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Association between urinary toxic and essential metals exposure and autism in children

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A B S T R A C T

Autism has a multifactorial origin, with genetic factors playing a significant role, while various environmental factors, including early exposure to toxic or essential metals, have been associated with an increased likelihood in children. The current study analysed the association between 15 different essential and heavy metals and autism among Spanish school children. Urine samples from a total of 108 children (autism group: N = 42; non-autism group: N = 66) between the ages of 5–16 years were analysed. Logistic regressions, adjusted for sex, age, BMI, SES, and diet quality, were performed using a categorical comparison of metal concentrations in quartiles, with the first quartile as a reference. Significant associations were found in the fourth quartile for copper (Cu) (aOR 9.45; CI: 1.89–48.02), third quartile for lead (Pb) (aOR 5.59; CI 1.36–22.94) and in the 75th percentile for manganese (Mn) (aOR 12.50; CI 3.27–47.72). Furthermore, Mn exposure was associated with more pronounced differences in social cognition, as measured by the Social Responsiveness Scale-2nd edition ($\beta = 22.04$, $p = .038$). However, no significant associations were found with other essential or heavy metals. This study provides significant insights into the association between metal exposure and autism in school-aged children, emphasising the need for further research to better understand and mitigate the impact of environmental influences on children's health and development.

Introduction

Autism is an early-onset, lifelong neurodevelopmental condition with an estimated prevalence of at least 1 % globally and 1.5 % in the Spanish population (Morales-Hidalgo et al., 2021; Zeidan et al., 2022). Although it is a heterogeneous condition, its core traits mainly involve differences in social interaction and communication as well as restrictive and repetitive behaviour patterns throughout a child's development (APA, 2022). During early development, the underlying factors contributing to autism include not only genetics

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but also environmental factors such as metal exposure, primarily through contaminated water and food (Heyer & Meredith, 2017; Tartaglione et al., 2024). Heavy metal exposures and poor diet can impact epigenetic factors related to the epidemiological increase of autism (Dufault et al., 2023). Due to underdeveloped metabolic pathways and immature detoxification systems, children are considered the most vulnerable population (Carroquino et al., 2012; Spungen, 2019).

Previous studies have investigated the role of heavy or essential metals during childhood and their association with autism. Higher concentrations of cadmium (Cd), lead (Pb), mercury (Hg) and arsenic (As) have been found in urine and hair samples from autistic children compared to a group without autism (Amadi et al., 2022; Rafi'i et al., 2024). The results of a systematic review show that the majority of studies found an association between elevated Hg levels and autism, particularly in those exposed to a polluted environment in the postnatal period (Netto et al., 2024). In addition, Hawari et al. (2020) suggested that a higher ratio of lead (Pb) to manganese (Mn) may be associated with an increased likelihood of autism. However, although Mn is an essential element for cognitive development, excessive exposure to it can be toxic and may affect cognitive processes. This has been linked to neurodevelopmental differences, including attention deficit hyperactivity disorder (ADHD) and autism (Aschner et al., 2024; Rahbar et al., 2021). Similarly, copper (Cu) plays a vital role in various physiological processes, including cell growth and development, but there is conflicting data regarding its levels. In this sense, some authors (Liu et al., 2023b; Ouisselsat et al., 2023) found an inverse association between hair Cu levels and autism, while other studies have found elevated Cu levels in autistic children compared to those without autism (Behl et al., 2020; Feng et al., 2023; Li et al., 2014). According to the Agency for Toxic Substances and Disease Registry (ATSDR, 2023), even some essential metals like zinc (Zn), when found in excess, can be harmful to health. Hence, a study by Rezaei et al. (2022) found higher levels of Zn in children diagnosed with autism.

Assessing exposure to environmental pollutants during childhood is crucial when studying autism for several reasons. First, childhood represents a period of rapid brain development, during which the cumulative effects of toxic exposures can have profound and lasting impacts (Nelson & Gabard-Durnam, 2020; Tartaglione et al., 2024). Many pollutants, such as heavy metals and endocrine-disrupting chemicals, are not fully eliminated from the body and can accumulate over time, leading to long-term biological changes (Liu et al., 2023a). These changes may not only contribute to the likelihood of autism diagnosis but also influence the intensity and manifestation of the core traits associated with the condition (Błażewicz & Grabrucker, 2022). Moreover, the diverse characteristics of autism, which often include a spectrum of cognitive and behavioural differences, might be intensified by ongoing exposure to metals (Blaurock-Busch et al., 2011; Błażewicz et al., 2022). Therefore, analysing the connection between early-life metal exposure and the intensity of autistic traits could provide valuable insights for better understanding the condition and its prognosis.

Although earlier studies have investigated the relationship between metals and autism, there remains a gap in comprehensive research that considers a wide range of heavy and essential metals with both autism diagnosis and the intensity of its core traits in

Table 1
Sociodemographic and clinical characteristics of children by diagnosis group.

Variables Sociodemographic and cognitive		Non-autism (N = 66) *n (%)*	Autism (N = 42) *n (%)*	*p*
Sex	Male	35 (53.0)	35 (83.3)	**.001**
	Female	31 (47.0)	7 (16.7)	
Ethnicity	Spain	60 (90.9)	41(97.6)	.167
	Other	6 (9.1)	1 (2.4)	
SES	Low	2 (3.2)	2 (4.8)	.317
	Medium	43 (69.4)	23 (54.8)	
	High	17 (27.4)	17 (40.5)	
		M ± ***SD***	***M*** ± ***SD***	
Age (years)		10.48 ± 1.07	9.62 ± 2.79	**.045**
BMI (kg/cm)		19.05 ± 3.70	18.46 ± 4.80	.145
SDQI		62.92 ± 7.01	59.73 ± 5.46	**.005**
IQ		110.77 ± 14.37	101.67 ± 19.98	.072
ADI-R		***M*** ± ***SD***	***M*** ± ***SD***	
Social interaction		1.93 ± 2.39	17.67 ± 7.74	< .001
Social communication		2.15 ± 2.43	11.67 ± 4.41	< .001
Repetitive behaviour		0.61 ± 0.96	5.67 ± 3.83	< .001
ADOS−2		***M*** ± ***SD***	***M*** ± ***SD***	
Social communication		1.00 ± 1.44	7.71 ± 2.28	< .001
Repetitive behaviour		.04 ± .19	1.71 ± 1.49	< .001
Total score		1.04 ± 1.51	9.43 ± 2.99	< .001
Social Responsiveness Scale−2		***M*** ± ***SD***	***M*** ± ***SD***	
Social awareness		-	64.10 ± 10.68	
Social cognition		-	71.42 ± 10.77	
Social communication		-	69.21 ± 12.67	
Social motivation		-	68.07 ± 11.19	
Restricted repetitive behaviours		-	73.16 ± 11.13	
SRS−2 total score		-	72.32 ± 11.01	

Chi-square tests were performed for categorical variables, whereas the Mann-Whitney *U* test was used for quantitative variables. Significant p values are highlighted in bold ($p < .005$). Abbreviations: ADI-R: Autism Diagnostic Interview-Revised; ADOS-2: Autism Diagnostic Observation Schedule, second edition; SES: socio-economic status; BMI: body mass index; SDQI: Spanish diet quality index; IQ: Intelligence quotient.

children. Therefore, our exploratory study aims to systematically examine the potential association between autism and the presence of toxic or essential metals individually, taking into account sociodemographic (sex, age, socioeconomic status) and nutritional (quality of diet and Body Mass Index) variables that can affect this association, as well as explore the association between these metal and core symptoms of autism.

Methods

Participants and the diagnosis procedure

The total sample comprised 108 children divided into the autism group (N = 42; mean age = 9 years) and the non-autism group (N = 66; mean age = 10 years), which included children with no diagnosis of ADHD or autism. Additional participant details are provided in the results section, Table 1. The DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition) criteria were used by psychologists and psychiatrists involved in the study for the diagnosis. The assessment procedure was comprehensive, with only the instruments used in the current study described below. Autism characteristics were assessed using the Autism Diagnostic Interview-Revised (ADI-R; Rutter et al., 2003) and the Autism Diagnostic Observation Schedule, second edition (ADOS-2; Lord et al., 2012), and administered to the parents and children, respectively. Parents in the autism group further completed the Social Responsiveness Scale–2nd edition (SRS-2) to provide information about the intensity of autistic characteristics. The Wechsler Intelligence Scales for Children (WPPSI-IV, WISC-IV or WISC-V) (Wechsler, 2005, 2014) were utilised to assess children's Intelligence Quotient (IQ).

Participants were recruited from the EPINED and PRONED projects, two initiatives focused on autism and ADHD in school-aged populations. The EPINED (Epidemiological Research Project on Neurodevelopmental Disorders) project was a two-phase cross-sectional epidemiology study conducted among a representative sample of the school population in Tarragona province, Spain, between 2015 - 2022. Detailed procedures and protocols for autism and ADHD assessments were previously outlined by Morales-Hidalgo et al. (2021) and Canals Sans et al. (2021). Ethical approval for the study was granted by the Reus Sant Joan University Hospital Ethics Committee (13–10-31/10proj5). Urine samples used in the present study were collected from the participating children during the second phase of EPINED, which involved individual evaluations of the children and their families. On the other hand, the PRONED project (Nutritional Intervention in Children with Autism and/or ADHD: Randomized Controlled Trial with Probiotics), conducted between 2019 - 2021, was a controlled, double-blind, and randomized trial using dietary supplements, registered under ClinicalTrials.gov Identifier: NCT05167110. Participants were children from Tarragona province diagnosed with autism and/or ADHD, recruited from both specialized neurodevelopmental clinics and the previously described project. Probability sampling was employed to ensure that every individual in the target population had an equal chance of being selected, thereby enhancing the external validity and representativeness of the sample. The urine samples used in this study were obtained during the PRONED pre-intervention phase. Both studies were conducted by the same research team using consistent data collection protocols, ensuring reliability and consistency throughout.

Urine collection and analysis procedure

Urine samples from the children were collected by their parents early in the morning using 12 ml transparent polystyrene tubes and kept refrigerated until delivery to the lab. At the research laboratory, the samples were centrifuged at 600 g for 10 min at 4 °C. A 1 ml sterile screw-cap cryotube was used to transfer the supernatant, which was stored at −80 °C until delivery to a specialised laboratory for metals detection and urine creatinine concentration analysis. Following heavy and essential metals were detected from the urine samples: antimony (Sb), arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and nickel (Ni) as well as essential trace metals including cobalt (Co), copper (Cu), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se), vanadium (V), and zinc (Zn).

Urine samples were diluted 1:10 in ultrapure water (Milli-Q, Merck, Darmstadt, Germany) containing 2% HNO_3 (nitric acid; Merck) and 1% HCl (hydrochloric acid; Merck) using appropriate metal standard solutions (Agilent Technologies, Santa Clara, CA, USA). Multi-element analyses were conducted using an Agilent 8900 triple quadrupole ICP mass spectrometer (ICP-MS; Agilent Technologies). Before analysis, the instrument was tuned, and performance parameters were verified. To ensure quality, a multi-element 400 μg/L internal standard solution containing Sc, Ge, Ir, and Rh was added online to the samples (ISC Science, Oviedo, Spain). Additionally, a certified reference material, Seronorm Trace Elements Urine L-1 (reference 210605, Sero, Billingstad, Norway), along with a blank and an intermediate calibration standard, was reanalyzed every 12 samples to maintain accuracy. The National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) Trace Elements in Natural Water Standard Reference Material SRM 1640a was also used as a certified reference material and analyzed at the beginning and end of each sequence. The limits of detection (LOD) in units of μg/L for each metal were as follows: Pb – 0.08, As – 0.5, Hg – 0.05, Cd – 0.03, Cr – 0.2, Cu – 0.9, Co – 0.005, Sb – 0.03, Zn – 4, Se – 1, Mg – 5, V – 3, Mn – 0.05, Ni – 0.4, and Mo – 0.03. All metals were assessed against their respective detection limits, with LOD/2 used as a threshold where applicable. For Pb, Mn, Sb and V, values falling below the LOD/2 were identified, and in these cases, imputed values were assigned using LOD/2. This approach was taken to maintain data integrity and limit imputations. Urinary creatinine levels were measured using the ADVIA 1800 Chemistry System and the ADVIA 2400 Chemistry System (Warren et al., 2014). To account for variation due to urine dilution, heavy metal concentrations were adjusted by dividing by their corresponding creatinine levels (mg/dL).

Covariates

Covariates were selected for inclusion in the statistical analyses as potential confounders based on published literature, biological plausibility, sex, and other descriptive data obtained from parents' responses to a sociodemographic questionnaire, (Bauer et al., 2020; Hashemi et al., 2023; Y. Li et al., 2020; Lozano et al., 2023). Directed Acyclic Graph (DAG) demonstrating the associations between exposure, outcome, and covariates can be found in supplementary figure 1. Several nutritional instruments were utilized to assess dietary quality and potential dietary confounders in the study. The Spanish Diet Quality Index (SDQI) for children was derived from the Food Consumption Frequency Questionnaire (FCFQ), which was validated in the Spanish population and answered by parents (Trinidad Rodríguez et al., 2008). The SDQI (Norte Navarro & Ortiz Moncada, 2011) is scored on a scale from 0 to 100 with the following cut-off points: ≥ 80 indicates a "healthy diet", 50–79 indicates a diet that "needs improvement", and ≤ 49 indicates an "unhealthy diet".

Statistical analysis

The Shapiro-Wilk test indicated that the data were non-parametric; therefore, the Mann-Whitney *U* test was conducted to compare heavy metal differences between the two groups. Significant heavy metals were stratified into quartiles or percentiles, with the first quartile (the lowest) used as a reference. Mn and Mg were stratified into < 50th, 75–50th, and > 75th percentiles and V was stratified into < 90 and > 90 percentiles, due to values close to LOD/2. Unadjusted and adjusted (for sex, age, BMI, SES and SDQI) logistic regressions were performed to analyse the association between metals and autism, reporting odds ratios (ORs) with 95% confidence intervals (CI) examined at $p < 0.05$. Finally, a multiple linear regression was performed to explore the relationship between SRS-2 total and subscales scores. Given that SRS-2 scores were only available for the autism group (n = 42), metals were analyzed as continuous metal levels (without quartile stratification) to preserve the statistical power. Statistical analyses were conducted using SPSS IBM (version 29).

Community involvement

Regarding community involvement, the school coordinators provided us with the necessary resources to assess the students and their families at their centres. Additionally, the teachers played an active role in encouraging maximum family participation in the project.

Results

Sample demographics and metal distribution

Table 1 presents the descriptive data of the sample. The children's ages ranged from 5 to 16 years, with a predominance of males in the autism group (83.3%). Most children were from medium SES families (63.5%) and Spanish ethnicity (93.5%). Although the diet quality (SDQI) of the non-autism group was better than those of children diagnosed with autism, both groups fell within the "need for improvement" range. Intelligence quotient levels were within the average range in both groups. Children with autism were in the range of the "autism" category according to the ADOS-2 criteria.

Table 2
Urinary heavy metals association between diagnosis groups.

Metals (μg/l)	Non-autism group (N = 66) Median ± SD	Autism group (N = 42) Median ± SD	*p*
Pb	.18 ± 0.30	0.22 ± .14	.799
V	1.07 ± .67	1.20 ± 1.15	.885
Cr	.35 ± .24	.32 ± .43	.453
Mn	.04 ± .09	.13 ± .18	**< .001**
Cd	.04 ± .05	.06 ± .03	0.52
Sb	.02 ± .08	.04 ± .23	.060
Hg	.35 ± .41	.30 ± .49	.950
Co	.40 ± 1.23	.44 ± .53	.614
Cu	3.92 ± 3.99	5.58 ± 4.03	**< .001**
Ni	2.47 ± 2.08	2.55 ± 1.24	.777
Mo	43.97 ± 31.21	52.34 ± 33.97	.465
Se	30.78 ± 14.20	33.16 ± 14.58	.821
As	20.41 ± 56.12	22.32 ± 116.99	.668
Zn	408.50 ± 325.19	486.63 ± 252.12	**.023**
Mg	11.52 ± 3.05 (x 10^4)	11.55 ± 3.98 (x 10^4)	.512

Significant *p* values are highlighted in bold ($p < .05$).
Abbreviations: Pb: Lead, V: Vanadium, Cr: Chromium, Mn: Manganese, Cd: Cadmium, Sb: Antimony, Hg: Mercury, Co: Cobalt, Cu: Copper, Ni: Nickel, Mo: Molybdenum, Se: Selenium, As: Arsenic, Zn: Zinc, Mg: Magnesium.

Table 2 presents metal concentration differences between the two groups. Only Cu, Mn, and Zn were significantly different and higher in children within the autism group compared to those in the non-autism group. Spearman correlation between heavy metals showed that the highest correlations involved Cu, Cd, and Mn (0.51 – 0.59) (see Supplementary Figure 2).

Logistic regressions (unadjusted and adjusted models) were performed for all 15 metals (see Supplementary Table 1). Fig. 1 shows significant metals results, where a positive linear association was found for Cu and Mn; and non-linear for Pb with the presence of autism. Children with higher Cu levels (4th quartile) aOR of 9.45 (CI: 1.89, 48.02) have times greater odds of being diagnosed with autism. For Mn, the highest for autism diagnosis was observed above the 75th percentile with an aOR of 12.50 (CI: 3.27, 47.72). For Pb, children in the 3rd quartile had aOR of 5.59 (CI: 1.36, 22.94) for autism diagnosis. In contrast, no significant associations were observed for Zn after adjusting for covariates. Furthermore, age as a covariate was not significant, whereas both the SDQI (indicating the lowest diet quality) and male sex were significant. As a result, 'metal*sex' and 'metal*diet' interactions were tested as covariates, but neither interaction was significant. This suggests that sex, age and diet are independently associated with an autism diagnosis, rather than interacting with metal exposure.

Additionally, multiple regression models exploring the association between heavy metals and SRS-2 scores (total and subscales) in the autism group revealed an association only between Mn and greater intensity of social cognition differences ($\beta = 24.28$, CI: 1.39, 47.18, $p = .038$). No significant associations were observed for the remaining subscales or metals. Supplementary Table 2 presents all 15 metals βs with SRS-2 scores.

Discussion

The current study explored the association between exposure to heavy and essential metals and the likelihood of autism in children. Metal exposure was assessed through urine analysis, which reflects both recent and accumulated exposure. Only Cu, Mn and Pb were found to be significantly associated with autism. Additionally, higher levels of Mn were linked to greater intensity of social cognition differences, as indicated by the SRS-2 subscale score.

Copper is considered an essential element for biological development, playing a critical role in the central nervous system, particularly in neuronal function and nerve signal transmission. However, elevated Cu levels in the brain can lead to motor deficits and a slight decrease in dopamine concentration in certain brain regions (Bulcke et al., 2017; Lutsenko et al., 2019). In our study, we observed that the likelihood of an autism diagnosis increased with exposure to Cu. While some studies on prenatal exposure to pollutants have suggested a non-monotonic relationship between Cu and ADHD in children, this association does not extend to autism (Skogheim et al., 2021; Zebbiche et al., 2024). In contrast, postnatal studies have shown that elevated Cu levels are associated with autism in children (Russo & deVito, 2011; Siddiqi et al., 2023; Tinkov et al., 2019). Conversely, additional research has found lower levels of Cu in hair among autistic children compared to those without any diagnosis (Liu et al., 2023b; Ouisselsat et al., 2023) and reported no differences in blood Cu levels (Liu et al., 2023b).

Manganese, with its biphasic dose-response nature, is considered essential yet neurotoxic when excessive levels accumulate in the basal ganglia of the brain, disrupting cognitive and motor functions. (Ramírez-Ayala & Azcona-Cruz, 2017; Vollet et al., 2016). The

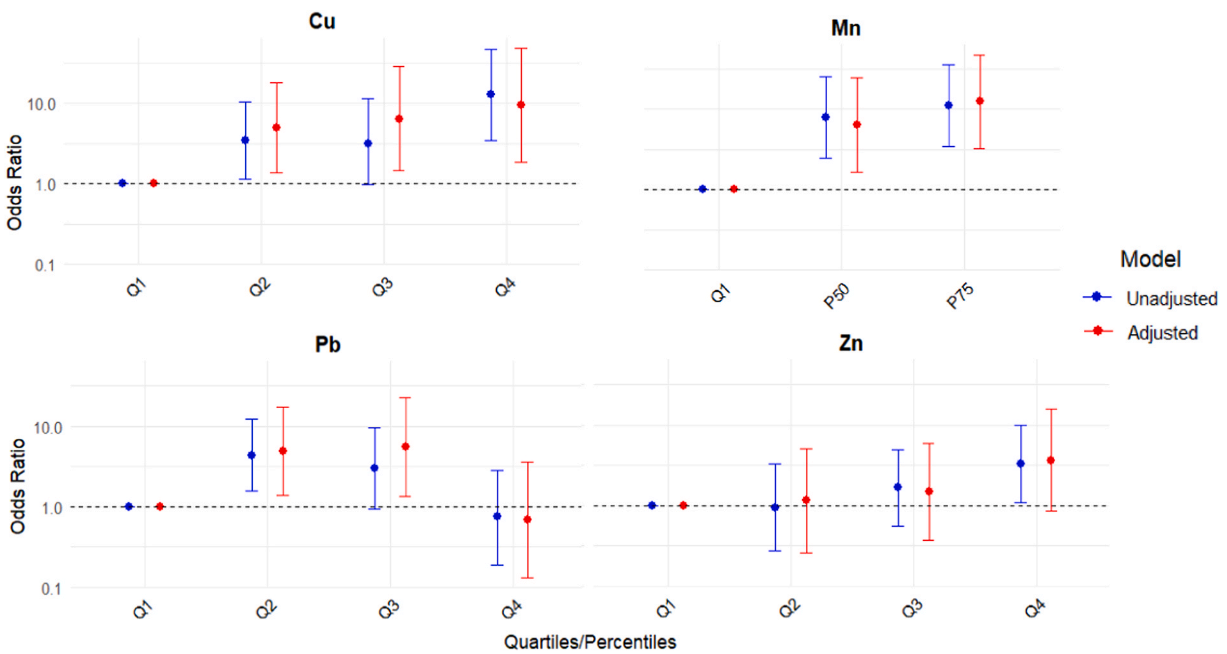

Fig. 1. Adjusted and unadjusted logistic regression ORs graphs for the autism vs non-autism groups.

primary source of Mn exposure is food, specifically through beans, nuts, grain or other Mn-containing supplements (Ramírez-Ayala & Azcona-Cruz, 2017). Our study found an association between Mn exposure and the likelihood of autism diagnosis, along with increased social cognition differences in the current assessment, in line with the results of Rossignol et al. (2014). Similarly, previous research has indicated that high Mn exposure may influence neurodevelopment in children (Freire et al., 2018; Mora et al., 2018). Some authors, conversely, have observed lower Mn levels in autistic children when compared to those without the condition (Hawari et al., 2020; Al-Ayadhi, 2005).

Lead is one of the toxic metals that not only poses significant risks to physical health but also adversely affects children's neurodevelopment by inducing oxidative stress, neuroinflammation and DNA methylation (Amadi et al., 2022; Awadh et al., 2023; Błażewicz & Grabrucker, 2022). The primary sources of Pb exposure include environmental contamination from anthropogenic emissions, ingestion of contaminated food or drinking water (particularly through Pb-piped systems), as well as exposure to Pb in soil, dust, and Pb-based paint (ATSDR, 2023). In our study, we have found a non-linear association between Pb and diagnosis of autism in children, however, this association is only observed until the third quartile, whereas in the fourth, we did not obtain significant results. This could be due to Pb's toxic nature, which may exert harmful effects regardless of exposure levels (Vrijheid et al., 2016). Our results are similar to the previous research that demonstrates Pb exposure is associated with autism diagnosis (Amadi et al., 2022; Nabgha-e-Amen et al., 2020; Rafi'i et al., 2024; Stojsavljević et al., 2023). While our results contribute to the growing body of evidence on Pb's neurotoxic effects, further research is needed to account for additional environmental factors that may influence autism development in children.

Zinc, an essential trace element for human biology and brain development, is found in soil, water, air, and various foods. However, according to the Agency for Toxic Substances and Disease Registry (ATSDR, 2023), consuming excessive amounts of Zn can negatively impact health. Our research did not identify any association between Zn levels and autism in children. Similarly, a systematic review by do Nascimento et al. (2023) reported no significant difference in Zn levels between autistic children and adolescents and those without autism, although they found lower Zn concentrations than us in the autism group. Conversely, other studies, such as Rezaei et al. (2022), suggested an association with higher Zn levels in autistic children compared to those without. These conflicting results highlight the need for further investigation to clarify the relationship between Zn and autism.

In the context of the covariates introduced in the multivariable models, our analysis did not find a significant interaction between sex and exposure to metals with autism, unlike what was suggested by Gade et al. (2021) and Roberts et al. (2013). This discrepancy may be due to our smaller sample size and the lower proportion of girls compared to boys in our study. Similarly, we did not observe a significant interaction between metal exposure and diet, contrary to suggestions by other authors (Dufault et al., 2023). However, it is important to note that certain metals, whether introduced through diet or other environmental sources, have the potential to alter the gut microbiota, as highlighted by Chouari et al. (2024). The gut microbiota plays a crucial role in overall health, including brain function, and its disruption could potentially intensify traits associated with autism (Fattorusso et al., 2019). Moreover, autistic children often have impaired metabolism and detoxification processes, which can make them more susceptible to the effects of toxins. This may result in higher levels of toxins in the blood rather than in the urine (Netto et al., 2024). Based on the results of this study, it is recommended that future research take these factors into account to better understand the additional layer of complexity that metal exposure, along with sex and diet, may add to the likelihood and course of autism.

Strengths and limitations

One of the main strengths of our study is that we analysed the urine levels of a broad range of essential and heavy metals in the diagnosis of autism in school-age children, in comparison with children without this condition. The findings of this study are based on a smaller sample size of autistic children with mild intensity of autistic traits, as indicated by ADOS-2 scores, mainstream school attendance, and IQ scores. Consequently, these results are not generalizable to children with more intense autistic traits or higher support needs. The cross-sectional nature of this study limits our ability to establish causality between metal exposure and autism, unlike studies that have evaluated exposure during prenatal stages or early neurodevelopmental periods. Additionally, our analysis did not consider various potential factors related to autism, such as genetic influences or other environmental pollutants beyond metals. Moreover, while urine is a commonly used biomarker for metal exposure, it may not adequately reflect long-term exposure for most metals, except for cadmium, which has a longer biological half-life (ATSDR, 2023). The presence of heavy metals in the food supply is particularly concerning for autistic children, as epigenetic interactions may affect the metabolism and excretion of these metals, resulting in higher blood levels and potentially intensifying cognitive and social differences (Dufault et al., 2021; Netto et al., 2024). However, further longitudinal studies are required to better understand the directionality of these associations and whether these metal levels could be influenced by the condition itself. Therefore, the results should be interpreted with caution, and future research should explore multiple exposure models such as Bayesian Kernel Machine regression (BKMR) or g-computation, which can be valuable for assessing the combined effects of the essential and non-essential metals. Additionally, using different biomarkers is recommended to better understand the association between toxic or essential metals and the likelihood of autism in children.

Conclusion

The current study provides valuable insights into the association between metal exposure and autism in school-aged children. We observed significant associations between Cu, Mn and Pb exposure and the likelihood of autism diagnosis. Additionally, Mn exposure was linked to more pronounced social cognition differences. These findings underscore the complex interaction between environmental factors, such as metal exposure, and neurodevelopmental conditions. Further research using diverse biomarkers and longitudinal designs is essential to better understand and mitigate the impact of environmental influences on children's health and development.

CRediT authorship contribution statement

Kaur Sharanpreet: Writing – review & editing, Writing – original draft, Formal analysis. **Canals-Sans Josefa:** Writing – review & editing, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Morales-Hidalgo Paula:** Writing – review & editing, Supervision, Data curation. **Arija Victoria:** Writing – review & editing, Supervision.

Ethical approval and informed consent statements

Ethical approval for the study was granted by the Reus Sant Joan University Hospital Ethics Committee (13–10-31/10proj5). PRONED project was registered under ClinicalTrials.gov Identifier: NCT05167110. Informed consent was obtained from all individual participants included in the study.

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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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reia.2025.202616.

Data Availability

The data that has been used is confidential.

References

Agency for Toxic Substances and Disease Registry (ATSDR). (2023, September 14). *Agency for Toxic Substances and Disease Registry*. ⟨https://www.atsdr.cdc.gov/index.html⟩.

Al-Ayadhi, L. Y. (2005). Heavy metals and trace elements in hair samples of autistic children in central Saudi Arabia. *Neurosciences (Riyadh, Saudi Arabia), 10*(3), 213–218.

Amadi, C. N., Orish, C. N., Frazzoli, C., & Orisakwe, O. E. (2022). Association of autism with toxic metals: A systematic review of case-control studies. *Pharmacology, Biochemistry, and Behavior, 212*, Article 173313. https://doi.org/10.1016/j.pbb.2021.173313

APA. (2022). *Diagnostic and statistical manual of mental disorders (5th ed., text rev.)*.

Aschner, M., Martins, A. C., Oliveira-Paula, G. H., Skalny, A. V., Zaitseva, I. P., Bowman, A. B., Kirichuk, A. A., Santamaria, A., Tizabi, Y., & Tinkov, A. A. (2024). Manganese in autism spectrum disorder and attention deficit hyperactivity disorder: The state of the art. *Current Research in Toxicology, 6*, Article 100170. https://doi.org/10.1016/j.crtox.2024.100170

Awadh, S. M., Yaseen, Z. M., & Al-Suwaiyan, M. S. (2023). The role of environmental trace element toxicants on autism: A medical biogeochemistry perspective. *Ecotoxicology and Environmental Safety, 251*, Article 114561. https://doi.org/10.1016/j.ecoenv.2023.114561

Bauer, J. A., Fruh, V., Howe, C. G., White, R. F., & Henn, B. C. (2020). Associations of metals and neurodevelopment: A review of recent evidence on susceptibility factors. *Current Epidemiology Reports, 7*(4), 237–262. https://doi.org/10.1007/s40471-020-00249-y

Behl, S., Mehta, S., & Pandey, M. K. (2020). Abnormal Levels of Metal Micronutrients and Autism Spectrum Disorder: A Perspective Review. *Frontiers in Molecular Neuroscience, 13*. https://www.frontiersin.org/articles/10.3389/fnmol.2020.586209.

Blaurock-Busch, E., Amin, O. R., & Rabah, T. (2011). Heavy metals and trace elements in hair and urine of a sample of arab children with autistic spectrum disorder. *Maedica, 6*(4), 247–257.

Błażewicz, A., & Grabrucker, A. M. (2022). Metal profiles in autism spectrum disorders: A crosstalk between toxic and essential metals. *International Journal of Molecular Sciences, 24*(1), 308. https://doi.org/10.3390/ijms24010308

Błażewicz, A., Grywalska, E., Macek, P., Mertowska, P., Mertowski, S., Wojnicka, J., Durante, N., & Makarewicz, A. (2022). Research into the association of cadmium and manganese excretion with thyroid function and behavioral areas in adolescents with autism spectrum disorders. *Journal of Clinical Medicine, 11*(3), 579. https://doi.org/10.3390/jcm11030579

Bulcke, F., Dringen, R., & Scheiber, I. F. (2017). Neurotoxicity of Copper. In M. Aschner, & L. G. Costa (Eds.), *Neurotoxicity of Metals* (pp. 313–343). Springer International Publishing. https://doi.org/10.1007/978-3-319-60189-2_16.

Canals Sans, J., Morales Hidalgo, P., Roigé Castellví, J., Voltas Moreso, N., & Hernández Martínez, C. (2021). Prevalence and epidemiological characteristics of ADHD in pre-school and school age children in the province of Tarragona, Spain. *Journal of Attention Disorders, 25*(13), 1818–1833. https://doi.org/10.1177/1087054720938866

Carroquino, M. J., Posada, M., & Landrigan, P. J. (2012). Environmental toxicology: Children at risk. *Environmental Toxicology*, 239–291. https://doi.org/10.1007/978-1-4614-5764-0_11

Chouari, R., Loubna, L., Elarabi, F., & Bour, A. (2024). Environmental impact of the heavy metal intoxication on metabolic, physiological and nutritional profiles in children with autism spectrum disorder in Morocco. *Ecological Engineering & Environmental Technology, 25*(7), 150–161. https://doi.org/10.12912/27197050/188057

do Nascimento, P. K., da, S. B., Oliveira Silva, D. F., de Morais, T. L. S. A., & de Rezende, A. A. (2023). Zinc status and autism spectrum disorder in children and adolescents: A systematic review. *Nutrients, 15(16), Article*, 16. https://doi.org/10.3390/nu15163663

Dufault, R. J., Crider, R. A., Deth, R. C., Schnoll, R., Gilbert, S. G., Lukiw, W. J., & Hitt, A. L. (2023). Higher rates of autism and attention deficit/hyperactivity disorder in American children: Are food quality issues impacting epigenetic inheritance? *World Journal of Clinical Pediatrics, 12*(2), 25–37. https://doi.org/10.5409/wjcp.v12.i2.25

Dufault, R. J., Wolle, M. M., Kingston, H. M. S., Gilbert, S. G., & Murray, J. A. (2021). Connecting inorganic mercury and lead measurements in blood to dietary sources of exposure that may impact child development. *World Journal of Methodology, 11*(4), 144–159. https://doi.org/10.5662/wjm.v11.i4.144

Fattorusso, A., Di Genova, L., Dell'Isola, G. B., Mencaroni, E., & Esposito, S. (2019). Autism spectrum disorders and the gut microbiota. Article 3 *Nutrients, 11*(3). https://doi.org/10.3390/nu11030521.

Feng, J., Shan, L., Miao, C., Xue, Y., Yue, X., & Jia, F. (2023). The association of vitamin A, zinc and copper levels with clinical symptoms in children with autism spectrum disorders in Jilin Province, China. *BMC Pediatrics, 23*(1), 173. https://doi.org/10.1186/s12887-023-03987-2

Freire, C., Amaya, E., Gil, F., Fernández, M. F., Murcia, M., Llop, S., Andiarena, A., Aurrekoetxea, J., Bustamante, M., Guxens, M., Ezama, E., Fernández-Tardón, G., & Olea, N. (2018). Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: The Environment and Childhood (INMA) Project. *Science of The Total Environment, 621*, 340–351. https://doi.org/10.1016/j.scitotenv.2017.11.273

Gade, M., Comfort, N., & Re, D. B. (2021). Sex-specific neurotoxic effects of heavy metal pollutants: Epidemiological, experimental evidence and candidate mechanisms. *Environmental Research, 201*, Article 111558. https://doi.org/10.1016/j.envres.2021.111558

Hashemi, M., Rajabi, S., Eghbalian, M., Suliburska, J., & Nasab, H. (2023). Demographic and anthropometric characteristics and their effect on the concentration of heavy metals (arsenic, lead, chromium, zinc) in children and adolescents. *Heliyon, 9*(2). https://doi.org/10.1016/j.heliyon.2023.e13621

Hawari, I., Eskandar, M. B., & Alzeer, S. (2020). The role of lead, manganese, and zinc in autism spectrum disorders (ASDs) and attention-deficient hyperactivity disorder (ADHD): a case-control study on Syrian Children Affected by the Syrian Crisis. *Biological Trace Element Research, 197*(1), 107–114. https://doi.org/10.1007/s12011-020-02146-3

Heyer, D. B., & Meredith, R. M. (2017). Environmental toxicology: Sensitive periods of development and neurodevelopmental disorders. *NeuroToxicology, 58*, 23–41. https://doi.org/10.1016/j.neuro.2016.10.017

Li, S., Wang, J., Bjørklund, G., Zhao, W., & Yin, C. (2014). Serum copper and zinc levels in individuals with autism spectrum disorders. *Neuroreport, 25*(15), 1216–1220. https://doi.org/10.1097/WNR.0000000000000251

Li, Y., Cha, C., Lv, X., Liu, J., He, J., Pang, Q., Meng, L., Kuang, H., & Fan, R. (2020). Association between 10 urinary heavy metal exposure and attention deficit hyperactivity disorder for children. *Environmental Science and Pollution Research International, 27*(25), 31233–31242. https://doi.org/10.1007/s11356-020-09421-9

Liu, D., Shi, Q., Liu, C., Sun, Q., & Zeng, X. (2023a). Effects of endocrine-disrupting heavy metals on human health. *Toxics, 11*(4), 322. https://doi.org/10.3390/toxics11040322

Liu, H., Huang, M., & Yu, X. (2023b). Blood and hair copper levels in childhood autism spectrum disorder: a meta-analysis based on case-control studies. *Reviews on Environmental Health*. https://doi.org/10.1515/reveh-2022-0256

Lord, C., Rutter, M., DiLavore, P., Risi, S., Gotham, K., & Bishop, S. (2012). *Autism diagnostic observation schedule (2nd edition (ADOS-2)*. Los Angeles. CA: Western Psychological Corporation,.

Lozano, M., Broberg, K., Soler-Blasco, R., Riutort-Mayol, G., Ballester, F., González, L., Murcia, M., Grimalt, J. O., Gil, F., Olmedo, P., Braeuer, S., Casas, M., Guxens, M., Irizar, A., Lertxundi, N., Zubero, M. B., Marina, L. S., & Llop, S. (2023). Prenatal Metals Exposure and pre-adolescents' Emotional and Behavioral Problems. *Exposure and Health*. https://doi.org/10.1007/s12403-023-00585-6

Lutsenko, S., Washington-Hughes, C., Ralle, M., & Schmidt, K. (2019). Copper and the brain noradrenergic system. *Journal of Biological Inorganic Chemistry: JBIC: A Publication of the Society of Biological Inorganic Chemistry, 24*(8), 1179–1188. https://doi.org/10.1007/s00775-019-01737-3

Mora, A. M., Córdoba, L., Cano, J. C., Hernandez-Bonilla, D., Pardo, L., Schnaas, L., Smith, D. R., Menezes-Filho, J. A., Mergler, D., Lindh, C. H., Eskenazi, B., & van Wendel de Joode, B. (2018). Prenatal mancozeb exposure, excess manganese, and neurodevelopment at 1 year of age in the Infants' Environmental Health (ISA) Study. *Environmental Health Perspectives, 126*(5), Article 057007. https://doi.org/10.1289/EHP1955

Morales-Hidalgo, P., Voltas Moreso, N., & Canals Sans, J. (2021). Autism spectrum disorder prevalence and associated sociodemographic factors in the school population: EPINED study. *Autism, 25*(7), 1999–2011. https://doi.org/10.1177/13623613211007717

Nabgha-e-Amen, Eqani, S. A. M. A. S., Khuram, F., Alamdar, A., Tahir, A., Shah, S. T. A., Nasir, A., Javed, S., Bibi, N., Hussain, A., Rasheed, H., & Shen, H. (2020). Environmental exposure pathway analysis of trace elements and autism risk in Pakistani children population. *Science of The Total Environment, 712*, Article 136471. https://doi.org/10.1016/j.scitotenv.2019.136471

Nelson, C. A., & Gabard-Durnam, L. (2020). Early adversity and critical periods: Neurodevelopmental consequences of violating the expectable environment. *Trends in Neurosciences, 43*(3), 133–143. https://doi.org/10.1016/j.tins.2020.01.002

Netto, B. B., da Silva, E. P., de Aguiar da Costa, M., de Rezende, V. L., Bolan, S. J., Ceretta, L. B., Aschner, M., Dominguini, D., & Gonçalves, C. L. (2024). Critical period of exposure to mercury and the diagnostic of autism spectrum disorder: A systematic review. *Journal of Neurochemistry*. https://doi.org/10.1111/jnc.16076

Norte Navarro, A. I., & Ortiz Moncada, R. (2011). Calidad de la dieta española según el índice de alimentación saludable. *Nutrición Hospitalaria, 26*(2), 330–336.

Ouisselsat, M., Maidoumi, S., Elmaouaki, A., Lekouch, N., Pineau, A., & Sedki, A. (2023). Hair trace elements and mineral content in moroccan children with autism spectrum disorder: A case–control study. *Biological Trace Element Research, 201*(6), 2701–2710. https://doi.org/10.1007/s12011-022-03365-6

Rafi'i, M. R., Ja'afar, M. H., Wahil, M. S. A., & Hanif, S. A. M. (2024). Urine manganese, cadmium, lead, arsenic, and selenium among autism spectrum disorder children in Kuala Lumpur. *PeerJ, 12*, Article e17660. https://doi.org/10.7717/peerj.17660

Rahbar, M. H., Samms-Vaughan, M., Saroukhani, S., Lee, M., Zhang, J., Bressler, J., Hessabi, M., Shakespeare-Pellington, S., Grove, M. L., & Loveland, K. A. (2021). Interaction of blood manganese concentrations with GSTT1 in relation to autism spectrum disorder in Jamaican Children. *Journal of Autism and Developmental Disorders, 51*(6), 1953–1965. https://doi.org/10.1007/s10803-020-04677-z

Ramírez-Ayala, R., & Azcona-Cruz, M. I. (2017). Efectos tóxicos del manganeso. *Revista Dėlelőtt especialidades Médico-quirúrgicas, 22*(2), 71–75.

Rezaei, M., Rezaei, A., Esmaeili, A., Nakhaee, S., Azadi, N. A., & Mansouri, B. (2022). A case–control study on the relationship between urine trace element levels and autism spectrum disorder among Iranian children. *Environmental Science and Pollution Research, 29*(38), 57287–57295. https://doi.org/10.1007/s11356-022-19933-1

Roberts, A. L., Lyall, K., Hart, J. E., Laden, F., Just, A. C., Bobb, J. F., Koenen, K. C., Ascherio, A., & Weisskopf, M. G. (2013). Perinatal air pollutant exposures and autism spectrum disorder in the children of nurses' health study II participants. *Environmental Health Perspectives, 121*(8), 978–984. https://doi.org/10.1289/ehp.1206187

Rossignol, D. A., Genuis, S. J., & Frye, R. E. (2014). Environmental toxicants and autism spectrum disorders: A systematic review. *Translational Psychiatry, 4*(2), Article e360. https://doi.org/10.1038/tp.2014.4

Russo, A. J., & deVito, R. (2011). Analysis of copper and zinc plasma concentration and the efficacy of zinc therapy in individuals with Asperger's syndrome, Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) and autism. *Biomarker Insights, 6*, 127–133. https://doi.org/10.4137/BMI.S7286

Rutter, M., Le Couteur, A., & Lord, C. (2003). *ADI-R: Autism Diagnostic Interview-Revised (ADI-R)*. Los Angeles: Western Psychological Services,.

Siddiqi, U. R., Begum, S., Shahjadi, S., Afroz, S., Mahruba, S. N., Parvin, J., & Rahman, M. M. (2023). Plasma zinc, copper and serum ceruloplasmin levels of autism spectrum disorder children in Bangladesh. *Heliyon, 9*(8), Article e18624. https://doi.org/10.1016/j.heliyon.2023.e18624

Skogheim, T. S., Weyde, K. V. F., Engel, S. M., Aase, H., Surén, P., Øie, M. G., Biele, G., Reichborn-Kjennerud, T., Caspersen, I. H., Hornig, M., Haug, L. S., & Villanger, G. D. (2021). Metal and essential element concentrations during pregnancy and associations with autism spectrum disorder and attention-deficit/hyperactivity disorder in children. *Environment International, 152*, Article 106468. https://doi.org/10.1016/j.envint.2021.106468

Spungen, J. H. (2019). Children's exposures to lead and cadmium: FDA total diet study 2014-16. *Food Additives & Contaminants: Part A, 36*(6), 893–903. https://doi.org/10.1080/19440049.2019.1595170

Stojsavljević, A., Lakićević, N., & Pavlović, S. (2023). Does lead have a connection to autism? A systematic review and meta-analysis. *Toxics, 11*(9), 753. https://doi.org/10.3390/toxics11090753

Tartaglione, A. M., Camoni, L., Calamandrei, G., Chiarotti, F., & Venerosi, A. (2024). The contribution of environmental pollutants to the risk of autism and other neurodevelopmental disorders: A systematic review of case-control studies. *Neuroscience & Biobehavioral Reviews, 164*, Article 105815. https://doi.org/10.1016/j.neubiorev.2024.105815

Tinkov, A. A., Skalnaya, M. G., Simashkova, N. V., Klyushnik, T. P., Skalnaya, A. A., Bjørklund, G., Notova, S. V., Kiyaeva, E. V., & Skalny, A. V. (2019). Association between catatonia and levels of hair and serum trace elements and minerals in autism spectrum disorder. *Biomedicine & Pharmacotherapy, 109*, 174–180. https://doi.org/10.1016/j.biopha.2018.10.051

Trinidad Rodríguez, I., Fernández Ballart, J., Cucó Pastor, G., Biarnés Jordà, E., & Arija Val, V. (2008). Validación de un cuestionario de frecuencia de consumo alimentario corto: Reproducibilidad y validez. *Nutrición Hospitalaria, 23*(3), 242–252.

Vollet, K., Haynes, E. N., & Dietrich, K. N. (2016). Manganese exposure and cognition across the lifespan: Contemporary review and argument for biphasic dose-response health effects. *Current Environmental Health Reports, 3*(4), 392–404. https://doi.org/10.1007/s40572-016-0108-x

Vrijheid, M., Casas, M., Gascon, M., Valvi, D., & Nieuwenhuijsen, M. (2016). Environmental pollutants and child health—A review of recent concerns. *International Journal of Hygiene and Environmental Health, 219*(4), 331–342. https://doi.org/10.1016/j.ijheh.2016.05.001

Warren, J. A., Siefring, G., & Morjana, N. (2014). Development of application protocols of the Emit® II Plus 6-Acetylmorphine Assay on the ADVIA® 1800 and 2400 Chemistry Systems. *Forensic Science International, 244*, 122–127. https://doi.org/10.1016/j.forsciint.2014.08.014

Wechsler, D. (2014). *Wechsler preschool and primary scale of Intelligence* (Fourth ed.). Pearson C & T Assessment,.

Zebbiche, Y., Stambouli, B., Kaddour-Benkada, I., Amziane, A., Chebli, A. I., Achouri, M. Y., Djidjik, R., & Alamir, B. (2024). Trace element levels and autism spectrum disorder in a sample of Algerian children: A case-control study investigation. *Research in Autism Spectrum Disorders, 110*, Article 102287. https://doi.org/10.1016/j.rasd.2023.102287

Zeidan, J., Fombonne, E., Scorah, J., Ibrahim, A., Durkin, M. S., Saxena, S., Yusuf, A., Shih, A., & Elsabbagh, M. (2022). Global prevalence of autism: A systematic review update. *Autism Research, 15*(5), 778–790. https://doi.org/10.1002/aur.2696