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## Abstract

Potentially toxic elements (PTEs) pose a threat to human health as they can easily enter the human body via ingestion of polluted soil/sediment. In order to estimate the bioavailability and assess the health impact on people, measurement of the oral bioaccessibility of a pollutant is crucial. Various laboratory based in vitro tests which mimic human gastrointestinal tract conditions can be used. In order to set up the method for analysing the bioaccessibility of pollutants in soil samples in the Laboratory for the analysis of geological materials at the Department of Mineralogy, Petrology and Mineral resources (Faculty of Mining, Geology and Petroleum Engineering, University of Zagreb - RGNF), with regards to the available equipment, an orientation survey was carried out in collaboration with the Department of Biochemical Engineering (Faculty of Food Technology and Biotechnology, University of Zagreb - PBF). The digestion of two different samples in synthetic fluids (gastric and intestinal fluid) was performed simultaneously at the RGNF laboratory and PBF laboratory under different extraction conditions according to each laboratory's ability. Prior to the analysis of bioaccessibility, detailed mineralogical and chemical characterization of the samples was performed. The comparison of two experiments showed that there is a relatively good correlation between concentrations obtained after digestion of the samples in different laboratories, under different conditions. As a result of this study, an efficient and relatively inexpensive method for determining bioaccessibility was set up at Faculty of Mining, Geology and Petroleum Engineering, which makes this kind of test more accessible and enables a new approach in risk assessment studies.

## Keywords

bioaccessibility, ingestion, health assessment, potentially toxic elements, polluted soil

## 1. Introduction

One of the major exposure routes for potentially toxic elements (PTEs) in soil/sediment is through ingestion. A crucial step in the estimation of bioavailability and evaluation of the risk to human health is the measurement of the oral bioaccessibility of a pollutant, defined as the fraction that is soluble in the gastrointestinal tract and is available for absorption (Paustenbach, 2000). Bioaccessibility is commonly confused in the literature with bioavailability, which is defined by toxicologists to be the fraction of an administered dose of a toxicant that is absorbed via an exposure route, reaches the bloodstream, and is transported in the body to a site of toxicological action (Plumlee et al., 2006). A number of laboratory based in vitro tests which mimic the biochemical conditions in the human gastrointestinal tract are used to assess the degree of metal solubility i.e. oral bioaccessibility (DIN, 2000, Rodriguez et al., 1999, Hamel et al., 1999, Hamel, 1998, Medlin, 1997, Rotard et al., 1995, Ruby et al., 1993, Molly et al., 1993, XII, 1990) (reviewed in (Dean and Ma, 2007, Intawongse and Dean, 2006, Wragg and Cave, 2003)). The human gastrointestinal tract consists of a number of compartments where ingested soil/sediment undergoes a series of reactions in which fluid composition, pH and reaction time all vary. The analogue digestive tract methods use extractions that mimic a combination of one or more of the compartments (Oomen et al., 2002). These methods can be divided into two types: 1) the batch test, where the sample and reagents are reacted in a single container with additional chemicals being added and samples removed as

stated in each specified protocol, and 2) the flow through a reactor system, in which the reaction takes place under flowing conditions, designed to simulate the actual transit characteristics of the gastrointestinal tract (Wragg and Cave, 2003).

As reported in several reviews (Dean and Ma, 2007, Intawongse and Dean, 2006, Wragg and Cave, 2003), the most commonly used batch extraction methods are as follows: the physiologically based extraction test (PBET) originally developed by Ruby et al. (1993); the *in vitro* gastrointestinal method (IVG) (Rodriguez et al., 1999); the Dutch National Institute for Public Health and the Environment method (RIVM) (Versantvoort and Rempelberg, 2004) and the relative bioaccessibility leaching procedure (RBALP) (Drexler and Brattin, 2007, Drexler, 1999). The Bioaccessibility Research Group of Europe (BARGE) developed *in vitro* physiologically based ingestion bioaccessibility procedure for soils, based on the RIVM methodology, known as the Unified BARGE Method (UBM) (Wragg et al., 2011). *In vitro* gastrointestinal protocols provide a rapid and inexpensive means to determine the bioaccessibility of a given potentially toxic element present in soil and represent appropriate alternatives to animal testing.

In order to set up the method for analysing bioaccessibility of pollutants in soil samples in the Laboratory for the analysis of geological materials at the Department of Mineralogy, Petrology and Mineral resources (Faculty of Mining, Geology and Petroleum Engineering, University of Zagreb - RGNF), with regards to the available equipment, an orientation survey was carried out in collaboration with the Department of Biochemical Engineering (Faculty of Food Technology and Biotechnology, University of Zagreb - PBF).

The digestion of two different samples in synthetic fluids (gastric and intestinal fluid) was performed simultaneously at the RGNF laboratory and the PBF laboratory under different extraction conditions according to each laboratory's ability. Prior to the analysis of bioaccessibility, detailed mineralogical and chemical characterization of the samples was performed.

## 2. Materials and Methods

Two different types of samples were used in this study: (1) residential soil; and (2) tailings. The analysed samples were collected in the area of an abandoned lead-zinc-vanadium mine Berg Aukas in Namibia.

Samples were air dried and sieved to fraction < 250 µm, because this particle size is representative of that which adheres to children's hands (Agency, 2008). Mineral content of the samples was determined using X-ray diffraction (Philips diffractometer PW1710). In addition to the body fluids extraction, pseudo-total metal content (*aqua regia* extraction) as well as sequential extraction were performed. All extracts were analysed using flame atomic absorption spectroscopy (AAAnalyst 700 Perkin Elmer). Samples with analytical methods performed are listed in Table 1.

**Table 1** Studied samples and analyses

Sample label	Sample type	XRD	Extraction type*
4578	tailings	+	XRD, AR, SEA, BF
4590	soil	+	XRD, AR, SEA, BF

\*XRD – X-ray diffraction, AR – *aqua regia* extraction, SEA – sequential extraction, BF – body fluids extractions

### 2.1. *Aqua regia* extraction

The pseudo-total Cd, Cu, Pb and Zn contents were obtained by a standardized *aqua regia* extraction protocol according to ISO Standard 11466 (ISO, 1995). *Aqua regia* soluble metal concentrations in analysed soil and tailings were compared with the Canadian Soil Quality Guidelines (CCME, 1999).

## 2.2. Sequential extraction

Different binding sites of metals in samples were analysed using sequential extraction, whereby a series of single reagents are used to extract operationally-defined phases (the selectivity depends on such factors as chemicals employed, the time and nature of contact, and the sample to volume ratio) in a defined sequence from 1 g of the sample. BCR (Community Bureau of Reference) sequential extraction procedure (Rauret et al., 2001) was used to give four fractions (Table 2): (1) acid (bound to carbonates); (2) reducible (bound to iron and manganese oxides, hydroxides and oxyhydroxides); (3) oxidisable (bound to organic matter and/or sulphides); and (4) residual (bound to chemically resistant minerals).

**Table 2** The BCR sequential extraction procedure (Rauret et al., 2001)

Step number	Fraction	Label	Reagent/concentration/pH
1	Exchangeable, acid-and water-soluble	CARB	Acetic acid CH <sub>3</sub> COOH (0.11 mol/L)
2	Reducible	FEMN	Hydroxylammonium chloride NH <sub>2</sub> OH×HCl (0.5 mol/L) pH 2
3	Oxidisable	OR/SUL	Hydrogen peroxide H <sub>2</sub> O <sub>2</sub> (8.8 mol/L) Ammonium acetate NH <sub>4</sub> OOCCH <sub>3</sub> (1.0 mol/L) pH 2
4	Residual	RES	<i>Aqua regia</i> : Hydrochloric acid HCl (12.0 mol/L) Nitric acid HNO <sub>3</sub> (15.8 mol/L)

## 2.3. Body fluids extractions

Simulated gastric and intestinal fluids were prepared according to Kos and Goreta (2000), with some modifications. Briefly, simulated gastric fluid was prepared by dissolving pepsin (AppliChem, Germany) (3 g/L) in a sodium chloride solution (0.5%) adjusting the pH to  $1 \pm 0.2$  with concentrated HCl. Simulated intestinal fluid was prepared by suspending pancreatin (AppliChem, Germany) (1 g/L) and bile salts (Difco, USA) (3 mg/mL ox gall) in a sodium chloride solution (0.5%) and the pH was adjusted to 8.0 with 30% NaOH. Both gastric and intestinal fluids were prepared fresh.

Digestion in synthetic fluids was performed simultaneously at the RGNF laboratory and the PBF laboratory under different extraction conditions, as described below and shown in Table 3, along with an overview of the different *in vitro* digestion models described in the literature. For each sample, two extracts were collected: one at the end of the gastric phase and another at the end of the gastro-intestinal phase. In this study, the gastric phase (G) is a digestive extract collected after 1 hour of agitation with simulated gastric fluid. The gastro-intestinal phase (GI) is a digestive extract collected after 1 hour of agitation with gastric fluid followed by 4 hours agitation with simulated intestinal fluid.

Table 3 Bioaccessibility test methods

Method	Compartments*	pH	T °C	S/L ratio	Residence time	Metals tested
RGNF	S	1.2	25-27	0.5/18	1 h	Cd, Cu, Pb, Zn
	SI	8		0.5/47	4 h	
PBF	S	1.2	37	0.5/18	1 h	Cd, Cu, Pb, Zn
	SI	8		0.5/47	4 h	
UBM (Oomen et al. 2002)	OC	6.5	37	0.6/9	10 s	As, Cd, Pb
	S	1.1		0.6/22.5	1 h	
	SI	7.4		0.6/58.5	4 h	
PBET (Ruby et al. 1996)	S	2.5	37	1/100	1 h	As, Pb
	SI	7			4 h	
SBET (Drexler 1999)	S	1.5	37	1/100	1 h	As, Cd, Pb
DIN (DIN 2000)	OC	6.4	37	1/15	0.5	As, Cd, Pb, Cr, Hg
	S	2		1/50	2 h	
	SI	7.5		1/100	6 h	
SHIME (Molly et al. 1993)	S	5.2	37	1/2.5	3 h	As, Cd, Pb
	SI	6.5		1/4	5 h	
RIVM (Rotard et al. 1995)	OC	6.5	37	1/15	5 min	As, Cd, Pb
	S	1.1		1/37.5	2 h	
	SI	5.5			2 h	
IVG (Rodriguez and Basta 1999)	S	1.8	37	1/150	?	As
	SI	5.5			?	
US P (Hamel et al. 1998)	S	ca. 1	37	1/1000	2 h	Pb, Cr, As, Cd, Ni
MB & SR (Hamel et al. 1999)	OC	ca. 1	37	1/160	5 s	Pb, Cr, As, Cd
	S			1/2160	2 h	
	SI			1/4770	4 h	

\*OC – Oral Cavity, S – Stomach, SI – Small Intestine

The digestion was started by suspending 0.5 g of dry soil sample in 18 mL of gastric fluid. This mixture was rotated end-over-end at room temperature (25-27°C) for 1 h at the RGNF laboratory, while agitated in a benchtop water bath shaker at 37°C for 1 h at the PBF laboratory. For the replicate samples, this was followed by an addition of 29 mL of intestinal fluid and agitation for another 4 hours. Subsequently, the samples of gastric and gastro-intestinal phase were centrifuged at 3800 rpm for 10 min and filtered, and 67% HNO<sub>3</sub> was added (0.5 mL and 1 mL, respectively) to the supernatants before they were stored for analysis. The pH of the extracts was measured at the end of digestion for each phase. Bioaccessibility was calculated per digestion according to the following equation:

$$\% \text{ bioaccessible} = \frac{\text{concentration of bioaccessible metal (mg kg}^{-1}\text{)}}{\text{concentration of total metal in sample (mg kg}^{-1}\text{)}} \times 100 \quad (1)$$

### 3. Results and Discussion

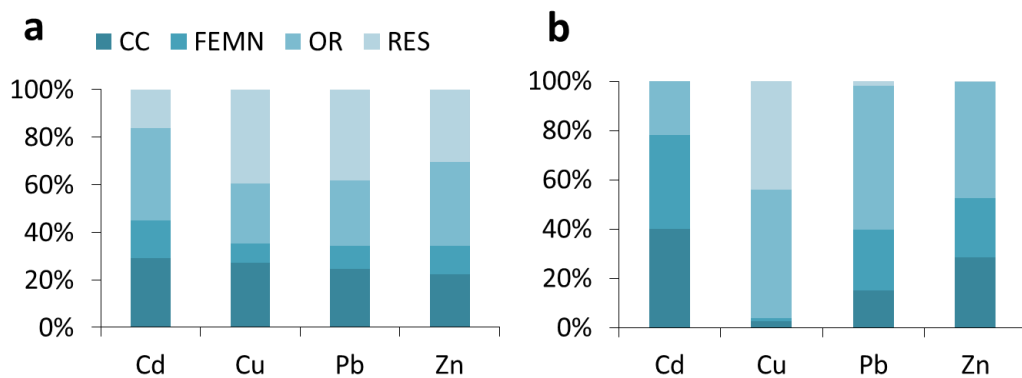
The dominant mineral in the tailings sample is dolomite, followed by calcite, quartz, willemite (zinc silicate mineral -  $Zn_2SiO_4$ ), illitic material and traces of K-feldspar. There is indication of the presence of descloizite (lead and zinc vanadate -  $(Pb,Zn)_2(OH)VO_4$ ). The soil sample contains dominantly quartz, followed by calcite, dolomite and K-feldspar.

Potentially toxic elements measured in *aqua regia* extracts of the samples are Cd, Cu, Pb and Zn. Pseudo-total concentrations of PTEs in the sample of tailings are 290 mg/kg, 153 mg/kg, 10,636 mg/kg and 43,783 mg/kg, while those in sample of soil are 23 mg/kg, 25 mg/kg, 1,199 mg/kg and 5,919 mg/kg, respectively. Metal concentrations in the analysed soil (except Cu concentration) and tailings exceed Canadian Soil Quality Guidelines (CCME, 1999) which are defined as the concentrations recommended to provide a healthy, functioning ecosystem capable of sustaining the existing and likely future uses of the site by ecological receptors and humans (Table 4).

**Table 4** Soil quality guidelines for the protection of environmental and human health (CCME, 1999)

Element	Concentration (mg/kg dry weight)			
	Agricultural	Residential/parkland	Commercial	Industrial
Cadmium	1.4	10	22	22
Copper	63	63	91	91
Lead	70	140	260	600
Zinc	200	200	360	360

Metal concentrations in *aqua regia* extracts cannot be the sole indicator of the environmental risk caused by polluted soil/sediment, since they can be tightly bound in a mineral lattice of minerals and although present in significant amounts do not affect the environment and human health. Hence, sequential extraction analysis was performed to determine the binding sites of the analysed metals. Distribution of metals in different soil phases, determined by sequential extraction analysis, is shown in Figure 1. It appears that approximately 20-40% of total concentration in the sample is bound in organic as well as in residual fraction for most of the analysed metals in the sample of tailings, whereas in the soil sample nearly 50% of metals are bound to organic fraction, with cadmium as an exception. The main binding sites for Cd are carbonate and reducible fraction.



**Figure 1** Distribution of cadmium, copper, lead and zinc in sequential fractions of the (a) tailings sample (4578), and (b) soil sample (4590). Legend: CC – carbonate fraction, FEMN – reducible fraction (iron and manganese oxides, hydroxides and oxyhydroxides), OR – oxidisable fraction (organic matter/sulphides), RES – residual fraction.

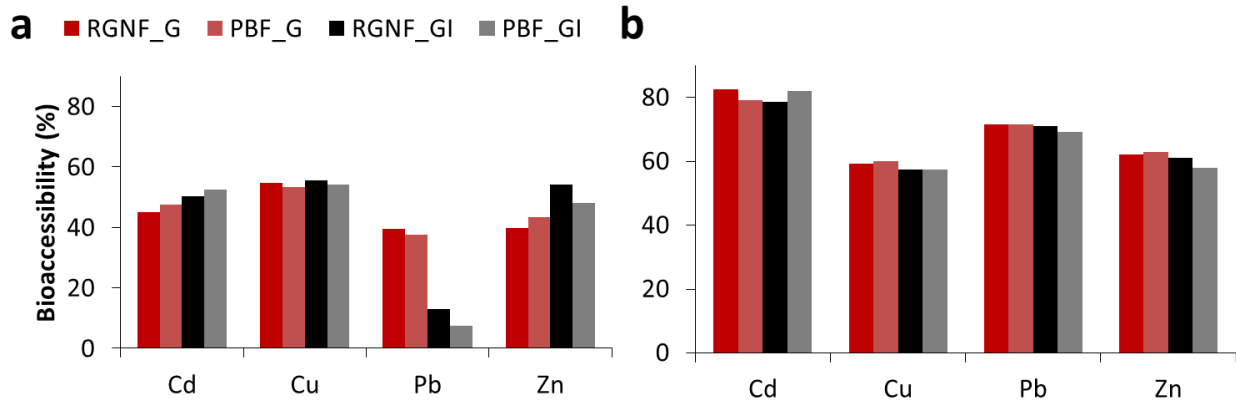
To assess the possible environmental risk posed by samples, they were classified according to the Risk Assessment Code (RAC) introduced by **Perin et al. (1985)**. It gives an indication of the available fraction of the elements by taking into account the percentage of element concentrations present in the exchangeable and carbonate fractions (< 1% no risk; 1 – 10% low risk, 11 – 30% medium risk, 31 – 50% serious risk and > 50% high risk). The tailings pose a medium risk to the environment in regards to all measured elements, while the risk of the soil sample is low for Cu, medium for Pb and serious for Cd and Zn.

### 3.1. Bioaccessibility of Cd, Cu, Pb and Zn in analysed samples

Bioaccessible concentrations (mg/kg) of Cd, Cu, Pb and Zn measured in the samples after digestion in simulated gastric and intestinal fluids, as part of the orientation study simultaneously carried out at RGNF and PBF, are given in **Table 5**. For each digestive phase, there is a relatively good correlation between concentrations obtained after digestion of samples in different labs, under different conditions. This is also obvious from **Figure 2**, which shows relative bioaccessibilities (%) of measured potentially toxic elements in the samples. The concentration of lead in the gastrointestinal phase of the tailings sample is significantly lower than in the gastric phase, possibly due to poor sample homogeneity. Even so, the values obtained in different labs differ only slightly. Hence, the reported results show that it is likely that differences in the experimental design of the tests i.e. agitation procedure and temperature conditions, do not cause significant variation in the bioaccessibility values of the measured elements.

**Table 5** Values of bioaccessible concentrations (mg/kg) of metals in the gastric (G) and gastrointestinal (GI) phases for the analysed samples measured at the *Faculty of Mining, Geology and Petroleum Engineering* (RGNF) and at the *Faculty of Food Technology and Biotechnology* (PBF).

Element	Gastric phase			
	(4578) tailings		(4590) soil	
	<i>RGNF</i>	<i>PBF</i>	<i>RGNF</i>	<i>PBF</i>
<b>Cd</b>	130	138	19	18
<b>Cu</b>	83	82	15	15
<b>Pb</b>	4,205	4,001	857	860
<b>Zn</b>	17,447	18,962	3,678	3,730
Element	Gastro-intestinal phase			
	<i>RGNF</i>	<i>PBF</i>	<i>RGNF</i>	<i>PBF</i>
<b>Cd</b>	146	152	18	19
<b>Cu</b>	85	83	14	14
<b>Pb</b>	1,368	790	854	830
<b>Zn</b>	23,666	21,022	3,620	3,433



**Figure 2** Relative bioaccessibility (%) of potentially toxic elements (PTEs) in the (a) tailings sample (4578), and (b) soil sample (4590). Legend: RGNF – measured at the Faculty of Mining, Geology and Petroleum Engineering, PBF – measured at the Faculty of Food Technology and Biotechnology, G – gastric phase, GI – gastrointestinal phase.

The acidic environment of the stomach compartment causes metals to mobilize from soil, which usually causes high bioaccessibility values in this phase. However, it can be observed that values in the intestinal phase for the tailings sample are higher compared to the gastric phase, except for Pb. This can be explained with more neutral pH levels of the intestinal compartment, where the metals can form new complexes. Furthermore, the pH values of the compartments are not fixed so they are dependent on the matrix of ingestion i.e. tailings/soil. The comparison of results of these two experiments enabled the decision to perform bioaccessibility determination at RGNF. The bioaccessibility test was repeated on samples as described previously and they were characterised in regards of their potential health risk as follows.

To estimate the potential risk of PTEs on humans, the Maximum Permissible Risk (MPR) values according to **Baars et al. (2001)** were quantified, herein expressed as tolerable daily intake (TDI). TDI is defined as the estimated amount of the chemical that humans can ingest daily during their entire lifetime without resultant adverse health effects. Values for the elements analysed in this study are as follows: Cd – 0.5 g/kg tt/day; Cu – 140 µg/kg tt/day; Pb – 3.6 µg/kg tt/day; Zn – 500 µg/kg tt/day; furthermore recalculated for body weight of 5 kg, 10 kg, 20 kg, 30 kg and 40 kg (see **Table 6**). An impact of PTEs is calculated based on the assumption that a child ingests 100 mg of soil per day by frequent hand to mouth activity, as suggested by **Van Wijnen et al. (1990)**. Calculated values (see **Table 7**) were compared to the maximum daily intake. Health risk assessment of potentially toxic elements showed that the elements of greatest concern in the tailings sample are lead and cadmium, exceeding the maximum allowable daily intake for children weighing up to 40 kg and up to 10 kg, respectively. The soil sample has a high bioaccessible concentration of lead which could pose a risk for children weighing up to 10 kg.

**Table 6** Tolerable daily intake (TDI) (µg/day) for different body weights

	TDI			
	<i>Cd</i>	<i>Cu</i>	<i>Pb</i>	<i>Zn</i>
<b>5 kg</b>	2,5	700	18	2,500
<b>10 kg</b>	5	1,400	36	5,000
<b>20 kg</b>	10	2,800	72	10,000
<b>30 kg</b>	15	4,200	108	15000
<b>40 kg</b>	20	5,600	144	20,000

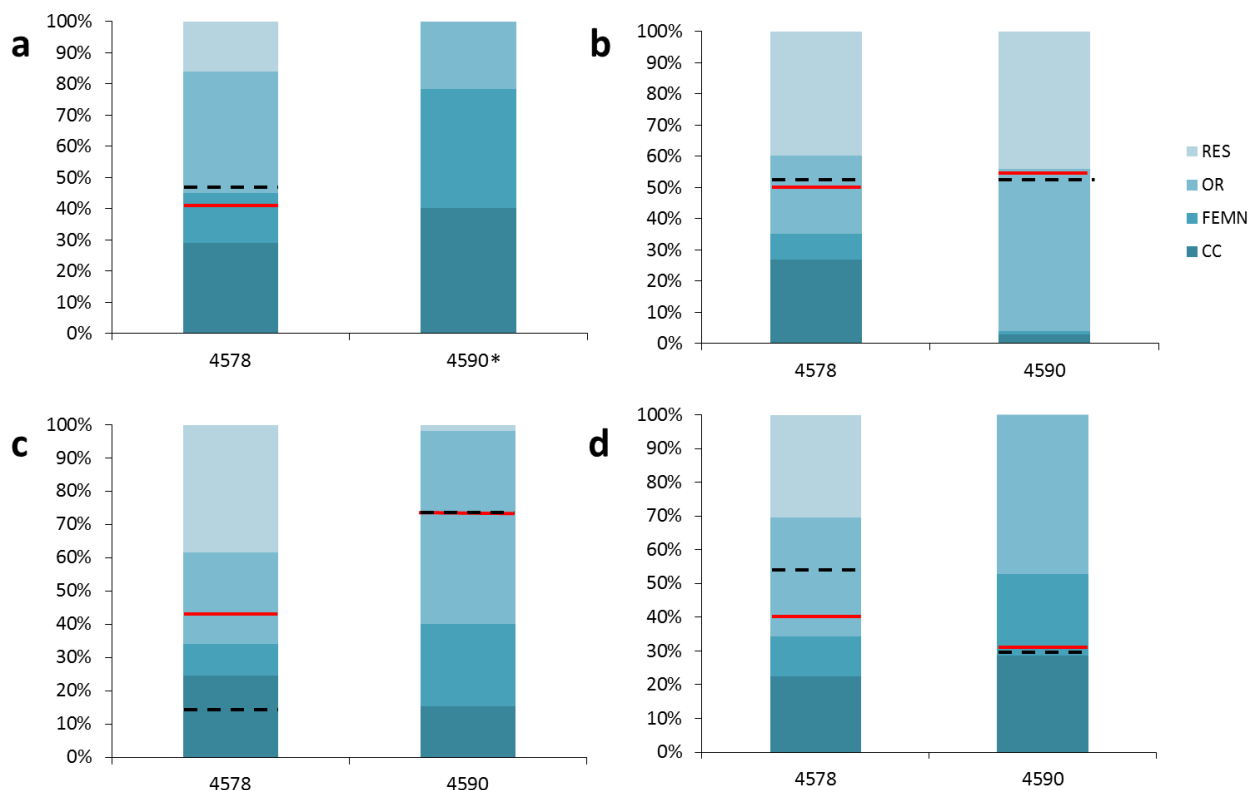


**Table 7** Concentration of bioaccessible fraction of PTEs in 100 mg of soil/tailings

Sample	Gastric fluid ( $\mu\text{g}/100 \text{ mg}$ )			
	<i>Cd</i>	<i>Cu</i>	<i>Pb</i>	<i>Zn</i>
(4578) tailings	7.6	6.7	265	1,715
(4590) soil	1.5	0.9	55	264
	Gastrointestinal fluid ( $\mu\text{g}/100 \text{ mg}$ )			
	<i>Cd</i>	<i>Cu</i>	<i>Pb</i>	<i>Zn</i>
(4578) tailings	7.7	7.3	30	1,369
(459) soil	1.3	0.9	13	139

### 3.2. Impact of binding sites of metals on bioaccessibility

The bioaccessibility of measured potentially toxic elements in samples was plotted in relation to the sum of their concentrations in soil/tailings fractions, obtained by sequential extraction (see **Figure 3**). It must be noted that the concentration of cadmium extracted by simulated body fluids from the soil sample (4590) was higher than its concentration extracted by sequential extraction, and therefore the percentage of bioaccessible Cd was calculated as > 100% and lines excluded from the graph. It can be observed that the bioaccessible concentration of all measured elements is higher than their concentration in a carbonate fraction of samples. This leads to the conclusion that the risk assessment code (RAC) cannot be used to assess the potential risk of elements to human health, as it gives lower values compared to concentrations which are extractable in simulated body fluids. Furthermore, there is no obvious correlation between the concentration extracted by gastric fluids and the concentration in soil/tailings sequential fractions of samples. Hence, it appears that the use of the sequential extraction analysis for assessing the risk of soil/sediment ingestion on health would be a difficulty.



**Figure 3** Bioaccessibility of potentially toxic elements (a) cadmium, (b) copper, (c) lead, and (d) zinc, following extraction in body fluids (BF) for a tailings (4578) and soil (4590) sample, in relation to the sum of their concentrations in soil fractions (CC – carbonate fraction, FEMN – reducible fraction (iron and manganese oxides, hydroxides and oxyhydroxides), OR – oxidisable fraction (organic matter/sulphides), RES – residual fraction), obtained by sequential extraction (SEA). Red line – bioaccessibility in the gastric phase (G), black dashed line – bioaccessibility in the gastrointestinal phase (GI). \*Concentration of Cd extracted by BF from the soil sample was higher than its concentration extracted by SEA, therefore the % of bioaccessible Cd is > 100% and lines are excluded from the graph.

#### 4. Conclusion

In conclusion, the comparison of these two experiments enabled the decision to perform bioaccessibility determination at RGNF. As a result of this study, an efficient and relatively inexpensive method for determining the bioaccessibility was set up at the Faculty of Mining, Geology and Petroleum Engineering, which makes this kind of test more accessible and enables a new approach to risk assessment studies.

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## 6. Acknowledgments

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## Sažetak

### Procjena utjecaja ingestije zagađenoga tla/sedimenta na zdravlje

Potencijalno toksični elementi (PTE) prijetnja su ljudskomu zdravlju jer lako mogu ući u ljudski organizam preko zagađenoga tla/sedimenta. Kako bi se procijenila biopristupačnost i utjecaj na ljudsko zdravlje, ključno je mjerenje oralne biopristupačnosti zagađivala. Biopristupačnost je definirana kao udio u tijelo unesene toksične tvari iz nekoga materijala koja se izlužila u tjelesnim tekućinama i dostupna je za apsorpciju u organizmu. U tu svrhu mogu se koristiti različiti laboratorijski *in vitro* testovi koji simuliraju ljudski probavni sustav. Oni omogućavaju brzo i povoljno određivanje biopristupačnosti te su odgovarajuća alternativa testiranju na životinjama. S ciljem uvođenja metode za analizu biopristupačnosti zagađivala u uzorcima tla u Laboratoriju za analizu geoloških materijala u Zavodu za mineralogiju, petrologiju i mineralne sirovine (Rudarsko-geološko-naftni fakultet, Sveučilište u Zagrebu – RGNF), uzimajući u obzir raspoloživu laboratorijsku opremu, izvedeno je orijentacijsko istraživanje u suradnji sa Zavodom za biokemijsko inženjerstvo (Prehrambeno-biotehnološki fakultet, Sveučilište u Zagrebu – PBF). Izluživanje dvaju različitih uzoraka u sintetičkim tekućinama (želučanoj i crijevnoj tekućini) izvedeno je istovremeno u obama laboratorijima pod različitim uvjetima ekstrakcije prema mogućnostima laboratorija. Obavljena je i detaljna mineraloška i kemijska karakterizacija uzoraka. Mineralni sastav uzoraka određen je rendgenskom difrakcijom. Osim izluživanja u tjelesnim tekućinama uzorci su otapani u zlatotopci kako bi se odredila ukupna koncentracija potencijalno toksičnih elementa, dok je za identifikaciju glavnih mjesta vezanja metala u tragovima u različitim mineraloškim frakcijama uzorka korištena sekvencijska ekstrakcijska analiza. Elementi u tragovima analizirani su u ekstraktima metodom plamene atomske apsorpcijske spektrometrije. Usporedba dvaju eksperimenata izvedenih sa sintetičkim tekućinama pokazala je da postoje neznatna odstupanja između koncentracija dobivenih nakon izluživanja uzoraka u različitim laboratorijima, pod različitim uvjetima. Kao rezultat ovoga istraživanja na Rudarsko-geološko-naftnome fakultetu uvedena je efikasna i relativno jeftina metoda za određivanje biopristupačnosti, koja čini ovu vrstu testa pristupačnijom i omogućuje novi pristup u studijama rizika za okoliš i zdravlje od zagađivala.

### Ključne riječi

biopristupačnost, ingestija, procjena utjecaja na zdravlje, potencijalno toksični elementi, zagađeno tlo

