Iron deficiency and cognitive impairment in children with low blood lead levels

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Abbreviations: ID, Iron deficiency; CMA, children in a mining area; CG, control group; BDT, bell dam test; DST, digit span test; IS, iron status; CBC, blood count; BLLs, blood lead levels; SAA-FG, graphite furnace atomic absorption spectrophotometer; VA, visual attention; WM, working memory; BMI, body mass index.

Contents lists available at ScienceDirect
Toxicology Reports
journal homepage: www.elsevier.com/locate/toxrep

1. Introduction

Environmental pollution by lead (Pb) and its effects on health among children remains a global problem, especially in developing countries. The Institute of Health Metrics and Evaluation (IHME) claims that the long-term effects of Pb exposure resulted in 1.06 million deaths and 24.4 million Disability-Adjusted Life Years (DALYs) lost [1]. Low- and middle-income countries are the most affected sites. Children are particularly vulnerable to the neurotoxic effects of Pb since its effects are significant on the developing brain. Their bodies absorb 50 % of ingested Pb and exhibit toxicity at lower levels of exposure than adults [2]. According to the Centers for Diseases Control and Prevention [3], cognitive
disorders are detectable in children at very low levels of contamination (blood Pb levels less than 5 \( \mu \text{g/dL} \)). Pb concentrations is usually measured in blood since BLLs are considered a key factor in the well-being of children worldwide [4]. Furthermore, it has been suggested that children of workers exposed to Pb should be targeted for blood Pb screening [5].

At exposure levels that do not cause obvious symptoms, Pb can impair multiple organ systems in a variety of ways. In particular, it can affect children’s brain growth, resulting in lower intelligence quotient (IQ), behavioral changes (reduced ability to concentrate, and increased antisocial behavior) and lower school performance [6]. In fact, there is no known blood exposure level under which no effects are expected [7].

Recently, evaluative studies conducted in Marrakech region in recent years have highlighted a decrease in blood Pb concentrations transiting from 12 \( \mu \text{g/dL} \) [8] to 3.14 \( \mu \text{g/dL} \) [9]. Nevertheless, the Pb risk exists given this metal’s environmental burden [10–12]. Indeed, Pb constitutes one of the chemicals of public health concern.

According to the French Health and Environment Association [13], Pb can also act in synergy with other metals and pollutants. Its toxicity is multifactorial, often entering organisms by molecular mimicry, using inherent transporters for essential elements such as iron, calcium, and zinc [14]. Sometimes, low essential element status can increase the risk of toxic effects of non-essential trace elements such as Pb and Cd [15]. ID is the most common nutritional deficiency in infants and young children [16]. The absorption of Pb is increased, mainly in people with this deficiency [17]. In addition, a downward trend in hemoglobin content with increased blood Pb levels in children has been reported [18]. ID and anemia are related to cognitive performance, especially attention and working memory [19]. Several symptoms have been described as a consequence of ID, even in the absence of anemia: apathy, drowsiness, irritability, decreased attention, inability to concentrate, and memory loss [20]. The harmful effects of ID on the neurocognitive abilities of children exposed to low levels of Pb have been partially elucidated. Therefore, our general hypothesis is that ID and/or anemia may increase the effect of low BLLs on neurocognitive function in children. For such a reason, the present work aims at assessing the possible interaction between iron status and low BLLs and its effects on neurocognitive performance in school children living from birth in a Pb contaminated area near Marrakech. The association with several co-variables was taken into account.

![Geographical map showing the two studied sites in Marrakech (Mining area: Saada zone and Control area: Chouiter zone).](image-url)
2. Materials and methods

2.1. Participants

The study was conducted over a six-month period and involved 90 children of both sexes (35.6% boys and 64.4% girls), aged from 6 to 10 years, whose parents or guardians agreed to participate in the study. These children were enrolled in the second year of primary school (PS). The results of biological (creatinine, blood glucose, TSH, iodine, transaminase) and clinical examination carried out by the consulting physicians ruled out the presence of any chronic pathology in selected children. Excluded from the participant population were children with heart or kidney disease, neurological disorders, mental retardation, dyslexia, diabetes and attention deficit hyperactivity disorder (ADHD). Visual processing tests will not be performed in children with poor and uncorrected near visual acuity. Rather, they will be performed after correction.

2.2. The setting of study

This cross-sectional prospective study took place in Marrakech, a city situated roughly in the center of Morocco (Fig. 1). Two sub-urban areas were concerned by this study: a) SAADA area due to the presence of a mining site called 'Drâa Lasfar' and located approximately 13 km west of Marrakech city; b) CHOUTIER area located approximately 20 km southeast of Marrakech. Local studies have shown that the CHOUTIER area is a clean area away from any type of environmental pollution that could expose children to Pb contamination [9, 11]. We randomly selected a school belonging to each of the areas: Tazakourte School from mining area (SAADA, Douar Ouled El Guern; n = 30) and Chouiter School from control area (CHOUTIER, Douar Moulay Jaber; n = 60).

2.3. Ethical considerations

The study protocol was approved on October 29th, 2019 by the Ethics Committee of the Mohammed VI University Hospital Center (UHC) in Marrakech. Written informed consent was obtained from the children’s parents, respecting their right to privacy and the confidentiality of personal information. As the study benefited from the logistical support of the Ministry of Health, children with anemia were given a corresponding dose of blood transfusion, in accordance with standard operating procedures. Samples were collected (between 8 and 11 a.m.) at the community health center by a team of nurses under the supervision of the consulting physician.

b) Hematological and biochemical assays

The samples were transported, in an isothermal bag, the same day of the sampling to the laboratory of biological analyses of the UHC Mohammed VI of Marrakech to carry out the hematological and biochemical assays. At the laboratory, the whole blood collected in the first row of EDTA tubes was gently mixed and read immediately with a Sysmex XT 4000i blood count (CBC) machine, according to the manufacturer’s instructions. The non-hemolyzed serum was analyzed on the Cobas 6000 Analyzer device. The parameters assayed were ferritin, transferrin, and serum iron. The second row of EDTA tubes was stored at −4 °C for transport and analysis within the same week at the mineral assay center at the pharmaceutical UFR in Nantes, France.

c) Blood Pb Assessment

The determination of blood Pb concentrations was performed using the Atomic Absorption Spectrometer (Perkin-Elmer A Analyst 600, reference LN/032398-20689) with graphite furnace (GFAAS) and Zeeman background correction. The method of calibrated additions with chemical depletion with HNO₃ 1 M is used according to the preparatory and analytical technique [21], as modified and adapted to the concept of the temperature stabilized platform furnace [22]. Standard solutions (1 M HNO₃: 500 ng/mL) were used to build the calibration curve (50 µL, 100 µL, 150 µL). Then, a volume of 200 µL of blood was diluted in 800 µL of nitric acid at 1 M and centrifuged (8000 rpm) for 10 min. The supernatant was then poured into wells for Pb determination. The accuracy of the measurement method was guaranteed using certified reference material (Seronorm: Trace Blood L-1 1702821, L-2 1702825, L-3 1702826) from the French Blood Establishment (FBE). This reference material is certified HIV and HBS negative. Certified reference materials obtained from Quebec City laboratory with known Pb concentrations and having the same origin (ADM, New York, Quebec). During this period, samples LOO-01 to L00-090 yielded a correspondence of our results ranging from 89% to 102.7%. The detection and quantification limits were 0.4 and 0.8 µg/100 mL respectively.

2.4. Neurocognitive function assessment

A two-part cognitive assessment battery was used to test children’s cognitive abilities. The cognitive function tested in group was visual attention (VA). The test used is the Bell Dam Test (BDT) in which each child looks for targets among distractors. It allows the evaluation of visual attentional deficits [23]. This deficit as defined by the DSM-IV classification (Diagnostic and Statistical Manual of Mental Disorders, 4th edition, 1994) corresponds to a child who does not pay attention to details. It turns out that he has difficulty maintaining his attention on a particular task or a game. He often has difficulty organizing his work and is easily distracted by external stimuli, or often forgets things during daily activities. Performing the bells test requires both visual processing (visual spatial scanning) and maintaining attention (selective attention) in a conflicting task [23]. The test consists of presenting the child with a sheet containing 112 drawings of objects (saw, apple, horse, carriage, cloud, etc.) including 35 bells. He must, during 2 min, surround as many bells as possible. The child assigned a number to each circled target to allow evaluation of his or her own bell scanning strategy. At the end of the test, the score (number of bells circled) is noted as well as the scanning strategy followed. Completion time is not a valuable indicator of success or neglect. If a subject has a total score of less than 29 (more
than 6 omissions), then he or she should be suspected of having an attentional deficit [24].

The task in which participants were tested individually was the working memory (WM) task following the use of the Digit Span Test (DST), which is a subtest of WISC III [25]. WM is the neurocognitive system that supports the temporary maintenance and manipulation of information necessary for many demanding cognitive activities [26]. The child was asked to repeat a series of numbers in the order (right-side up) or in the reverse order in which they were stated (upside down) with a one second gap between each number. The child repeated the numbers in the same order. We stop when the child fails twice for a sequence of the same number of digits. Responses were recorded in the corresponding columns. The right-hand span or right side digit span (RSDS) is the number of digits in the longest sequence given correctly. The reverse span or reverse digit span (RDS) is the number of digits of the longest sequence repeated in reverse order without error.

2.5. Data analysis

The collected data were entered and analyzed using SPSS software 20th version. The normal distribution of the variables was studied by conformity tests (Skewness, Kurtosis, Kolmogorov smirnov) and homogeneity of variances (Levene’s test). For Quantitative variables that satisfy normal distribution, the following statistics were performed: (a) centrality and dispersion parameters for univariate analysis (mean and standard deviation), (b) Student and ANOVA tests to compare means, and (c) Chi-square test for qualitative variables. Frequencies were expressed in number and in percentage for categorical variables. Concerning variables asymmetrically distributed, data are expressed as median and interquartile range (IQR). Paired-group comparison was performed using non-parametric Mann-Whitney U test. Data were log-transformed in order to produce distributions that better approximated a normal distribution.

Stepwise multiple linear regression was performed for significant items. Children’s characteristics were used as covariates. The significance threshold is retained for a p < 0.05.

3. Results and discussion

3.1. Demographic, nutritional, and school characteristics

The mean age of the children in the mining area (CMA) is 7.66 ± 0.41 years (range 7.51–7.84 years) and shows no statistical difference (U = 891.5, p = 0.942) with that of the control group (CG) 7.65 ± 0.78 years (range 7–8.1 years), see Table 1. The sex ratio is 0.53. Females are overrepresented in selected schools (70 % for CMA and 61.7 % for CG). Nevertheless, there is no association between gender and the type of study population (Chi square of Pearson: X² = 0.606; p = 0.436). Thus, the two groups were matched by sex and age.

Table 1 illustrates that 73.3 % of the CMA’s both parents are illiterate compared to 50 % of those in the CG. The average monthly income (AMI) for almost half of the families is between 100 and 200 euros (€) while 26.7 % of the parents of the children in the control zone have an average income of less than 100 €. To study the association between the 4 levels of monthly income and the two groups of families, Chi square test (X²) was used. It turned out that the monthly income level of families does not depend on the type of study group (X² = 5.443; p = 0.142). Therefore, there is no statistical difference between the two groups in terms of AMI. Survey results indicate that 46.7 % of CMA is exposed to indirect Pb contamination. This is probably happened due to the dust tracked into houses through the shoes and clothing of mining fathers [5,27]. It seems that the majority of mothers from mining area (93.3 %) applied the traditional cosmetic product “kholt” to their children’s eyes during the first months of birth with statistically significant association (X² = 4.057; p = 0.044). Pica syndrome (the compulsive, habitual consumption of nonfood items) is manifested in 20 % in CMA versus 26 % in the CG with a statistically significant difference (X² = 4.235; p = 0.044). Soil is the most common form of geophagy in our population (25.6 %), not shown. This behavior has long been recognized as a risk factor for elevated BLL because children with pica may also ingest Pb [28]. Indeed, the hand-to-mouth activity manifested in toddlers is a more common source of Pb ingestion [29,30]. Hence, Pica could be a source of Pb exposure, particularly for children living in a

Table 1 Demographic, nutritional, and school characteristics of CMA in comparison with CG.

| Population characteristics | CMA | CG | Test value; p value |
|----------------------------|-----|----|---------------------|
| Gender                     | Female | 21(70 %) | 37(61.7 %) | X² = 0.606; p = 0.436 |
|                            | Male (M) | 9(30 %) | 23(38.3 %) |                          |
| Average age                | Median (IQR) | 7.67 | 7.66(7.81) | U = 891.5; p = 0.942 |
|                            | Both parents’ illiteracy | Yes | 22(73.3 %) | 30(50 %) | X² = 6.051; p = 0.049 |
|                            | AMI (in euros) < 100 € | 26.7 | 16(26.7 %) |                          |
|                            | 100; 200 € | 16(53.3 %) | 28(46.7 %) |                          |
|                            | > 300 € | 6(20 %) | 9(15 %) |                          |
| Exposure of children to contamination by fathers’ clothing from the mine | Yes | 6(20 %) | 7(11.7 %) |                          |
|                            | No | 14(46.7 %) | 0(0 %) |                          |
|                            | Yes | 16(53.3 %) | 60(100 %) |                          |
| Use of traditional cosmetic product “kholt” | Yes | 28(93.3 %) | 44(73.3 %) | X² = 4.057; p = 0.044* |
|                            | No | 26(6.7 %) | 16(26.7 %) |                          |
| Pica syndrome              | Yes | 6(20 %) | 26(43.3 %) | X² = 4.752; p = 0.028* |
|                            | No | 24(80 %) | 34(56.7 %) |                          |
| Parity                     | Median (IQR) | 3(12.5%) | 3(12.4%) | U = 810.5; p = 0.427 |
| Breastfeeding mode         | Exclusive | 16(53.3 %) | 30(55 %) | X² = 7.487; p = 0.024* |
|                            | Mixed | 8(26.7 %) | 25(41.7 %) |                          |
|                            | Artificial | 6(20 %) | 23(3.3 %) |                          |
| Duration of breastfeeding (per month) | Median (IQR) | 12(5.5–19.5) | 3(17.2–17.5) | U = 678.50; p = 0.056 |
| Irregular consumption of breakfast (sometimes or rarely) | Yes | 24(80 %) | 42(70 %) | X² = 14.017; p = 0.003* |
|                            | No | 6(20 %) | 18(30 %) |                          |
| BMI (kg m²)               | Median (IQR) | 13.87 | 16.16 | U = 255.00; p = 0.001*** |
|                            | Underweight (yes) | M | 2(22.2 %) | 0(0 %) |                          |
|                            | Daily tea consumptiona | Median (IQR) | 1(1) | 1(1) | U = 821.00; p = 0.464 |
|                            | Weekly tea consumptiona | Median (IQR) | 7(16.75) | 7(4.75) | U = 876.00; p = 0.785 |
|                            | Daily milk consumptiona | Median (IQR) | 1(1) | 1(1) | U = 541.00; p = 0.0001*** |
|                            | Pre-school education | Yes | 30(100 %) | 53(83.3 %) | X² = 3.795; p = 0.051 |
|                            | No | 0(0 %) | 7(11.7 %) |                          |
| Duration of pre-school education (per month) | Mean ± SD | 25.67 ± 9.12 | 23 ± 12.3 | t = 1.028; p = 0.307 |
| Academic performance     | Mean ± SD | 6.3 ± 1.30 | 6.6 ± 0.95 | t = – 1.110; p = 0.270 |
| Repetition rate           | Yes | 23(76.7 %) | 52(86.7 %) | X² = 1.440; p = 0.230 |

* The correlation is significant at the 0.05 level (two-tailed). ** The correlation is significant at the 0.005 level (two-tailed). *** The correlation is significant at the 0.001 level (two-tailed). IQR: Interquartile range. a: Expressed in number of glasses.
mining area. The pregnancy frequency (parity) is equal among the mothers of CMA and CG (p > 0.05). Exclusive breastfeeding is the mode adopted by almost half of the mothers in the two areas (53.3 % for the CMA and 55 % for the CG). Moreover, the average duration of breastfeeding shows no difference between the two groups (U = 678.50; p = 0.056). Breakfast intake was irregular (sometimes or rarely) in 80 % of the CMA compared to 70 % in the CG with statistically significant association (X² = 14.017; p = 0.003). The body mass index (BMI), expressed as the median (IQR), was 13.87 (13.5–15.61) kg m⁻² in the CMA and 16.16 (15.51–17.4) kg m⁻² in the CG. This disparity is statistically significant (U = 255.00; p < 0.001). Thus, the majority of child population is of normal weight, whereas 22.2 % of boys and 19 % of girls in the mining zone are underweight. This could be due to the known anorectic effect of Pb [31,32]. This finding has been confirmed by studies indicating the influence of Pb on food intake and body weight [33]. The average daily milk consumption showed a significant difference between the children of the two sites (p < 0.001). On the contrary, no significant difference is revealed for the average tea consumption (p > 0.05).

With regard to school characteristics, our results reveal that CMA were unanimously enrolled in preschool education compared to 88.3 % in the CG with no statistically significant association (X² = 3.795; p = 0.051). The duration of pre-school education does not show a significant difference between the two groups of children (t = 1.028; p = 0.307). The academic performance, represented in this study by the mean obtained during the first semester, indicates no significant difference between the two groups. Although CMA have a higher repetition rate (23.3 %) than that recorded in the CG (13.3 %), this difference remains insignificant (X² = 1.440; p = 0.230) and is not reflected in their academic performance (t = – 1.110; p = 0.270).

A binary logistic regression model was used to examine the relationship between gender and child characteristics. Repetition rate (R = 7.746, p = 0.005), preschool education (R = – 6.266, p = 0.041) and breakfast intake (R = – 4.093, p = 0.043) were found to be associated with female gender. The most important determinant is repetition rate, which was less frequent among girls (p < 0.001).

### 3.2. Iron status

According to World Health Organisation standards [34], a hemoglobin (HBG) < 11.5 g/dL indicates anemia. However, ferritin remains the most useful marker of iron status. It allows us to classify the children in the study into two categories: a) those with ferritin < 15 µg/L have ID and, b) those with ferritin ≥ 15 µg/L have no ID. The calculation of transferrin saturation coefficient (TSC) allows the evaluation of the transferrin saturation rate according to the following two Formulas:

\[
\text{TSC} = \frac{[\text{Plasma Iron}] \times 100}{\text{CTF}} \tag{1}
\]

\[
\text{CTF} (\text{µmol/L}) = 106 \times 2 \times [\text{TF g/L}] \times 80000 \times [\text{TF g/L}] \times 25 \tag{2}
\]

In the case of ID, the amount of transferrin rises, increasing its binding capacity. Yet, with the insufficient amount of iron to be transported, the saturation rate decreases significantly [35].

Table 2 presents the results of the biological and toxicological assessment parameters. The mean hemoglobin values for the CMA and CG are 12.8 ± 1.1 g/dL and 12.6 ± 1.1 g/dL, respectively. The mean corpuscular volume (MCV) of all children is less than 82 fl signifying the presence of microcytic anemia in the participant. The mean corpuscular hemoglobin concentration (MCHC) indicates normochromic anemia as it is greater than 32 g/dL in children in both areas. Serum ferritin, expressed in median (IQR), was 25.5 (14.7–38) ng/mL and 24 (16–34) ng/mL respectively in CMA and in CG. The TSC was higher (19.2 ± 10.9 %) in CMA if compared to CG (17.8 ± 11.1 %) while no significant difference was established between the two groups (p > 0.05).

In addition, it appears that 10 % of the CMA have anemia (all of them are girls 14.3 %) compared to 11.7 % of the CG. This result does not seem to be very comparable with other studies conducted in the same sites. Empirically, a higher prevalence was reported, reaching 18.18 % in the mining area and 21.7 % in the control area [36] . ID is particularly prevalent in this study compared to anemia. It affected 23.3 % of the CMA and 21.7 % of the CG. There has been no significant difference was reported, the saturation rate decreases significantly [35].

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The mothers reported that their children consume tea at all meals. Given that he bioavailability of iron in a plant food depends on what comes with it, a cup of tea taken during the same meal would divide into four predefined by the second version of the ODEDYS (4.3 ± 2.1 g/L) compared to only 3.3 % in CG (represented entirely by girls). The comparison of BLLs according to gender has not revealed any significant difference for the two populations groups, even for the group with BLLs above the cut-off value (p > 0.05). The existence of a base metal mine in the mining area reported as a source of elevated Pb levels in nearby water and soil [10], may explain these significantly elevated Pb levels in CMA. Furthermore, half of CMA is exposed to Pb contamination through their fathers’ occupational clothing taken home for washing and drying. A meta-analysis [5] mentioned that household members who work in Pb contaminated environments or engage in particular hobbies can carry Pb to their houses through clothes or shoes they used at work. This supports the results of studies that found occupational exposure to be the most potent risk factor correlated with increased levels of chromium (Cr), cadmium (Cd), and Pb [27]. In this study, medical consultations revealed no symptoms related to chronic Pb toxicity whilst the concentrations are lower than those reported by Uruguayan children (4.2 ± 2.1 µg/dL) [37]. However, they remain higher than the levels recorded by the National Health and Nutrition Examination Survey (NHANES) in American children (1.3 µg/dL) [38] and in those from Cartagena (1.7 ± 0.3 µg/dL) [4]. It should be noted that BLLs have been decreased compared to those observed recently in the same group (4.3 µg/dL) [36]. This decline could be a consequence of the awareness of families after several studies. For BLLs similar to those found in our study, it is advisable to take preventive measures to reduce or eliminate children’s environmental Pb exposure before such exposure occurs [39].

3.4. Children’s neurocognitive performance

3.4.1. Visual attention and scanning strategy

The mean score of the VA test (BDT) is 20.87 ± 5.68 out of 35 for the CMA versus 21.02 ± 5.29 for the CG (Fig. 2: A). The test made on students showed no statistically significant difference in children’s scores between the two groups (t = − 0.259; p = 0.796). However, a comparison of mean BDT scores of the two groups with the calibrations predefined by the second version of the ODEDYS [40] and the Fourth version of BSEDS 5-6 [23] indicates a very significant difference (T-test /CMA: t = − 7.899, p < 0.001; CG: t = − 11.699, p < 0.001). Indeed, the average reference score given to second year of PS is 29.4 ± 1.08 for the CMA and, CG (VA: t = − 0.718, p = 0.476; WM (RDS): t = − 1.296, p = 0.200; WM (RDS): t = − 1.071, p = 0.289).

3.5. Association between IS, BLLs, and neurocognitive results

To explain the children’s low scores on neurocognitive tests, a bivariate analysis was conducted first.

It should be noted that, in this study, the independent variables related to BLLs and IS (RBCs, HBG, HCT, VGM, MCHC, Serum Ferritin, TSC) show no correlation (p < 0.05) with scores of the two tested cognitive functions (VA and WM). Moreover, bivariate analysis has showed no difference in mean BLLs between the students who adopt the disorganized scanning strategy compared to the others whose method is organized for both CMA (t = − 0.508, p = 0.616) and CG (t = − 0.014, p = 0.989) (Fig. 2: B). We infer that the low BLLs did not disrupt the scanning strategies adopted by these children. Our result is inconsistent with studies that demonstrated dose-dependent impairment of attention-related behavior, inability to organize and inability to follow instructions [46], and adolescents exposed to Pb in early childhood had concentration and attention problems, which probably contributed to global cognitive delays [47].

In addition, ID (Serum Ferritin < 15 µg/L) was not proven to affect the organizational strategies (visual spatial abilities) adopted by children in either group (CMA: X² = 1.969, p = 0.161; CG: X² = 1.013, p = 0.314) (not shown). On the one hand, our results corroborate the work of other studies that found no significant differences in cognitive performance between the two groups even after iron treatment [36,49]. Similarly, it was demonstrated that the effects of ID on child development appear to be independent of the effects of Pb [19,50]. On the other
hand, it was stated that cognitive performance, including attention, decreased in anemic schooled girls [50]. This discrepancy can be explained by the high prevalence of anemia (63 %) and ID (50 %) in their population.

Subsequently, multivariable linear regression analysis was performed and regression model included only items characterized by significant correlation with cognitive scores among the two study groups. Given the significant difference elucidated in the WM scores (RSDS and RDS) according to the BLL by the cut-off value (≥ 5 μg/dL), this categorical variable was entered into the first step of the regression analysis. Next, each of the selected variables was tested to see whether its inclusion in the regression model altered the relation of the BLL by cut-off value to the WM scores. Regarding the VA scores, the BLL by cut-off was included in the linear regression model after demonstrating an increase in the predictive ability of the model (from 23 % to 24 %).

Since some variables (age, BMI, daily and weekly tea consumption, daily milk consumption, parity, duration of breastfeeding, Serum Ferritin) have been shown to be asymmetrically distributed, we log-transformed them to produce an approximate normal distribution before incorporating them into the regression analysis.

Here, we present the results of the final step obtained following the progression of multiple linear regression according to the stepwise method.

### 3.5.1. Association between IS, BLLs, and VA abilities

The model in Table 3 is significant and its predictive potential explains 23 % and 31 % of the variability in VA scores for CMA and CG, respectively. A positive association was found with daily milk consumption in CMA. The regression model presented here is the only one that visualizes at least one association between an explanatory variable and VA scores in CMA. Indeed, every 1 % increase in daily milk consumption was associated with a 9.202 higher VA scores (95 % confidence interval [CI], 3.175–15.229). Milk is animal product which constitute a source of micronutrients such as iron, zinc, iodine and vitamin B12 known to be linked to the cognitive processes in infant and young children [51]. The nature of polyunsaturated fatty acids (in particular omega-3) present in milks conditions the visual and cerebral abilities, including intellectual abilities [20].

#### Table 3

Summary of the multiple linear regression models with children’s VA scores as a dependent variable.

| Outcome measure | Parameters | CMA | | | | CG | | |
|-----------------|------------|-----|---|---|-----------------|-----|---|---|---|
|                 |            | β   | p value | 95 % CI | β   | p value | 95 % CI |
| VA scores       | BLL by cut-off value (≥ 5 μg/dL) | – | 0.007 | 0.967 | VE | – | 0.903 | 0.411 | VE |
|                 | Daily milk consumption | 9.202 | 0.004** | 3.175, 15.229 | 0.012 | 0.915 | VE | | |
|                 | AMI [200; 300] | 0.037 | 0.827 | VE | 5.870 | 0.001** | 2.667, 9.074 | | |
|                 | WM (RDS) | 0.208 | 0.221 | VE | 1.342 | 0.017* | 0.253, 2.432 | | |
|                 | Age | 0.057 | 0.734 | VE | 29.595 | 0.028* | 3.258, 55.932 | | |
|                 | Multiple R | 0.509 | 0.586 | | | | | |
|                 | Multiple R² | 0.259 | 0.344 | | | | | |
|                 | Adjusted R² | 0.232 | 0.309 | | | | | |
|                 | p for model | 0.004** | < 0.001*** | | | | |

The covariates in Model were: BLL by cut-off value, Daily milk consumption, AMI, WM (RDS) and Age. *p < 0.05, **p < 0.005, ***p < 0.001. VE: variables were excluded from the equation.

![Fig. 2. Comparison of mean scores on the BDT (A: VA scores and B: visual scanning strategy) and the WM test (DST) between the two study areas (C: Mean scores on the RSDS (right-side digit span). D: Mean scores on the RDS (reverse digit span)).](image-url)
In the CG, the VA scores are positively influenced by the AMI and are increased by 5.870 (95 % CI, 2.667–9.074) once the family income reaches a level between 200 and 300 €. According to the literature, children from low-income families have lower cognitive test scores, smaller cortical volume and cortical area [52]. Age and WM (RDS) slightly influence VA scores. The positive association between WM and VA scores have been already predicted, in fact, correlations between capacity in WM and the efficiency of controlled attention revealed links between the two constructs [53].

### 3.5.2. Association between IS, BLLs, and WM abilities

#### a) WM (RDS)

Multiple regression analysis was also performed to assess the impact of BLL above the cut-off value (≥ 5 μg/dL) on WM (RDS) skills and to detect a potential modifying effect of the parameters: AMI, academic performance, and WM (RDS) on the relationship between BLL by cut-off value and outcome measure (Table 4). This adjusted model increased the predictive ability of the model to 44 %. In particular, the BLL above 5 μg/dL was a significant negative predictor of WM (RDS) impairment in CMA (β = −1.181, p = 0.003, 95% CI, −1.895 to −0.466). Our result is consistent with the existing literature which states that learning and memory are two processes that are most affected in children following chronic exposure to environmental amounts of Pb during development [54]. This confirms the finding that Pb, even at low levels, harms the brains of developing children [55,56] and results in poorer outcomes [57,58]. Previous study has suggested that working memory is a cognitive domain especially vulnerable to the effects of Pb exposure. Indeed, prenatal Pb exposure is related to decreased allocation of resources for working memory [59]. This neurobehavioral changes induced by Pb may underlie the alterations in serotoninergic and dopaminergic system [60], and at this level of blood Pb (5 μg/dL), intervention actions are recommended to decrease Pb exposure [61].

For the CG, it seems that WM (RDS) and AMI between 100 and 200 € are positively associated with children’s WM (RDS) abilities.

#### b) WM (RDS)

The β coefficients (Table 5) indicate that an increase of parity is strongly associated with elevated scores of WM (RDS) among CMA (β = 3.640, p = 0.002, 95 % CI, 1.479–5.801). However, BLL by cut-off value does not remain so after adjustment and parity is the most determining factor. Of other sociodemographic variables such as academic performance, AMI, use of cosmetic product K-hol, and duration of preschool education didn’t show any significant association with WM (RDS) abilities.

Thus, the more multiple births mothers have, the better their children perform on WM (RDS) tests. This suggests that the child probably interacts better when surrounded by family members and thus develops working memory skills. In this sense, a recent meta-analysis supports the idea that the quality of social interaction is one of the stress factors that impact the development of cognitive abilities during the early childhood period [62].

The multiple regression revealed that WM (RDS) was significantly decreased in CG when BLL cut-off is above 5 μg/dL (β = −1.427, p = 0.079, 95 % CI, −2.763 to −0.091) and when Pica syndrome is manifested (β = −0.550, p = 0.028, 95 % CI, −1.039 to −0.061). Nevertheless, it is shown that academic performance is strongly associated with WM (RDS) performances (β = 0.443, p = 0.001, 95 % CI, 0.197–0.689). This results is in line with another study which indicates a strong correlation between WM and academic performance [63]. Since WM is used to manipulate information in order to perform neurocognitive tasks such as reasoning and comprehension, its deterioration can compromise the neurocognitive performance in classroom mental activities [26].

### 4. Conclusion

In the present paper, we have noted the presence of normochromic microcytotic anemia in a small group of children. Although ID has been more prevalent, no differences were elucidated between the two groups either in gender or in other parameters of IS. We have not recorded any serious cases of Pb poisoning. Despite the high significant difference in BLLs between CG and CMA, the prevalence of anemia or ID is far from being able to significantly influence the VA skills of CMA. Children’s VA abilities were positively influenced by daily milk consumption. The principal result of our study is that BLL cut-off value of 5 μg/dL, when it is reached, negatively influences WM (RDS) performances among CMA. Therefore, early recognition of Pb exposure and associated developmental neurocognitive disorders could allow the implementation of specific techniques to improve children’s cognitive trajectories. To do so, a longitudinal study that follows children from school age to adolescence would be useful for detecting the long-term effect of low Pb contamination in the presence of malnutrition issues and for planning specific interventions. In addition, targeted screening is recommended for parents of children working at the mine site to determine the degree of Pb contamination of their clothing and footwear.

Although the sample size is relatively small, the point estimate of the magnitude of the impact of low BLLs on working memory in this population of children from the mining area is relevant and the reduction of environmental insults associated with lead exposure risk may confer a greater advantage to children at high risk of exposure and thus improve their outcomes.

### CRediT authorship contribution statement

Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, S.M, C.R.O, M.O, A. M, M.L, N. L, A.A, A.P, A.S. Writing – original draft, S.M. Writing – review & editing, Visualization, Supervision, A.A, A.P, A.S. Project

### Table 4

Summary of the multiple linear regression models with children’s WM (RDS) scores as a dependent variable.

| Outcome measure | Parameters | CMA | | | CG | | |
|-----------------|------------|-----|-----|-----|-----|-----|-----|
| WM scores (RDS) | BLL by cut-off value (≥ 5 μg/dL) | β = −1.181 | 0.003** | 95 % CI, −1.895 to −0.466 | β = 0.169 | 0.141 | VE |
| WM (RDS) | β = 0.253 | 0.024* | 0.036, 0.409 | β = 0.292 | 0.001** | 0.133, 0.450 |
| AMI [100; 200 €] | β = −0.211 | 0.216 | VE | β = 0.591 | 0.001** | 0.294, 0.933 |
| Academic performance | β = 0.202 | 0.292 | VE | β = 0.123 | 0.318 | VE |
| Multiple R | β = 0.706 | 95 % CI, 0.564 |
| Multiple R² | β = 0.498 | 0.318 |
| Adjusted R² | β = 0.443 | 0.294 |
| p for model | β = 0.002** | < 0.001*** |

The covariates in Model were: BLL by cut-off value, Academic performance, AMI and WM (RDS). *p < 0.05, **p < 0.005, ***p < 0.001. VE: variables were excluded from the equation.
administration, M.I., N. I., A.A., A.P., A.S. Funding acquisition, S.M. All authors have read and agreed to the published version of the manuscript.

Funding
This work was supported by resources available to the Mohamed VI University Hospital of Marrakech in Morocco and the Center for the Determination of Mineral Elements at the Pharmaceutical UFR of Nantes in France.

Declaration of Competing Interest
The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sana Maidoumi reports equipment, drugs, or supplies was provided by Mohammed VI University Hospital Centre Nantes Faculty of Pharmacy. Sana Maidoumi

Data Availability
Data will be made available on request.

Acknowledgments
The authors would like to thank all the work teams, especially Mr. Hakkoum Ahmad Taoufik for his assistance during the biological analyses and Mr. Yannick François for his technical supervision for the determination of mineral elements.

Conflict of interest
The author(s) declare that there is no conflict of interest.

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Table 5
Summary of the multiple linear regression models with children’s WM (RDS) scores as a dependent variable.

| Outcome measure       | Parameters | CMA   | CG   |
|-----------------------|------------|-------|------|
|                       |            | β     | p value | 95% CI | β     | p value | 95% CI |
| WM scores (RDS)       |            | 0.005 | 0.961 | VE     | 1.427 | 0.037* | −2.763, −0.091 |
| Parity                |            | 3.640 | 0.002** | 1.479, 5.801 | 0.082 | 0.476 | VE     |
| Academic performance  |            | 0.283 | 0.123 | VE     | 0.443 | 0.001** | 0.197, 0.689 |
| AMI < 100€            |            | −0.341 | 0.066 | VE     | −0.002 | 0.987 | VE     |
| Pica syndrome         |            | −0.161 | 0.445 | VE     | −0.550 | 0.028* | −1.039, −0.061 |
| Use of cosmetic product k-hol | | −0.147 | 0.427 | VE     | 0.014 | 0.907 | VE     |
| Duration of preschool education | 0.015 | 0.934 | VE     | 0.173 | 0.156 | VE     |
| Multiple R            |            | 0.629 | 0.558 | VE     |
| Multiple R²           |            | 0.395 | 0.312 | VE     |
| Adjusted R²           |            | 0.364 | 0.274 | VE     |
| p for model           |            | 0.002** | < 0.001** | VE     |

The covariates in Model were: BIL by cut-off value, BMI, pica syndrome, duration of pre-school education, parity, Academic performance, and use of cosmetic product k-hol. *p < 0.05, **p < 0.005, ***p < 0.001. VE: variables were excluded from the model.
