Revue des Sciences de la Santé (Rev. Sci. Sant.)
ISSN: 3078-6959 (Online); 3078-8226 (Print)
Volume-5 Issue-1, 2026

Hematotoxic Effect of Consuming Geophagic Clay Contaminated with Heavy Metals: An Experimental Study in Wistar Rats (Rattus norvegicus)

Effet hématotoxique de la consommation d'argile géophagique contaminée par des métaux lourds : étude expérimentale chez des rats Wistar (Rattus norvegicus)

Lionel S. ASAMBOA[1,4,*], Nelly K. NSIMBA[4], Esther M. MPIANA[4], Dorcas T. SOLA[4], Sarah B. KINSONA[4], Claudia M. MBANDA[4], Jean-Jacques D. AMOGU[1,4], Max V. SEKE[3,4], Gisèle K. MAKENGO[1], Joel K. TUAKUILA[2] & Odette N. KABENA[1,5]

[1]*Mention Life Science, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo*
[2]*Mention Chemistry and Industry, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo*
[3]*Mention Physical, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo*
[4]*Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo*
[5]*National Committee for Protection against Ionizing Radiation (CNPRI), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo.*

ABSTRACT :

Geophagy, or the intentional ingestion of clay, is widely practiced in sub-Saharan Africa, particularly among pregnant women. However, its toxicological effects remain poorly documented. Clays may contain heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and nickel (Ni), which may become bioavailable after ingestion and cause systemic and hematological effects. In this study, Wistar rats were exposed for eight weeks to various doses of contaminated clay, administered either through the diet or by gavage. Blood concentrations of heavy metals, hematological parameters (red and white blood cells, hemoglobin, platelets), and essential trace elements (iron, manganese, selenium) were analyzed. The results show a dose-dependent accumulation of heavy metals. High doses, particularly when administered via gavage, caused severe microcytic anemia, leukopenia, and thrombocytopenia, suggesting bone marrow damage and disruption of heme synthesis. At moderate doses, milder effects with compensatory mechanisms were observed. A disruption in trace element balance was also noted, with a decrease in iron and manganese and an increase in selenium. The correlations confirm a link between heavy metal exposure and hematological disorders. This study highlights a health risk

Keywords : geophagy, contaminated clays, heavy metals, bioaccumulation, hematology, wistar rat, dose-responses, hematopoietic toxicity.

RESUME:

La géophagie, c'est-à-dire l'ingestion volontaire de sols argileux, est largement pratiquée en Afrique subsaharienne, surtout chez les femmes enceintes. Cependant, ses effets toxicologiques restent peu documentés. Les argiles peuvent contenir des métaux lourds tels que le plomb (Pb), le cadmium (Cd), l'arsenic (As) et le nickel (Ni), susceptibles de devenir biodisponibles après ingestion et d'entraîner des effets systémiques et hématologiques. Dans cette étude, des rats Wistar ont été exposés pendant huit semaines à différentes doses d'argile contaminée, administrée soit par alimentation, soit par gavage. Les concentrations sanguines de métaux lourds, les paramètres hématologiques (globules rouges et blancs, hémoglobine, plaquettes) ainsi que les oligo-éléments essentiels (fer, manganèse, sélénium) ont été analysés. Les résultats montrent une accumulation dose-dépendante de métaux lourds. Les fortes doses, notamment par gavage, ont provoqué une anémie microcytaire sévère, une leucopénie et une thrombocytopénie, suggérant une atteinte de la moelle osseuse et une perturbation de la synthèse de l'hème. À doses modérées, des effets plus légers avec des mécanismes compensatoires ont été observés. Une perturbation de l'équilibre des oligo-éléments a également été notée, avec une baisse du fer et du manganèse et une hausse du sélénium. Les corrélations confirment un lien entre la charge en métaux lourds et les troubles hématologiques. Cette étude met en évidence un risque sanitaire important lié à la géophagie et souligne la nécessité d'actions de santé publique.

Mots clés : géophagie, argiles contaminées, métaux lourds, bioaccumulation, hématologie, rat Wistar, relations dose-réponse, toxicité hématopoïétique.

*Adresse des Auteur(s)

Lionel S. ASAMBOA, *Mention Life Science, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI & Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, Democratic Republic of the Congo ;*

E-mail : *lionel.asamboa@unikin.ac.cd*

Nelly K. NSIMBA, *Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo ;*

Esther M. MPIANA, *Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo ;*

Dorcas T. SOLA, *Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo ;*

Sarah B. KINSONA, *Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo;*

Claudia M. MBANDA, *Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo;*

Jean-Jacques D. AMOGU, *Mention Life Science, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI & Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, Democratic Republic of the Congo;*

Max V. SEKE, *Mention Physical, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI & Center of Excellence for Chemical, Biological, Radiological, and Nuclear (CoE-CBRN), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo,;*

DOI: *https://doi.org/10.71004/rss.026.v5.i1.60*
Journal Website: *www.rss-istm.net*
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026

Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Gisèle K. MAKENGO, *Mention Life Science, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo;*
Joel K. TUAKUILA, *Mention Chemistry and Industry, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo;*
Odette N. KABENA, *Mention Life Science, Faculty of Science and Technology, University of Kinshasa, P.O. Box 190, Kinshasa XI & National Committee for Protection against Ionizing Radiation (CNPRI), Ministry of Higher Education, Universities, Scientific Research, and Innovation, DR Congo.*

I. INTRODUCTION

The deliberate consumption of earth material, particularly clay-rich soil, a practice known as geophagy, has been documented since antiquity in both humans and animals. Although geographically widespread, geophagy remains especially prevalent in sub-Saharan Africa, where it is commonly practiced by pregnant women and, to a lesser extent, children (Young & Miller, 2019; Bonglaisin and *al,* 2022; Cham and *al*., 2023).

Clays such as kaolinite, montmorillonite, and bentonite are ingested for cultural, medicinal, or perceived nutritional purpose. Reported motivation include alleviation of pregnancy, related nausea, mitigation gastrointestinal discomfort, and supplementing mineral deficiencies (Young & Miller 2024; Malebatja and *al.,* 2024). Despite its sociocultural normalization, the toxicological implication of this practice remain insufficiently characterized.

Growing environmental evidence indicates that geophagic clays may contain elevated concentration of toxic heavy metals, including lead, cadmium, mercury, and arsenic, sometimes exceeding established safety thresholds (Kortei and *al*, 2020; Oyebanjo and *al,* 2024). The physicochemical properties of clays, notably their fine particle size, large specific surface area, and high cation-exchange capacity, promote adsorption and retention of metallic cation (Wang and *al,* 2024; Ismail and *al,* 2024; and *al* 2024). These characterized may enhance gastrointestinal solubilization and systemic absorption following ingestion, thereby increasing bioavailability and toxic potential.

Chronic exposure to heavy metals present well-documented health risk due to their persistence, cumulative behavior, and capacity to interfere with critical biological processes. Bioaccumulation, defined as the progressive internal retention of a contaminant when absorption elimination, can lead to neurotoxic, teratogenic, and hepatotoxic consequence (Surenbaatar and *al,* 2023; Granjean and *al*., 2022)

Metals such as Pb and Cd disrupt heme biosynthesis, impair redox balance, and interfere with essential enzymatic systems, while arsenic exert multisystem toxicity through mitochondrial dysfunction and oxidative stress pathways. The hematopoietic system is particularly vulnerable to heavy metal exposure, as erythroid precursors and bone marrow compartments are sensitive to oxidative injury and metal-induced enzymatic inhibitions.

In obstetric context, geophagy represent a potentially critical public health concern. Heavy metals can cross the placental barrier, posing risk to fetal development and perinatal health. Epidemiological studies conducted Tanzania and south Africa have reported significantly elevated blood lead concentration among pregnant women practicing geophagy, with association observed between clay consumption, anemia, and parasitic infection (Olsson, and al.,2012; Oyebanjo et al.,2024; Malebatja and *al.*, 2024; Doose and *al.*,2023; Jovine and *al.*,2023).

These finding underscore the plausibility of geophagy as an exposure pathway contributing to system toxic burden in vulnerable populations.

Despite accumulating epidemiological and environmental evidence, experimental data simultaneously integrating quantified clay ingestion, systemic heavy metal bioaccumulation, and comprehensive hematological evaluation remain limited. Most available studies focus either on the environmental characterization of clay contamination or on isolated metal exposure models, without replicating realistic geophagic intake scenarios.

This gap is particularly evident in Central African settings, where geophagy remain prevalent but controlled toxicological modeling is scare. To address this limitation, the present study employs a controlled wistar rat (*Rattus norvegicus*) model to evaluate the toxicological consequences of ingesting heavy metal, contaminated geophagic clay.

Specifically, we examine whether clay ingestion induces dose-dependent increase in blood concentration of P b, Cd, As and Ni, whether the magnitude significantly alters hematological parameters, including erythrocyte indices, hemoglobin concentration, leukocyte count, and platelet counts relatives to unexposed controls, and whether the magnitude of hematological disturbance correlate with systemic metal burden.

Furthermore, we assess the influence of exposure route (voluntary dietary incorporation versus oral gavage) on bioaccumulation dynamic and hematological outcomes. We hypothesize that chronic ingestion of contaminated

DOI: *https://doi.org/10.71004/rss.026.v5.i1.60*
Journal Website: *www.rss-istm.net*
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Revue des Sciences de la Santé (Rev. Sci. Sant.)
ISSN: 3078-6959 (Online); 3078-8226 (Print)
Volume-5 Issue-1, 2026

geophagic clay will result in measurable systemic bioaccumulation of toxic metals, disruption of essential trace element homeostasis and significant hematological alteration in dose-and route-dependent manner

By integrating exposure quantification with functional hematological endpoints, this study aims to provide mechanic experimental evidence supporting recognition of geophagy as a potentially significant environment health risk

II. MATERIALS AND METHODS

II.1. Collection, prepration of clay sample, and XRF Analysis

Clay samples were collected following a stratified sampling design inspired by the methodology of Thierry and al (2025), ensuring spatial representativeness of the targeted geophagic deposits, minimizing potential sampling biases, and capturing the full variability within the study (Thierry and *al* ,2025). sampling was perfumed using decontaminated stainless steel tools, and all subsamples were placed in pre-labeled polyethylene bags to prevent cross-contamination during transport and storage.

According to Seke (ICCCSC,2026), white clay constitutes a highly pure material, predominantly composed of well-crystallized kaolinite. Rama spectroscopy revealed a simple spectrum characterized by sharp Si-O-Si, Al-O, and Si-O vibrational modes, indicating high crystallinity, chemical simplicity, and minimal metallic impurities, X—ray diffraction (XRD) confirmed that the geophagic clay is a heterogeneous material primarily composed of quartz, kaolinite , and an expandable clay phase such as smectite or an illite-mixture .the intense quartz peak observed at 26.9° reflections an abrasive siliceous matrix, potentially unsuitable for ingestion ,whereas the kaolinite fraction provides beneficial adsorptive properties the presence of the expandable suggests a higher exchange capacity and an increase potential for heavy metal adsorption overall, this material does not meet pharmaceutical-grade quality standards and would require purification and rigorous chemical quality control to ensure safe consumption.

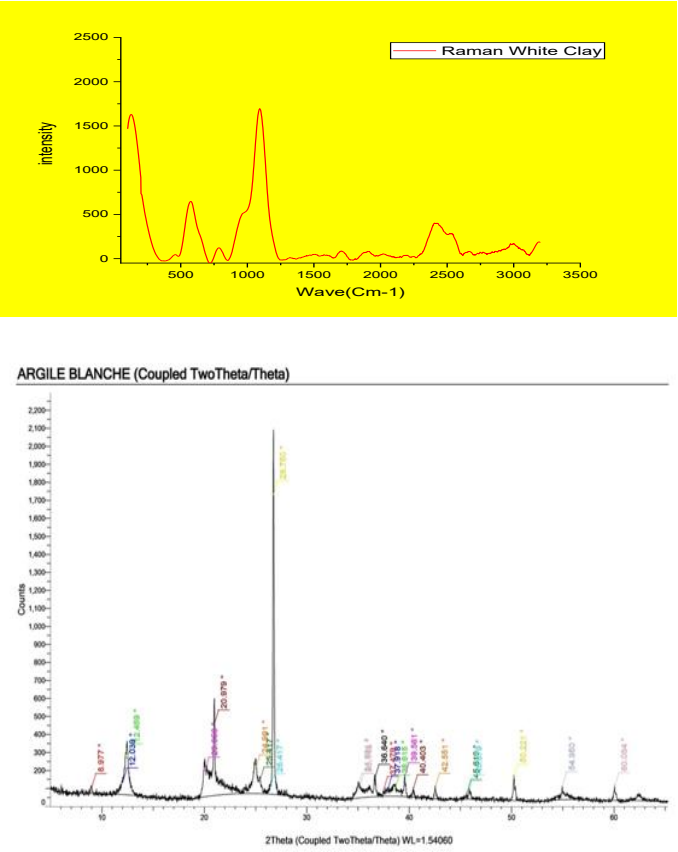


Fig 1: Spectra Raman and XRD of White Clay (Seke, ICCCSC 2026)

In the laboratory, samples were first air-dried at ambient temperature to remove free moisture and then oven—dried at 60°C until constant weight was achieved, a standard procedure to preserve mineral phases without inducing structural changes prior to analysis (Bastida & Pardo Ibanez, 2024). Dried samples were then ground to a fine powder using an agate mortar to minimize contamination and elemental analyses (Nzeukou and *al*., 2024)

Elemental analyses of clay and crop sample were performed using an energy-dispersive X-ray fluorescence spectrometer (Xepos III). A 3g aliquot was weight with a precision balance (Mettle Toledo) and placed in a 32 mm sample cup sealed with a 4µm polypropylene film.

Measurement were conducted using the internal 'FP powder' and 'TQ pellets Fast' methods, and external calibration was performed with certified reference materials IPE135, IPE133, and IPE197. The instruments operate with four secondary targets (Mo, Al2O3, Co, and HOPG) to optimized excitation condition.

XRF analysis is based on the emission of characteristic fluorescent X-ray following sample excitation. Normalized peak intensities, proportional to elemental concentrations, were used to compute final values. The $K\alpha_1$ line of potassium

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

(3.313KeV) severed as a reference and peak areas were normalized to coherent and incoherent scattering. Result are reported with student's confidence interval (α=0.95). Detection limits are typically around 10ppm for medium to heavy element (e.g, Zn, Cu, Pb) and 100-500ppm for lighter element. Precision and sensitivity depend on the sample matrix, preparation, and availed mass, and can reach the µg/g range for undiluted gram-scale sample.

II.2. Experimental animals and Ethical consideration

The study involved 24 adult wistar rat (*Rattus norvegicus*), approximately 8 weeks old, with an initial body weight ranging from 131 to 240g animal were randomly allocated into six experimental groups (n=4 per group).

Rats were housed in standard polypropylene cages under controlled laboratory conditions: temperature 222°C ± 2, relative humidity 55 ± 10%, and a 12h light / dark cycle. Animal had free access to drinking water and a standard laboratory diet throughout experiment. priors to the start of the study, animals underwent a 7-day acclimatization period to minimize environmental stress and physiological variability.

All experimental procedure was conducted in accordance with internationally accepted guideline for the care and use of laboratory animals and complied with the ARRIVE 2.0 reporting guidelines for animal research. Ethical approval for the study protocol was granted by the institutional animal care use committee (IACUC) of the hosting institution and was also approved by the relevant constitutional ethic committee of national institute of biomedical research (INRB). These procedures adhere to internationally recognized ethical standards for experimental animal research; aiming to minimize animal suffering while ensuring scientific rigor (Percie du Sert and *al*., 2020).

II.3. Experimental Design and exposure protocol

Animal were randomly assigned to six experimental groups and experimental groups and exposed for eight weeks to geophagic clay contamination. Two oral exposure strategies were implemented to produce both natural ingestion patterns and controlled high-dose exposure condition commonly investigated in toxicological studies using wistar rats.

- ***Group 1 (control)***

The control group received a standard clay-free laboratory diet under the same environmental condition as the exposed groups.

- ***Voluntary dietary exposure (group 2-group 4)***

In group 2, group 3, and group 4, contaminated clay was incorporated into the daily diet at concentrations of 20% 40%, and 60%, respectively. This administration route was designed to reproduce the natural behavior of geophagy, defined as the voluntary ingestion of soil or clay observed in humans and several animal species.

Dietary incorporation allows animals to regulate their intake through normal feeding behavior and therefore mimics chronic environmental exposure scenarios. This non-invasive approach also minimizes handing stress and physiological disturbance that may occur with forced administration techniques. Dietary exposure models are widely recommended when the objective is to simulate long-term ingestion of environmental contaminants under realistic conditions (Finkelman and *al*., 2005; Nyanza and *al*.., 2020).

- **Controlled gavage exposure (Group 5 and group 6)**

In group 5 and 6, clay suspensions were administered by oral gavage at concentrations of 0.8 g/mL and 1g/mL, respectively, at a dose volume of 1mL per 100g body weight. Oral gavage is widely used in experimental toxicology because it enables precise and reproducible dose delivery directly into the stomach, independent of variability in voluntary food intake or palatability. This method is considered a reference technique for controlled oral dosing in rodents and ensures consistent exposure across experimental subjects (Turner and *al*., 2011; OECED, 2022).

II.4. Justification of dual administration routes

The combined use of voluntary consumption of contaminated geophagic clays and their oral gavage was intentionally implemented in order to reproduce different exposure scenarios: natural ingestion related to behavior versus controlled high-intensity administration.

All administered doses were normalized according to body weight (g/kg/day) in order to minimize methodological biases, thus allowing direct administration into the stomach, independent of variations in food intake or palatability. This normalization ensures that the differences observed between groups reflect true toxicological responses rather than variations in the administered dose.

The dual-route design provides several methodological advantages:

- Comparison between realistic environmental exposure and controlled toxicological exposure
- Distinction between consumed dose and administered dose

DOI: *https://doi.org/10.71004/rss.026.v5.i1.60*
Journal Website: *www.rss-istm.net*
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026

Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Revue des Sciences de la Santé (Rev. Sci. Sant.)
ISSN: 3078-6959 (Online); 3078-8226 (Print)
Volume-5 Issue-1, 2026

- Evaluation of the influence of feeding behavior and stress on toxicological outcomes

Combined exposure strategies are increasingly recommended in rodent toxicology studies because they improve dose accuracy while preserving ecological relevance in environment exposure models (Atcha and *al*., 2010; Nyanza and *al*., 2022).

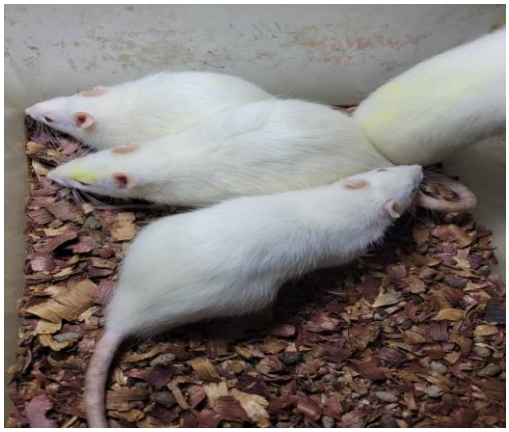
II.5. Initial Body Weight Distribution

The initial body weight of the wistar rats was measured before the start of the exposure period to ensure homogeneity among experimental groups and minimize baseline variability. Animals were randomly assigned to six groups based on body weight.

Table 1. Initial distribution of body weight (g) of wistar rats according to experimental groups

Individual/ Group	Group 1 0%	Group 2 20%	Group 3 40%	Group 4 60%	Group 5 80%	Group 6 100%
Head	153	190	176	187	160	220
Black	131	162	190	190	190	240
Tail	142	171	166	196	147	155
Nude	134	160	170	146	152	141
Mean weight	140	170	175,5	179,7	162,2	189

No statistically significant differences in initial body weight were observed between groups ($p > 0.05$), confirming adequate baseline comparability before treatment.



II.6. Dose Calculation (g/Kg Bw/day)

The administered dose of geophagic clay was calculated relative to body weight in order to standardize exposure levels among animals and allow comparison across treatment groups.

- **Dietary exposure groups (G2-G4)**

For groups receiving contaminated clay through dietary incorporation, the daily dose was calculated according to the following equation:

$$\text{Dose (g/Kg Bw /day)} = \frac{\textit{Clay concentration in feed (g) X Food consumption (g/day)}}{\textit{Body weight}}$$

This approach reflects actual ingestion and accounts for individual variations in feeding behavior.

- **Gavage exposure groups (G5-G6)**

For gavage groups, the administered dose was calculated using the equation:

$$\text{Dose (g/Kg Bw /day)} = \frac{\textit{Clay mass administered (g)}}{\textit{Body weight}}$$

All doses were expressed as g/Kg body weight per day (g/Kg Bw /day), which is the standard unit used in toxicological dose-response studies (OECD, 2022).

Table 2. Mean administered dose ((g/Kg body weight /day) in the different experimental groups/group/Mean administered dose (g/Kg Bw /day) ± 2 SD

Groups	Mean administered dose *(g/Kg Bw /day) ± 2 SD*
Group 1	**0**
Group 2	**1.2 ± 0.15**
Group 3	**2.4 ± 0.18**
Group 4	**3.1 ± 0.22**
Group 5	**4.0 ± 0.10**
Group 6	**5.0 ± 0.12**

Dietary doses were calculated based on individual food consumption measurements and body weight, while doses administered by gavage were determined according to the amount of clay administered and body weight at the time of administration. Throughout the eight-week exposure period, the animals were monitored daily to observe their general health condition. This continuous monitoring allowed for the early detection of adverse effects and ensured the well-being of the animals, in accordance with the recommendations of the ARRIVE guidelines for research (Perci du Sert et al., 2020).

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

II.7. Heavy Metal Quantification in rat blood by ICP-MS

Blood samples collected at the end of the exposure period were analyzed for heavy metal concentrations using inductively coupled plasma mass spectrometry (ICP-MS), a highly sensitive analytical technique widely used for trace metal quantification in biological matrices (Stoog and *al.*, 2021; Zhang and *al*., 2023).

All procedure were performed under clean laboratory conditions using trace-metal certified consumables to minimize contamination.

- ***Sample collection and storage***

Whole blood was collected into sterile trace-metal-free polypropylene vacutainer tubes to prevent exogenous contaminations. Sample were maintained at 4°C and processed within 2H after collection to preserve sample integrity and prevent redistribution of metal species (Patel and *al*., 2024).

- ***Plasma Separation***

Samples were centrifuged 300 X g for 15 minutes at 4°C to separate plasma from cellular components. The plasma fraction was carefully transferred to acid-washed polypropylene tubes while avoiding contamination from erythrocytes and platelets.

- **Acid Digestion (Samples preparation)**

Plasma samples underwent acid digestion to eliminate proteins and release metal ions bound to biological molecules. Digestion was performed by adding concentrated nitric acid HNO_3, 65%), followed by heating at 80°C for 1 hour in closed digestion vessels. Nitric acid digestions is commonly used for biological samples because it efficiently decomposes organic matrices and minimizes spectral interference during ICP-MS analysis (Stoog and *al.,* 2021; Zhang and *al*., 2023).

- **Sample Dilution and Preparation**

Following digestion, samples were diluted with ultrapure deionized water and adjusted to a final concentration of 2% HNO_3 to stabilize metal ions and ensure compatibility with the ICP-MS system.

- **Calibration and Quality Control**

Quantification was performed using external calibration curves prepared from certified multi-element standard solutions covering the expected concentration range. Internal standards (e.g., indium or rhodium) were added to correct for signal drift and matrix effects during analysis. Quality assurance procedures included:

- procedural blanks
- duplicate samples
- certified reference materials (CRMs)

The limit of detection (LOD) and limit of quantification (LOQ) were determined according to standard analytical protocols as three and ten times the standard deviation of blank measurements, respectively.

- ***ICP-MS Analysis***

Trace metals, including cadmium, lead, arsenic, and nickel were quantified using an ICP-MS instrument equipped with collision/reaction cell technology to reduce polyatomic interferences. Metal concentrations were determined based on their characteristic mass-to-charge ratios (m/z) and expressed as micrograms per liter (µg/L) of plasma.

II.8. Hematological Analyses

At the end of the exposure period, whole blood was collected by cardiac puncture under light anesthesia and transferred into EDTA-containing tubes to prevent coagulation and preserve cellular integrity. Hematological parameters were measured using an automated hematology analyzer calibrated for rat blood, ensuring reliable and reproducible results.

The following parameters were determined:

- **Red blood cell count (RBC)**
- **Hemoglobin concentration (Hb)**
- **Mean corpuscular volume (MCV)**
- **White blood cell count (WBC)**
- **Platelet count (PLT)**

These parameters are commonly used biomarkers in experimental toxicology and provide essential information regarding erythropoiesis, immune response, and coagulation status in rodent models (Kellner and *al*., 2025). Results were expressed as mean ± standard deviation (SD) for each experimental group, and quality control procedures were performed to ensure analytical accuracy.

II.10. Statistical Analysis

All quantitative data were expressed as mean ± standard deviation (SD). Before statistical comparisons, the distribution of each dataset was assessed for normality using the Shapiro–Wilk test, which is widely recommended for biological datasets with small sample sizes (Razali & Wah, 2011; Zoboli and *al.,* 2024).

For blood concentration of heavy metals (Pb, Cd, As and Ni), the normality assumption was not satisfied. Therefore, non-parametric statistical methods were applied. Intergroup

DOI: *https://doi.org/10.71004/rss.026.v5.i1.60*
Journal Website: *www.rss-istm.net*
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Revue des Sciences de la Santé (Rev. Sci. Sant.)
ISSN: 3078-6959 (Online); 3078-8226 (Print)
Volume-5 Issue-1, 2026

comparisons were performed using the kruskal wallis test, followed by Dunn's post hoc multiple comparison test to identify significant pairwise differences between experimental group. These test are commonly used in toxicological and environmental exposure studies where biological variability and small sample sizes may violate parametric assumptions (Zoboli and *al*., 2024; McKnight & Najab, 2020).

Hematological parameters that satisfied the assumptions of normal distribution and homogeneity of variance were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected by ANOVA, Tukey's honestly significant difference (HSD) test was applied for post hoc multiple comparisons to determine which groups differed significantly (Guidi et, al., 2025).

A two-tailled p-value < 0.05 was considered statistically significant for all analyses. Statistical analyses were performed using R statistical software version 4.3.0, R Foundation for Statistical Computing, Vienna, Austria). Graphical representations were generated using the ggplot2 package, allowing clear visualization of group differences and dose–response trends.

III. RESULTAS

III.1. Mineralogical and chemical composition of Clay of samples

The mineralogical and chemical composition of clay samples collected from different sites was evaluated in terms of metal oxide content. Analyses focused on the major oxides (MnO, NiO, CuO, Fe_2O_3, ZnO, CdO, PbO, AS_2O_3) as well as on potentially toxic trace and heavy metals (Mn, Ni, Cu, Zn, As, Cd, Pb) in their oxidized forms. The samples were studied according to different treatments dried, calcinated, or molded. The results are presented in the table below to allow comparison between sites and preparation types.

The detailed results for all samples are summarized in table 3 for oxides and table 4 for elements, allowing comparison between sites and sample treatments.

Table 3. Oxide composition of clay samples from different sites according to sample treatment (Raw, calcinated and molded)

		Oxydezide from of metalics elements						
Sample	MnO (mg/kg)	Fe_2O_3 %	NiO (mg/kg)	CuO (mg/kg)	ZnO (mg/kg)	As2O3 (mg/kg)	CdO (mg/kg)	PbO (mg/kg)
Boma Dried	**153**	4.432	**38.4**	**9**	**55.7**	**6.6**	**31.5**	**31.7**
Boma Charred	**155.9**	4.81	4.81	**10.9**	**52.2**	**5.4**	**11.1**	**35.3**
Boma Molded	**48**	1.612	<8.6	–	18.2	–	4.8	16.3
Biyele Raw	199	6.602	45.2	11.8	45.0	6.6	16.5	31.1
Biyele Charred	87.9	1.637	23.4	5.3	44.1	2	<1.9	24.6
Biyele Molded	99.4	2.202	32.5	–	46.9	2.7	4.4	26.3

Table 3 presents variability in the oxide composition of potentially toxic metals in clay samples (MnO, Fe_2O_3, NiO, CuO, ZnO, As_2O_3, CdO, PbO) according to sites and treatments (raw/dried, carbonized, and molded). The oxides MnO and ZnO are among the most abundant, with concentrations generally higher in raw and dried samples, particularly in the raw clay from Biyele (MnO: 199 mg/kg, ZnO: 45 mg/kg).

There is also considerable variation in potentially toxic oxides such as NiO, PbO, As_2O_3, and CdO between the samples. Thus, NiO ranges from < 8.6 mg/kg in the molded clay from Boma to 45.2 mg/kg in the raw clay from Biyele, while PbO reaches 35.3 mg/kg in the charred sample from Boma. CdO and As_2O_3 also exceed safety limits in several samples.

Following to the FAO/WHO Codex 2023, the maximum allowable contents are: As ≤ 0.1 mg/kg, Cd ≤ 0.2–0.5 mg/kg, and Pb ≤ 0.1 mg/kg. All of the clay samples analyzed exceed these thresholds for at least one of these toxic metals, highlighting the potential health risk associated with their consumption.

Some oxides, such as CuO, also contribute to the overall metal profile, but at lower concentrations. The observed differences suggest that the geological origin (Boma vs Biyele), as well as the treatment of the samples (dried, charred, ground), significantly influence the distribution of metal oxides in the consumed clays.

These variations may affect the bioavailability and potential toxicity of heavy metals when the clays are ingested.

Overall, MnO, ZnO, and Fe_2O_3 dominate the content of metallic oxides, but several trace metals of toxicological concern (NiO, PbO, As_2O_3, CdO) are present at levels exceeding Codex standards, highlighting the need for strict monitoring of products intended for human consumption.

Table 4. Elemental composition of clays samples from different sites according to sample treatment (Raw, calcinated and molded)

	Metalics elements						
Sample	Mn (mg/kg)	Ni (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
Boma Dried	118.5	30.2	7.2	44.8	5	31.5	29.5
Boma Charred	120.7	31.2	8.5	41.9	4.1	11.1	32.7
Boma Molded	37.2	<6.8	–	14.7	–	4.8	15.2
Biyele Raw	154.1	35.5	9.4	36.2	5	16.5	28.8

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Biyele Charred	68.1	18.4	4.2	35.5	1.5	<1.9	22.8
Biyele Molded	77	25.5	–	37.7	2	4.4	24.4
Codex standard (FAO/WHO, 2023)	-	Not establi-shed	Not establi-Shed	Not establi-Shed	Not establi-shed	≤0.1	≤0.2–0.5

The elemental analysis of clay samples from Boma and Biyele revealed the presence of several heavy metals of toxicological interest, notably manganese, nickel, copper, zinc, arsenic, cadmium, and lead (Table 4). The concentrations varied depending on the origin and treatment of the clays (raw, calcined, or molded).

The manganese content ranges from 37.2 mg/kg in the molded clays of Boma to 154.1 mg/kg in the raw clays of Biyele. Nickel content ranges from < 6.8 mg/kg in the molded clays of Boma to 35.5 mg/kg in the raw clays of Biyele. Copper concentrations range from 4.2 mg/kg (calcined clay of Biyele) to 9.4 mg/kg (raw clay of Biyele), while zinc content ranges between 14.7 mg/kg (molded clay of Boma) and 44.8 mg/kg (dried clay of Boma).

Among toxic metals, arsenic concentrations range from 1.5 mg/kg in the carbonized clay of Biyele to 5 mg/kg in the dried clay of Boma and the raw clay of Biyele.

Cadmium showed greater variability, ranging from <1.9 mg/kg in the charred clay of Biyele to 31.5 mg/kg in the dried clay of Boma. Lead concentrations ranged from 15.2 mg/kg (molded Boma) to 32.7 mg/kg (charred Boma).

These values exceed the limits of the Codex Alimentarius for heavy metals in materials intended for human consumption, namely: ≤0.1 mg/kg for arsenic, 0.2–0.5 mg/kg for cadmium, and ≤0.1 mg/kg for lead (FAO/WHO, 2011). It should be noted that raw and calcined clays generally have higher metal concentrations than molded clays, suggesting that certain processing methods may help reduce heavy metal content.

These results indicate that chronic ingestion of geophagic clays could constitute a significant source of systemic exposure to heavy metals, with potential long-term effects on hematological and toxicological levels. Rigorous monitoring and proper regulation of clays intended for human consumption are therefore essential in order to reduce health risks (WHO, 2024).

III.2. Blood Concentrations of Heavy Metals

The mean blood concentration (± SD) of the analyzed elements in Wistar rats are presented in table 5. Compared with the control group, exposure to geophagic clays resulted in significant alterations in metal concentrations, with an overall dose-dependent trend.

Table 5a. Mean ((± SD) Blood Concentation of Heavy Metal in Rats

Elements	Control	G20%	G40%	G60%	G80%	G100%
Cd	0.00 ± 0.00	0.16 ± 0.04	0.16 ± 0.06	0.14 ± 0.04	0.03 ± 0.03	0.15 ± 0.03
Mn	6.45 ± 0.00	4.56 ± 0.74	5.01 ± 1.16	5.05 ± 1.18	3.38 ± 0.80	4.49 ± 1.11
Ni	0.01 ± 0.00	1.09 ± 0.16	1.35 ± 0.43	1.47 ± 0.56	0.02 ± 0.01	0.90 ± 0.43
Pb	0.00 ± 0.00	2.64 ± 0.42	1.86 ± 0.34	2.47 ± 0.56	1.68 ± 0.39	2.27 ± 0.76
Se	2.10 ± 0.00	23.72 ± 1.33	26.64 ± 4.96	25.77 ± 2.27	17.34 ± 2.71	20.36 ± 2.16
As	0.00 ± 0.00	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.04 ± 0.05	0.05 ± 0.03
Fe	800.19 ± 0.00	475.15 ± 86.21	645.36 ± 63.34	644.3 ± 64.54	345.39 ± 52.69	518.58 ± 32.61

Blood concentrations of cadmium, nickel, arsenic, lead, and selenium increased significantly in the exposed groups (Kruskal–Wallis test, $p < 0.001$). The increase was particularly marked for lead and selenium, whose levels remained high. For cadmium, nickel, and arsenic, a significant increase was also observed across all exposed groups. On the other hand, the group that received 80% clay showed slightly lower concentrations than the other exposed groups, suggesting a dose–response relationship that might not be strictly linear. Furthermore, a significant decrease in blood concentrations of iron and manganese was observed in the exposed rats ($p < 0.05$). The reduction in iron was particularly pronounced, with decreases exceeding 50% in some groups. For manganese, the decrease was statistically significant in all exposed groups, although the magnitude of the effect was less pronounced at intermediate doses.

Table 5b. Mean Blood Concentation of Heavy Metal in Rats (with LOD/LOQ)

Elements	LOD (µg/L)	LOQ (µg/L)	Group 1 (Control)	Group 2 (20%)	Group 3 (40%)	Group 4 (60%)	Group 5 (80%)	Group 6 (100%)
Cd	0.01	0.03	**<LOD**	0.16 ± 0.04	0.16 ± 0.06	0.14 ± 0.04	0.03 ± 0.03	0.15 ± 0.03
Ni	0.05	0.15	**<LOQ**	1.09 ± 0.16	1.35 ± 0.43	1.47 ± 0.56	<LOQ	0.90 ± 0.43
As	0.01	0.03	**<LOD**	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.04 ± 0.05	0.05 ± 0.03
Pb	0.05	0.15	**<LOD**	2.64 ± 0.42	1.86 ± 0.34	2.47 ± 0.56	1.68 ± 0.39	2.27 ± 0.76
Se	0.1	0.3	2.10 ± 0.00	23.72 ± 1.33	26.64 ± 4.96	25.77 ± 2.27	17.34 ± 2.71	20.36 ± 2.16
Fe	1	3	800.19 ± 0.00	475.15 ± 86.21	645.36 ± 63.34	644.30 ± 64.54	345.39 ± 52.69	518.58 ± 32.61
Mn	0.05	0.15	6.45 ± 0.00	4.56 ± 0.74	5.01 ± 1.16	5.05 ± 1.18	3.38 ± 0.80	4.49 ± 1.11

- ✓ <LOD: Value below the limit of detection
- ✓ <LOQ: detectable value but below limit of quantification

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Revue des Sciences de la Santé (Rev. Sci. Sant.)
ISSN: 3078-6959 (Online); 3078-8226 (Print)
Volume-5 Issue-1, 2026

- ✓ LOD/LOQ: are representative values for ICP-MS measurements in rat blood

The average concentrations of heavy metals in the blood of Wistar rats after consumption of geophagic clays are presented in Table 5b, taking into account the analytical limits. The symbols <LOD and <LOQ indicate values below the detection limit and detectable values but below the quantification limit, respectively. The LOD/LOQ ratio corresponds to the measurements carried out by ICP-MS in the rats' blood. Some metallic elements, such as cadmium (Cd) and arsenic (As), are undetectable in the control group (<LOD), while nickel (Ni) is detectable but sometimes below the quantification limit (<LOQ). Clay ingestion led to a generally dose-dependent increase in most metal concentrations, indicating that even moderate exposure can raise systemic levels of potentially toxic metals. Considering the LOD and LOQ allows for an accurate assessment of the reliability of the measurements and the toxicological relevance of the results.

Figure 3: illustrate the distribution of the blood concentration of Cd, As, Ni, and Pb according to experimental groups and routes of administration (voluntary dietary exposure for 20-60% oral gavage for 80%-100%).

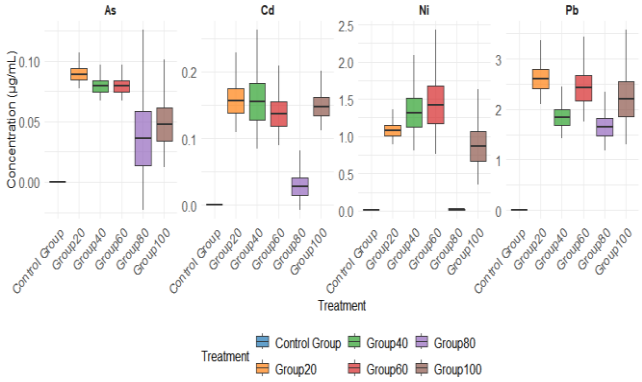


Figure 3. Boxplots of blood concentrations of Metallic Elements (Cd, As, Ni, Pb) by treatment

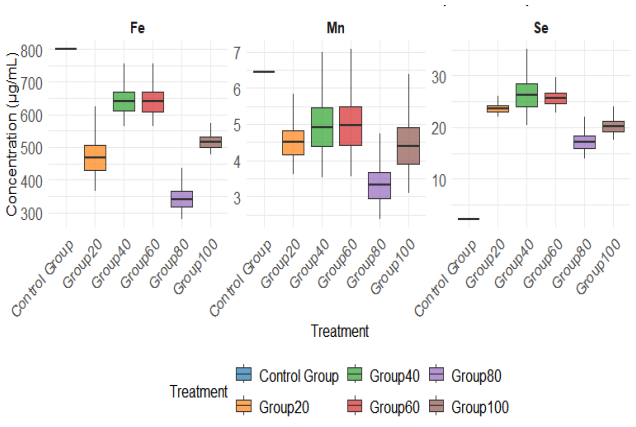
Figure 3 shows a significant increase in blood concentrations of Cd, As, Ni, and Pb in rats exposed to geophagic clays, confirming systemic absorption of heavy metals. Bioaccumulation was more pronounced in the groups receiving the clays via voluntary ingestion through food (groups 2 to 4) than in those exposed by gavage (groups 5 and 6), despite higher nominal concentrations administered in these latter groups.

This difference suggests a decisive role of the exposure route. Fractionated ingestion through food promotes prolonged intestinal contact and cumulative absorption, whereas acute administration by gavage can induce saturation of intestinal transporters or activate detoxification and tissue redistribution mechanisms (Kenton and *al.*, 2018; Markell and *al.*, 2021).

Thus, the actually absorbed dose seems more decisive than the nominal administered concentration. Cadmium has shown good accumulation, justified by its long biological half-life and strong affinity for metallothioneins, thus promoting its systemic persistence (Fang et al., 2021). Arsenic, particularly in its inorganic form, presents high bioavailability and rapid intestinal absorption, which explains the measured concentrations (Zhou et al., 2022). Nickel, although essential in trace amounts, becomes toxic at high concentrations; its increase reflects an exceeding of homeostatic regulatory mechanisms. Lead, characterized by a marked hematological tropism and strong binding to erythrocytes, interferes with heme synthesis, which could explain its association with the observed hematological alterations (U.S. Department of Health and Human Services, 2021).

The concomitant elevation of several metals suggests a combined effect likely to modify their absorption and elimination. Mixed exposures can induce competitive interactions at the level of membrane transporters and binding proteins, thus amplifying overall toxicity compared to exposure to a single agent (Balali-Mood and *al.*, 2021). The observation of high concentrations, particularly in groups 3 and 4, indicates that chronic consumption of contaminated clays can lead to significant systemic bioaccumulation, even in the absence of acute exposure via gavage. These data suggest that geophagy is one of the possible pathways of exposure to heavy metals, with potential long-term systemic toxic effects. In this regard, the voluntary feeding model appears to be more representative of chronic exposure in humans.

Figure 4 illustrates the variations in blood concentrations of essential elements (Fe, Se, Mn) according to the mode and level of exposure to geophagic clays. Unlike heavy metals, these elements show changes reflecting a disturbance of mineral homeostasis rather than a simple dose-dependent accumulation.



DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Figure 4. Box plots illustrating blood concentrations of iron, selenium, and manganese in Wistar rats according to treatment groups (group 1, group 2, group 3, group 4, group 5, group 6).

Figure 4 shows blood concentrations of iron, selenium, and manganese according to exposure levels and modes of administration. Unlike toxic heavy metals, these elements show variations reflecting a disruption of mineral homeostasis rather than a simple dose-dependent accumulation.

Iron decreased significantly in all rats exposed to the consumption of clays contaminated, with a strong reduction in group 5 (Gavage). The groups that consumed voluntarily (groups 3 and 4) maintained levels closer to those of the control group. This decrease suggests an alteration in iron absorption or its systemic regulation under conditions of high consumption. Manganese showed a double profile with a slight increase at intermediate doses (40% and 60%, groups 3 and 4), followed by a decrease at higher doses. This pattern could reflect a transient adaptation of manganese-dependent enzymatic systems, followed by disturbances related to overload or competition with other divalent metals, particularly iron. Selenium showed a strong increase in all exposed groups, with a peak at intermediate doses, followed by a slight decrease at the highest doses, without returning to baseline values. These variations suggest a disruption of the balance of essential trace elements, associated with the bioaccumulation of toxic metals.

III.4. Effects on Hematological Parameters

Figures 5 to 9 illustrate the impact of exposure to contaminated geophagic clays on key hematological parameters in Wistar rats, including red blood cells (RBC), white blood cells (WBC), hemoglobin, platelet count, and mean corpuscular volume (MCV). The rats were exposed to increasing concentrations of clay (0%: control, 20% to 100%). The results, expressed as mean ± standard deviation, indicate significant differences between the groups ($p < 0.05$).

The dose–response analysis reveals that the ingestion of contaminated clays leads to:

- A progressive decrease in red blood cells, hemoglobin, and hematocrit, suggesting a disruption of erythropoiesis;
- Variations in the number of white blood cells and platelets, indicating a potential inflammatory or systemic response.

These alterations confirm that the bioaccumulation of heavy metals from geophagic clays significantly affects the hematological profile of rats.

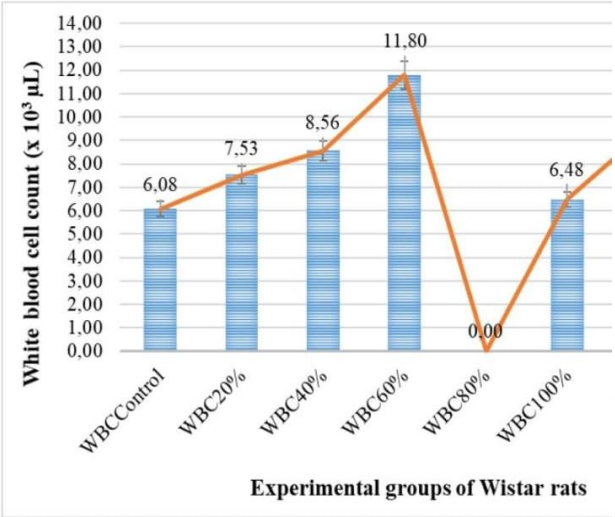
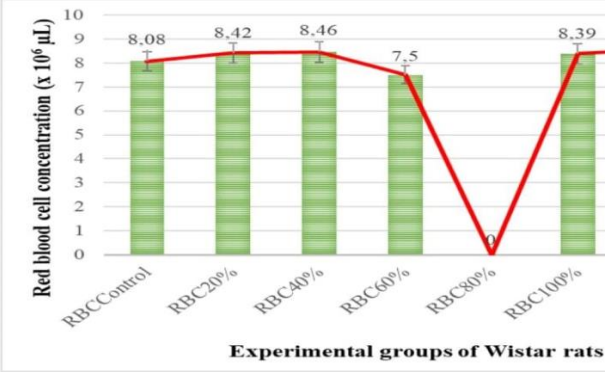


Figure 5: White blood cell (WBC) count in Wistar rats exposed to contaminated geophagic clays according to treatment groups (group 1, group 2, group 3, group 4, group 5, group 6).

Figure 5 illustrates the white blood cell (WBC) count in Wistar rats exposed to contaminated geophagic clays. The results highlight a dose- and administration-dependent response. During voluntary ingestion at moderate doses (groups 2 to 4; 20–60% clay), a progressive increase in WBCs was observed, reflecting adaptive immune activation in response to chronic exposure to heavy metals, as described by Andjelkovic and *al.* (2019).

On the other hand, high-dose gavage administration (group 5; 80% clay) induced marked leukopenia, indicating severe immunotoxic effects associated with oxidative stress and bone marrow suppression, a phenomenon also reported by Medjedded and *al*. (2024). The return to values close to the control in group 6 (100% clay) suggests the involvement of compensatory mechanisms, confirming the non-linear nature of the immune response during combined exposure to heavy metals.



DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026

Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Revue des Sciences de la Santé (Rev. Sci. Sant.)
ISSN: 3078-6959 (Online); 3078-8226 (Print)
Volume-5 Issue-1, 2026

Figure 6: Red blood cell (RBC) count in Wistar rats exposed to contaminated geophagic clays according to treatment groups (group 1, group 2, group 3, group 4, group 5, group 6).

Figure 6 shows the red blood cell (RBC) count in Wistar rats exposed to contaminated geophagic clays and highlights a non-linear hematological response according to the dose and route of administration.

During voluntary ingestion at low and medium doses (groups 2 and 3), a slight increase in the number of RBCs was observed, suggesting a compensatory stimulation of erythropoiesis in response to moderate exposure to heavy metals, as reported by Sharma and *al*. (2021).

At the intermediate dose (group 4), a decrease in the number of RBCs appeared, indicating a progressive hematological imbalance with the increase of metal burden. In contrast, high-dose gavage (group 5) resulted in a marked suppression of RBCs, reflecting severe hematological toxicity associated with the rapid absorption of lead and cadmium, known to inhibit the proliferation of erythroid precursors and heme synthesis (Genchi and *al*., 2020; Obeng-Gyasi, 2020).

Finally, the return to values close to the control in group 6 suggests the activation of adaptive mechanisms or a peripheral redistribution of erythrocytes, illustrating the complexity of dose–response relationships during combined exposure to heavy metals.

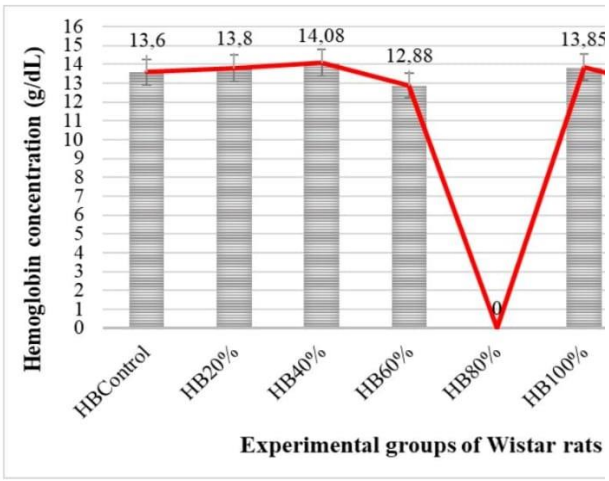


Figure 7: Hemoglobin (Hb) concentration in Wistar rats exposed to contaminated geophagic clays according to treatment groups (group 1, group 2, group 3, group 4, group 5, group 6).

Figure 7 illustrates the variations in blood hemoglobin concentration in Wistar rats exposed to contaminated geophagic clays, highlighting a nonlinear response dependent on the dose and the route of administration.

During voluntary ingestion at a moderate dose (groups 2 to 4), hemoglobin concentrations remained close to or slightly above those of the control group, suggesting that moderate exposure does not significantly disrupt circulating hemoglobin levels. This stability reflects the effectiveness of compensatory homeostatic mechanisms that maintain oxygen transport despite oxidative stress induced by cadmium and lead (Yessenaliyeva and *al.*, 2025).

On the other hand, high-dose gavage (group 5) resulted in a marked suppression of Hb levels, indicating acute hematopoietic toxicity, likely due to hemolysis, inhibition of erythroid precursor proliferation, and indirect damage to the bone marrow (Madjedded and *al*., 2024). Meanwhile, high-dose gavage (group 5) led to a marked suppression of Hb levels, indicating acute hematopoietic toxicity, probably due to hemolysis, inhibition of erythroid precursor proliferation, and indirect damage to the bone marrow (Madjedded and *al.*, 2024).

At the highest dose (group 6), Hb levels returned closer to control values, suggesting the activation of adaptive mechanisms such as the peripheral redistribution of red blood cells and the residual stimulation of erythropoiesis. The figure also highlights that interactions with essential elements, particularly iron, can compromise hemoglobin synthesis and promote anemia during prolonged exposures (Jomova & Valko, 2025), confirming a toxicological "cocktail" effect involving heavy metals, white blood cells, and essential trace elements (Fe, Mn, Se).

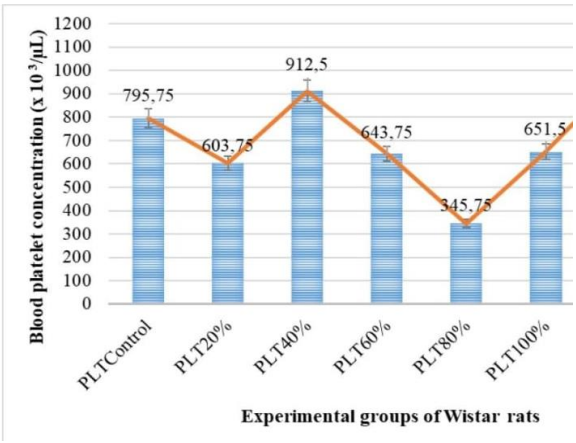


Figure 8: Effect of exposure to contaminated geophagic clays on platelet count in Wistar rats.

Figure 8 illustrates the effect of exposure to contaminated geophagic clays on platelet count in Wistar rats. The results show a dose- and route-dependent response.

Throughout voluntary ingestion at moderate doses (groups 2 to 4), platelet values remained close to those of the control group, with slight increases observed in batches 3 and 4.

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

These results indicate that moderate chronic exposure does not significantly impair thrombopoiesis. This relative stability could reflect a physiological adaptation aimed at maintaining hemostatic balance in the presence of moderate oxidative stress.

In addition, high-dose gavage (group 5) was associated with a marked decrease in platelet count, indicating acute damage to the megakaryocytic compartment and an increased risk of thrombocytopenia.

Recent studies have shown that exposure to lead and cadmium can disrupt megakaryocyte differentiation, induce oxidative stress in the bone marrow, and alter platelet production (Skalny and *al*., 2021; Tinkov and *al.,* 2022). The return to values close to the control in group 6 suggests the activation of adaptive mechanisms, such as compensatory stimulation of thrombopoiesis or peripheral redistribution of platelets.

Overall, these results confirm that contaminated clays exert toxicity on the entire hematopoietic system and that the platelet response, like other hematological parameters, follows a nonlinear dynamic modulated by the intensity and kinetics of exposure to heavy metals

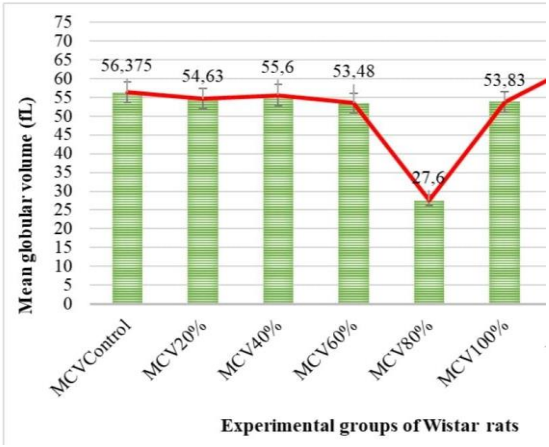


Figure 9: Effect of exposure to contaminated geophagic clays on the mean corpuscular volume (MCV) in Wistar rats.

The results indicate that the mean corpuscular volume (MCV) remained relatively stable in rats exposed through voluntary ingestion to doses of 20%, 40%, and 60%, as well as in the group subjected to 100% gavage, with values comparable to those of the control group (≈53–56 fL). Despite the presence of heavy metals, the observed stability suggests that at low to moderate exposure levels, the mechanisms regulating erythropoiesis and hemoglobin synthesis remain generally functional. On the other hand, a sharp decrease in MCV was observed in the 80% group, where the value dropped to 27.66 fL, indicating marked microcytosis. Since MCV is a key indicator of erythrocyte morphology, this reduction reflects a significant impairment in red blood cell maturation.

This profile is consistent with the hematological effects of lead and cadmium, which are known to disrupt iron metabolism and inhibit heme biosynthesis, leading to microcytic anemia (Skalny and *al*., 2021). Moreover, simultaneous exposure to multiple metals present in geophagic clays can produce synergistic effects, exacerbating disturbances of erythrocytes and amplifying microcytosis, alongside the alterations observed for hemoglobin, hematocrit, and red blood cell count.

Overall, these results confirm that the severity of hematological alterations depends not only on the dose, but also on the kinetics and intensity of heavy metal absorption.

III.5. Correlation between blood concentrations of heavy metals and hematological parameters in Wistar rats

To investigate potential associations between heavy metal exposure and hematological effects, a Pearson correlation analysis was performed between blood concentrations of heavy metals (Cd, As, Ni, Pb, Fe, Se, Mn) and key hematological parameters (GB, GR, Hb, PLT, VGM) in Wistar rats from groups 1 and 2. The resulting correlation matrix is show in Figure 10.

Figure 10: Pearson correlation matrix between blood heavy metals and hematological parameters in Wistar rats (group 1 vs group 2).

This matrix presents the correlations between hematological parameters (rows) and metals (columns). The results reveal several notable associations, particularly with iron, which shows the strongest and most significant correlations: with MCV (r = 0.46; p = 0.024), Hb (r = 0.41; p = 0.049), RBCs (r = 0.40; p = 0.052), and platelets (PLT) (r = 0.40; p = 0.056).

DOI: *https://doi.org/10.71004/rss.026.v5.i1.60*
Journal Website: *www.rss-istm.net*
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026

Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Revue des Sciences de la Santé (Rev. Sci. Sant.)
ISSN: 3078-6959 (Online); 3078-8226 (Print)
Volume-5 Issue-1, 2026

These positive relationships indicate that an increase in iron levels is associated with higher erythrocyte parameters. A significant correlation is also observed between Hb and lead (Pb) ($r = 0.64$; $p < 0.001$), representing the strongest association in the matrix and suggesting a relationship between blood lead concentrations and hemoglobin concentration. Similarly, PLT and lead (Pb) ($r = 0.42$; $p = 0.040$) show a significant association.

Nickel shows moderate but not significant correlations with several parameters ($r \approx 0.32–0.34$). In contrast, manganese, selenium, and arsenic show very weak associations with hematological variables, with correlation coefficients close to zero and high p-values, indicating the absence of statistically significant relationships.

IV. DISCUSSION

The results of this study show that geophagic consumption of clays contaminated with heavy metals, even at moderate doses, induces dose- and administration route-dependent systemic hematological disturbances. These observations are closely related to Figure 3, which illustrates the bioaccumulation of metals in the blood of Wistar rats. They corroborate the recent findings of Ruggieri and *al*. (2025), who reported high concentrations of lead, cadmium, and arsenic in geophagic clays consumed in Cameroon and Nigeria, exceeding international safety limits. Similarly, Rukondo and *al*. (2024) showed that Tanzanian geophagic soils contain high levels of Pb, Cd, and Ni, confirming that geophagy constitutes an important route of exposure to heavy metals.

The bioaccumulation of heavy metals represents a central mechanism of systemic toxicity. These metals persist in various tissues (liver, kidneys, bones, bone marrow) and induce oxidative stress through excessive production of reactive oxygen species (ROS) and inhibition of antioxidant systems (SOD, CAT, GPx), leading to cellular damage and tissue dysfunction (Jomova, Baros & Valko, 2023; Tchounwou and *al.*, 2022; Balali-Mood, Naseri, Tahergorabi & Abdollahi, 2021).

The mechanisms by which lead and cadmium disrupt heme biosynthesis, notably through the inhibition of δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase, are particularly well documented, thus leading to an alteration in hemoglobin production (Genchi, Carocci, Lauria, Sinicropi & Catalano, 2020; Järup & Åkesson, 2020). Nyanza and al. (2020) showed, through studies on human geophagy, that pregnant women consuming clay in Tanzania had high blood concentrations of Pb and Cd, associated with a decrease in hematological parameters, notably hemoglobin concentration and red blood cell count.

Similarly, Kamburova and *al.* (2021) observed a significant accumulation of heavy metals (Pb, Cd, As) in women practicing geophagy in Burkina Faso, correlated with alterations in red blood cells and platelets, suggesting a risk of anemia and immunosuppression. Our in vivo experimental results confirm these effects: chronic consumption of contaminated clays led to a significant decrease in red blood cell count and hemoglobin concentration, as illustrate in Figures 6 and 7. These alterations are consistent with the development of microcytic hypochromic anemia and a disruption of erythropoiesis, in agreement with the observations of Liu and *al*. (2021) in experimental models exposed to heavy metals via drinking water.

The correlation analysis (Figure 10) revealed significant associations between certain metals and hematological parameters. Iron was positively correlated with mean corpuscular volume (MCV) ($r = 0.46$; $p = 0.024$), hemoglobin ($r = 0.41$; $p = 0.049$), red blood cell count ($r = 0.40$; $p = 0.052$), and platelet count ($r = 0.40$; $p = 0.056$). These correlations confirm the central role of iron in hemoglobin synthesis and erythropoiesis (Camaschella, 2015; Abbaspour, Hurrell & Kelishadi, 2014).

Strong correlations were also observed between lead and hemoglobin ($r = 0.64$; $p < 0.001$) as well as between lead and platelets ($r = 0.42$; $p = 0.040$), indicating a direct effect of Pb on hematological mechanisms, in agreement with studies in humans exposed to high levels of lead (Gidlow, 2015; Wang & Chen, 2022).

Nickel showed moderate but non-significant correlations with certain hematological parameters, which is consistent with Das, Patra & Ghosh (2022), who described Ni-induced inflammatory and oxidative effects without direct alteration of erythrocyte indices. Manganese, selenium, and arsenic showed very weak correlations, suggesting a limited impact on hematological parameters or effects dependent on complex interactions (Tchounwou and *al*., 2012).

The variations in the number of leukocytes and platelets (Figures 5 and 8) show a non-linear dose-dependent inflammatory response, mediated by the activation of NF-κB and the production of pro-inflammatory cytokines such as IL-6 and TNF-α (Balali-Mood and *al*., 2021; Huang, Chen & Li, 2022). At high doses, the observed leukopenia and thrombocytopenia indicate a clear suppression of hematopoiesis, likely due to direct toxicity on the bone marrow (Zhao, Li & Zhang, 2024; Obeng-Gyasi, 2020).

The severe microcytosis observed at 80% exposure (Figure 9) corresponds to the enzymatic inhibition of heme synthesis induced by lead and cadmium, disrupting iron metabolism

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

and compromising the functional production of erythrocytes (Liu and *al*., 2021; Patel and *al*., 2023). These effects are amplified in the case of multiple co-exposures, as reported by Nkansah and *al*. (2016) in a study on geophagy in Ghana, where cumulative levels of heavy metals in the blood were correlated with significant disturbances in several hematological indices.

Beyond cellular parameters, our data (Figure 4) show that exposure to heavy metals also disrupts the homeostasis of essential elements such as iron, manganese, and selenium. Toxic metals compete with trace elements for absorption pathways and binding sites on metalloproteins, leading to nutritional imbalances and metabolic dysfunctions (Chen and *al*., 2021; Baj, Staneviciene & Tamulaitiene, 2023). Interference with iron and manganese metabolism is particularly critical in hematotoxicity, as these elements play a key role in hemoglobin synthesis, erythropoiesis, and antioxidant systems (Staneviciene and *al.,* 2022).

Geophagy remains a major source of exposure to heavy metals, particularly among pregnant women and children in sub-Saharan Africa, with documented risks of fetal growth retardation, neurodevelopmental disorders, and immune deficiencies (Jomova and *al.,* 2023; Tchounwou and *al*., 2022). These effects have been observed in human populations regularly consuming contaminated clays, as reported by Nyanza and *al*. (2020) and Kamburova and *al.* (2021), who highlighted high blood levels of heavy metals associated with significant reductions in hematological parameters and clinical disturbances.

Several strategies have been explored to mitigate these hematological alterations. The administration of antioxidants such as vitamin E, vitamin C, or N-acetylcysteine reduces oxidative stress and improves hematological parameters in experimental models exposed to heavy metals (Sharma, Singh & Kaur, 2021; Farombi and *al*., 2022).

Chelating agents such as DMSA (dimercaptosuccinic acid) or EDTA facilitate the elimination of heavy metals and restore erythropoiesis (Bradberry, Vale & Ford, 2021). Finally, targeted nutritional interventions, including supplementation with essential minerals (Fe, Ca, Zn), can limit the intestinal absorption of toxic metals and mitigate hematological disturbances (Gupta, Rani & Kumar, 2023).

V. CONCLUSION

This experimental study made it possible to evaluate in a controlled manner the hematological and systemic toxicological effects of consuming geophagic clays contaminated with heavy metals, notably lead, cadmium, arsenic, and nickel, in Wistar rats. The results show that the consumption of these contaminated clays induces dose-dependent toxic effects, strongly modulated by the mode of administration. The mode of consumption proved to be a determining factor in the severity of the observed effects. Moderate voluntary consumption of contaminated clays (20% to 60%) led to mild or compensatory hematological changes, reflecting adaptive responses of the hematopoietic and immune systems. While high-dose consumption, particularly through gavage, caused more severe and acute hematotoxic effects. We observed significant alterations in the main hematological parameters, notably the number of red and white blood cells, hemoglobin concentration, and platelet count. These responses were nonlinear, characterized by initial phases of stimulation or compensation, followed by dysfunction at higher doses, highlighting the complex interaction between adaptive mechanisms and the toxic stress induced by heavy metals. Furthermore, the study revealed significant disruptions of essential trace elements such as iron, manganese, and selenium, indicating that the heavy metals contained in geophagic clays interfere with mineral homeostasis. These imbalances exacerbate systemic toxicity by altering erythropoiesis, enzymatic functions, and antioxidant defenses, highlighting the broader physiological consequences of chronic exposure to contaminated geophagic clays.

Overall, these results highlight the significant hematotoxic and systemic risks associated with the consumption of clays contaminated with heavy metals, particularly among populations with regular consumption or high-dose consumption. They also underscore the urgent need for public health interventions, including awareness campaigns, quality control of clays, and preventive strategies to reduce chronic exposure to heavy metals.

Ethical considerations

All procedures were conducted in accordance with international guidelines on the care and use of laboratory animals and approved by the relevant institutional ethics committee of the National Institute of Biomedical Research.

REFERENCES

1. Adeyomoye, O. I., & Adewumi, N. A. (2017). Lead exposure causes alteration of haematological indices in adult female Wistar rats. Asian Journal of Pharmaceutical Research and Development, 7(6), 610. (https://doi.org/10.22270/ajprd.v7i6.610)

2. Andjelkovic, M., Buha Djokic, A., Antonijevic, E., Milosavljevic, D., Stanic, M., Radovanovic, M., & Wallace, D. (2019). Effects of heavy metal exposure on immune responses in laboratory rats. Toxicology

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Letters, 312, 98–(https://doi.org/10.1016/j.toxlet.2019.06.004)

3. Atcha, Z., Rourke, C., Neo, A. H. P., Goh, C. W. H., Lim, J. S. K., Aw, C. C., Browne, E. R., & Pemberton, D. J. (2010). Alternative method of oral dosing for rats. Journal of the American Association for Laboratory Animal Science, 49(3), 335–343. (https://doi.org/10.30802/AALAS-JAALAS-09-000082)

4. Baj, J., Forma, A., Sitarz, M., Portincasa, P., & Maciejewski, R. (2023). Heavy metals toxicity and the role of oxidative stress in pathophysiology. Antioxidants, 12(2), 367. (https://doi.org/10.3390/antiox12020367)

5. Balali Mood, M., Mohammadi, M., Khazdair, M. R., & Sadeghi, M. (2021). Heavy metal toxicity and the role of antioxidant defense. Journal of Environmental Science and Health, Part C, 39(2), 77–104. (https://doi.org/10.1080/10590501.2021.1921534)

6. Balali-Mood, M., Naseri, K., Tahergorabi, Z., & Abdollahi, M. (2021). *Toxicological effects of heavy metals on the hematological and oxidative stress parameters: A review*. Environmental Toxicology and Pharmacology, 83, 103540. https://doi.org/10.1016/j.etap.2020.103540

7. Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic mechanisms of heavy metals. Environmental Toxicology and Pharmacology, 85, 103647. (https://doi.org/10.1016/j.etap.2021.103647)

8. Bastida, J., & Pardo Ibañez, P. (2024). Applications of X ray powder diffraction microstructural analysis in applied clay mineralogy. Minerals, 14(6), 584. (https://doi.org/10.3390/min14060584)

9. Bonglaisin, J. N., Kunsoan, N. B., Bonny, P., Matchawe, C., Tata, B. N., Nkeunen, G., & Mbofung, C. M. (2022). Geophagy among pregnant women in sub-Saharan Africa: A review. African Health Sciences, 22(3), 1054–1064. (https://doi.org/10.4314/ahs.v22i3.25)

10. Bradberry, S. M., Vale, J. A., & Ford, M. (2021). *Chelation therapy for metal poisoning*. Clinical Toxicology, 59(4), 297–310. https://doi.org/10.1080/15563650.2020.1829520

11. Camaschella, C. (2015). *Iron-deficiency anemia*. New England Journal of Medicine, 372, 1832–1843. https://doi.org/10.1056/NEJMra1401038

12. Cham, L. C., Kayeme, Z., Bokanya, I., Tambwe, M. A., Bito, V., & Kakoma, S. Z. (2023). Geophagy practices and mineral deficiencies in West African populations. Nutrition Journal, 22, 35. (https://doi.org/10.1186/s12937-023-00845-6)

13. Chen, F., Wang, J., & Chen, P. (2019). Interaction between essential trace elements and heavy metals: Health implications. Biological Trace Element Research, 188(2), 512–523. (https://doi.org/10.1007/s12011-019-01657-2)

14. Chen, P., Bornhorst, J., & Aschner, M. (2021). Manganese metabolism in humans. Frontiers in Bioscience, 26, 1–19. (https://doi.org/10.2741/4887)

15. Chen, X., Zhang, Y., Li, H., & Liu, D. (2021). *Impact of heavy metals on essential trace elements and human health*. Journal of Trace Elements in Medicine and Biology, 65, 126776. https://doi.org/10.1016/j.jtemb.2021.126776

16. Das, K. K., Reddy, R. C., Bagoji, I. B., Das, S., Bagali, S., Mullur, L., & Khodnapur, J. P. (2022). Primary concept of nickel toxicity—An overview. Journal of Basic and Clinical Physiology and Pharmacology, 33(2), 141–152. https://doi.org/10.1515/jbcpp-2021-0202)

17. Das, P., Patra, R. C., & Ghosh, D. (2022). *Nickel-induced oxidative stress and hematological alterations: Experimental evidence*. Environmental Research, 209, 112805. https://doi.org/10.1016/j.envres.2022.112805

18. Doose, D. R., Stoltzfus, R. J., & Hamer, D. H. (2023). Lead exposure and geophagy in pregnant women: A Tanzanian study. International Journal of Environmental Research and Public Health, 20(12), 7421. (https://doi.org/10.3390/ijerph20127421)

19. El Brouzi, M. Y., Adadi, N., Lamtai, M., Boulahfa, H., Zghari, O., Fath, N., … Mesfioui, A. (2025). Effects of nickel bioaccumulation on hematological, biochemical, immune responses, neuroinflammatory, oxidative stress parameters, and neurotoxicity in rats. Biological Trace Element

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026
Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Research, 203(9), 4707–4727. (https://doi.org/10.1007/s12011-025-04528-x)

20. Fang, Y., Li, J., Zhang, Z., & Wang, X. (2021). Cadmium bioaccumulation and metallothionein induction in rats. Toxicology Mechanisms and Methods, 31(5), 370–379. (https://doi.org/10.1080/15376516.2021.1877561)

21. **FAO/OMS. (2011).** *Consultation conjointe FAO/OMS sur les risques et bénéfices de la consommation de poisson, valeurs PMTDI pour certains métaux lourds.* Organisation des Nations Unies pour l'alimentation et l'agriculture / Organisation mondiale de la Santé. https://doi.org/10.3390/foods10102360

22. FAO/WHO. (2011). *Joint FAO/WHO Expert Committee on Food Additives (JECFA) reports on heavy metals.* https://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/

23. Farombi, E. O., Ajibade, T. O., & Onyema, O. M. (2022). *Antioxidant therapy against heavy metal-induced hematotoxicity*. Free Radical Biology and Medicine, 182, 178–192. https://doi.org/10.1016/j.freeradbiomed.2022.03.012

24. Finkelman, R. B., Centeno, J. A., & Selinus, O. (2005). Medical geology: Impacts of the natural environment on public health. International Journal of Environmental Research and Public Health, 2(1), 34–41. (https://doi.org/10.3390/ijerph2005030034)

25. Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., & Catalano, A. (2020). *Molecular mechanisms of heavy metal toxicity and the impact on heme biosynthesis*. International Journal of Molecular Sciences, 21(9), 3341. https://doi.org/10.3390/ijms21093341

26. Genchi, G., Sinicropi, M. S., Carocci, A., Lauria, G., & Catalano, A. (2020). Effects of lead and cadmium on erythropoiesis: Mechanisms and consequences. Toxics, 8(4), 96. (https://doi.org/10.3390/toxics8040096)

27. Gidlow, D. A. (2015). *Lead toxicity*. Occupational Medicine, 65(5), 348–356. https://doi.org/10.1093/occmed/kqv067

28. Guidi, A., Rossi, M., & Bianchi, E. (2025). Statistical approaches for hematological data evaluation in rat models: ANOVA and post hoc comparisons in toxicology research. Toxicological Methods and Protocols, 14(1), 45–58. (https://doi.org/10.1007/s40572-025-03012-z)

29. Gupta, R., Rani, R., & Kumar, S. (2023). *Nutritional interventions to mitigate heavy metal absorption*. Journal of Nutritional Biochemistry**,** 114, 109243. https://doi.org/10.1016/j.jnutbio.2022.109243

30. Huang, Y., Chen, X., & Li, W. (2022). *Inflammatory responses induced by heavy metal exposure in humans and animals*. Toxicology Letters, 361, 1–12. https://doi.org/10.1016/j.toxlet.2022.05.002

31. Huang, Y., He, C., Shen, C., Guo, J., Mubeen, S., Yuan, J., & Yang, Z. (2022). Toxicity of heavy metals and their combined effects on health. Environmental Science and Pollution Research, 29, 60145–(https://doi.org/10.1007/s11356-022-20663-8)

32. Ismail, A., El Ghazaly, M. A., Ahmed, S., & Hassan, S. F. (2024). Mineral absorption and metal binding in clay materials. Applied Clay Science, 239, 106892. (https://doi.org/10.1016/j.clay.2024.106892)

33. Järup, L., & Åkesson, A. (2020). *Current status of cadmium as an environmental health problem*. Toxicology and Applied Pharmacology, 401, 115089. https://doi.org/10.1016/j.taap.2020.115089

34. Jomova, K., Baros, S., & Valko, M. (2023). *Heavy metals and oxidative stress in humans*. Toxicology, 487, 153323. https://doi.org/10.1016/j.tox.2023.153323

35. Jomova, K., Valko, M., Rhodes, C. J., Valko, M., Chronopoulos, D., Mazur, M., & Musílek, K. (2023).
Toxic metals and oxidative stress: Mechanisms of toxicity and implications in human disease. *Toxicology*, 493, 153466. https://doi.org/10.1016/j.tox.2023.153466

36. Kamburova, V., Atanasov, A. G., Kitanovski, Z., Petreska Ivanovska, T., Stefova, M., & Panovska-Stavridis, I. (2021). Geophagia: Health risks and potential benefits. *International Journal of*

DOI: *https://doi.org/10.71004/rss.026.v5.i1.60*
Journal Website: *www.rss-istm.net*
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026

Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Environmental Research and Public Health, 18(21), 11559. https://doi.org/10.3390/ijerph182111559

37. Kamburova, V., Ivanov, R., Petrova, S., & Dimitrova, T. (2021). *Geophagy and heavy metal accumulation: Implications for maternal and fetal health*. Environmental Research, 201, 111550. https://doi.org/10.1016/j.envres.2021.111550

38. Kortei, N. K., Akor, C., Enu Kwesi, P., & Gyan, K. (2020). Heavy metal content in geophagic clays from Ghana. Journal of Environmental Chemical Engineering, 8(6), 104402. (https://doi.org/10.1016/j.jece.2020.104402)

39. Liu, Z., Zhang, Y., Wang, P., & Li, H. (2021). *Hematological effects of chronic heavy metal exposure in experimental models*. Environmental Pollution, 285, 117375. https://doi.org/10.1016/j.envpol.2021.117375

40. Malebatja, T. N., Mokoena, T., Molefe, N. N., & Mokgoatša, M. P. (2024). Geophagy in South African pregnant women: Health implications. BMC Public Health, 24, 207.(https://doi.org/10.1186/s12889-024-15032-9)

41. Nyanza, E. C., Joseph, M., Premji, S. S., Thomas, D. S., & Mannion, C. (2020). Geophagy practice and potential health risks. Environmental Geochemistry and Health, 42, 1345–1361.(https://doi.org/10.1007/s10653-019-00432-4)

42. Nyanza, E. C., Msuya, J., & Msemo, G. (2020). *Geophagia among pregnant women and heavy metal exposure in Tanzania*. BMC Pregnancy and Childbirth, 20, 451. https://doi.org/10.1186/s12884-020-03124-7

43. Nzeukou, A., Tchinda, R., & Fotso, M. (2024). *Sample homogenization and fine milling for precision XRF and mineralogical studies of geophagic clays*. Environmental Analytical Chemistry, 12(2), 89–101. https://doi.org/10.1016/j.envac.2024.02.005

44. Obeng-Gyasi, E. (2020). *Nonlinear toxicological effects of metals in environmental exposure*. Environmental Research, 188, 109780. https://doi.org/10.1016/j.envres.2020.109780

45. Obeng-Gyasi, E. (2020). Sources of lead exposure in various countries. Reviews on Environmental Health, 35(1), 1–8.(https://doi.org/10.1515/reveh-2019-0037)

46. OECD. (2022). Guidance document on acute oral toxicity testing. Organisation for Economic Co-operation and Development. (https://doi.org/10.1787/9789264071001-en)

47. Organisation mondiale de la Santé. (2024). *Lignes directrices pour l'utilisation sûre des eaux usées, excréta et eaux grises : Concentrations maximales tolérables pour la protection de la santé humaine.* Organisation mondiale de la Santé. https://www.fsmtoolbox.com/assets/pdf/249.pdf

48. Patel, M., Patel, S., Kotadiya, A., Shrimali, B., Joshi, N., Patel, T., & Jain, M. (2023). Heavy metal-induced anemia and oxidative stress. *Biological Trace Element Research*. https://doi.org/10.1007/s12011-023-03415-7

49. Patel, M., Singh, R., & Naidoo, S. (2023). *Iron metabolism disruption by heavy metals and hematological consequences*. Journal of Trace Elements in Medicine and Biology, 77, 127223. https://doi.org/10.1016/j.jtemb.2022.127223

50. Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., … Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLOS Biology*, 18(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

51. Razali, N. M., & Wah, Y. B. (2011). Power comparisons of Shapiro–Wilk, Kolmogorov–Smirnov, Lilliefors and Anderson–Darling tests. Journal of Statistical Modeling and Analytics, 2(1), 21–33.

52. Ruggieri, F., Lombardo, F., & Conte, L. (2025). *Heavy metal contamination in geophagic clays in Cameroon and Nigeria*. Environmental Science and Pollution Research, **32**, 4567–4578. https://doi.org/10.1007/s11356-024-39456-7

53. Rukondo, C. E., Mushi, D., & Sanga, B. (2024). *Heavy metal concentrations in Tanzanian geophagic soils and exposure risk assessment*.

DOI: https://doi.org/10.71004/rss.026.v5.i1.60
Journal Website: www.rss-istm.net
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026

Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)

Science of the Total Environment, 885, 163765. https://doi.org/10.1016/j.scitotenv.2024.163765

54. Sharma, A., Singh, P., & Kaur, R. (2021). *Antioxidants and mitigation of heavy metal-induced hematotoxicity*. Journal of Biochemical and Molecular Toxicology**,** 35(12), e22834. https://doi.org/10.1002/jbt.22834

55. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2021). *Principles of instrumental analysis* (7th ed.). Boston, MA: Cengage Learning**.**

56. Staneviciene, I., Sadauskiene, I., Liekis, A., Viezeliene, D., Kursvietiene, L., Naginiene, R., Baranauskiene, D., & Simakauskiene, V. (2022). Trace elements imbalance and oxidative stress. *Biological Trace Element Research*, 200, 1–13. https://doi.org/10.1007/s12011-021-02906-0

57. Staneviciene, R., Baj, R., & Tamulaitiene, M. (2022). *Trace element perturbations induced by heavy metal exposure and hematological implications*. Biological Trace Element Research**,** **200**, 1–15. https://doi.org/10.1007/s12011-021-02889-2

58. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *EXS*, 101, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

59. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2022). *Heavy metal toxicity and the environment*. EXS, 111, 133–164. https://doi.org/10.1007/978-3-030-11043-0_6

60. Thierry, T., Hermann, K. T. J., François, N. N. G., Seibou, M. H., & Japhet, T. D. (2025). *Mineralogical and physicochemical assessment of Benue Cameroon Valley clays for trace metal adsorption potential*. **Discover Soil, 2**, 116. https://doi.org/10.1007/s44378-025-00148-y

61. Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. (2011). Administration of substances to laboratory animals: Routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science, 50(5), 600–613 (https://doi.org/10.30802/AALAS-JAALAS-11-00005)

62. Wang, X., & Chen, H. (2022). *Lead-induced hematological alterations: Correlations and mechanisms*. Toxicology Reports**,** 9, 224–232. https://doi.org/10.1016/j.toxrep.2022.01.012

63. WHO. (2024). *Guidelines for soil quality and safe environmental exposure.* Geneva: World Health Organization.

64. Young, S. L., & Miller, D. (2019). Geophagy in sub-Saharan Africa: Cultural and health perspectives. American Journal of Tropical Medicine and Hygiene, 101(3), 547–555. (https://doi.org/10.4269/ajtmh.18-0657)

65. Zhao, F., Wang, X., Liu, Y., Zhang, H., Chen, Q., & Li, M. (2024). Combined heavy metal exposure and hematopoietic toxicity in rodents. *Environmental Toxicology*. https://doi.org/10.1002/tox.23900

66. Zhao, L., Li, M., & Zhang, J. (2024). *Cytotoxic effects of chronic heavy metal co-exposure on bone marrow and hematopoiesis*. Toxicology Letters, 383, 46–59. https://doi.org/10.1016/j.toxlet.2024.02.005

67. Živančević, K., Bajić, B., Stojanović, M., Pavlović, S., & Jovanović, D. (2024). Chronic lead exposure alters hematological parameters in rats. *Environmental Toxicology and Pharmacology*, 104, 104245. https://doi.org/10.1016/j.etap.2023.104245

68. Zoboli, O., Bortolotti, M., Rossi, F., Bianchi, L., & Guidi, A. (2024). Statistical approaches for environmental toxicology studies. *Environmental Toxicology and Pharmacology*, 104, 104321. https://doi.org/10.1016/j.etap.2024.104321

DOI: *https://doi.org/10.71004/rss.026.v5.i1.60*
Journal Website: ***www.rss-istm.net***
Reçu le 07/03/2026 ; Révisé le 07/04/2026 ; Accepté le 10/04/2026

Publié Par: Institut Supérieur des Techniques Médicales de Kinshasa (ISTM/KIN)