, and executive functioning, accompanied by neuropsychiatric symptoms and functional impairment in daily living (WHO, 2019). The pathological hallmarks of AD include the accumulation of extracellular β-amyloid (Aβ) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, and widespread neuroinflammation and synaptic dysfunction (Liu et al., 2024).

Single-domain amnestic mild cognitive impairment (aMCI) is recognized as a transitional stage between normal aging and AD. This subtype is characterized by clearly measurable memory deficits that exceed age-related expectations, although they do not significantly affect functional autonomy (Mian et al., 2024). Individuals with aMCI are at particularly high risk of progressing to AD, making this population a primary target for early detection and the development of preventive strategies (Petersen et al., 2014; Edmonds et al., 2024).

In recent years, the gut microbiota (GMB) has received considerable attention as a potential modulator of neurodegenerative processes (Schneider et al., 2024). Through the gut–brain axis, the GMB participates in several biological processes, including immune system regulation and metabolic modulation (Loh et al., 2024). The gut-brain axis is a bidirectional communication pathway, meaning that microbial signals can influence neuronal function, while the brain can, in turn, release molecules that alter gut microbial composition. Dysbiosis, defined as an imbalance in gut microbial composition, such as reduced diversity, loss of beneficial commensal species, or overrepresentation of pro-inflammatory taxa, has been associated with heightened inflammatory activity and alterations in the central nervous system, both of which are characteristic features of neurodegeneration in AD (Wallace et al., 2020; Leblhuber et al., 2018).

Environmental toxicants, particularly metals, which include highly toxic metals and metalloids, can influence both central and peripheral biological systems, including the GMB (Livingston et al., 2020). Due to their widespread presence in the environment, primarily resulting from anthropogenic activities, humans are chronically exposed to metals through air, water, food, and consumer products (Sanchini et al., 2022). Metals such as copper (Cu) and selenium (Se) are essential elements due to their involvement as cofactors in metabolic processes (Nascimento et al., 2021; Tsang et al., 2021).

However, metals like iron (Fe) and lead (Pb) have been shown to foster the proliferation of pro-inflammatory bacterial taxa while reducing the abundance of beneficial anti-inflammatory species in population-based studies (Ahmed et al., 2020; Eggers et al., 2019). In addition, other metals such as cadmium (Cd) can increase the permeability of the blood–brain barrier in mouse models, facilitating their entry into the brain and triggering neuroinflammatory and neurodegenerative cascades (Huang and Ren, 2022). In AD, accumulations of Fe or aluminum (Al) have been observed in brain regions typically affected by the disease, including the hippocampus (Jakubowski et al., 2021; Kenkhuis et al., 2021), where they colocalize with β-amyloid plaques and may contribute to the aggregation or stabilization of these hallmark structures. In parallel, animal studies have shown that exposure to metals can disrupt gut microbial diversity and composition, while also inducing neurotoxic effects, including oxidative stress, synaptic damage, and cognitive impairment (Porru et al., 2024; Wang et al., 2024; Richardson et al., 2018). While some studies have explored associations between metal burden and neurodegenerative processes in humans, current evidence directly linking fecal metal excretion, GMB alterations, and accumulation in target tissues—particularly the brain—remains scarce and is limited by small sample sizes, heterogeneous study designs, and variability in the biological matrices analyzed (Mateo et al., 2023; Liu et al., 2020; Yabe et al., 2018). Taken together, these observations suggest that microbiota–metal interactions could play a role in the early pathophysiological processes underlying the transition from aMCI to AD.

The present pilot study was aimed at investigating whether exposure to metals could modulate the relationship between gut microbiota composition and clinical outcomes in individuals with aMCI and AD, collectively classified as cognitive impairment (CI). We characterized the differences in microbiota diversity and metal concentrations in stool samples across individuals with aMCI, AD, and those who are cognitively healthy. The hypothesis is that individuals with aMCI and AD should exhibit reduced richness and alpha diversity, as well as significant differences in beta diversity in their fecal microbiota. Additionally, we expected altered levels of silver (Ag), Al, arsenic (As), barium (Ba), Cd, cerium (Ce), cobalt (Co), cesium (Cs), copper (Cu), Fe, mercury (Hg), lanthanum (La), lithium (Li), magnesium (Mg), manganese (Mn), Pb, platinum (Pt), rubidium (Rb), antimony (Sb), selenium (Se), tin (Sn), strontium (Sr), thallium (Tl), uranium (U) and zinc (Zn) in stool samples, compared to cognitively healthy individuals. Furthermore, we hypothesized that shifts in metal concentrations could contribute to changes in microbial diversity, given that several metals can act as microbial stressors or modulators, potentially altering gut ecological balance and influencing pathways relevant to neuroinflammation and cognitive decline. The present study includes both well-established neurotoxic metals and less-studied elements like Ag, Li, and Pt to explore a broader range of potential environmental and therapeutic exposures.

2. Materials and methods

2.1. Study population

Single-domain aMCI patients (n = 12), AD patients (n = 18), and age- and sex-matched healthy controls (HC, n = 30) were enrolled by recruitment hospitals/centers in Tarragona County, Spain, from May 2021 to June 2022. HC were recruited as relatives of the patients and through hospital advertising campaigns. These centers included Verge de la Cinta Hospital, Xarxa Santa Tecla Hospital, and Lerín Neurocognitive Institute. Inclusion criteria for the aMCI and AD group were: (1) AD and single-domain aMCI diagnosis by the neurology department following NIA-AA 2011 criteria (Albert et al., 2011; McKhann et al., 2011); (2) aged between 60 and 85 years; and (3) a Global Deterioration Scale Fast (GDS-FAST) (Reisberg, 1988) score of 3 for aMCI patients, and 4 or 5 for AD patients. Inclusion criteria for the control group were: (1) healthy men and women; and (2) between 60 and 85 years of age. Exclusion criteria for both groups were: (1) diagnosis of or comorbidity with other neurological diseases; (2) use of antibiotics or corticosteroids in the 6 months prior to study enrollment; (3) treatment with immunosuppressants or immunostimulants in the 6 months before collecting the stool sample; (4) gastrointestinal illnesses; (5) consumption of large doses of commercial probiotics (greater than or equal to 10^8 CFU per organism per day); or (6) illiteracy.

Informed consent was obtained from all patients and their families prior to their participation. Participants received stool collection kits and were scheduled for testing their cognitive status within one week. This investigation was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Research Committee (CEIm) of the Pere Virgili Institute for Health Research (IISPV, Ref. CEIM: 183/2020). The study has also been included in ClinicalTrials.gov (ID: NCT05943925). This study is part of the DEM-BIOTA Project (PID2019–103888RB-I00).

2.2. Fecal sample collection and DNA isolation

Fecal samples were collected at home using the Stool Nucleic Acid Collection and Preservation System kit (Norgen Biotek Corporation®, Canada) within three days before the interview. Participants stored the

kits at room temperature or in the refrigerator until the day of the interview. Aliquots of approximately 500 mg were placed into Eppendorf tubes and then frozen and stored at −80 °C. One aliquot per participant was analyzed at the Centre for Omic Sciences (COS). DNA was extracted using the Fast Stool DNA Mini Kit (Qiagen, Germany) with prior lysis of approximately 200 mg of sample in 100–200 µL of nuclease-free water at 95 ºC, following the kit instructions (QIAGEN. 2020. *QIAamp Fast DNA Stool Mini Kit Handbook*. Hilden, Germany: QIAGEN). The final DNA concentration and purity were assessed using the Qubit 4.0 fluorometer and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific®, Massachusetts, USA) according to the manufacturer's instructions. 2019. *Qubit 4 Fluorometer User Guide* and *Qubit dsDNA HS Assay Kit Product Information Sheet*. Waltham, MA, USA: Thermo Fisher Scientific®.

2.3. Shotgun metagenomics and quality control

The gut microbiome was assessed at COS using a shotgun metagenomic approach. DNA was extracted from samples using the DNA Prep with Tagmentation kit, according to the manufacturer's protocol (Illumina, California, USA, catalog no. 20018705). Sequencing library concentration was determined with a Qubit 4.0 fluorometer and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific®, Massachusetts, USA). Additionally, sequencing library length was checked using the Agilent TapeStation and the Agilent High Sensitivity DNA kit (Agilent Technologies, California, USA). Sequencing libraries with concentrations below 750 pM or outside the 400–600 bp range were excluded. Final sequencing libraries were pooled at 750 pM and sequenced on the NextSeq 2000 system (Illumina, California, USA) with 2 × 150-bp paired-end reads. Samples with fewer than 7.5 million reads were discarded. Relative abundances of microbial species were profiled from the shotgun metagenomic reads by mapping them to k2_pluspfp_16gb_20220607's database, which includes bacteria, archaea, protozoa, fungi, viruses, plasmids, human sequences, and UniVec Core (vector sequences) using Kraken2 [36]. Unidentified reads were excluded.

2.4. Exploratory assessment

All enrolled participants completed a 30-minute sociodemographic assessment at the recruitment centers, which included questionnaires on medication use and risk factors for AD. For patients, these questionnaires were completed with the assistance of their relatives, whereas HC completed them independently. The following parameters were assessed: (1) demographic characteristics, including age, sex (male/female), nationality, profession, years of education, civil status, and cohabitants at home; (2) the presence of risk factors, including family history of aMCI or AD, hearing loss, hypertension (measured as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg), diagnosis of dyslipidemia (total cholesterol ≥190 mg/dL, LDL cholesterol ≥115 mg/dL, triglycerides ≥150 mg/dL, and/or HDL cholesterol <40 mg/dL in men or <46 mg/dL in women), diagnosis of diabetes mellitus (type I or II), Body Mass Index (BMI), stroke, exposure to toxics, smoking habits (never, previous or current, measured in quantity of cigarettes), and alcohol consumption (never, previous or current, considering one alcoholic drink/day); and (3) use of medication or supplements. Additionally, patients and their families completed the Mediterranean Lifestyle Index (MEDLIFE), a standardized 28-item self-report questionnaire on adherence to the Mediterranean diet and related habits (Sotos-Prieto et al., 2015).

2.5. Cognitive functions, emotional, neuropsychiatric, and functionality assessment

Cognitive functions were evaluated by trained researchers with a duration of approximately two hours, using the following tests in order of administration: (1) cognitive domain, which includes Temporal, Spatial, and Personal Orientation from the Barcelona-2 Test (Peña-Casanova, 2019), Mini Mental State Examination (MMSE, Folstein et al., 1975), Memory Impairment Screen (MIS, Buschke et al., 1999), Digit Span from the Barcelona-2 Test (DS, Peña-Casanova, 2019), Free and Cued Selective Reminding Test (FCSRT) (Buschke, 1984), Trail Making Test Parts A and B (TMT-A and TMT-B, Reitan, 1958), Clock Drawing Test with Cacho et al. correction (CDT, Cacho-Gutiérrez et al., 1999), Construction Praxis from the Barcelona-2 Test (PCTB2, Peña-Casanova, 2019), Frontal Assessment Battery (FAB, Dubois et al., 2000; Heredia et al., 2025), Cognitive Reserve Scale (CRS, León-Estrada et al., 2017), Abbreviated Boston Naming Test version C (Boston-C, Kaplan et al., 2001), and Category Fluency from the Barcelona-2 Test (CEF, Peña-Casanova, 2019); (2) emotional domain, which includes the Goldberg Anxiety and Depression Scale (Goldberg et al., 1988) and the Life Events Questionnaire adapted from the PREDIMED-PLUS study (LEQ, Soldevila-Domenech et al., 2021); (3) neuropsychiatric domain, which includes Neuropsychiatric Symptomatology from the Barcelona-2 Test (NPTB2—major neuropsychiatric symptoms—and NPETB2—supplementary neuropsychiatric and behavioral symptoms, Peña-Casanova, 2019); (4) functionality, including the Activities of Daily Living from the Barcelona-2 Test (ADL, Torrente et al., 2025). A detailed description of each test is provided in Supplementary Table 1.

2.6. Determination of metal concentrations in fecal samples

Concentrations of chemical elements, including Ag, Al, As, Ba, Cd, Ce, Co, Cs, Cu, Fe, Hg, La, Li, Mg, Mn, Pb, Pt, Rb, Sb, Se, Sn, Sr, Tl, U, and Zn, in fecal samples were quantified following closed-vessel microwave-assisted digestion using a Start D system (Milestone, Italy). Analyses were performed at the Analytical and System Toxicology Group (ASTox), School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil, during July–August 2022. Fecal specimens were collected, transported under controlled conditions, and stored at −80 °C until analysis. Approximately 75–100 mg of each sample was placed into a pre-cleaned digestion vessel, and 8 mL of 50 % (v/v) sub-boiling HNO_3 was added. The digestion protocol comprised two steps: 100 °C at 1100 W for 10 min, followed by 200 °C at 1100 W for 30 min. The digested solution (1 mL) was diluted with 10 mL of deionized water and analyzed by inductively coupled plasma mass spectrometry (ICP-MS, NexION 2000, PerkinElmer, Norwalk, CT, USA) under high-purity argon (99.999 %), as described previously by Batista et al. (2009) and Souza et al. (2023).

To ensure analytical reliability, all samples were processed in duplicate, and reagent blanks (1 per 20 samples) were prepared using the same acid solution. Calibration curves spanned 2–500 ppb, with correlation coefficients consistently above 0.99, confirming the robustness of the calibration model. Only analytical-reagent grade chemicals were employed, and nitric acid was further purified by quartz sub-boiling distillation (Kürner Analysentechnik). All solutions were prepared under clean laboratory conditions in a class 100 laminar-flow hood. Plastic containers used for sample storage were pre-cleaned by soaking in 10 % (v/v) HNO_3 for 24 h, rinsed five times with Milli-Q water, and air-dried in a laminar-flow hood. All procedures were conducted in a class 1000 clean-room environment.

2.7. Statistical analysis

Statistical analysis was conducted using Jamovi v.2.4.1 and RStudio v4.4.1. Continuous variables are expressed as the median (with interquartile range, IQR), while categorical variables are expressed as counts (with percentages). Normality and homogeneity were assessed using the Shapiro-Wilk and Levene tests. Variables were then compared between the cognitive impairment (CI) and healthy control (HC) groups using two-sample *t*-tests for normally distributed data and Mann-Whitney U tests for non-normally distributed data. For multiple comparisons, p-

values were adjusted using the Holm–Bonferroni method (Holm, 1979). For fecal metal levels, detection frequency (DF) was calculated as counts (percentage). Metals were treated as: (i) continuous variables when DF > 80 %, (ii) included in bivariate analyses (Chi^2) when DF ranged between 10 % and 80 %, and (iii) excluded from analysis when DF < 10 %.

Richness (Chao1) and alpha-diversity (Shannon Index) metrics were calculated at the OTU level by performing rarefaction with 10 iterations of random subsampling to 1788 reads (the lowest number of sequences from any participant). Variables were then compared between cognitive impairment and healthy control groups using two-sample *t*-tests for normally distributed data and Mann-Whitney U tests for non-normally distributed data. Beta-diversity metrics were computed from normalized OTU-level data, including Bray-Curtis dissimilarity. To determine statistically significant differences in beta-diversity metrics between groups, we employed permutational multivariate analysis of variance (PERMANOVA) using the vegan R package (Oksanen et al., 2025).

Associations between GMB and metal concentrations were assessed using multivariable linear models implemented in the Maaslin2 R package (Mallick et al., 2020). The multivariate linear models were defined as:

$$g(Y_i) = \beta_0 + \beta_1 \cdot Phylum_{1,i} + \ \ldots \ + \beta_p \cdot Phylum_{p,i} + \beta_{(P+1)} \cdot Age_i + \beta_{(P+2)} \cdot BMI_i + \beta_{(P+3)} \cdot Education_i + \varepsilon_i$$

Relative abundances at the phylum level were included as predictors, while metals, psychological test scores, richness (Chao1), and alpha-diversity (Shannon index) were considered as outcomes (Y_i). Models were adjusted for age, BMI, and years of education as covariates. These associations encompassed both richness and alpha-diversity, along with relative abundance at the phylum level, applying minimum detection and prevalence thresholds of 0.2/100 and 50/100, respectively, to enhance robustness. To account for false positives, a false discovery rate (FDR) correction (q-value < 0.1) was implemented using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

Statistical significance for all tests was established at a p-value < 0.05.

3. Results

3.1. Cohort characteristics and cognitive functions, emotional, neuropsychiatric and functionality assessment

Sixty participants initially met the eligibility criteria and were classified into three groups: 30 individuals in the HC group, 12 in the single-domain aMCI group, and 18 in the AD group. Due to the unequal sample sizes, participants were subsequently categorized into two groups: the CI group, comprising individuals with aMCI and AD (n = 30), and the HC group (n = 30). A detailed flow chart of each aggrupation is provided in Supplementary Figure 1. Importantly, the results for the original three-group classification (HC, aMCI, AD) are also reported in the Supplementary Material for transparency and completeness.

The CI group did not differ significantly from the HC group in age, BMI, years of education, smoking habits, or alcohol consumption. Similarly, no significant differences were observed in the various components of the MEDLIFE questionnaire (Table 1 and Supplementary Table 2). Other variables collected, such as sociodemographic parameters and clinical features, were also assessed but did not differ significantly between groups (data not shown).

The cognitive, emotional, neuropsychiatric, and functional characteristics of the participants are presented in Table 2, Table 3, Supplementary Table 3 and Supplementary Table 4. The results observed in cognitive domains, neuropsychiatric symptoms, and functional impairment are consistent with a diagnosis of CI.

Table 1
Cohort characteristics by cognitive impairment diagnosis (N = 60).

	HC (n = 30)		CI (n = 30)		p-value	p-value corrected[a]
Age (years)	67	5	74	5	**< 0.01**	0.09
Sex						
Male	16	52 %	13	48 %	0.45	1.00
Female	14	48 %	17	52 %		
BMI (kg/m^2)	26.1	3.2	24.7	3.6	0.17	1.00
Education (years)	12.5	5.4	9.9	4.6	0.05	0.35
Smoking status						
Current	4	12 %	2	4 %	0.18	1.00
Former	10	32 %	7	32 %		
Never	16	56 %	21	64 %		
Alcohol consumption						
Current	8	28 %	3	12 %	0.55	1.00
Former	0	0 %	4	8 %		
Never	22	72 %	23	80 %		
MEDLIFE						
Food consumption	9	2	9	2	0.96	1.00
Dietary habits	5	1	5	1	0.30	1.00
Physical and social activity	4	1	3	1	**0.01**	0.09

Displayed as mean and standard deviation for continuous data, and as count and percentage for categorical data. BMI: Body Mass Index, CI: Cognitive impairment, HC: Healthy controls, MEDLIFE: Mediterranean Lifestyle Index Interview (Sotos-Prieto et al., 2015).

[a] Corrected using the Holm–Bonferroni method (Holm, 1979).

3.2. Metals determination

The concentrations of metals in stool samples are presented in Table 4 and Supplementary Table 5. Several metals —Al, Ba, Cd, Ce, Co, Cs, Cu, Fe, Hg, La, Li, Mg, Mn, Rb, Sn, Sr, Tl, U, and Zn —showed DF > 80 %. In addition, Ag, Pb, Pt, and Sb had DF between 60 % and 80 %. However, DF < 10 % and Se were not detected. Therefore, both were excluded from subsequent analyses. Interestingly, significantly elevated levels of Ag (p-value < 0.001), Li (p-value < 0.001), and Pt (p-value < 0.001) were observed in the CI group compared to the HC group (Figs. 1, 2, and 3, and Supplementary Figures 2, 3, and 4).

3.3. Microbial diversity and multivariable linear models

No significant differences were observed in microbial richness or alpha-diversity between the HC and CI groups (Chao1, p-value = 0.134; Shannon Index, p-value = 0.218, Figs. 4 and 5, respectively). The corresponding analyses for the three original groups (HC, aMCI, and AD) are provided in Supplementary Figures 5 and 6. Similarly, beta-diversity analysis did not reveal any statistically significant differences between the two groups. Furthermore, no associations were found between metals (Ag, Pt, and Li) and alpha-diversity measures or cognitive functions (Supplementary Table 6).

4. Discussion

Our pilot study investigated the relationship between GMB composition, fecal metal concentrations, and CI in individuals with aMCI and AD.

The cognitive decline observed in participants with CI is a defining feature of conditions like aMCI and AD. This deterioration encompassed memory, attention, language, executive functions, and visuoconstructive abilities. These findings are in line with the literature, which consistently reports similar profiles of impairment in mild-to-moderate stages of cognitive decline (Vasileiou et al., 2025; Rouch et al., 2020). This decline is typically associated with structural and functional brain changes and frequently overlaps with alterations in other domains (Ávila-Villanueva et al., 2022). In our cohort, the CI group exhibited more neuropsychiatric symptoms and a trend toward higher

Table 2
Cognitive and emotional characteristics by cognitive impairment diagnosis (N = 60).

	HC (n = 30)		CI (n = 30)		p-value	p-value corrected[a]
OTB2						
Personal orientation	90.00	0.00	68.10	36.95	**0.003**	**0.027**
Spatial orientation	90.00	0.00	37.13	40.98	**< .001**	**0.024**
Temporary orientation	90.00	0.00	27.63	38.32	**< .001**	**0.024**
MMSE	29.50	1.28	23.57	4.18	**< .001**	**0.024**
MIS	7.14	1.30	1.76	2.18	**< .001**	**0.024**
DS						
Forward span	11.60	2.51	10.40	2.46	0.078	0.468
Backward span	11.37	2.38	10.60	2.69	0.340	1.000
FCSRT						
1st free recall						
Total free recall	10.27	2.95	3.83	3.76	**<.001**	**0.024**
Total recall	9.77	3.13	1.86	3.03	**< .001**	**0.024**
Delayed free recall	9.77	2.97	2.07	3.51	**< .001**	**0.024**
Total delayed recall	10.87	3.25	1.50	2.87	**< .001**	**0.024**
TMT A	12.20	4.54	2.11	4.18	**< .001**	**0.024**
TMT B	10.23	2.76	6.77	3.71	**< .001**	**0.024**
CDT	9.07	3.59	3.62	3.83	**< .001**	**0.024**
PCTB2	9.55	0.84	6.45	3.41	**< .001**	**0.024**
FAB	88.67	5.07	72.10	31.53	**0.013**	0.091
CRS	17.33	1.09	13.03	4.60	**< .001**	**0.024**
Boston-C	14.33	4.70	10.93	3.87	**0.005**	**0.040**
CEF	11.80	2.81	8.60	3.39	**< .001**	**0.024**
Semantic fluency	10.48	2.67	8.60	3.49	**< .001**	**0.024**
Formal fluency	9.73	2.77	7.48	3.49	0.319	1.000
GOLDBERG						
Anxiety scale	1.30	2.09	1.83	2.20	0.305	1.000
Depression scale	0.63	1.27	1.00	1.93	0.661	1.000
LEQ	13.200	6.03	10.72	5.55	0.122	0.610

Displayed as mean and standard deviation for continuous data. Boston-C: Abbreviated Boston Naming Test version C (Kaplan et al., 2001), CDT: Clock Drawing Test with Cacho et al. correction (Cacho et al., 1999), CEF: Categorial Evocation Fluency from Barcelona-2 Test (Peña-Casanova, 2019), CI: Cognitive Impairment, CRS: Cognitive Reservation Scale (León-Estrada et al., 2017), DS: Digit Span from Barcelona-2 Test (Peña-Casanova, 2019), FAB: Frontal Assessment Battery (Dubois et al., 2000), FCSRT: Free and Cue Selective Reminding Test (Buschke, 1984), GOLDBERG: Anxiety and Depression Scale (Goldberg et al., 1988), HC: Healthy Controls, LEQ: Life Events Questionnaire (Soldevila-Domenech et al., 2021), MIS: Memory Impairment Screen (Buschke et al., 1999), MMSE: Mini Mental Score Examination (Folstein et al., 1975), OTB2: Orientation from Barcelona-2 Test (Peña-Casanova, 2019), PCTB2: Construction Praxis from Barcelona-2 Test (Peña-Casanova, 2019), TMT A: Trail Making Test A (Reitan, 1958), TMT B: Trail Making Test B (Reitan, 1958).

[a] Corrected using the Holm–Bonferroni method (Holm, 1979).

Table 3
Neuropsychiatric and functionality characteristics by cognitive impairment diagnosis (N = 60).

	HC (n = 30)		CI (n = 30)		p-value	p-value corrected[a]
NPTB2	0.20	0.55	5.70	5.34	**< .001**	**0.004**
NPETB2	0.00	0.00	0.33	0.80	**0.022**	**0.022**
ADL						
Basic activities	0.00	0.00	1.40	3.19	**< .001**	**0.004**
Instrumental activities	0.07	0.37	27.27	17.68	**0.001**	**0.004**

Displayed as mean and standard deviation for continuous data. ADL: Activities of Daily-Living from Barcelona-2 Test (Torrente et al., 2025), CI: Cognitive Impairment, HC: Healthy Controls, NPETB2: Complementary Neuropsychiatric Symptomatology from Barcelona-2 Test (Peña-Casanova, 2019), NPTB2: Neuropsychiatric Symptomatology from Barcelona-2 Test (Peña-Casanova, 2019).

[a] Corrected using the Holm–Bonferroni method (Holm, 1979).

dependency in activities of daily living, although emotional symptoms did not differ significantly between groups. This lack of differences may be explained by the heterogeneity of our sample, which included participants at varying stages of CI. Kida et al. (2016) have shown that emotional alterations tend to become more pronounced as cognitive decline progresses. However, other authors have highlighted the variability of such alterations, noting that they can be influenced by individual factors such as personal resilience, social support, and baseline psychological characteristics (Warren et al., 2024; Yu et al., 2022).

Regarding GMB alterations in aMCI and AD patients, no significant differences in alpha- or beta-diversity indices were observed between the CI and HC groups in our sample, contrasting with findings reported by other authors (Li et al., 2019; Duan et al., 2021). These results may be explained by the heterogeneity of the CI group and the small sample size, as this was a pilot study. In another study from our group with a larger cohort of AD patients, reductions in richness and alpha-diversity were indeed observed (Mateo et al., 2024). Findings on microbial diversity in neurodegenerative diseases vary considerably across populations, depending on factors like diet, geography, methodology, and disease severity (Vogt et al., 2017; Saji et al., 2019; Jemimah et al., 2023).

Furthermore, our results revealed significantly higher concentrations of Ag, Li, and Pt in the stool of individuals with CI compared to HC. Although these metals are not typically included in standard panels of neurotoxic agents, evidence suggests that exposure to certain trace elements, even at low concentrations, may contribute to neurodegeneration (Budi et al., 2022).

Strużyńska, Skalska., (2018)) reported that Ag nanoparticles can cross the blood-brain barrier and accumulate in the brain, leading to neurotoxic effects such as oxidative stress, inflammation, and neuronal damage. These effects can contribute to the cognitive decline observed in CI patients. More recently, Gong et al. (2025) demonstrated that these nanoparticles could alter the GMB composition, potentially disrupting the gut-brain axis and exacerbating neuroinflammation. In our study, the elevated fecal concentrations of Ag in CI participants may reflect clearance mechanisms aimed at minimizing systemic damage, a hypothesis primarily supported by animal studies and yet to be confirmed in humans (Park et al., 2011).

For Li, the current findings reflect an increased intestinal excretion, which may indicate reduced systemic and cerebral bioavailability. Recently, decreased endogenous Li levels have been reported in the prefrontal cortex of individuals with aMCI and AD, suggesting that Li deficiency could be an early pathogenic event in both diseases (Aron et al., 2025). We therefore hypothesize that the elevated fecal Li levels we observed may reflect a systemic dysregulation of Li homeostasis, potentially leading to a functional deficiency in the brain. Experimental models further demonstrated that Li deficiency promotes amyloid-β deposition, tau phosphorylation, synaptic dysfunction, and cognitive decline, whereas Li supplementation exerts neuroprotective effects (Aron et al., 2025; Shen et al., 2024). Beyond its direct effects on the brain, Li has been shown to modulate GMB composition and short-chain fatty acid production, thereby influencing gut–brain axis communication and neuroinflammatory responses (Ortega et al., 2023). Taken together, these observations raise the possibility that the elevated fecal Li concentrations may reflect a state of systemic deficiency, with potential implications for both gut–brain axis dynamics and neurodegenerative processes.

On the other hand, elevated fecal concentrations of Pt may reflect a protective mechanism against systemic and neural toxicity. Although Pt is not an essential element, both environmental and therapeutic exposures, particularly from chemotherapeutic agents, can lead to systemic absorption and accumulation. Studies in rats have shown that Pt compounds can cross the blood–brain barrier, induce oxidative stress, trigger

Table 4
Metal determination in our cohort by cognitive impairment diagnosis (N = 60).

Element	DF (n (%))	Median (P25-P75)[a]		p-value	p-value corrected[b]
		HC	CI		
Ag	36 (60)	$5.00e^{-4}$ ($5.00e^{-4}$ – 4.32)	78.8 (25.3 – 108)	**< .001**	**0.023**
Al	56 (93)	5.43 (3.01 – 15.1)	7.48 (4.00 – 9.57)	0.706	1.000
As	1 (2)	ND	33.2 (33.2 – 33.2)	-	-
Ba	59 (98)	2.10 (1.26 – 4.33)	2.72 (0.924 – 3.93)	0.832	1.000
Cd	60 (100)	33.5 (14.5 – 67.8)	31.4 (15.7 – 70.2)	0.686	1.000
Ce	49 (82)	7.35 (2.53 – 18.2)	9.09 (2.08 – 14.5)	0.970	1.000
Co	58 (97)	53.5 (13.2 – 135)	64.5 (38.0 – 130)	0.404	1.000
Cs	60 (100)	5.75 (3.94 – 8.91)	6.88 (4.09 – 9.59)	0.775	1.000
Cu	60 (100)	3.05 (2.05 – 5.49)	3.42 (1.90 – 4.56)	0.959	1.000
Fe	51 (85)	20.9 (10.5 – 41.0)	41.8 (18.4 – 55.3)	0.063	1.000
Hg	59 (98)	9.39 (3.85 – 22.4)	12.4 (6.30 – 30.9)	0.109	1.000
La	57 (95)	5.62 (2.74 – 11.7)	6.08 (2.75 – 12.5)	0.877	1.000
Li	53 (88)	3.65 (0.308 – 7.63)	15.5 (8.31 – 26.7)	**< .001**	**0.023**
Mg	60 (100)	741 (416 – 1417)	708 (384 – 923)	0.381	1.000
Mn	60 (100)	7.44 (2.83 – 10.8)	6.77 (3.41 – 9.86)	0.901	1.000
Pb	37 (62)	$2.39e^{-4}$ ($2.39e^{-4}$ – 37.2)	21.6 (2.19 – 39.7)	**0.017**	0.340
Pt	37 (62)	0.002 (0.002 – 0.208)	0.658 (0.200 – 1.06)	**< .001**	**0.023**
Rb	60 (100)	1.61 (1.08 – 2.72)	2.13 (0.975 – 2.83)	0.562	1.000
Sb	46 (77)	1.65 ($4.88e^{-4}$ – 3.10)	2.88 (0.936 – 6.36)	0.067	1.000
Se	0 (0)	ND	ND	-	-
Sn	60 (100)	0.075 (0.012 – 0.469)	0.041 (0.021 – 0.076)	0.467	1.000
Sr	60 (100)	7.03 (3.32 – 11.2)	6.55 (3.15 – 9.75)	0.686	1.000
Tl	60 (100)	0.797 (0.488 – 1.71)	1.39 (0.811 – 2.01)	0.130	1.000
U	60 (100)	13.5 (5.83 – 25.3)	9.37 (5.77 – 18.3)	0.213	1.000
Zn	60 (100)	21.9 (10.7 – 28.7)	19.4 (9.96 – 31.1)	0.971	1.000

[a] Values are expressed as ng/g for Ag, As, Cd, Ce, Co, Cs, Hg, La, Li, Pb, Pt, Sb, Tl and U, and as µg/g for Al, Ba, Cu, Fe, Mg, Mn, Rb, Se, Sn, Sr and Zn.
[b]Corrected using the Holm–Bonferroni method (Holm, 1979).
Displayed as median (IQR) for continuous data, and as count (percentage) for categorical data. Ag: silver, Al: aluminum, As: arsenic, Ba: barium, Cd: cadmium, Ce: cerium, CI: Cognitive Impairment, Co: cobalt, Cs: cesium, Cu: copper, DF: Detection Frequency, Fe: iron, HC: Healthy Controls, Hg: mercury, La: lanthanum, Li: lithium, Mg: magnesium, Mn: manganese, ND: Not detected, Pb: lead, Pt: platinum, Rb: rubidium, Sb: antimony, Se: selenium, Sn: tin, Sr: strontium, Tl: thallium, U: uranium, Zn: zinc.

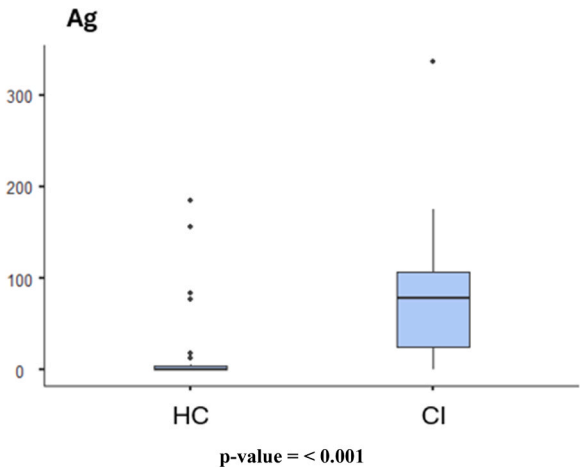


Fig. 1. Silver (Ag) levels (ng/g) in our cohort.

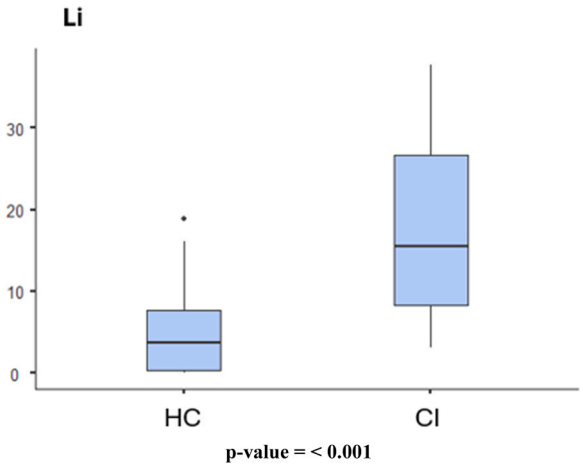


Fig. 2. Lithium (Li) levels (ng/g) in our cohort.

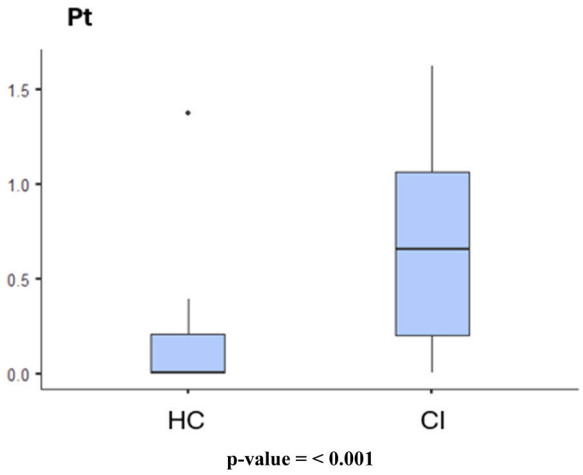


Fig. 3. Platinum (Pt) levels (ng/g) in our cohort.

neuroinflammation, and impair cognitive function (Alhowail, 2025; Kanat et al., 2017). While these findings support a possible neurotoxic role of Pt, further studies are needed to fully elucidate its relevance in humans.

Finally, studies integrating metals, GMB, and cognitive function are limited and controversial. Cd exposure has been linked to reductions in anaerobes and *Lactobacillus* and increases in Lachnospiraceae, potentially promoting gut inflammation (Guo et al., 2022). In turn, Fe supplementation has been associated with a decrease in short-chain fatty acid-producing bacteria, such as *Firmicutes* and *Faecalibacterium prausnitzii*, which may alter gut–brain signaling (Ahmed et al., 2020). However, Pb exposure has been linked to increased α-diversity and elevated

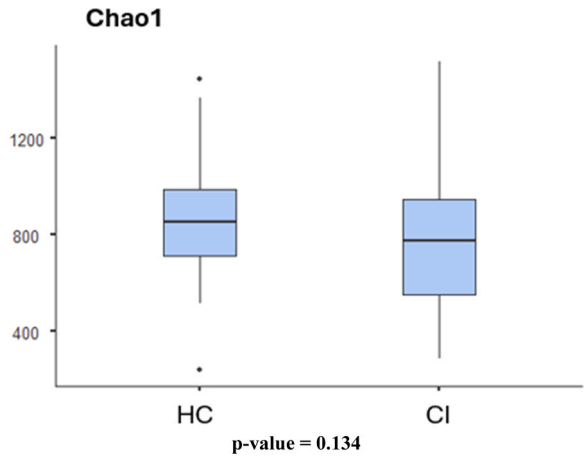


Fig. 4. Microbial richness values expressed as the Chao1 index in our cohort.

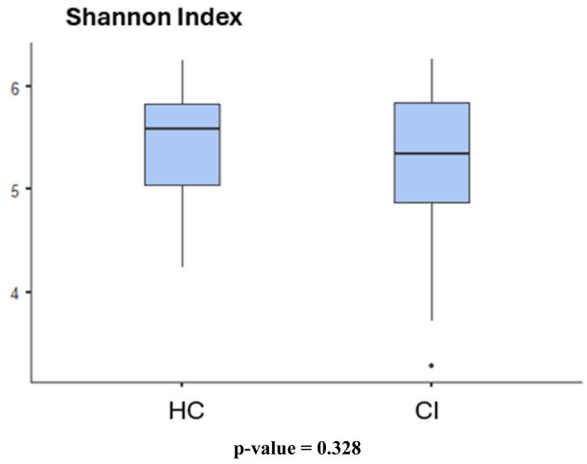


Fig. 5. Alpha-diversity values assessed using the Shannon index in our cohort.

Proteobacteria, which could disrupt microbial balance and influence neuroinflammation (Eggers et al., 2019). While these studies highlight the potential for metal-GMB interactions, particularly for metals like Cd, Fe, and Pb, in our pilot study, no significant associations were observed between metal concentrations, gut microbiota, and cognitive outcomes. This absence of associations may again be due to the limited sample size or the heterogeneous clinical stages of participants. Overall, these findings highlight the heterogeneous and context-dependent effects of metals on GMB, suggesting a possible contribution to cognitive impairment, although the underlying mechanisms remain poorly understood.

4.1. Strengths and limitations

A key strength of the present study lies in its multidimensional novel approach: combining microbiota sequencing, metal quantification, and cognitive, neuropsychologic, neuropsychiatric, and functional assessment. This integrative perspective allows us to explore potentially synergistic relationships that are rarely examined together. Importantly, this approach provides a foundation for future studies aimed at identifying early biomarkers of cognitive decline, which could inform early diagnosis, risk stratification, and potential interventions targeting both GMB and metal exposure.

Despite its strengths, the present study has several limitations. First, the sample size was small, which may have limited the detection of subtle associations between metals, microbiota, and cognitive outcomes. However, this small sample size is explained by the pilot nature of the study. Second, the CI group included participants at different stages of CI, introducing heterogeneity that may have masked group-level differences. Third, although this was not the case for most participants, some HC and CI individuals were relatives, which may introduce shared environmental factors that could partially influence microbial biodiversity. Finally, while fecal metal concentrations provide valuable insight into intestinal excretion, they do not fully reflect systemic or cerebral metal burden, which may be more directly relevant to neurodegenerative processes; this limitation could be addressed in future studies by assessing metal levels in additional biological matrices. Although the study offers promising preliminary insights, further research with larger, more homogeneous cohorts are needed to assess the clinical utility of integrating microbiota and metal measurements for early diagnosis, risk stratification, and potential interventions in CI.

5. Conclusions

Contrary to our initial hypothesis, no significant differences in microbial richness, alpha-diversity, or beta-diversity were observed between groups. However, CI participants exhibited higher fecal levels of Ag, Li, and Pt compared with HC, suggesting potential alterations in intestinal metal handling. These differences may reflect changes in metal absorption, excretion, or host–microbiome interactions, although differences in exposure between groups cannot be excluded. Overall, these findings underscore the complexity and heterogeneity of the relationships between metals, GMB, and cognitive status. Larger, longitudinal studies will be necessary to determine whether these associations are causal, to clarify underlying mechanisms, and to assess their potential relevance as early biomarkers of cognitive decline.

Funding

This research was supported by the Spanish "Ministerio de Ciencia e Innovación and Agencia Estatal de Investigación" through the project DEM-BIOTA (PID2019–103888RB-I00) and the Agència de Gestió d'Ajuts Universitaris i de Recerca through the pre-doctoral grant (FI_B00314) funded by AGAUR. Montse Marquès acknowledges support from the Ramón y Cajal fellowship (RYC2022–038179-I), funded by the Agencia Estatal de Investigación (AEI-MICIU).

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used GPT-5 mini in order to improve translation accuracy and enhance the readability of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the

published article.

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.

Acknowledgements

The authors appreciate the collaboration of the volunteers and healthcare staff from the participating hospitals. Without them, this type of study would not be possible.

We would also like to thank the Centre for Omic Sciences (COS), Mixed Unit of Rovira i Virgili University and Eurecat, considered Singular Scientific and Technical Infrastructure (ICTS), for the metagenomic analysis of the samples studied.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2026.103390.

Data availability

Data will be made available on request.

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