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RESEARCH ARTICLE

In vitro gastro-intestinal method for the assessment of heavy metal bioavailability in contaminated soils

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Abstract
Introduction Balya and its associated villages which is a town of the Balikesir region of Turkey have very rich zinc, lead, and manganese mines. These mines have been operating since the thirteenth century and now there is heavy metal contamination in both the soil and natural waters in these areas.
Materials and methods Soils were collected from Sarı su, Enverpaşa, and Hastanetepe which are in Balya town and Kadıköy, Kaşıkcı, Müstecap, Patlak, Çakallar, and Bengiler which are the villages near Balya and the mine areas. Nine trace analytes (As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, and Zn) were determined using an acid extraction procedure as well as from PBET in vitro gastro-intestinal experiments using ICP-OES.
Results and discussion The results showed that high As, Ba, Pb, Zn, and Cd concentrations were found in these soils. The amounts ingested by pica behavior of children at the rate of 10 $g\,day^{-1}$ are calculated using the results of in vitro intestinal bio-accessibility experiments.
Conclusion The results showed that the amount of As, Pb, Ba, and Cd levels ingested by pica behavior are substantially higher than tolerable daily intake values in most of the soils. When normal ingestion is taken into account, the tolerable daily limits are only exceeded for one element (Pb) and even then, only at two sites.

Keywords Risk assessment · Soil · Bio-accessibility heavy metals · Children

1 Introduction

The major sources of heavy metals in the environment are metal mining and smelting activities. These activities can result in considerable soil contamination (Fanfani et al. 1997; Sutherland and Tack 2000). Soils containing metals and other contaminants pose a particular hazard to children because of activities that involve frequent hand-to-mouth behavior and the subsequent ingestion of the soil (Hamel et al. 1998; Schroder et al. 2003). The daily amount of soil ingested has been estimated to be in the range of 50–200 $mg\,day^{-1}$ (Van Wijnen et al. 1990). When assessing risks for children who are not expected to exhibit soil-pica or geophagy behavior, the recommended central tendency soil + dust ingestion estimate is 100 mg/day for children aged 1 to <6 years by the US EPA (2008). Deliberate ingestion of large amounts of non-nutritive substances is termed pica and is classified as an eating disorder when the ingestion continues for a period of at least 1 month and the person is above 2 years of age. Pica behavior includes the ingestion of several non-food materials and when soil is ingested in a pica manner this is termed geophagy. As stated above, ingestion of non-nutritive substances via hand-to-mouth transfer is common in children between 18 months to 2 years and pica behavior is not considered pathological at that age (Ellis and Schnoes, 2009). When assessing risks for children who may exhibit soil-pica behavior, or a group of children that includes individual children who may exhibit soil-pica behavior, the soil-pica ingestion estimate for children

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up to age 14 ranges from 400 to 41,000 mg/day (US EPA 2008). Calabrese et al. (1989) presented a study in which one child displayed soil-pica behavior, where the soil intake ranged from 10 to 14 $g day^{-1}$ during the second week of observation. In the developed world, pica behavior is the most common eating disorder seen in adult individuals with developmental disabilities (Ellis and Schnoes 2009).

Unfortunately, children have the ability to absorb higher proportions of metals through the digestive tract than adults. Once they have entered the systemic circulation system, the potentially toxic analytes may make the children subject to greater adverse health effects (Miller et al. 1981; Calabrese and Stanek 1995).

Bioavailability is the amount of a contaminant that is absorbed into the blood stream and redistributed around the body (Ruby et al. 1999). It can only be determined with costly and ethically controversial animal testing. Therefore, the concept of bio-accessibility, i.e., the amount of a contaminant that is liberated into an aqueous form within the gastro-intestinal tract and is thus available for uptake in the bloodstream, has been introduced (Ruby et al. 1999). Bio-accessibility tests are used as an approximation for bioavailability and take the form of in vitro gastro-intestinal simulations, involving temperature, agitation, pH, and enzyme/chemical conditions that are similar to those found in the human body during digestion (Ruby et al. 1993; Ruby et al. 1996; Rodriguez et al. 1999; Oomen et al. 2002). Several in vitro approaches have been developed which have attempted to mimic the effects of the human digestion process. They are commonly described in the scientific literature under the specific name of the physiologically based extraction test (PBET) or more generally, a simulated in vitro gastro-intestinal extraction procedure. All of the PBET models involve simulated gastric extraction with pepsin and with a mixture of pancreatin, amylase, and bile salts in the intestinal stage. Researchers have shown that the results from the in vitro studies can be correlated to bioavailability determined by in vivo studies (Ruby et al. 1996). The approaches can be simple, relatively rapid, and low in cost and may provide insights not achievable in whole animal studies (Miller et al. 1981). Although the small intestine is the main site within the gastro-intestinal tract where food products including fats, carbohydrates, proteins, calcium, iron, vitamins, water, and electrolytes are adsorbed; the hydrochloric acid environment of the stomach is important since it will allow dissolution of labile mineral oxides, sulfides, and carbonates, thereby aiding dissolution of the foodstuffs (Dean 2007). The PBET protocol followed in this work utilizes both the stomach phase dissolution/digestion and the comparatively less aggressive conditions found in the intestines to mimic the gastro-intestinal tract as a whole.

The region around Balya (a town of the Balikesir region of Turkey) is very rich in zinc, lead, and manganese ores, and has a long-standing mining history. These mines were operating since the thirteenth century and now there is heavy metal contamination in the soil and natural waters in the area. The aim of this work is to identify the bioaccessible heavy metal amounts using an in vitro bioaccessible gastric and intestinal digestion method if the soils are eaten by children. The ingested heavy metal amounts were estimated from the assumptions of a soil ingestion rate of 10 $g day^{-1}$ for the children having pica behavior or deliberate ingestion and 100 $mg day^{-1}$ for the children who are not expected to exhibit pica behavior for different soil samples calculated from values taken by in vitro intestinal bioaccessible results. These results were compared with tolerable daily intake (TDI) values that were presented together with the background exposure (BE) (Baars et al. 2001) in micrograms per day for a child weighing 10 kg.

2 Experimental

2.1 Apparatus

An ICP-OES instrument (Varian 725-ES, Melbourne, Australia) was used for the determination of As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, and Zn in the extracts. Operating conditions for the ICP-OES instrument were: forward power 1.4 kW, coolant gas flow rate 14 $L min^{-1}$, auxiliary gas flow rate 1.5 $L min^{-1}$, and nebulizer gas flow rate 0.68 $L min^{-1}$. Analytes were determined at the following wavelengths (nm): As 188.980; Ba 455.403; Ca 317.933 and 422.673; Cd 214.439; Cr 267.716; Cu 327.395; Fe 239.563; Mg 279.800; 285.213; Mn 257.610; Ni 231.604; Pb 220.353; and Zn 213.857. A centrifuge (Thermo IEC) was used for the complete separation of the extracts. A Hanna instruments model 221 pH-meter was used to monitor pH values of the solutions. A controlled temperature shaking water bath (Nuve ST model 402) was employed for the PBET experiment.

2.2 Reagents and solutions

All chemicals were of analytical grade. All solutions were prepared using analytical-reagent grade deionized water. All glassware and PTFE containers were previously soaked in 10% (*v*/*v*) nitric acid for at least 24 h and then rinsed with deionized water.

Calibration solutions for metals were reagent-matched (i.e., made up with the corresponding extracting solutions) and were prepared by serial dilution of 1,000 mg/L Merck standard solutions of the appropriate elements as nitrate salts (except for As which was as dissolved As_2O_3). A certified reference material (NIST SRM 2711 Montana soil) was used to verify the accuracy of the results.

2.3 Procedures

2.3.1 Sample collection and preparation

Soil samples were collected from Sarı su, Enverpaşa, and Hastanetepe which are in Balya town and Kadıköy, Kaşıkcı, Müstecap, Patlak, Çakallar, and Bengiler that are the villages near to Balya and the mine areas (Fig. 1). The surface soil samples were collected from depths not exceeding 3 cm from these areas. Surface soils were especially chosen because these are where the children play and are therefore most likely to be ingested. All samples were air-dried for 2–3 days at a temperature of 25°C and sieved to collect the particle size fraction <200 μm that is available for incidental ingestion. This particle size was chosen as a compromise between the very fine material that has a much greater surface area and hence may have greater extraction efficiency (but is present at only a relatively small proportion of the bulk soil) and the bulk soil itself that will contain much larger particles and small stones. The fraction used would include the portion that could become airborne dusts that may settle on un-washed fruit/vegetables and also within the home. Soils were thoroughly homogenized/ mixed prior to use and stored in secured, airtight polyethylene containers.

2.3.2 Aqua regia digestion

Pseudo-total metal content was determined by digestion with aqua regia (Zemberyova et al. 2006). Soil (0.5 g) was weighed into a pre-cleaned beaker, 0.5–1.0 mL of water was added to obtain a slurry, then 7.0 mL of 12.0 $molL^{-1}$ HCl followed by 2.3 mL of 15.8 $molL^{-1}$ HNO_3 were added drop by drop to reduce foaming. The reaction vessels were allowed to stand for 16 h (overnight) at room temperature for slow oxidation of the organic matter of the soil. The contents of each beaker were covered with a watch glass and the samples boiled gently on a laboratory hot-plate until digestion was complete. This process took approximately 2 h. After cooling the reaction vessel to room temperature, the digests were filtered through Whatman filter paper into pre-cleaned 25-mL volumetric flasks, the insoluble residue on the filter paper was washed with a 0.5 $molL^{-1}$ HNO_3 and the washings added to the volumetric flask. The samples were then diluted to volume using 0.5 $molL^{-1}$ HNO_3.

2.3.3 Procedure for physiologically based in vitro test

The physiological based in vitro test used in this study was a modified test proposed by Ruby et al. (1996). Glass reaction vessels were approximately 80% submerged in a temperature controlled shaking water bath maintained at body temperature (37°C). Anaerobic conditions were created by constantly diffusing nitrogen gas at 1 L/min through the solution, and the solution pH was monitored constantly and adjusted to the selected pH as necessary throughout the procedure. Constant mixing was performed

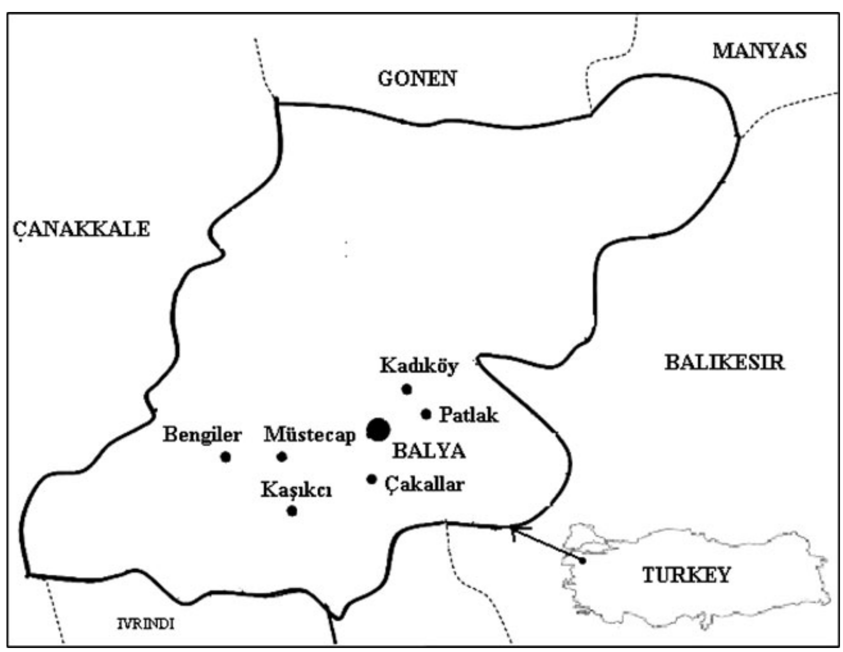

Fig. 1 Location of the Balya and its associated villages in Balikesir, Turkey

throughout the procedures to simulate gastric mixing using a temperature controlled sideways shaking water bath at a speed of approximately 100 rpm. The flow of nitrogen through the mixture would also have an additional mixing effect.

The gastric solution was prepared by dissolving 1.25 g porcine pepsin, 0.50 g sodium citrate, 0.50 g sodium malate, 420 µL lactic acid, and 500 µL of acetic acid in deionized water to a volume of 1 L. The pH was adjusted to 1.8 using concentrated HCl. Soil (0.5 g) was mixed with 50 mL of gastric solution in the reactor vessel. After 1 h, 5 mL of sample was collected using a syringe. The supernatant was filtered through a 0.45-µm nitrate fiber filter. The sample was preserved by adding concentrated HNO_3 (0.1 mL) and maintained under refrigeration until analysis. The sample solution volume removed from the sample was compensated for by adding an equal amount of original gastric solution.

After 1 h of gastric phase digestion, the gastric solution was modified by adjusting the pH to 7 with a saturated $NaHCO_3$ solution followed by the addition of 87.5 mg bile salts and 25 mg pancreatin to each reaction vessel. This mixture represented the intestinal digestion solution. After 4 h of digestion by the intestinal phase, 5 mL of sample was removed using a syringe. This 5-mL sub-sample was filtered through a 0.45-µm nitrate fiber filter and was preserved by adding concentrated HNO_3 (0.1 mL) and maintained under refrigeration until analysis. All in vitro tests were performed in triplicate for each soil sample.

2.3.4 Sample analysis

Concentrations of the analytes As, Cu, Zn, Pb, Mn, Ni, Cd, Cr, and Ba in each fraction were determined by ICP-OES. All results are the mean of the three replicates and are quoted on a dry weight basis.

For in vitro results, bioaccessible metal concentrations are calculated by dividing the metal ions' concentrations measured in the in vitro stomach phase or the in vitro intestinal phase solutions by the pseudo-total soil metal ion as described by the following equation (Intawongse and Dean 2008):

$$\text{In vitro bioaccessible metal ion, } \% = \frac{[\text{in vitro metal}]}{[\text{total metal}]} \times 100$$

3 Results and discussion

The "pseudo-total" metal results obtained using the aqua regia digestion of the certified material were in good agreement with certified values except for Ba and Cr (Table 1). The data in Table 1 are, in general, slightly lower than certified values (typically 85–95% of certified value). This is to be expected since an aqua regia digest was used rather than a full decomposition method (e.g., fusion or HF digest). The small proportion that was not extracted using aqua regia is unlikely be bio-available and hence the overall conclusions will be unaffected. The results do show, however, that the aqua regia extracts give a good "pseudo-total" value. Much of the Ba and Cr should be present in the crystalline structure of the soil and would therefore require a complete digestion, e.g., using HF treatment. The proportion of these analytes retained in the crystalline structure of the soil would therefore also not be bio-available. The good agreement for the majority of the analytes with certified values gives an indication that the analytical data is valid.

Sarı su, Enverpaşa, and Hastanetepe are in Balya town. Kadıköy, Kaşıkcı, Müstecap, Patlak, Çakallar, and Bengiler are the villages near to Balya and the mine areas. The pH values and percentage values of iron, calcium, and magnesium in these soil samples are given in Table 2. The pH of the soil is important for the evaluation of soil pollution. This is because there are different limit allowance values at pH values below and higher than 6 in the Turkish Environmental Agency Soil Pollution Allowance report by Turkish Environmental and Forestry Ministry to decide any possible soil pollution (Turkish Environmental and Forestry Ministry 2005). These values are lower in acidic soil in the range of pH 5 and 6. The pHs of all soil samples are between 6 and 7 (Table 2). The limit pollution values (µg/g) given by Turkish Environmental and Forestry Ministry for the soil that their pHs are higher than 6 are 20 for As; 200 for Ba; 3 for Cd; 100 for Cr; 140 for Cu; 75 for Ni; and 300 for Zn and Pb (Turkish Environmental and Forestry Ministry 2005). There is not any pollution value given for manganese. The manganese concentrations vary widely in soils ranging from <20 to >3,000 µg/g soil (Burhan Kaçar 2009). The

Table 1 The trace metal concentrations for NIST SRM 2711 Montana soil (n=3)

Metal ion	Aqua regia (µg/g)	Certified values (µg/g)
As	90.9±1.5	105±8
Cu	96.3±0.7	114±2
Zn	308.1±6.7	350.4±4.8
Pb	1,071.1±18.1	1,162±31
Mn	507.0±8.8	638±28
Ni	21.9±0.9	20.6±1.1
Cd	36.4±0.9	41.7±0.25
Cr	24.85±0.95	47
Ba	209.3±6.2	726±38

Table 2 The pH values and the percentage of Fe, Ca, and Mg in soils (mean and standard deviation; n=3)

	pH	% Fe	% Ca	% Mg
Sarı su	7.10	2.93±0.12	3.50±0.13	0.28±0.01
Hastanetepe	7.18	3.67±0.06	1.45±0.02	0.46±0.01
Enver Paşa Mahallesi	7.10	3.44±0.04	1.82±0.04	0.36±0.01
Kadıköy	7.03	3.62±0.12	1.96±0.06	0.40±0.02
Patlak	7.05	1.69±0.04	13.77±0.58	0.31±0.02
Bengiler	6.82	2.83±0.12	2.14±0.08	0.42±0.01
Çakallar	6.80	2.65±0.13	4.17±0.28	0.38±0.02
Müstecap	6.27	3.65±0.19	0.93±0.06	0.42±0.04
Kaşıkçı	6.77	4.93±0.08	0.76±0.01	0.38±0.004

average results and standard deviations for aqua regia experiments obtained from the ICP-OES analyses show that nearly all elements' concentrations except Cr and Ni are higher than these limit values for Sarısu soil (Table 3). Especially, concentrations of As, Cu, Cd, and Pb were elevated and relatively great concentrations of Mn and Zn were measured in Hastanetepe soil. As, Zn, Pb, and Ba concentrations are higher than these limit values in Hastanetepe soil. The soils collected from Enverpaşa, Kadıköy, and Kaşıkcı have greater concentrations of As, Zn, Pb, Cd, and Ba. Especially the soil collected from Kaşıkcı also contained substantial heavy metal contamination, especially for Zn (the greatest value), as well as Cu, Ba, Cd, Ni, Pb, Mn, and Cr. The Ba concentration is only greater than these limit values in Müstecap and Bengiler soils while Müstecap soil contained the greatest concentration of Ba. The soil collected from Patlak and Çakallar have greater concentrations of As, Zn, and Pb than the limit values. Also the Çakallar sample contained the greatest concentrations of Ni and Cr but still lower than the limit values given for these elements. Concentrations of some metals in different soils (As, Zn, Pb, Cd, Ba, Cr, Ni, Cu, and Mn) were such that they raised concerns regarding potential health impacts on children if they should be ingested, e.g., during play (Table 3). It must be emphasized though, that the "pseudo-total" concentration of an analyte does not necessarily indicate the toxicity of that sample because although present at high concentrations, the analytes may simply not be bio-available. In an attempt to identify the proportion of the trace metals that are bio-available, an in vitro gastro-intestinal method was applied to determine the metal concentrations that can dissolve in gastric and intestinal solutions (Table 3).

Although the pseudo-total concentrations are substantially higher than the levels recommended by the Turkish Environmental and Forest Ministry, the data also indicates that the vast proportion of this is not bio-available (Table 3). The Turkish Environment and Forest Ministry's recommended maximum values assume that 100% of the analyte will be available (i.e., a worst case scenario is assumed). Therefore, once the bio-available fraction is taken into account, few of the analytes at few of the sampling sites are above the recommended maxima. Daily As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, and Zn ingestion amounts have been calculated based on a soil ingestion rate of 10 $\mathrm{g\,day^{-1}}$ (pica behavior, deliberate ingestion) using the in vitro intestinal bioavailability results given in Table 3 (Table 4). Ingested As and Pb amounts by pica behavior of children are greater than tolerable daily intake values given by Baars et al. for all soil samples. Zn amounts digested by children is greater for the Sarı Su and Kaşıkçı soil samples while the Cr level is only greater in Enverpaşa soil compared with TDI values. Mn and Cd levels are greater in all samples except Patlak and Müstecap. The amounts of Ba ingested by pica behavior are greater in all samples except Patlak and Çakallar while Ni levels are lower than TDI values in all samples. All of the soil samples collected from children's play areas contain large amounts of heavy metals. The amount of As, Pb, Ba, and Cd levels are significantly greater than TDI values. The results show that if these soils are ingested by children, they might cause a great risk to children's health. It should be emphasized that although the levels for many of the analytes are high (in terms of micrograms per day of each analyte), this does relate to children exhibiting pica behavior and it assumes that 10 g of soil is ingested per day. Clearly, most children, even those suffering from pica will not ingest amounts this high, whereas a minority will ingest greater amounts. The figures given are therefore a guide to the overall safety of the soil.

Table 4 also shows the amount of available metal ions calculated for children who do not have pica behavior at a soil ingestion rate of 100 $\mathrm{mg\,day^{-1}}$ recommended by US EPA (2008). Only, Pb levels are much greater and only in Sarı Su and Kaşıkçı soil than the TDI values given by Baars et al. All other analytes are below the TDI values and can therefore be regarded as being less of a risk to normal children. The results show that the lead pollution in these soils may potentially pose a risk even at very low amounts of soil ingestion. However, the overall risk can be minimized if the children are encouraged to wash their

Table 3 Concentrations of elements obtained using in vitro gastro-intestinal experiments in soils (mean and standard deviation; *n*=3)

Sample	Metal ion	Aqua regia (µg/g)	Gastric phase (µg/g)	Intestinal phase (µg/g)	In vitro bio-available gastric phase (%)	In vitro bio-available intestinal phase (%)
Sarı su	As	400.6±11.7	56.17±7.76	59.04±1.94	14.0	14.7
	Cu	163.1±5.1	25.24±4.57	54.21±9.28	15.5	33.2
	Zn	1,998.1±1.2	797.46±36.81	601.85±91.53	39.9	30.1
	Pb	4,611.6±201.8	1,337.87±35.82	1,280.37±72.09	29.0	27.8
	Mn	1,293.9±52.4	553.38±54.19	585.96±7.59	42.8	45.3
	Ni	14.7±2.7	2.21±0.21	3.13±0.65	15.0	21.3
	Cd	13.40±0.74	8.75±0.81	5.59±0.96	65.3	41.7
	Cr	12.14±0.89	0.54±0.04	2.36±0.16	4.4	19.4
	Ba	314.7±14.0	87.93±4.94	76.11±1.38	27.9	24.2
Hastanetepe	As	101.7±1.9	12.46±1.96	8.39±1.65	12.3	8.3
	Cu	33.6±2.5	9.87±0.41	11.68±0.36	29.4	34.8
	Zn	519.1±10.4	337.27±15.19	193.61±14.78	64.9	37.3
	Pb	502.9±10.6	294.13±7.93	161.08±4.94	58.5	32.0
	Mn	898.0±22.2	453.47±22.32	391.81±16.53	50.5	43.6
	Ni	16.9±1.7	3.09±0.17	1.16±0.09	18.3	6.9
	Cd	2.59±0.11	2.51±0.09	2.44±0.17	96.9	94.2
	Cr	17.17±0.42	0.46±0.04	0.42±0.10	2.7	2.5
	Ba	269.3±6.5	76.67±3.89	60.70±2.94	28.5	22.5
Enver Paşa Mahallesi	As	170.3±4.0	25.09±0.84	22.63±8.95	14.7	13.3
	Cu	66.3±3.2	25.57±1.06	27.34±2.08	38.6	41.2
	Zn	1,187.6±5.2	789.97±78.47	473.73±12.53	66.5	39.9
	Pb	1,098.0±35.1	595.40±2.40	319.31±5.20	54.2	29.0
	Mn	1,139.7±34.6	655.59±67.28	539.84±16.49	57.5	47.4
	Ni	16.9±0.6	4.63±1.28	3.84±0.22	27.4	22.7
	Cd	5.74±0.31	5.38±0.36	3.99±0.11	93.7	69.5
	Cr	17.80±0.16	0.72±0.16	1.15±0.03	4.0	6.5
	Ba	282.1±11.6	81.45±3.88	55.32±2.53	28.9	19.6
Kadıköy	As	105.3±11.1	12.98±0.75	9.68±2.61	12.3	9.2
	Cu	49.8±1.5	9.94±2.69	16.70±2.28	20.0	33.5
	Zn	1,274.9±6.3	701.08±97.77	384.29±21.61	55.0	30.1
	Pb	822.2±23.6	281.99±49.95	139.36±14.34	34.3	17.0
	Mn	929.9±28.1	325.33±70.70	299.96±58.64	35.0	32.2
	Ni	15.9±1.2	3.34±0.49	0.86±0.11	21.0	5.4
	Cd	5.57±0.68	4.97±0.84	3.56±0.19	89.2	63.9
	Cr	17.19±0.69	0.15±0.03	0.54±0.04	0.9	3.1
	Ba	267.9±10.2	70.96±7.31	57.17±5.06	26.5	21.3
Patlak	As	85.3±2.9	12.31±1.56	5.52±2.24	14.4	6.5
	Cu	37.4±1.0	1.95±0.74	8.82±1.13	5.2	23.6
	Zn	780.2±38.0	174.36±14.31	0.52±0.01	22.4	0.07
	Pb	377.1±19.5	38.77±7.71	12.96±5.06	10.3	3.4
	Mn	1,058.2±53.0	376.85±25.83	34.78±9.15	35.6	3.3
	Ni	23.1±1.9	2.73±0.15	0.90±0.23	11.8	3.9
	Cd	2.33±0.14	1.04±0.12	0.14±0.04	44.6	6.0
	Cr	21.94±1.04	0.47±0.07	0.40±0.01	2.1	1.8
	Ba	169.4±9.3	59.70±4.25	10.80±0.54	35.2	6.4
Bengiler	As	10.3±1.4	5.74±1.29	1.35±0.32	55.7	13.1
	Cu	20.9±0.7	2.99±0.55	9.63±3.04	14.3	46.1

Table 3 (continued)

Sample	Metal ion	Aqua regia (μg/g)	Gastric phase (μg/g)	Intestinal phase (μg/g)	In vitro bio-available gastric phase (%)	In vitro bio-available intestinal phase (%)
	Zn	115.9±2.5	37.30±4.55	19.14±3.23	32.2	16.5
	Pb	24.8±4.0	5.42±1.47	18.12±5.10	21.9	73.1
	Mn	487.8±12.6	205.87±14.12	179.50±15.26	42.2	36.8
	Ni	15.1±0.9	3.34±0.43	2.05±0.32	22.1	13.6
	Cd	0.68±0.03	0.67±0.01	0.54±0.06	98.5	79.4
	Cr	22.55±0.64	1.37±0.03	3.35±0.21	6.1	14.9
	Ba	284.1±10.1	68.95±1.29	58.60±1.28	24.3	20.6
Çakallar	As	57.5±4.6	7.65±1.28	2.79±0.81	13.3	4.9
	Cu	32.6±0.6	4.13±0.62	10.14±0.99	12.7	31.1
	Zn	423.2±34.2	126.20±22.82	17.33±1.92	29.8	4.1
	Pb	348.8±32.1	92.27±11.45	37.23±15.59	26.5	10.7
	Mn	925.6±62.9	311.04±8.57	213.14±14.52	33.6	23.0
	Ni	39.9±2.3	5.08±0.91	4.72±0.13	12.7	11.8
	Cd	1.81±0.04	0.90±0.05	0.52±0.08	49.7	28.7
	Cr	39.55±0.79	0.75±0.02	1.54±0.03	1.9	3.9
	Ba	196.3±16.6	38.22±0.42	13.46±1.86	19.5	6.9
Müstecap	As	15.3±1.5	4.07±1.27	2.61±0.71	26.6	17.1
	Cu	31.2±0.9	4.15±0.57	8.19±1.24	13.3	26.3
	Zn	83.0±0.9	21.86±2.41	6.83±0.64	26.3	8.2
	Pb	27.2±2.9	18.05±1.05	13.52±4.82	66.4	49.7
	Mn	265.8±15.8	135.24±19.67	115.56±10.55	50.9	43.5
	Ni	11.1±0.9	2.60±0.57	2.51±0.43	23.4	22.6
	Cd	0.35±0.01	<LOD	<LOD	–	–
	Cr	20.29±1.60	0.18±0.01	0.47±0.03	0.9	2.3
	Ba	494.3±20.1	38.72±3.43	25.18±2.14	7.8	5.1
Kaşıkçı	As	90.0±8.6	15.50±2.50	11.50±1.90	17.2	12.8
	Cu	100.1±5.2	37.08±2.99	40.21±0.49	37.0	40.2
	Zn	2,694.6±9.6	1,204.49±33.78	865.60±83.32	44.7	32.1
	Pb	2,206.5±60.2	1,440.55±62.20	964.37±92.93	65.3	43.7
	Mn	1,507.3±16.4	1,137.22±43.07	993.71±16.51	75.5	65.9
	Ni	21.9±0.1	4.28±0.92	4.07±0.33	19.5	18.6
	Cd	5.17±0.39	3.54±0.56	2.84±0.34	68.5	54.9
	Cr	24.66±0.32	0.25±0.01	0.78±0.01	1.0	3.2
	Ba	323.1±2.7	140.19±0.85	91.24±2.45	43.4	28.2

hands prior to eating and all fruit/vegetables are washed prior to consumption.

4 Conclusions

Since Balya and its associated villages are very rich in ores, the soil in the area contains very high concentrations of heavy metals, especially As, Cd, Pb, Zn, and Ba. All of the soil samples collected from children's play areas contained elevated amounts of heavy metal ions. Amounts of soil ingestion as a result of pica behavior of 10 $g day^{-1}$ were used to calculate using in vitro intestinal bio-available results. The results showed that the amount of As, Pb, Ba, and Cd levels are much greater than TDI values in most of the soils. The results show that if these soils are ingested by children suffering from pica, they might cause a great risk to children's health. It should be emphasized, however, that the amounts of these analytes ingested by non-pica children are below TDI in all cases except for Pb in soil from Sarısu and Kaşıkçı and therefore even these contaminated soils can be regarded as relatively harmless for non-pica children.

Table 4 Amounts (μg) of metal ingested from the assumptions of a soil ingestion rate of 10 g day^{-1} (pica behavior, deliberate ingestion) and the 100 mg day^{-1} soil ingestion rate for different soil samples calculated from values taken by in vitro intestinal bioavailability results

	Soil ingestion rate (g day^{-1})	Sarısu (μg/day)	Hastanetepe (μg/day)	Enverpaşa (μg/day)	Kadıköy (μg/day)	Patlak (μg/day)	Bengiler (μg/day)	Çakallar (μg/day)	Müstecap (μg/day)	Kaşıkçı (μg/day)	BE (μg/day)	TDI (μg/day)	RDI (μg/day)
As	10	590	84	226	97	55	14	28	26	115	3	10	ND
	0.1	5.9	0.8	2.3	1.0	0.6	0.1	0.3	0.3	1.2			
Cu	10	542	117	273	167	88	96	101	82	402	300	1,400	440
	0.1	5.4	1.2	2.7	1.7	0.9	1.0	1.0	0.8	4.0			
Zn	10	6,018	1,936	4,737	3,843	5	191	173	68	8,656	3,000	5,000	5,000
	0.1	60.2	19.4	47.4	38.4	0.1	1.9	1.7	0.7	86.6			
Pb	10	12,804	1,611	3,193	1,394	130	181	372	135	9,644	20	36	ND
	0.1	128.0	16.1	31.9	13.9	1.3	1.8	3.7	1.4	96.4			
Mn	10	5,860	3,918	5,398	3,000	348	1,795	2,131	1,156	9,937	–	–	1,500
	0.1	58.6	39.2	54.0	30.0	3.5	18.0	21.3	11.6	99.4			
Ni	10	31	12	38	9	9	21	47	25	40.7	40	500	ND
	0.1	0.3	0.1	0.4	0.1	0.1	0.2	0.5	0.3	0.4			
Cd	10	56	24	40	36	1.4	5	5.2	–	28	2.2 (males), 1.7 (females)	5	ND
	0.1	0.6	0.2	0.4	0.4	0.0	0.1	0.1	–	0.3			
Cr	10	24	4	115	5	4	34	15.4	4.7	7.8	10 (Cr(III))	50	15
	0.1	0.2	0.0	0.1	0.1	0.0	0.3	0.2	0.0	0.1			
Ba	10	761	607	553	572	108	586	135	252	912	90 (soluble)	200 (soluble)	–
	0.1	7.6	6.1	5.5	5.7	1.1	5.9	1.3	2.5	9.1			

Tolerable daily intake (TDI) values are presented together with the background exposure (BE) (Baars et al. 2001) in micrograms per day for a child weighing 10 kg. The given value for manganese is recommended dietary allowance for 4–8 years old child (USDA 2009)

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