

Original article

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## Bioaccessibility of lead in sand intended for playground sandboxes in Slovenia: a preliminary study

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Slovene press has recently expressed concern for the health of children who ingest playground sand contaminated with lead. However, current assessments may be overestimating the health risk, as they assume that human body absorbs the whole amount of a contaminant. The aim of our study was to see how much lead in sand intended for playground sandboxes in Slovenia really is absorbed and what health risk it may present. To do that, we ran bioaccessibility tests to simulate metabolism and see how digestion affects lead levels. Our results show low bioaccessibility of lead from sand (0.01-36.84 %). Taking into account lead bioaccessibility, we ran several scenarios of sand ingestion in children and established that in general the risk is negligible, except for pica behaviour with extremely high (20 g) ingestion of sand with highly mobilised lead (2.69 µg g<sup>-1</sup>). Further research should assess real-life exposure to lead from playground sand in children and perhaps update these preliminary bioaccessibility data.

KEY WORDS: *exposure scenarios; intake; in vitro model; risk assessment; toxic metal*

Children playing in sandboxes often expose themselves to lead in the sand (1-8). In small children this exposure is the highest through unintentional or intentional ingestion (the so called pica behaviour) (9). On average, children can ingest between 50 and 200 mg of sand per day. In case of pica behaviour, ingestion soars to between 1 and 20 g per day (10-12).

However, not all of the ingested toxic metal content will be absorbed by the human body. Absorption will depend on age, stomach content, chemical compound containing the metal, and its bioaccessibility (13). In simple terms, *bioaccessibility* is the absorption potential - that fraction of a substance that is dissociated from soil (or sand for that matter) by gastrointestinal juices and available for absorption. An even more accurate parameter of exposure is *bioavailability* or the potential of a substance to reach circulation. However, determining it requires complex, expensive, and time-consuming *in vivo* analysis, which raises practical and ethical issues when it comes to humans and animals. Bioaccessibility, in turn, is a much simpler *in vitro* method generally simulating gastric and small intestinal digestion, which, depending on the specific method used, correlates with bioavailability (14).

The primary aim of our study was to analyse lead concentrations in sand intended for playground sandboxes in Slovenia and to assess its bioaccessibility. Our secondary aim was to simulate different scenarios of oral sand intake

in children and compare our exposure assessment to current tolerable intake values.

### METHODS

First we identified the producers of sand intended for playgrounds; then we assessed lead bioaccessibility in sand samples by simulating metabolism *in vitro* and analysing the effects of digestion on absorption; and finally we included our findings in different ingestion scenarios to assess the risk of exposure.

#### *Producers of sand intended for sandboxes*

In 2014, the Health Inspectorate of the Republic of Slovenia analysed sand samples from 75 kindergarten sandboxes as part of the regular monitoring across the nation. From kindergarten books the Inspectorate identified five suppliers of sand of different types (three limestone and two quartz).

Our bioaccessibility and risk assessment were based on 26 sand samples obtained from these five producers, all providing sand originating in Slovenia.

#### *Sample preparation and data analysis*

To assess the bioaccessibility of lead in sand samples, we simulated sand metabolism *in vitro* to see how digestion affects lead absorption. The experiment followed the standards ISO/TS 17924 (15) and DIN 19738 (16) under realistic worst-case conditions. It was based on the

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properties of the human digestion process, the contaminants, and geochemistry and mineralogy of soils. At first 2 g of sampled sand were treated with synthetic saliva (50 mg L<sup>-1</sup> NaCl, 15 mg L<sup>-1</sup> NaSCN, 55 mg L<sup>-1</sup> Na<sub>2</sub>SO<sub>4</sub>, 15 mg L<sup>-1</sup> NaHCO<sub>3</sub>, 45 mg L<sup>-1</sup> KCl, 60 mg L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 15 mg L<sup>-1</sup> CaCl<sub>2</sub>·2H<sub>2</sub>O, 75 mg L<sup>-1</sup> mucin, 25 mg L<sup>-1</sup> α-amylase, 15 mg L<sup>-1</sup> urea, and 1 mg L<sup>-1</sup> uric acid) for 30 minutes. In the next step, synthetic stomach juices were added to suspension (290 mg L<sup>-1</sup> NaCl, 70 mg L<sup>-1</sup> KCl, 27 mg L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 100 mg L<sup>-1</sup> pepsin, 300 mg L<sup>-1</sup> mucin and HCl for pH correction at 2.0) and mixed with an agitator at 4,500 g for 2 hours. Whole milk powder (10 g L<sup>-1</sup>) was added to stomach juices to simulate the influence of food on the mobilisation of lead. After that, we added synthetic intestinal juices (30 mg L<sup>-1</sup> KCl, 50 mg L<sup>-1</sup> CaCl<sub>2</sub>·2H<sub>2</sub>O, 20 mg L<sup>-1</sup> MgCl<sub>2</sub>·6H<sub>2</sub>O, 100 mg L<sup>-1</sup> NaHCO<sub>3</sub>, 30 mg L<sup>-1</sup> trypsin, 900 mg L<sup>-1</sup> pancreatin, 900 mg L<sup>-1</sup> lyophilized bile, and 30 mg L<sup>-1</sup> urea) and set the pH to 7.5 using a phosphate buffer. Digestion continued for another 6 hours. The temperature was controlled by means of a water bath at 37 °C.

With digestion completed, the samples were centrifuged at 7000 g for 10 minutes, after which the supernatant was decanted. The residual pellet was stirred in 30 mL of distilled water for 20 min, centrifuged again, and the supernatant decanted. The decanted solutions were combined for analysis. Figure 1 shows a detailed flow chart of the experiment.

We also wanted to see if particle size had any influence on the amount of lead, so we analysed sand particles of five different sizes (less than 100 µm; 100-200 µm; 200-315 µm; 315-500 µm; 500-630 µm).

Lead concentration was analysed with the inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7500a (Agilent Technologies Inc., Palo Alto, CA, USA) before and after digestion. This method allows direct measurements in the supernatant, without any further preparation and manipulation. In sampling, analysis, traceability of results, and determination of the boundaries we followed the DIN 32645 (17) and ISO 17025 (18) standards. Each series of digestion experiments was controlled with a digestion tube without soil to exclude the presence of lead in the test solution, and none of the control measurements exceeded the detection limit for lead.

The amount of mobilised lead ( $w_{mob}$ ) was calculated as follows:

$$w_{i,mob} = \frac{\rho \cdot V}{m} \quad (1)$$

where  $w_{i,mob}$  is the mass fraction of mobilised lead (µg g<sup>-1</sup>),  $\rho$  is the concentration of lead in test solution (µg L<sup>-1</sup>),  $V$  is the volume of test solution (L), and  $m$  is the mass of soil sample (g).

Bioaccessibility of lead was calculated using following equation:

$$R_i = \frac{w_{i,mob} \cdot 100}{w_{i,total}} \quad (2)$$

where  $R_i$  is bioaccessibility (%),  $w_{i,mob}$  is the mass fraction of mobilised lead (µg g<sup>-1</sup>), and  $w_{i,total}$  is the total amount of lead in the original sample before digestion (µg g<sup>-1</sup>).

#### Simulation of sand ingestion

Having obtained the results for bioaccessibility, we simulated several ingestion scenarios in which children ingest between 50 mg and 200 mg of sand per day (average ingestion) and between 1 and 20 g (in pica behaviour). Then we compared the simulation findings with the current provisional tolerable lead intake (36 µg per day) for children weighing 10 kg (19).

## RESULTS AND DISCUSSION

#### Lead concentrations in sand and bioaccessibility

Table 1 shows lead levels before digestion and those obtained with the gastrointestinal simulation model for different particle sizes and sand producers (Table 1). We found large variability in lead concentrations (4.90-19.20 µg L<sup>-1</sup>) between the five sand suppliers for kindergartens. Our results also indicate that the amount of lead is not related to particle size. One of the reasons may be that the sand particles we analysed were relatively coarse, as several studies with fine particles reported results contrary to ours: that heavy metal concentration in soil is related to particle size (20-22).

Our bioaccessibility test with the ingestion model has shown that the relation between the amount of mobilised lead and lead levels in soil is not linear. Take the outstanding example of sand samples from Limestone sand producer 2, whose mass fraction was as high as 10,685 µg g<sup>-1</sup> (10.69 mg g<sup>-1</sup>), yet only a small amount of lead (2.69 µg g<sup>-1</sup> or 0.03 %) was mobilised. Limited bioaccessibility of metals may be related to their solubility (23). This suggests that the health risks from poorly soluble soil contaminants are in many cases overestimated because bioaccessibility has not been taken into account.

Several *in vitro* gastrointestinal extraction protocols have been used to evaluate the bioaccessibility of toxic elements in soils. Oomen et al. (24) analysed the bioaccessibility of lead under controlled laboratory conditions, where artificial standard soil OECD medium was contaminated with 530 mg kg<sup>-1</sup> of lead, and found that only 23 % of lead was bioaccessible. Our results have shown a wider range of lead bioaccessibility from sand (0.01-36.84 %), but all these findings confirm that it is the amount of mobilised contaminant that counts in any risk assessment.

**Table 1** Results of the experiment using gastrointestinal simulation model for lead

Source of sand	Pb in original sample ( $w_{i,total}$ ) [ $\mu\text{g g}^{-1}$ ]	Fraction ( $\mu\text{m}$ )	Concentration of Pb in the test solution ( $\rho$ ) [ $\mu\text{g L}^{-1}$ ]	Amount of mobilised Pb ( $w_{i,mob}$ ) [ $\mu\text{g g}^{-1}$ ]	Bioaccessibility ( $R_i$ ) (%)
Limestone sand producer 1	4.10	100-200	7.70	1.44	35.21
		200-315	7.30	0.78	19.14
		315-500	6.60	0.87	21.33
		500-630	10.60	1.51	36.84
		630-1000	11.00	1.29	31.52
Limestone sand producer 2	10,685	100	5.40	0.90	0.01
		100-200	19.20	2.69	0.03
		200-315	19.20	2.54	0.02
		315-500	4.90	0.71	0.01
		500-630	18.10	2.35	0.02
Limestone sand producer 3	12.00	630-1000	8.00	1.06	0.01
		100	4.90	0.87	7.25
		100-200	4.90	0.66	5.51
		200-315	4.90	0.59	4.88
		315-500	4.90	0.65	5.41
Quartz sand producer 1	2.90	500-630	4.90	0.72	6.02
		100	4.90	0.56	19.43
		100-200	4.90	0.55	19.01
		200-315	4.90	0.60	20.70
Quartz sand producer 2	13.20	500-630	4.90	0.69	23.66
		100	8.00	1.34	10.15
		100-200	4.90	0.65	4.92
		200-315	4.90	0.70	5.29
		315-500	4.90	0.66	5.01
		500-630	4.90	0.66	5.01
		630-1000	4.90	0.64	4.83

$w_{i,total}$  is the total amount of lead in the original sample, based on pooled measurement before digestion ( $\mu\text{g g}^{-1}$ )

#### Simulation of sand ingestion taking into the account bioaccessibility of lead

Table 2 shows lead intake scenarios based on bioaccessibility results (amount of mobilised lead) for limestone and quartz sand, taking into account current tolerable intake. Our findings suggest that sand ingestion in normal behaviour would not lead to increased risk of exposure to lead, except in the worst case scenario with highest mobilised Pb levels and pica intake of 20 g of sand per day.

## CONCLUSIONS

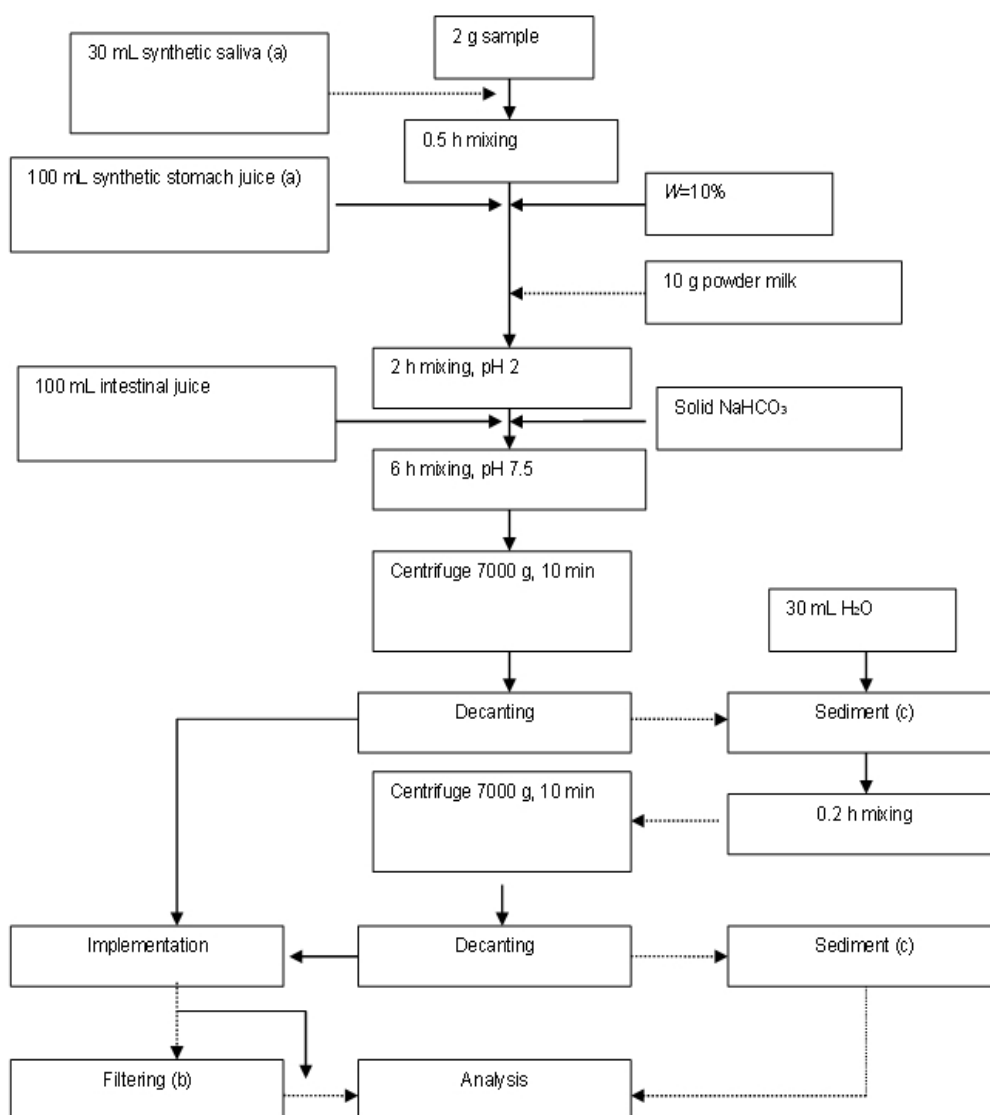
Toxic metal pollution of playgrounds has received wide public interest, and recent studies suggest that pollution and children's exposure to metals in these areas are of high concern. Our study is important because it shows that current assessments of risk from exposure to lead in children playing with sand, even those with pica disorder, may be overblown because they do not take into account bioaccessibility. The results of our study suggest that

bioaccessibility is more related to lead concentration in test solution ( $\rho$ ) than to its particle size. Moreover, only a small fraction of lead dissolves in digestive juices. Exposure of children via sand ingestion is therefore negligible in most scenarios.

There are several limitations to this preliminary study. Firstly, we did not do any statistical analysis due to too few samples. Secondly, our measurements start from the assumption that children are exposed to lead in sand as delivered by producers, which may exclude input from playground environment such as precipitation, leaching, etc. These can affect lead's bioaccessibility. Our future research will therefore address this shortcoming, and we will try to establish lead bioaccessibility in real-life conditions.

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**Figure 1** Flow chart of the experiment conducted to assess the bioaccessibility of lead in sand samples in simulated digestion conditions following the DIN ISO 11464 norm

a) The volume of synthetic stomach juice is reduced after adding synthetic saliva to 70 mL. Therefore, adding 30 mL of water is necessary; b) if applicable; c) measures for quality

**Table 2** Simulation of lead intake based on bioaccessibility of limestone and quartz sand and predicted sand intake for children

Scenarios	Amount of mobilised Pb ( $w_{i,mob}$ ) $\mu\text{g g}^{-1}$	PTI <sup>a</sup> $\mu\text{g day}^{-1}$	Ingestion of sand in children <sup>b</sup>				Ingestion of sand in children with pica behaviour <sup>b</sup>			
			50 mg day <sup>-1</sup>		200 mg day <sup>-1</sup>		1000 mg day <sup>-1</sup>		20,000 mg day <sup>-1</sup>	
			Pb dose $\mu\text{g day}^{-1}$	% of PTI	Pb dose $\mu\text{g day}^{-1}$	% of PTI	Pb dose $\mu\text{g day}^{-1}$	% of PTI	Pb dose $\mu\text{g day}^{-1}$	% of PTI
<b>Limestone sand</b>										
Lowest	0.59	36	0.03	0.083	0.12	0.3	0.6	1.6	11.7	32.7
Highest	2.69	36	0.13	0.37	0.54	1.5	2.7	7.5	53.8	<b>149.5</b>
<b>Quartz sand</b>										
Lowest	0.55	36	0.03	0.083	0.12	0.3	0.6	1.6	11.7	32.5
Highest	1.34	36	0.07	0.194	0.27	0.7	1.3	3.6	26.8	74.4

a - Provisional tolerable intake for 10-kg children. This is the limit above which adverse health effects are expected (19)

b - Young children have an oral intake between 50 and 200 mg day<sup>-1</sup>. About 5% of all children display pica behaviour; these children have an oral intake of soil between 1 and 20 g day<sup>-1</sup> (10, 12)

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### **Biolška dostopnost svınca v mivki, ki je namenjen otroškim peskovnikom v Sloveniji**

V zadnjem obdobju so se v Sloveniji pojavili številni pomisleki o varnosti mivke, ki je namenjena za otroške peskovnike. Ocena tveganja otrok, ki so izpostavljeni svincu temelji na predpostavki, da je celotna količina toksične kovine biološko dostopna. Zato je bil namen raziskave analizirati biološko dostopnost svınca v mivki, ki je namenjen za peskovnike otroških igrišč. Z simulacijo prebavnega trakta smo analizirali vpliv prebavnih sokov za izplavljanje svınca iz mivke. Rezultati kažejo na majhno biodostopnost svınca v mivki (0,01–36,84 %) slovenskih proizvajalcev. Na podlagi biološke dostopnosti smo simulirali različne scenarije vnosa in ugotovili, da je tveganje zanemarljivo, razen v primeru pica sindroma (zaužitje 20 g mivke) ter ob predpostavki največje dokazane biološke dostopnosti (2,69  $\mu\text{g g}^{-1}$ ). Raziskave v prihodnosti bodo vključevale realno izpostavljenost otrok svincu iz peskovnikov in bodo morda nadgradile preliminarno raziskavo.

KLJUČNE BESEDE: *in vitro model; ocena tveganja; scenariji izpostavljenosti; toksična kovina; vnos*