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Oral In Vitro-Bioaccessibility of Toxic Heavy Metals in Contaminated Soils: A Review of Methodological Approaches, Analytical Challenges, and Implications for Human Health Risk Assessment

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ABSTRACT

Heavy metal contamination of soil remains a global environmental public health concern due to industrialisation, indiscriminate e-waste disposal, illegal mining, urbanization, and agricultural activities. Researchers often assess the health risk using total heavy metal concentration. In this review of heavy-metal bioaccessibility in soil samples, we assessed the methodological and analytical challenges in evaluating human health risk from peer-reviewed journals published from 2020 to 2026, using search syntax that included heavy metals OR toxic heavy metals AND bioaccessibility OR oral bioaccessibility. We screened relevant peer-reviewed papers for inclusion and exclusion; the included papers show that the fraction of total metal dissolution that becomes accessible within the human gastrointestinal tract for absorption differs from total metal concentrations. This available metal fraction in the gastrointestinal tract is termed oral bioaccessibility. This review critically evaluates recent developments in bioaccessibility methodologies and analytical techniques, and the factors that influence quality assurance, including comparisons between total heavy metal concentrations in soil samples and the bio accessible fraction for assessing human health risk. The dynamic and static bioaccessibility is also discussed, along with the current research gap in the literature.

Keywords: Dynamic; Static; Bioaccessibility; Gastrointestinal Tract; Heavy Metals; Soil; Methodologist; Analytical Techniques; Contamination.

1.0. Introduction

Toxic heavy metals (THMs) are found in environmental matrices such as soil, sediments, dust, and water, posing public health risks worldwide [1]. Sources of this contamination include mining activities, agricultural revolutions, industrialization, and informal electronic waste recycling, which release toxic heavy metals into the environment. These metals include lead (Pb), arsenic (As), mercury (Hg), nickel (Ni), copper (Cu), cadmium (Cd), and zinc (Zn) [2].

The primary routes of exposure to these toxic heavy metals in humans are oral ingestion, dermal contact, and inhalation. These are absorbed into different target organs such as the kidney and liver, which results in adverse health diseases such as kidney dysfunction, developmental abnormalities, and carcinogenicity [3].

Researchers assessed the environmental health risk posed by these toxic heavy metals using total concentrations of these metals measured in environmental matrices [4-5-6]. However, total toxic heavy metal content does not reflect the soluble fraction of contaminated metals that is absorbed in the gastrointestinal tract, becomes available for uptake into the bloodstream, and is transported to target organs [7].

The emergence of bioaccessibility testing has proved a more accurate approach to evaluating health risk from human exposure than total concentration [8]. Therefore, bioaccessibility is the soluble portion of contaminants from the environmental matrix available in gastrointestinal fluid for absorption in the human digestive system [9].

1.1. Analytical Methods of Bioaccessibility Testing

There are various established methods to simulate human gastrointestinal conditions, such as the physiological basis extraction test (PBET), the solubility bioavailability research consortium (SBRC), the unified BARGE method (UBM), in vitro Gastrointestinal (IVG), and dynamic gastrointestinal simulation (DGS) [10].

Among these methods, they are widely used for testing the bioaccessibility of toxic substances in environmental matrices and for toxicological studies (Li et al., 2024). The challenges of this method start from inconsistencies in extraction process protocols. The inconsistencies among several re-searchers are in pH conditions, particle size selection, extraction duration, temperature, enzymatic composition, and analytical procedures [11]. As a result of these protocol inconsistencies, bioaccessibility results are reported by different researchers across the globe, making it difficult to compare inter-study bioaccessibility testing results [1-13].

Physiologically based extraction test (PBET): This test simulates the human digestive system. Mainly the gastric and intestinal phases, while the gastric phase, which is more acidic at pH 1.5 -2.5, contained some organic acids, pepsin, sodium hydroxide, and other body electrolytes [14]. The intestinal phase contained a pH of 5.5 -7 with some substances such as bile salts and pancreatin. The method dissolved toxic metals from the sample matrix into the simulated fluid and measured the amount dissolved by inductively coupled plasma mass spectrometry [15]. The challenges of this method included the non-universal protocol and the use of different pH levels during extraction [16].

Unified BARGE Method (UBM): Developed by the European Bioaccessibility Research Group (EBRG). It simulates mouth fluid, stomach, and intestinal phases. This involved the salivary, gastric, and intestinal phases, coupled with digestive enzymes, bile salts, malic acid, and citric acid. [17]. The complexity of this method is considered the main challenge, as it involves multiple reagents and procedures. It's also expensive because of the chemicals and enzymes involved. This method is sensitive to the pH and reagent preparation [18].

Solubility Bioaccessibility Research Consortium Method (SBRC): This method is widely adopted for contaminated soil samples because it measures the soluble contaminants in the stomach under acidic conditions. It simplifies gastrointestinal extraction, which includes the gastric and intestinal phases. The simulated fluid is a glycine solution at pH 1.5 and 37 °C. The method is validated for measuring Arsenic, Lead, and Cadmium. Therefore, this method has limited physiological relevance because it does not simulate a complete digestive system and is also sensitive to pH changes [19].

In vitro Gastrointestinal Method (IVG): The method uses sequential extraction to simulate gastro-intestinal digestion. The simulation fluid releases metals from soil samples during the digestion

Table 1. The Common Methods Used by the Researchers.

Common Method Used	Properties	Typical Area of Application
Physiologically Based Extraction Test (PBET)	The method is simple, with a pH 2.5 gastric phase and a pH 7.0 for the intestinal phase. Bile salts and pancreatin are used to break down fat into smaller droplets and to digest fat into fatty acids and glycerol, respectively.	Bioaccessibility of toxic metals in soil for the environmental risk Assessment

Unified Bioaccessibility method (UBN)	Started from the Saliva simulation, and more complex intestinal fluid simulation. The physiological gastric phase pH range is between 1.1 and 1.8	Recommended by BARGE for standard human health risk Assessment
German standard method	Simulate saliva; the gastric phase includes the duodenal phase. This is similar to a physiologically based test	Mostly used for the bioaccessibility of nutrients and pharmaceuticals
In vitro Gastrointestinal test (IVG)	This involves the addition of a food component such as casein. This process involves a lower Ph	Used to test the bioaccessibility of lipophilic contaminants.

The choice of methods used by several researchers sometimes moves from a simple method of ex-traction, which may be fast and less time-consuming, but it may be inaccurate because of the physio-logical changes of the system. These methods are mainly called static or dynamic physiological methods [20].

1.2. Static Test of Bioaccessibility

Static test for bioaccessibility refers to the fixed composition simulation of human digestion conditions. In this situation, the pH conditions and temperature are static during the experiment. Therefore, the digestive fluid is added to the samples consciously without changing the PH or adjusting the physiological conditions [21]. Therefore, the mimic extraction forms the stomach phase, and the intestinal phase is sequential without changing the conditions, but allows continuous flow [22].

The advantage of the static bioaccessibility test is that the method is simple, fast, and less expensive; it was reported to be good for screening and validation protocols. The limitation of the test is that there is no change in pH; static testing may underestimate bioaccessibility for the most sensitive pH-dependent samples for solubilization. This method is mostly used in interlaboratory harmonization for effective screening [23].

1.3. Dynamic Test for Bioaccessibility.

This test involves advanced bioaccessibility in an in vitro system for the continuous simulation of human digestion with changes in pH, use of enzymes, and intestinal transit during the experiment. The principle behind this test is to change or vary the pH and intestinal conditions to secrete digestive fluid. Test of time and the mixing rate [24]. This test follows the complete movement of sub-stances during digestion. Starting from the mouth the salivary phase, the stomach phase, with a gradual decrease in pH, and the small intestinal phase, where the stomach contents move slowly into the intestinal phase. In this phase, the addition of bile salts and pancreatic enzymes and changes in PH. [25]. The overall model of this test is the simulator of the human intestinal microbial ecosystem (SHIME). This involves the simulation of the stomach, small intestine, and colon, which is used to study the gut microbiota behaviour [18].

Table 2. Comparison of Dynamic and Static Bioaccessibility Test.

Condition of the Experiment	Static Test	Dynamic Test
Cost of experiment	Low cost	More expensive
Complexity	Very simple to carry out	Complex experiment

Simulation of digestion	Fixed throughout the experiment	Continuous changing
Ph	Fixed pH	Not Fixed
Emptiness of the gastric phase	Not considered	Simulated
Mouth phase	Saliva not simulated	Saliva simulated
Precision	Moderate	Very high
Area of uses	Common in environmental studies	Common in human digestion studies

Static bioaccessibility tests are widely used for contaminated soil samples because of the cost-effectiveness of large samples in environmental studies. Some of the adopted bioaccessibility tests are the Solubility Bioaccessibility Research Consortium SBRC, the Physiological Base Extraction Test PBET and the unified BARGE method UBM. Several environmental researchers prefer this method because it requires a common laboratory method and is easy to perform for a large number of samples [18]. Some regulatory environmental agencies have accepted the use of UBM and SBRC for environmental studies of bioaccessibility tests. Therefore, the use of dynamic bioaccessibility has created a gap in the study of bioaccessibility tests of environmental contaminants; it further creates a problem of correlation between bioaccessibility and the bioavailability test with an animal model [26].

Table 3. Major In Vitro Bioaccessibility Methods (2020–2026).

Method	Country	Gastrointestinal phases	Validation	Current use
PBET	Canada	Gastric + intestinal	Moderate	High
SBRC	USA	Gastric	High	Very high
UBM	United Kingdom	Gastric + intestinal	Excellent	Highest
IVG	Netherlands	Gastric	Moderate	Moderate
DIN	Germany	Gastric	Limited	Low

Recent publications increasingly recommend the Unified BARGE Method (UBM) because of its robust validation against animal studies and broad international acceptance.

1.4. Study Objectives

The objectives of the reviews study are as follows:

1) To examine the previous research work on bioaccessibility studies of toxic heavy metals in contaminated soils.

2) To evaluate the different methods of bioaccessibility studies used by various researchers used for heavy metals in the soils.

3) To examine the major factors, influence heavy metals bioaccessibility test.

4) To identified analytical challenges for the bioaccessibility test.

2.0. Methodology

In this review, various methods were adopted, including the use of PRISMA for accurate identification and screening of peer-reviewed journals and the inclusion of relevant journals.

A literature search was conducted from 2000 to 2026 across various databases, including Scopus, Web of Science, PubMed, Google Scholar, and ScienceDirect. These searches were conducted using the relevant keywords and Boolean operators. Some

examples of search syntax include OR and AND. As heavy metals OR toxic heavy metals, AND bioaccessibility OR oral bioaccessibility

2.1. Inclusion and Removal Criteria

The search articles were downloaded and exported to the reference management software to eliminate duplicates, and their titles and abstracts were evaluated for relevance during the review process. Then, the full papers were evaluated based on their eligibility for the present study

2.2. Quality Assessment Adopted in the Study

The methodology used in all the included studies was carefully evaluated to identify the sample matrix, heavy metals investigated, bioaccessibility model used, the extraction conditions, the analytical instrument used, the percentage of bioaccessibility reported, health risk prediction, and the major findings. All the studies that show the analytical quality control and the validated methodologies were selected in the studies.

Table 4. Results and Discussion.

Authors	Sample Matrices	County	Bioaccessibility Test	Extraction Protocol	Instrument Uses	Toxic Metals Consider	Risk Assessment
[1]	Mechanic workshop soil	Nigeria	SBET	Static	AAS	Mn and Cd	Mn and Cd are low
[2]	China mining soil	China	PBET	Static	ICP-MS	Cu, Cd, Pb, Cr, Ni	Cr fund underestimated
[3]	Soil sample from an urban area in eastern Canada	Canada	IVB	Static	ICP-MS	As, Cd, Cr, CO, Cu, Pb, Ni, Zn	Pb fund underestimated
[4]	Industrial site soil	China	PBET	Static	ICP-MS	Pb, Ni, Cu, Zn, Cd, Cr	High bioaccessibility in gastrointestinal phase
[5]	Mining site	China	PBET	Static	ICP-MS	As, Mn, Ni, Pb, Zn, Cu, Cr	The intestinal fraction of metals is lower than the gastric phase
[7]		China	BARGE and UBM	Dyna mic	ICP-MS	Zn, Ni, Cd, and Cu	Ni and Cd have high bioaccessibility
[8]	E-waste SITE soil	Ghana	PBET	Static	ICP-MS	Cu, As, Cd, Sb, Pb	Cu is more bio accessible than other metals
[28]	Topsoil	Ireland	UBM	static	ICP-MS	Cr, Ni, As, Cu, Pb, Zn	As, Cu, Pb, Zn have high bioaccessibility

[29]	Cultivated land soil	China	-	static	ICP -MS	Hg, AS, Cd and Zn	Cd and Hg show low bioaccessibility
[6]	Soil	China	PBET/ SHIME	Dynamic	ICP-MS	As, Cd, Cr, Cu, Ni, Fe	As and Cd have high bioaccessibility
[9]	Urban soil	China	Dermal bio-accessibility	-	ICP -MS	Cr, As, Cd, Pb, Cu	Cd has higher bioaccessibility
[10]	Arsenic-contaminated soil	China	SGIS	Static	N.M	As	As has bioaccessibility
[11]	Topsoil from greenhouse	China	RIVM	static	ICP MS	Cd, Cr, Pb, As	Cd, Cr, As vary in bioaccessibility
[12]	Soil from mining area	China	NM	static	ICP MS	Cd, Cu, Pb, Zn, CO, Cr, Ni, V	Cd shows the highest gastric bioaccessibility
[13]	Soil from school playground	Ghana	SBET	Static	AAS	Cu, Pb, Cu, As	Cu and Pb have higher bioaccessibility
[14]	Contaminated soil	China	PBET/ SHIME	Dynamic	NM	Cr, As, Cd, Pb, Cu	Nutrient-utter bioaccessibility
[18]	Paddy soil	China	SBET	Static	NM	Cd, Zn, Pb, Cu, Ni, Hg. As, Cr	Total concentration overestimated
[16]	E-waste soil	China	SBRC	static	NM	Zn, Cu, Cd, Pb	Total concentration overestimated
[17]	Mineral clay soil	USA	UBM/ $CaCO_2$	NM	ICP-MS	As, Cd, Pb	As and Pb show lower bioaccessibility than Cd
[19]	Mining soil	Nigeria	BRAI	Static	ICP-OES	Pb, Zn, Cd, Mn, Fe, Ba	Cd-highly polluted soil
[21]	Agricultural and mining soil	Nigeria	NM	static	ICP -MS	Hg	Low Bioaccessibility of Hg
[20]	Indoor dust	Nigeria	NM	Static	FAA	Cr, Pb, Cd, and Zn	High bioaccessibility
[28]	Soil and Dust	China	PBET	Static	ICP-MS	Zn, Cu, Mn, As, Pb, Cd, Cr, Ni, CO	Concentrations of heavy metals are higher in the gastric phase than in the intestinal phase.

Key: ICP-MS, AAS, FAA, ICP-OES: inductively coupled plasma-mass Spectroscope and optical emission spectrometer, atomic absorption Spectrometer, flame Atomic Absorption Spectrometer, NM: not mentioned. PBET, SHIME, UBM, SBET, IVB, RIVM, BRAI: Physiological Bases Extraction Test, Simulator of Human Intestinal Microbial Ecosystem, Unified BARAGE Method, Simple Bioaccessibility Extraction Test, In vitro Bioaccessibility, National Institute for Public Health and Environment, Bioaccessibility Research Assessment Index.

The review of different researchers from 2020 to 2026 is summarised as shown in Table 4. The reported authors, sample matrix, bioaccessibility method used by the different authors as reported, the selected toxic heavy metals, the area of sampling, and the bioaccessibility level. Different authors from different countries use different approaches to determine the bioaccessibility level for health risk determination.

One common conclusion among the authors is that using the total concentration of toxic heavy metals to determine the health risk of the toxic metal in both children and adults is usually overestimated compared to the use of the bioaccessibility of the same toxic heavy metals.

In the work of [8], they targeted the soil from Yellow River Subdistrict in the province China; among the toxic heavy metal analyses were Hg, As, Pb, Cu, and Zn. The author compared the total heavy metal concentration, chemical fractionation, and in vitro bioaccessibility for an accurate measurement of health risk assessment. In his findings, Cd and Hg are found to be the most concerning toxic metals, with a higher proportion in the extractable fractions. The research further reviews that the fractionation of metals from the sample's matrix combined with in vitro bioaccessibility provides a more accurate assessment of the health risk [29].

Investigating in vitro bioaccessibility of heavy metals in contaminated soil samples and the effect of microbiota was conducted by [6]. Using physiologically based extraction (PBET) and a simulator of the human intestinal microbial ecosystem (SHIME). The authors evaluated the bioaccessibility of selected heavy metals, As, Cd, Cr, Ni, and Fe, and compared the intestinal condition with the colon phases. The authors confirmed that there is an increase in the bioaccessibility of As, Cr, and Ni and a decrease in Cu bioaccessibility. Gut microbiota shows significant differences in metal bioaccessibility, which implies that gut microbiota plays a vital role in the dissolution of the metals and therefore, it can improve the accuracy in determination of human health risk in children and adults.

In another study, [8] also investigated the bioaccessibility with the fractionation of the soil heavy metals from soil obtained from Shandong province, China. The authors used total concentration compared to bioaccessibility dissolution to assess the health risk. Cd and Hg were the most concentrated contaminants, but Cd showed a high proportion in the exchangeable fraction of the soil extract. The author concluded that the pH of the soil and the organic matter are the main factors determining the bioaccessibility of heavy metals from soil to the intestinal phase.

Bioaccessibility of dermal and cytotoxicity of Cr, As, Cd, Pb, and Cu in four urban soil samples [9]. The author determined the bioaccessibility using artificial perspiration to simulate metal dissolution during skin contact. The bioaccessibility of Cd was higher than that of other metals. The author concluded that the calculated dermal absorption doses were below the corresponding derived dermal standard values. The extract from the soil has cytotoxic effects, and therefore, the conventional dermal bioaccessibility underestimated the dose-risk model.

[27] also investigated the bioaccessibility of heavy metals in the soil, physicochemical properties, and microbial communities. In his studies, the total heavy metal concentration and in vitro bioaccessibility of the heavy metals was also compared. The author concluded that metal dissolution was associated with the physicochemical properties and microbial environment. Therefore, metal bioaccessibility from soil provides more information than total concentrations for understanding interactions between soil properties and microbial communities.

3.0. Discussion

The review of literature about Bioaccessibility of Heavy Metals from Soil, the Methodologies, Analytical Challenges, and Human Health Risk Assessment from 2020 to 2026 shows a clear distinction from conventional total concentration assessment to bioaccessibility for determination of health risk. Bioaccessibility studies have improved the use of in vitro gastrointestinal methods to estimate the amount of dissolution of heavy metals in the gastrointestinal and intestinal phases, which is the actual concentration available for absorption [17].

Different researcher demonstrated in their work that bioaccessibility varies considerably among metals' dissolution and its effect on the microbial environment, including soil properties.

From another perspective, the studies of E-waste soil, agricultural and mining soils, including the physicochemical properties, such as the particles size and the organic matter also shows proved that the relying on the total concentrations alone can be overestimated health risks of the different heavy metals [8-16-21]. In other developments, the incorporation of chemical speciation and soil physicochemical characteristics into bioaccessibility assessment during the dissolution of heavy metals during digestion [14]. This review shows the relationship between bioaccessibility and different fractions, such as the exchangeable phase, carbonate-bound, organic matter, and residual phase. Also, the physicochemical properties such as pH and texture are explained by [8]. Particularly, the relevance of the study compared to the total concentrations of the heavy metals, the speciation combined with bioaccessibility shows that Cd and Hg were important contaminants in the samples and that the pH and the organic matter influence metal dissolution. Some researchers also incorporated gut microbiota and observed that the dissolution of metals in the intestinal gut also depends on the microbiota, which shows a better understanding of metal behaviour in the gastrointestinal system.

These advanced research and methodology gaps reviewed in this present review show that most researchers still use static in vitro digestion experiments; the static experiment bases the gastrointestinal phase on constant pH and other factors without considering the dynamic physiological changes of the intestinal phase transition. Therefore, the dynamic kinetic mechanism of dissolution of metals is poorly understood, which brings about a research gap in the studies of bioaccessibility.

The methodologist variability among the different researchers, and the modification methods in gastrointestinal protocol making the results of researcher’s difficulties to compare among the researcher output. Moreover, the chemical fractionation and the bioaccessibility studies are mainly investigated separately, as reviewed in the literature, except for a few researchers. The influences of ligands, microbial activities, and the physicochemical properties of the samples are not adequately incorporated into bioaccessibility studies.

The review of the literature, therefore, shows a move from static bioaccessibility to a dynamic mechanistic bioaccessibility approach. Kinetic model studies of bioaccessibility are under study among researchers in the literature review; however, dynamic factors in physiological studies are lacking in the literature. However, integrating the model of machine learning and artificial intelligence for the prediction of bioaccessibility of heavy metals in soil samples and validation in other environments. The main research gap from the present review of the

literature from 202-2026 is not the absence of heavy metal bioaccessibility studies, but the lack of integrated kinetic studies and how the soil physicochemical properties interfere with the dissolution of metals.

3.1. Challenges in Bioaccessibility Testing

The challenges of analytical testing in bioaccessibility testing, reviewed from the literature, include heterogeneity such as particle size, organic matter, and mineral composition of the samples. This affects the efficiency of the extraction procedures among different researchers and the dissolution of the metals. The use of inductively coupled plasma has also proved to have sensitivity and a low detection limit for the analysis of bioaccessibility of the heavy metals from the soil samples. Among these, ICP-MS demonstrates superior sensitivity and lower detection limits but requires careful matrix correction and calibration procedures.

4.0. Conclusion

The literature review has shown the gaps and the challenges of the analytical techniques among the researchers. Bioaccessibility testing has essential tools for the accurate risk health assessment of heavy metal pollutants. Although the various in vitro bioaccessibility study methodologies and analytical studies, are inconsistencies and limiting the standard methods for comparison by the findings of different researchers on bioaccessibility studies. The present review also demonstrated that a study in conjunction with total concentration, soil properties, speciation, dynamic gastrointestinal bioaccessibility, and kinetic modelling for the prediction of health risk will address the gap and provide a realistic health risk assessment of human exposure to the toxic heavy metals compared to conventional total concentration and static bioaccessibility studies.

The review also highlights the need for the harmonization of methodologies and validation of interlaboratory studies in order to ensure standard quality assurance among researchers. Therefore, a harmonization approach will improve the studies of risk assessment of heavy metal exposure and protection of public health globally.

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