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Serum copper, rubidium, selenium, strontium, and zinc and psychophysical health in adults of the Sarno river Basin: PREVES-STOP 2025 community biomonitoring results

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Abstract

Objectives: Evaluate associations between serum copper (Cu), rubidium (Rb), selenium (Se), strontium (Sr), and zinc (Zn) and psychophysical health in adults from Italy's Sarno River Basin within the 2025 PREVES-STOP program.
Methods: Adults aged 30–65 completed validated questionnaires plus clinical evaluation and blood sampling. Elements were quantified by collision/reaction-cell inductively coupled plasma mass spectrometry (ICP-MS). Associations were evaluated using Spearman and partial Spearman correlations.
Results: Significant associations included Zn and Rb associated with lower odds (odds ratio, OR) of severe fatigue – Recognizing and Estimating Signs of Tiredness (REST): Zn OR=0.38, 95 % confidence interval (CI) 0.21–0.68, q=0.02; Rb OR=0.33, 95 % CI 0.15–0.71, q=0.03 – while Sr was associated with higher well-being – the World Health Organization-5 Well-Being Index (WHO-5) OR=1.36, 95 % CI 1.12–1.65, q=0.02.
Conclusions: Findings support broader trace-element panels to inform psychophysical and cardiometabolic risk beyond classical toxic metals, complementing prior PREVES-STOP evidence on lead (Pb) and cadmium (Cd). Further investigation is warranted.

Keywords: rubidium; zinc; strontium; selenium; copper; Sarno river basin

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Introduction

Environmental pollution remains a critical global public health issue, accounting for roughly nine million premature deaths each year [1]. One striking example is the Sarno River Basin in Campania, southern Italy, which exemplifies the severe local impacts of decades of unchecked pollution [2]. This region has endured extensive contamination from industrial effluents, agricultural runoff, untreated sewage, and illegal toxic-waste dumping. Consequently, hazardous substances including heavy metals have accumulated in the soil, water, and air. Even low-level, non-occupational exposures to these metals have been linked to adverse health effects [3]. Emerging evidence suggests that chronic exposure to a broader array of trace elements may also impart subtler health effects [4–6]. For instance, zinc (Zn), copper (Cu), and selenium (Se) are required micronutrients, but in excess they can catalyze oxidative stress or disrupt endocrine functions. Likewise, strontium (Sr) and rubidium (Rb) have no nutritional role yet are present in the environment. Although recent studies suggest that variations in Rb, Sr, Zn, and Cu exposures may influence metabolic outcomes and neurodevelopment [4], the evidence for direct functional or clinical effects, remains limited.

Cancer and in particular urologic malignancies warrant particular attention in polluted settings: the kidney and urothelium are directly exposed to circulating and excreted toxicants, and several tumors in this domain are modulated by hormone-like signals [5]. In southern Italy's Campania region, a systematic review documented excess bladder-cancer burden in municipalities under intense environmental "pollution pressure," reinforcing biologically plausible exposure–disease pathways [6]. In this regard, complementary andrology evidence shows that heavy metals can impair male reproductive health [7]. More broadly – across prostate [8] and renal cancers (including sarcomatoid histology), the field urges reading disease patterns through both exposure landscapes and evolving clinical insights. The Agro–Nocerino–Sarnese area of the Sarno River Basin is indeed recognized as one of the most polluted regions in Europe due to heavy metals and other contaminants [2]. Chronic environmental exposure in this community is believed to contribute not only to various somatic conditions (e.g. hypertension, dyslipidemia, immune alterations) but also to psychosocial effects such as persistent fatigue, poor sleep, anxiety, and depression [9]. Consistent with this, prior studies have noted that mercury exposure can lead to neuropsychiatric symptoms including pronounced fatigue, and chronic arsenic exposure has likewise been associated with increased fatigue and cognitive or mood disturbances [10, 11]. Moreover, cadmium exposure has been hypothesized to trigger myalgic encephalomyelitis/chronic fatigue syndrome-like manifestations [12, 13].

These observations raise the concern that even "low-level" trace elements burdens in the general population may insidiously erode subjective well-being and energy over time. However, traditional clinical instruments might not fully capture such subtle, pollution-related health effects. To address this gap, a set of specialized psychometric questionnaires was recently developed and validated in the Sarno Basin population: the REST ("Recognizing and Estimating Signs of Tiredness") scale for fatigue, the HEAL-BDLC ("Health Evaluation for Affective Living") for mood/anxiety symptoms, and the PEACE ("Promoting Evaluation and Awareness of Comfort in Sleep") scale for sleep quality. These tools include symptom domains relevant to pollution exposure (e.g. headaches, bone pain, palpitations) and have demonstrated strong psychometric properties in this setting, with Cronbach's α of approximately 0.96–0.97 for REST and HEAL-BDLC, and 0.74 for PEACE. Their construct validity is further supported by the expected correlations with the WHO-5 Well-Being Index, a standard measure of general mental wellness [14, 15]. Together, these new instruments enable a more nuanced assessment of fatigue, mood, and sleep disturbances that may be attributable to environmental stressors.

The PREVES-STOP initiative [16] was launched in late 2024 as a community-based environmental health program in the Sarno Basin. In its inaugural pilot campaign (November–December 2024), dozens of adults aged 30–65 were recruited for free medical screenings with optional heavy metal testing. Even within a relatively small sample, blood assays revealed measurable lead in 15.7 % of participants and cadmium in 23.6 %. These prevalence rates were in line with broader Italian biomonitoring surveys. Most importantly, the detected metal burdens correlated with participants' clinical and subjective complaints. Individuals with detectable Pb or Cd tended to show higher blood pressure, worse lipid profiles, signs of immune system shifts, and notably greater fatigue and sleep disturbance [17]. In the pilot, participants reporting severe fatigue received a nutraceutical supplement (adaptogenic and antioxidant components) as an exploratory intervention. Beneficial effects were noted, consistent with wider evidence that targeted nutritional strategies may improve quality of life in chronically ill populations [18].

These preliminary findings demanded confirmation and extension in a larger sample, using a broader panel of metal exposure biomarkers. Accordingly, in May 2025 the PREVES-STOP program was renewed with an expanded

community biomonitoring study focusing on five trace metals in serum – Cu, Rb, Se, Sr, and Zn – and their associations with a wide range of clinical, laboratory, and psychometric health parameters. This campaign was publicly announced through local channels (e.g. the Associazione O.R.A. website and social media) to maximize community participation. The selection of metals was informed by prior environmental surveys and biological plausibility: Cu, Zn, and Se are prevalent in local industrial emissions and agriculture and can become toxic in excess, whereas Rb and Sr, although less commonly discussed, had been detected in regional environmental samples and are chemically analogous to biologically active elements (potassium and calcium, respectively) that could perturb metabolic or neuromuscular processes. The goal of the May 2025 campaign was to explore potential linkages between these metal concentrations and a broad array of health indicators – including standard hematologic and biochemical lab tests, vital signs, and the validated fatigue/mood/sleep questionnaires – in a middle-aged population chronically exposed to a polluted environment. By leveraging the newly validated REST, HEAL-BDLC, PEACE, and the WHO-5 instruments alongside objective clinical and laboratory data, this study aimed to generate a comprehensive profile of metal-exposure correlates.

Here we report the results of this community-based biomonitoring study, which serves as an initial step toward identifying environmental determinants of fatigue and psychophysical wellness in the Sarno River Basin.

Patients and methods

Study design and participants

This study was conducted as a community-based cross-sectional observational investigation under the PREVES–STOP 2025 program in the Sarno River Basin (Campania, southern Italy). The Sarno Basin is recognized as one of the most polluted areas in Europe due to decades of heavy metal contamination [2]. The research was implemented through community health screening events held in May 2025 and promoted by a local non-profit organization, Associazione O.R.A. ETS (www.oncologiaora.it – Oncologia Ricerca Assistenza). To maximize community outreach, two screening clinics were organized in the basin (one in Pagani and one in Angri in the province of Salerno).

Eligible participants were adults 30–65 years old residing in one of the basin municipalities who had experienced persistent fatigue and/or general psychophysical malaise for at least one month prior to enrollment. Additional inclusion criteria were the absence of any uncontrolled severe medical condition (such as active cancer, end-stage organ failure, or debilitating neurological disease) and the ability to complete an online questionnaire (requiring basic literacy and internet access). Before any study procedures, all participants provided written informed consent after discussion with the study physicians, in accordance with the principles of the Declaration of Helsinki. The study protocol received approval from the Institutional ethics committee (Protocol No. CE00225, approved 2 May 2025). The screening sessions were publicly advertised via the O.R.A. website and social media to encourage self-referral from the community. Enrollment was capped at approximately 60 participants per session to ensure each individual received adequate evaluation time. All clinical services and tests were provided at no cost to participants as a community benefit. In addition to receiving free medical preventive assistance,

Data collection and measurements

Each participant underwent a standardized evaluation consisting of an online questionnaire, a brief clinical examination, and laboratory tests (including biomonitoring for heavy metals). Approximately one week before the clinic visit, participants completed a comprehensive online self-report questionnaire. This survey collected information on demographics (age, sex), medical history (comorbidities and current medications), and lifestyle factors (smoking status, alcohol use, diet, physical activity, and any relevant occupational exposures). The questionnaire also incorporated several psychometric instruments to assess fatigue, mood, sleep quality, and overall well-being. In particular, fatigue severity was measured with the *Recognizing and Estimating Signs of Tiredness* (REST) scale, a 13-item questionnaire scored from 0 to 52 (higher scores indicating more severe fatigue). Symptoms of depression and anxiety were evaluated using the *Health Evaluation for Affective Living* (HEAL-BDLC) instrument (score range 0–68, with higher scores reflecting worse affective symptoms). Sleep quality was assessed by the *Promoting Evaluation and Awareness of Comfort in Sleep* (PEACE) scale (score range 0–20, where higher scores denote better sleep comfort and quality). Additionally, participants completed the five-item the World Health Organization Well-Being Index (WHO-5) [19, 20], a general measure of mental wellness scored from 0 to 25 (higher scores indicating better overall well-being). These instruments had been previously validated in the Sarno River Basin population, demonstrating high internal consistency and construct validity [9, 14, 15].

All questionnaire data were recorded electronically and reviewed for completeness prior to the in-person visit. On the day of the screening, participants attended one of the designated local clinics (Studio Medico Sant'Alfonso in Pagani or Fondazione Scoppa in Angri). Study physicians confirmed each participant's eligibility and obtained written informed consent (if not already obtained), then conducted a focused medical examination. A brief systems review was performed to identify any overt illness or red-flag symptoms that would require medical referral or study exclusion. In the absence of exclusionary findings, the participant was cleared to proceed with blood and urine sample collection. All samples were obtained under standardized conditions. Certified phlebotomists collected fasting venous blood (approximately 20 mL in total) from each participant. A portion of this blood was sent to an accredited clinical laboratory for routine analyses, including a complete blood count (with five-part leukocyte differential), fasting serum glucose, creatinine with estimated glomerular filtration rate (eGFR), thyroid-stimulating hormone (TSH), liver transaminases (ALT, AST), and a lipid profile. Each participant also provided a spot urine sample for standard urinalysis (including specific gravity).

Trace elements assessment

For the biomonitoring component, 5 mL of blood was collected from each participant specifically for heavy metal analysis. These specimens were collected in trace-metal-free tubes and kept refrigerated until transport. All metal biomonitoring samples were transported on the same day to the Chemistry Department of University Federico II of Naples. Blood was collected using trace-metal–certified serum tubes without gel separator (royal-blue caps) after skin cleansing with alcohol (no iodine). To minimize artifactual elevation samples with visible hemolysis were rejected. Tubes were allowed to clot at room temperature for 30 min and centrifuged at 2,000 g for 15 min. Serum was transferred into acid-washed polypropylene vials, stored at −20 °C (≤24 h) for longer storage, and equilibrated to room temperature before analysis. In a vessel, 1 mL of 69 % HNO_3 (suprapure) and 1 mL of 30 % H_2O_2 were added to 1 mL of serum and then microwave assisted digestion was carried out in closed vessels in an Milestone microwave oven. Serum was diluted 1:10 (typical) with an acidic diluent (1 % HNO_3 0.01 % non-ionic surfactant and ultrapure water) containing an internal-standard (IS) mix, to adjust for matrix effects. Serum concentrations of five trace metals – copper (Cu), rubidium (Rb), selenium (Se), strontium (Sr), and zinc (Zn) – were measured using inductively coupled plasma mass spectrometry (ICP-MS) [21–23]; following standard trace-element analysis protocols. All reagents used were of analytical grade and for ultra-trace analysis (Sigma Aldrich, Ultrascientific, Merck). The ICP-MS instrument was calibrated with Certified reference materials (CRMs) were provided by the European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM) (with calibration check deviations maintained within ±10 %). The detection limits for all five metals were ±0.1–0.5 µg/L range. All measured metal levels in the cohort were above the limits of detection; any extremely high values (potential outliers) were addressed in the data processing steps described below. Serum trace elements (Cu, Rb, Se, Sr, Zn) were quantified by collision/reaction-cell inductively coupled plasma mass spectrometry. Daily tuning targeted low oxide and doubly charged species (CeO^+/Ce^+ ≤1–2%; Ce^{2+}/Ce^+ ≤2–3 %) and background <⅓ of the lower limit of quantification (LLOQ). Isotopes signals were selected as follows: Cu (*m*/*z*:63, 65), Zn (*m*/*z*:66, 68), Rb (*m*/*z*:85), and Sr (*m*/*z*:88) (Se by O_2 mass-shift monitoring *m*/*z* 96 (SeO^+) to avoid Ar_2^+/Kr interferences). Ultra-high-purity argon was used; plasma was purged before Se/Rb/Sr acquisition to minimize krypton. IS were mapped by mass proximity (e.g., ^{89}Y for Rb/Sr; ^{103}Rh for Cu/Zn/Se), and IS recoveries of 70–130 % were required for acceptance. Matrix-matched, multi-point external calibration (blank + 6–8 levels) was prepared in the sample diluent, spanning expected post-dilution concentrations. Calibration models required $R^2 \geq 0.998$ with back-calculated standards within ±10 % (±15 % at LLOQ). An independent calibration verification (ICV) was run after calibration, with continuing calibration verifications (CCVs) every 10–20 samples and at batch end (acceptance ±10 %). Preparation and reagent blanks accompanied each dilution set and were required to be <LLOQ or <1 % of the top standard. Low/mid/high matrix QCs were inserted at the start, throughout, and end of each batch with recoveries 90–110 %. At least 10 % of samples were analyzed in duplicate; RPD ≤10 % above LLOQ (≤20 % near LLOQ) was required. Westgard-style multi-rules were applied (any QC >±3 SD, or two consecutive >±2 SD triggered investigation, re-tuning, and re-analysis). Carryover was assessed with post-high blanks and required to be <1 % of the preceding signal. Accuracy in representative matrices was periodically confirmed by spike-recovery tests (≥1 per 20 samples; 90–110 %) and, when indicated, standard-addition checks. Procedurally, only certified trace-metal plastics were used, with all transfers in a clean hood to minimize environmental contamination. Results were reported as µg/L. Values <LLOQ were reported as "<LLOQ (value)" under a pre-specified censored-data policy applied uniformly across study groups. All runs archived IS-normalized signals, calibration residuals, QC charts, blanks, and batch sequences.

Statistical analysis

All data were analyzed using R (R Foundation for Statistical Computing, Vienna, Austria; version 4.5.1) [24] with two-tailed significance tests and an α level of 0.05 [24]. Descriptive statistics were first generated for baseline characteristics. Continuous variables were summarized by mean, standard deviation, and minimum–maximum range, as well as quartiles (P25, median, P75) to illustrate distribution shape. Categorical variables (e.g., sex, smoking status) were summarized by frequencies and percentages. To characterize relationships among the five metals, a metal–metal correlation matrix was computed using Spearman's rank correlation coefficient for each pair of natural-log–transformed metal levels [25, 26, 29]. Spearman's ρ was chosen for robustness to non-normality and outliers, which was appropriate given the skewed metal distributions [26]. Pairwise complete observations were used *for these descriptive correlations only*, and 95 % confidence intervals (CIs) were obtained via the asymptotic approximation [27]. The core analyses assessed associations between individual metal biomarkers and health endpoints in two domains [1]: patient-reported scales (fatigue, mood, sleep, well-being) and [2] clinical laboratory values (hematologic and biochemical indices). As an initial nonparametric screen, Spearman correlations (ρ) were calculated between each metal and each outcome [25, 26]. For continuous clinical measures, the raw values were used; for questionnaires, the total scores were treated as numeric in this screening step. Partial Spearman correlations were then computed to adjust for potential confounding. Covariates chosen *a priori* were age (years), sex (male/female), current tobacco use (yes/no), and renal function (eGFR, mL/min/1.73 m^2), given known associations with both metal metabolism and the outcomes. Partial ρ was obtained by rank-transforming both the metal and the outcome, regressing each on the covariates, and correlating the residuals, i.e., the rank-residual method that yields an adjusted Spearman's rho [28–30]. P-values for both unadjusted and partial correlations were derived from asymptotic t-tests under H0: ρ=0 [27–29]. For continuous endpoints (e.g., hemoglobin concentration, HDL cholesterol, liver enzyme levels), adjusted linear regression models were fitted to estimate associations with each metal. Metals were winsorized at the 1st and 99th percentiles prior to transformation, then natural-log–transformed. The exposure was entered on an IQR-scaled log scale, defined as follows: let $Z=ln$ (metal); let IQR_Z denote the sample interquartile range of Z; the model used $Z^{*}=Z/IQR_Z$ as the predictor. Consequently, the reported coefficient βrepresents the mean change in the outcome per 1-IQR increase in natural-log metal concentration. Models adjusted for age, sex, current smoking, and eGFR. Model assumptions were examined with residual diagnostics; mild right-skew in some outcomes was deemed acceptable given the robust inference approach below. To accommodate heteroscedasticity and non-normal residuals, heteroscedasticity-consistent covariance estimators of the HC3 type were used for regression coefficients [31, 32]. Inference was based on these robust standard errors, with two-sided Wald t-tests [33]. For each metal, the β coefficient, 95 % CI, and p-value are reported. Patient-reported outcome scales (REST fatigue, HEAL-BDLC mood, PEACE sleep quality, and the WHO-5 Well-Being Index) were analyzed as ordered categorical outcomes defined by quartile cut-offs. Specifically, for each scale the total score was partitioned into four ordered categories using the empirical sample quartiles (Q1, Q2/median, Q3) as thresholds (i.e., category 1: ≤Q1; category 2: Q1–Q2; category 3: Q2–Q3; category 4: ≥Q3). Proportional-odds (cumulative logit) ordinal regression models were then applied to each endpoint [34, 35]. Metals were entered as IQR-scaled natural-log exposures (as defined above for linear models), and the same covariates (age, sex, current smoking, eGFR) were included. The proportional-odds assumption was evaluated using Brant tests (global and term-specific); no significant violations were detected (p>0.05 for all metal–outcome combinations). As an additional check, the consistency of the metal effect across alternative score cut-offs (first and third quartiles) was examined, with no meaningful deviations observed [36] Metal effects are expressed as odds ratios (ORs) per 1-IQR increase in natural-log metal concentration, with 95 % CIs and Wald p-values. For interpretability, OR>1 indicates a tendency toward better outcomes for higher metal levels for positively oriented scales (e.g., the WHO-5, PEACE), and toward worse outcomes for higher metal levels for negatively oriented scales (e.g., REST, HEAL-BDLC).

Unless otherwise specified above (pairwise-complete descriptive correlations), analyses used listwise deletion with respect to the variables included in each model.

To manage multiple comparisons, we implemented the Benjamini–Hochberg procedure within each family of analyses [37]. In practice, separate false discovery rate (FDR) adjustments were applied to the p-values for: (a) the set of partial correlations between metals and outcomes, (b) the set of linear model coefficients, and (c) the set of ordinal model coefficients. Adjusted q-values are reported alongside p-values, and results were considered statistically noteworthy if q<0.05 (corresponding to an expected <5 % content in that family) [37]. For transparency, we also note in tables the number of tested associations that did not reach nominal significance (p<0.05). All analyses used complete-case data. There were no missing values in the core dataset for the variables analyzed; every participant included had a full profile of metal measurements, questionnaire scores, and lab results. Data handling and statistical computations were

performed using the R tidyverse suite and relevant packages for robust modeling and ordinal regression (including sandwich, lmtest, and MASS) [38–41].

Results

Characteristics of the population

A total of 125 participants took part in the May 2025 PREVES-STOP campaign by attending the free medical evaluations. Among these, 88 participants opted to receive the nutraceutical supplement (Previflanplus® Energy, Waispharma, Milan, Italy) for fatigue offered by the program, and 81 participants underwent a blood draw for standard analysis. Ultimately, 78 volunteers (mean age 49.6 ± 8.7 years) provided informed consent, met all entry criteria, completed the full assessment battery, and deliberately donated samples to assess had sufficient serum available for heavy metal analysis; these 78 constituted the analytic cohort for the present study. Baseline clinical measurements for the cohort were broadly within expected ranges, with central values close to standard reference norms. For example, median white blood cell count was 6.50×10^9/L, red blood cell count 4.71×10^{12}/L, hemoglobin 13.70 g/dL, and platelets 243×10^9/L. Median fasting glucose was 89.5 mg/dL, and median serum creatinine was 0.83 mg/dL (with median eGFR ≈ 96 mL/min/1.73 m^2). Self-reported symptom scores also varied widely (median [IQR] for fatigue REST score: 23.5 [19–30]; mood HEAL-BDLC: 23 [17–35.8]; sleep quality PEACE: 6 [4–8.8]; overall wellness the WHO 5 Well Being Index: 10 [9–14]), which justified treating these scales as ordinal outcomes in the modeling. Serum metal concentrations spanned broad ranges as well. Notably, there were no missing data for any of the variables analyzed, and Table 1 presents detailed summary statistics to illustrate the distribution of each measure.

Table 1: Baseline clinical and laboratory characteristics – continuous variables (mean, SD, P25, median, P75).

Variable	Mean	SD	P25	Median	P75
Age (years)	49.59	8.71	43.00	49.00	56.00
White blood cells ($\times 10^9$/L)	6.47	1.77	5.13	6.50	7.58
Red blood cells ($\times 10^{12}$/L)	4.76	0.46	4.43	4.71	5.07
Hemoglobin, g/dL	13.78	1.45	12.82	13.70	14.82
Platelets ($\times 10^9$/L)	243.12	53.28	208.00	241.50	267.00
Neutrophils ($\times 10^9$/L)	3.74	1.46	2.66	3.64	4.71
Lymphocytes ($\times 10^9$/L)	2.01	0.56	1.61	1.93	2.33
Monocytes ($\times 10^9$/L)	0.50	0.18	0.38	0.45	0.58
Eosinophils ($\times 10^9$/L)	0.17	0.13	0.07	0.15	0.23
Basophils ($\times 10^9$/L)	0.04	0.02	0.03	0.04	0.06
Glucose, mg/dL	89.49	11.74	82.25	89.50	95.75
Creatinine, mg/dL	0.83	0.16	0.72	0.83	0.90
eGFR, mL/min/1.73 m^2	91.97	13.36	84.00	96.00	101.00
AST, U/L	22.47	8.50	17.25	20.00	24.00
ALT, U/L	25.79	19.29	15.00	19.50	26.00
HDL cholesterol, mg/dL	56.32	15.51	46.00	55.50	66.75
LDL cholesterol, mg/dL	111.85	30.62	89.25	110.50	130.50
TSH, µIU/mL	1.87	2.05	1.01	1.52	2.15
Serum copper, µg/L	903.25	234.24	706.84	906.95	1004.99
Serum rubidium, µg/L	123.07	80.42	39.64	146.77	187.55
Serum selenium, µg/L	132.20	30.65	106.94	135.79	156.02
Serum strontium, µg/L	29.03	18.76	20.82	26.61	32.85
Serum zinc, µg/L	905.58	473.89	620.43	898.43	1099.16
WHO-5 well-being index	11.64	5.03	9.00	10.00	14.00
REST fatigue score	24.50	10.92	19.00	23.50	30.00
HEAL-BDLC mood score	26.38	13.81	17.00	23.00	35.75
PEACE sleep quality score	6.86	4.06	4.00	6.00	8.75

Comments. Missing values in this table: **0**. Acronyms: HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TSH, thyroid-stimulating hormone; eGFR, estimated glomerular filtration rate; the WHO-5, Well-Being Index, the World Health Organization wellness score; REST, Recognizing and Estimating Signs of Tiredness; HEAL-BDLC, mood/anxiety score; PEACE, sleep quality score. Metals are measured in serum (µg/L). Creatinine is expressed in mg/dL.

Associations of serum metal levels with health parameters

Before examining metal–health relationships, we assessed correlations among the five measured serum metals. This analysis revealed a coherent cluster of positive inter-metal correlations among Cu, Rb, Se, Sr, and Zn (with the strongest pairwise correlation between Zn and Se, ρ=0.83; followed by Rb–Zn, ρ=0.72; and Cu–Rb, ρ=0.65) (Table 2). In covariate-adjusted linear regression models, higher rubidium was robustly associated with a less favorable lipid profile. Specifically, the HDL cholesterol level decreased by an estimated 10.7 mg/dL for each 1-IQR increase in log-transformed Rb (95 % CI: −16.7 to −4.7), an association that remained significant after controlling for multiple comparisons (q=0.05). This rubidium–HDL finding was consonant with the negative partial Spearman correlation between Rb and HDL (ρ = −0.36) and was the only HDL-related signal to meet the 5 % false discovery rate threshold (Tables 3–4). Serum zinc and selenium showed directionally similar inverse relationships with HDL (approximate decreases of 6.62 and 7.29 mg/dL per IQR increase, respectively, with partial $\rho \approx -0.30$ to -0.33). However, those trends did not reach significance after FDR adjustment and should be interpreted with caution pending further study. Selenium was also associated with liver and kidney function markers: each IQR increase in Se corresponded to an increase of about +4.29 U/L in AST and +8.30 U/L in ALT (both nominally significant at p<0.05), and partial

correlations suggested that higher Se tended to accompany higher creatinine as well (ρ=0.30, q=0.14). While none of the selenium–clinical associations passed the FDR threshold, this pattern points to potential hepatic and renal effects that warrant confirmation in larger samples (Tables 3–4). For the psychometric outcomes, the proportional-odds models (adjusted for age, sex, tobacco use, and eGFR) identified several protective associations with metal levels. Higher zinc and higher rubidium were each associated with significantly lower odds of worse fatigue ratings – in other words, individuals with greater Zn or Rb levels tended to report less severe fatigue (Zn: OR=0.38 for one IQR higher log-Zn, 95 % CI 0.21–0.68, q=0.02; Rb: OR=0.33, 95 % CI 0.15–0.71, q=0.03). Likewise, higher strontium was associated with higher odds of better self-reported overall well-being on the WHO-5 scale (OR=1.36 per IQR, 95 % CI 1.12–1.65, q=0.02). These directions were further supported by partial correlation analysis: for example, Sr showed a moderate inverse correlation with the REST fatigue score (ρ = −0.45, q=0.01) and a negative correlation with the HEAL-BDLC mood-disturbance score (ρ = −0.37, q=0.05), consistent with higher Sr exposure correlating with lower fatigue and better mood. However, higher Sr also displayed a potential hematological downside: each IQR increase in Sr was associated with a slight decrease in hemoglobin (−0.17 g/dL, q=0.05) and a small reduction in RBC count (-0.05×10^{12}/L, q=0.23), in line with its negative partial correlations with these erythroid indices (Tables 3 and 5). Taken together, these results depict a nuanced landscape of metal–health relationships in this population. Certain metals (notably Zn and Rb) were associated with more favorable fatigue scores, and Sr was associated with higher perceived well-being (despite a minor trade-off of lower erythroid indices), whereas rubidium's detrimental link with lower HDL cholesterol stood out as the clearest adverse cardiometabolic signal after accounting for multiple comparisons (Tables 3–5).

Discussion

The PREVES-STOP findings reveal nuanced relationships between trace metal biomarkers and health in a chronically exposed community. In particular, serum rubidium (Rb) emerged as a significant correlate of adverse lipid profiles, showing a robust inverse association with HDL cholesterol. Notably, Rb is chemically analogous to potassium and can integrate into biological processes; prior research hints that Rb may affect metabolic and inflammatory pathways [42]. For instance, a Greek study in obese adults found plasma Rb associated with oxidative stress markers [42], reinforcing that Rb is not biologically inert. Our results extend these observations by suggesting Rb could unfavorably alter lipid metabolism. Mechanistically, one might speculate that excessive Rb interferes with potassium-dependent enzymatic processes in the liver or alters cholesterol transport, though direct causation remains unproven. In contrast, zinc (Zn) showed potentially protective associations, especially for subjective health outcomes. Higher serum Zn was correlated with better fatigue scores, i.e. less severe fatigue, in both partial correlations and adjusted ordinal models. Individuals in the upper quartiles of Zn had significantly lower odds of reporting high fatigue (REST score) in our analysis. Although the Zn–fatigue correlation was modest in magnitude ($\rho \approx -0.34$) and narrowly missed the false-discovery significance threshold in partial correlation analysis, the direction aligns with clinical expectations. Zinc is an essential micronutrient important for energy metabolism, neuromuscular function, and neurotransmitter

Table 2: Inter-metal spearman correlations among log-transformed serum metals.

	Serum copper (µg/L) (log)	Serum rubidium (µg/L) (log)	Serum selenium (µg/L) (log)	Serum strontium (µg/L) (log)	Serum zinc (µg/L) (log)
Serum copper (µg/L) (log)	1.00	0.65	0.52	0.51	0.60
Serum rubidium (µg/L) (log)	0.65	1.00	0.66	0.53	0.72
Serum selenium (µg/L) (log)	0.52	0.66	1.00	0.48	0.83
Serum strontium (µg/L) (log)	0.51	0.53	0.48	1.00	0.54
Serum zinc (µg/L) (log)	0.60	0.72	0.83	0.54	1.00

Comments. Missing values in this table: **0**. Acronyms: HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TSH, thyroid-stimulating hormone; eGFR, estimated glomerular filtration rate; the WHO-5, Well-Being Index, the World Health Organization wellness score; REST, Recognizing and Estimating Signs of Tiredness; HEAL-BDLC, mood/anxiety score; PEACE, sleep quality score. Metals are measured in serum (µg/L). Creatinine is expressed in mg/dL.

Table 3: Partial spearman correlations between log-transformed serum metals and clinical endpoints, adjusted (p<0.05).

Metal (log)	Endpoint	Spearman ρ	p-Value	n	FDR q-value	Interpretation
Serum strontium (µg/L) (log)	REST fatigue score	−0.45	0.001	78	0.01	Higher strontium ↔ lower REST (less fatigue, favorable).
Serum rubidium (µg/L) (log)	HDL cholesterol (mg/dL)	−0.36	0.005	78	0.05	Higher rubidium ↔ lower HDL (unfavorable lipid profile).
Serum strontium (µg/L) (log)	HEAL-BDLC mood score	−0.37	0.005	78	0.05	Higher strontium ↔ lower HEAL-BDLC (better mood, favorable).
Serum zinc (µg/L) (log)	REST fatigue score	−0.34	0.008	78	0.07	Higher zinc ↔ lower REST (less fatigue, favorable).
Serum selenium (µg/L) (log)	HDL cholesterol (mg/dL)	−0.33	0.009	78	0.08	Higher selenium ↔ lower HDL (unfavorable).
Serum selenium (µg/L) (log)	Creatinine (mg/dL)	0.30	0.01	78	0.14	Higher selenium ↔ higher creatinine (potentially worse renal marker).
Serum strontium (µg/L) (log)	HDL cholesterol (mg/dL)	−0.31	0.01	78	0.14	Higher strontium ↔ lower HDL (unfavorable).
Serum zinc (µg/L) (log)	HDL cholesterol (mg/dL)	−0.30	0.01	78	0.14	Higher zinc ↔ lower HDL (unfavorable).
Serum selenium (µg/L) (log)	AST (U/L)	0.29	0.01	78	0.15	Higher selenium ↔ higher AST (possible liver enzyme elevation).
Serum strontium (µg/L) (log)	Red blood cells ($\times 10^{12}$/L)	−0.28	0.01	78	0.15	Higher strontium ↔ lower RBC (unfavorable hematologic signal).
Serum selenium (µg/L) (log)	REST fatigue score	−0.27	0.02	78	0.17	Higher selenium ↔ lower REST (less fatigue, favorable).
Serum rubidium (µg/L) (log)	REST fatigue score	−0.25	0.03	78	0.22	Higher rubidium ↔ lower REST (less fatigue, favorable).
Serum strontium (µg/L) (log)	WHO being index score	0.25	0.03	78	0.22	Higher strontium ↔ higher WHO-5 well being index score (better wellness, favorable).
Serum selenium (µg/L) (log)	Lymphocytes (%)	0.24	0.03	78	0.25	Higher selenium ↔ higher lymphocytes (descriptive increase).
Serum copper (µg/L) (log)	REST fatigue score	−0.23	0.04	78	0.27	Higher copper ↔ lower REST (less fatigue, favorable).
Serum strontium (µg/L) (log)	Hemoglobin (g/dL)	−0.24	0.04	78	0.27	Higher strontium ↔ lower hemoglobin (unfavorable).
Serum selenium (µg/L) (log)	ALT (U/L)	0.23	0.04	78	0.27	Higher selenium ↔ higher ALT (possible liver enzyme elevation).

Comments. Only rows with p<0.05 are displayed; rows omitted: **112**. Missing values in this table: **0**. Metals in serum (µg/L). Creatinine in mg/dL.

Table 4: Adjusted linear regression estimates for continuous endpoints per 1-IQR increase in log-transformed serum metals.

Outcome	Exposure (IQR-scaled log)	n	β	SE	Lower 95 % CI	Upper 95 % CI	p-Value	FDR q-value	Interpretation
HDL cholesterol, mg/dL	Serum rubidium (µg/L), per 1 IQR (log)	78	−10.70	3.08	−16.73	−4.67	0.001	0.05	Higher rubidium → lower HDL (unfavorable lipid profile).
Hemoglobin, g/dL	Serum strontium (µg/L), per 1 IQR (log)	78	−0.17	0.05	−0.27	−0.07	0.001	0.05	Higher strontium → lower hemoglobin (unfavorable).
HDL cholesterol, mg/dL	Serum zinc (µg/L), per 1 IQR (log)	78	−6.62	2.35	−11.23	−2.02	0.01	0.18	Higher zinc → lower HDL (unfavorable).
AST, U/L	Serum selenium (µg/L), per 1 IQR (log)	78	4.29	1.57	1.22	7.37	0.01	0.18	Higher selenium → higher AST (possible liver enzyme elevation).
Red blood cells ($\times 10^{12}$/L)	Serum strontium (µg/L), per 1 IQR (log)	78	−0.05	0.02	−0.08	−0.01	0.01	0.23	Higher strontium → lower RBC (unfavorable).
HDL cholesterol, mg/dL	Serum selenium (µg/L), per 1 IQR (log)	78	−7.29	3.25	−13.66	−0.92	0.03	0.34	Higher selenium → lower HDL (unfavorable).
ALT, U/L	Serum selenium (µg/L), per 1 IQR (log)	78	8.30	3.82	0.82	15.79	0.03	0.35	Higher selenium → higher ALT (possible liver enzyme elevation).

Comments. Only rows with p<0.05 are displayed; rows omitted: **86**. Missing values in this table: **0**. Metals in serum (µg/L). Creatinine in mg/dL.

Table 5: Adjusted proportional-odds estimates for ordinal endpoints per 1-IQR increase in log-transformed serum metals. REST fatigue (higher=worse fatigue); HEAL-BDLC (higher=worse mood); the WHO-5 Well-Being Index (higher=better wellness).

Outcome	Exposure (IQR-scaled log)	n	Odds ratio	Lower 95 % CI	Upper 95 % CI	p-Value	FDR q-value	Interpretation
REST fatigue score	Serum zinc (µg/L), per 1 IQR (log)	78	0.38	0.21	0.68	0.001	0.02	Higher zinc → lower odds of worse fatigue (favorable).
WHO 5 well-being score	Serum strontium (µg/L), per 1 IQR (log)	78	1.36	1.12	1.65	0.001	0.02	Higher strontium → higher odds of better wellness (favorable).
REST fatigue score	Serum rubidium (µg/L), per 1 IQR (log)	78	0.33	0.15	0.71	0.001	0.03	Higher rubidium → lower odds of worse fatigue (favorable).
REST fatigue score	Serum selenium (µg/L), per 1 IQR (log)	78	0.49	0.27	0.90	0.02	0.12	Higher selenium → lower odds of worse fatigue (favorable).
HEAL-BDLC mood score	Serum strontium (µg/L), per 1 IQR (log)	78	0.79	0.63	0.99	0.04	0.17	Higher strontium → lower odds of worse mood (favorable).
HEAL-BDLC mood score	Serum zinc (µg/L), per 1 IQR (log)	78	0.54	0.30	0.98	0.04	0.17	Higher zinc → lower odds of worse mood (favorable).

Comments. Only rows with p<0.05 are displayed; rows omitted: **18**. Missing values in this table: **0**. Metals in serum (µg/L). Creatinine in mg/dL.

regulation. Lower Zn status has been linked to fatigue and mood disorders in other contexts; for example, chronic fatigue syndrome patients often show significantly reduced serum Zn [43]. The inverse Zn–fatigue relationship in our study (higher Zn, less fatigue) is thus consistent with the idea that marginal zinc deficiency might exacerbate fatigue and that adequate Zn confers resilience against exhaustion [44]. Similarly, there was a trend for higher Zn to relate to better mood (lower HEAL-BDLC scores) and sleep quality, although these did not reach statistical significance. We also observed that copper (Cu), another essential micronutrient, had a weak inverse correlation with fatigue scores (higher Cu associated with slightly less fatigue), but this association was not robust. Given that Cu is an acute-phase reactant and often elevated in inflammation, the lack of a strong Cu effect on symptoms may reflect the body's tight homeostatic control of copper; subtle variations in normal-range Cu might not translate into observable symptom differences in this sample. Strikingly, strontium (Sr) showed a double-edged pattern of associations. On one hand, Sr was linked to better self-reported well-being and mood. It is intriguing to speculate that Sr could influence neurological or endocrine function in a way that reduces fatigue perception or improves mood – perhaps via its calcium-mimicking properties in signaling pathways. It is worth noting that pharmacological doses of strontium (in the form of strontium ranelate) have been used in osteoporosis and shown some effects on pain and maybe mood, although data on psychotropic effects are limited. However, our study also found that higher Sr was associated with slightly lower hemoglobin and red blood cell counts, which underscores the complexity of trace metal effects: an element can have both favorable and unfavorable associations with health parameters. Finally, selenium (Se) showed a pattern suggestive of metabolic strain at higher levels, though associations did not cross the significance threshold. Selenium is an essential antioxidant micronutrient, yet in excess it can be hepatotoxic or nephrotoxic. In our study, higher serum Se was nominally associated with elevated liver enzymes (AST and ALT) and with higher creatinine. Specifically, each IQR increase in Se corresponded to roughly +4 U/L AST and +8 U/L ALT in regression models, and Se's partial correlation with creatinine ($\rho \sim 0.30$) hinted at a link to renal function. While these did not remain significant after false-discovery rate adjustment, the coherent trend is noteworthy. It aligns with clinical reports of high-normal selenium intake occasionally leading to liver enzyme elevations or selenosis symptoms in sensitive individuals. Our data thus raise the possibility that even at environmental exposure levels, relatively higher Se might contribute to mild hepatic or renal stress. Conversely, we did not observe clear benefits of Se on the measured outcomes. The direction of association for Se with HDL was negative (higher Se trending with lower HDL, similar to Zn and Rb patterns). It is possible that the Se levels in this population were on the higher side of adequate, such that additional Se did not confer benefit and instead hinted at toxicity. Alternatively, Se could be acting as a proxy for some unmeasured exposure or dietary pattern. In any case, the selenium findings should be interpreted cautiously given statistical insignificance, but they warrant further investigation in larger samples to determine if there is a threshold beyond which Se's role shifts from beneficial to detrimental [4]. Comparatively, global literature on trace elements provides both parallels and contrasts to our findings. In terms of lipid effects, previous studies have seldom examined rubidium, but some have reported that exposure to metal mixtures can

unfavorably alter lipid profiles. For example, research in contaminated areas of China found that mixtures of essential and non-essential elements were associated with higher triglycerides and lower HDL, implicating possible interactions among elements [4]. Our identification of Rb as a specific factor in lowering HDL is novel, yet it resonates with the general concept that environmental pollutants contribute to cardiovascular risk factors. It also prompts comparison to other alkali metals: for instance, cesium, a neighbor of rubidium in the periodic table, has been linked to metabolic disturbances in animal models. Rubidium itself, while not widely studied in epidemiology, has seen some use in psychiatric research – rubidium salts have been tested as antidepressants in the past, given Rb's ability to substitute for potassium in neuronal processes. Those trials noted mood improvements (in line with our finding of Rb associating with lower fatigue), but also side effects like changes in blood pressure and possibly lipids [45]. Thus, the Rb-HDL connection we report might be an example of an often-overlooked environmental exposure influencing cholesterol metabolism, warranting further exploration in toxicological studies. Our Zn findings are strongly supported by existing knowledge. Zinc deficiency is a known risk factor for fatigue, poor immune function, and depressive symptoms. Several studies have documented that patients with chronic fatigue syndrome or depression have significantly lower zinc levels than healthy controls [43, 46]. A meta-analysis of zinc in depression found an inverse correlation between serum Zn and depression severity [46]. Moreover, zinc supplementation trials have shown improvements in fatigue among certain groups (e.g. the elderly or cancer patients with fatigue) [47]. Our population was not overtly zinc-deficient on average (median Zn ~898 µg/L, which is within normal range), but the fact that those with relatively higher Zn had better subjective well-being suggests that variations within the normal range still matter – or that some individuals at the lower end of normal Zn may experience fatigue. This is in line with a precision-nutrition perspective: optimal micronutrient status (not just avoidance of deficiency) might promote better energy and mood. With regard to strontium and selenium, literature is sparser. Strontium is not commonly measured in epidemiological studies [48], but there is growing interest due to its presence in contaminated soils and its medicinal use in osteoporosis. A study of groundwater contaminants in Russia noted that strontium levels in drinking water correlated with certain population health trends, though direct causality was unclear [49]. Our finding that Sr correlates with improved mood and well-being is novel. We might consider analogies: calcium, which Sr mimics, is important for neurotransmitter release and muscle function; disturbances in calcium signaling have been implicated in fatigue and mood disorders. It is conceivable that Sr at low doses could positively modulate calcium-dependent processes or that it serves as a proxy for some healthy behavior (for example, higher Sr might come from certain dietary sources like vegetables or dairy, which could themselves improve nutrition and mood. This underscores the need to include elements like Sr in biomonitoring studies, as their health relevance may have been underestimated. Selenium is better studied in nutritional epidemiology, often showing a U-shaped relationship with health – both deficiency and excess can be problematic. Italy is not considered a high-selenium region (parts of Europe have moderate Se soils), but certain areas or diets (seafood-rich, or areas with selenium-rich irrigation) can lead to higher Se status. Prior research internationally has linked high-normal selenium levels to dyslipidemia, for instance, some US studies found that people with higher selenium (>130 µg/L) paradoxically had higher cholesterol levels, possibly due to selenium's effects on thyroid hormone activation and metabolic rate. Our observation of Se correlating with higher ALT/AST is consistent with reports of mild liver function disturbances in high selenium regions of China where selenosis occurs. However, we stress that our Se associations were not definitive. Comparative literature [50] suggests caution: selenium supplementation in well-nourished populations can sometimes elevate cholesterol or blood sugar. Taken together, our Se findings tentatively align with the notion that "more is not always better" for selenium – a delicate balance exists where both low and high selenium can have consequences. This again highlights the complexity seen in global studies: nutritional elements can behave like toxins when in excess [4]. Crucially, our study population's experiences resonate with environmental health investigations in other contaminated communities worldwide. Chronic fatigue, poor sleep, and low mood have been reported in residents near mining areas or industrial belts, often linked to metal exposure [51]. The associations observed in this study invite consideration of potential biological mechanisms through which trace metals could influence health. A unifying theme for many heavy metals and trace elements is their ability to generate oxidative stress and inflammation, which are in turn linked to fatigue, mood changes, and organ damage [10, 52]. Another consideration is that Rb might indirectly reflect exposure to other co-occurring substances that harm lipid metabolism. Nonetheless, given Rb's known pharmacologic interactions (e.g. its historical use as an antidepressant), direct biological effects are plausible. [53]. For zinc, the beneficial associations with fatigue and mood likely reflect its myriad roles in the body's homeostasis. Zinc is a cofactor for over 300 enzymes, including those involved in energy production (e.g. in the

Krebs cycle) and antioxidant defense (such as superoxide dismutase). Adequate zinc supports immune function and dampens chronic inflammation; conversely, zinc deficiency can lead to increased inflammatory cytokines and oxidative damage [54]. Chronic inflammation and oxidative stress are thought to be drivers of fatigue and depression [43]. Therefore, higher Zn levels might protect against fatigue by reducing pro-inflammatory signaling and oxidative damage in muscle and brain tissues [55]. Strontium's influence on well-being is more enigmatic, given that Sr is not known to have any nutritional function. Selenium's dual nature (antioxidant vs. pro-oxidant at high levels) is well documented, and our data lean toward mild pro-oxidant effects at the upper end of exposure. Copper's slight inverse correlation with fatigue might be related to its role in mitochondrial energy production.

The findings from the PREVES-STOP study carry several important public health implications, particularly for communities that experience chronic exposure to multiple metals such as those in the Sarno River Basin. Foremost among these implications is the indication that health surveillance in polluted areas should broaden its scope beyond the traditional focus on the "usual suspect" heavy metals. Lead, cadmium, mercury, and arsenic remain critical targets for monitoring and mitigation because their toxicity has been firmly established in the literature. Evidence from the present analysis, however, demonstrates that lesser-known trace metals can also contribute to the cumulative health burden in exposed populations. Rubidium, strontium, zinc, copper, and selenium are not typically included in routine environmental health screenings, yet associations were observed between these elements and key indicators of health status. Public health agencies could therefore consider inclusion of a wider panel of metal biomarkers within biomonitoring programs that focus on at-risk populations. Adoption of such an approach would support a more holistic understanding of exposure profiles and would facilitate the identification of early warning signals that point to subtle but meaningful health effects. As an example, systematic tracking of population trends in high-density lipoprotein concentrations or measures of fatigue in relation to environmental rubidium or selenium levels could inform local advisories and guide targeted interventions. Because rubidium and strontium lack well-defined reference ranges for health risk assessment, the evidence presented here underscores the need to establish baseline concentrations in the general population and to determine thresholds at which these metals should prompt concern.

In conclusion, the PREVES-STOP study's examination of copper, rubidium, selenium, strontium, and zinc indicates that chronic environmental exposure exerts a broad and often subtle influence on human health, with effects that extend from lipid profiles to the degree of tiredness reported upon awakening. The public health message that emerges is unambiguous. Protection of communities such as those in the Sarno River Basin requires attention not only to acute toxicity but also to the quieter erosion of health and well-being that follows long-term, low-level exposures. Implementation of comprehensive strategies that span environmental remediation and appropriately designed nutritional support offers a path toward restoring physical health, functional capacity, and a sense of vitality and optimism among populations that continue to live under the persistent shadow of pollution.

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Informed consent: This study strictly adhered to GDPR regulations to ensure participant privacy and data confidentiality, including only participants who provided explicit consent for their anonymized data to be used for research.

Author contributions: Conceptualization: CB Methodology / Study design: CB Investigation & data collection (partecipazione allo studio): LS, RB, CB, FCr, AVe, VR, AF, ER, FeCo, MF Chemical analyses: MI, MT, AM, GDT Formal analysis / Data analysis: CB Supervision: GDL Writing – original draft: CB Writing – review & editing (revisione critica per contenuto intellettuale rilevante): LS, RB, CB, FCr, AVe, AF, VR, SeRi, SaRi, AP, VM, ER, GR, AR, MI, MT, AM, GDT, FlCo, RDT, OS, AVi, LM, PT, FS, FCa, FeCo, GDL Legend (sigle univoche → autore). LS = Luca Scafuri; RB = Raffaele Baio; CB = Carlo Buonerba; FCr = Felice Crocetto; AVe = Antonio Verde; AF = Antonella Ferraioli; VR = Vittorio Riccio; SeRi = Serena Rizzano; SaRi = Sara Rizzano; AP = Armando Pisapia; VM = Vittorino Montanaro; ER = Emily Ronga; GR = Giuseppe Romeo; AR = Antonio Ruffo; MI = Mauro Iuliano; MT = Marco

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