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Lead (II) detection and contamination routes in environmental sources, cookware and home-prepared foods from Zimatlán, Oaxaca, Mexico

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ABSTRACT

An interdisciplinary investigation, involving environmental geochemists, epidemiologists, nurses, and anthropologists, was undertaken to determine the contamination source and pathway of an on-going outbreak of lead poisoning among migrants originating from Zimatlán, Oaxaca, Mexico and living in Seaside, California, and among their US-born children. An initial investigation in Seaside identified grasshopper foodstuff ("chapulines") imported from Mexico and consumed as snacks, as containing alarmingly high lead concentrations (up to 2300 mg/kg). The focus in the present work concentrates on the Oaxacan area of origin of the problem in Mexico, and two potential sources of contamination were investigated: wind-borne dusts from existing mine residues as potential contaminants of soil, plant, and fauna; and food preparation practices using lead-glazed ceramic cookware.

Over a three year period, sampling was conducted in Oaxaca using community-level sampling and also targeted sampling with families of cases with lead poisoning in California. In addition to fresh field chapulines, we analyzed for total lead: soil, water, mine residues, and plant materials, both from areas adjacent to or at an abandoned waste site containing mine tailings, and from fields where chapulines are collected; foodstuffs gathered in community markets or in a food transport business; and foodstuffs and cookware gathered from relatives of case families in California. Also, selected new and used lead-glazed clay cookware was extracted for lead, using 0.02 M citric acid and with 4% acetic acid.

The results indicated significant presence of lead in mine wastes, in specific foodstuffs, and in glazed cookware, but no extensive soil contamination was identified. *In-situ* experiments demonstrated that lead incorporation in food is made very efficient through grinding of spices in glazed cookware, with the combination of a harsh mechanical action and the frequent presence of acidic lime juice, but without heating, resulting in high but variable levels of contamination.

1. Introduction

An on-going outbreak of lead poisoning that began in the late 1990s among patients at a community-based clinic in Seaside, California, belonging to the Monterey County Health Department, was investigated and determined to be disproportionately affecting migrants from Zimatlán, Oaxaca, Mexico who resided in Seaside and reported eating imported homemade foods sent from their families back home (Handley and Grieshop, 2007; Handley et al., 2007). An important source of lead was identified in imported dried grasshopper ("chapulines") foodstuff from Mexico (with levels ranging from 3 to 2300 mg Pb/kg), although few other foods were tested for lead. The findings raised the concern that there was a significant source of lead in the Zimatlán community that had not been identified previously.

Lead is absorbed principally via respiration and ingestion. Poisoning in humans is considered by the Centers for Disease Control when blood lead levels reach 10 μg/dL or higher (Roper et al., 1991; Gordon et al., 2004), and children are particularly sensitive to this poisoning because of their lead-vulnerable developing nervous system (Gerba, 1996; Siegel, 2002).

Although some natural sources for lead exist, such as soil erosion and volcanic emissions, mining and industrial activities contribute to about 90% of lead environmental pollution, where lead-containing dust particles contaminate air and soils (Siegel, 2002; Spiro and Stigliani, 2002), causing this element to border on ubiquitous (Gerba, 1996).

The worldwide decline in the use of leaded gasoline and fuels has gradually eliminated this important past source of lead pollution in air in many countries. However, lead is still widely used and pervasively causes environmental contamination from activities that range from production and use of car batteries, paints and pigments, and glazed earthenware, to lead solder and piping in water distribution systems (Gerba, 1996; Siegel,

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Table 1
Sampling description list (Zim = Zimatlán, SP = San Pablo, SI = Santa Inés)

Type of sample	Number of samples	Source
Sampling 1: February 12–14, 2004		
Fresh chapulines from fields	2 sets	West of Zim, different fields
	2 sets	Northwest of Zim, different fields
Soil	4	Northwest of Zim, different fields
	2	South of Zim, different fields
	1	East of Zim (discharge Fe mine)
	1	From SP
	1	From Zim (deposit of Fe mine)
Water	1	Atoyac river
Fresh plants from fields	2	South of Zim, different fields
	1	West of Zim, different fields
Chapulines prepared for eating	1	Only cooked, from Zim
	2	Cooked and seasoned, from Zim
Mole (foodstuff)	2	Zim weekly market
Pumpkin seeds	2	Zim weekly market
Garlic, fresh	1	Zim weekly market
Chocolate	1	Zim weekly market
Tortilla (foodstuff)	1	Dough from Zim weekly market
	1	Dry from Zim weekly market
	1	Fresh from Zim weekly market
Lime	2	For tortilla cooking, from Zim weekly market
Sampling 2: April 19–23, 2005		
Chapulines prepared for eating	10 sets	Cooked and seasoned, from Zim weekly market
Food coloring	2	Red and green from Zim weekly market
Soil	1	In Zim
Mine residues	1	From a deposit in SI
Sampling 3: October 29, 2005		
Chapulines prepared for eating	4 sets	Different markets in Oaxaca City
	2 sets	From Zim fixed market
Sampling 4: February 4–13, 2006		
Chapulines prepared for eating	1 set	Weekly market in Ayoquezco
	8 sets	Weekly market in Villas de Etla
Outside the study area	1 set	Fixed market in Zaachila
	5 sets	Fixed market in Ocotlán
Sampling 5: October 9–11, 2006		
Chapulines prepared for eating	13 sets	From transport business in SP
	1 set	From weekly market in Zim
	1 set	From private home in Zim
Chapulines, fresh	1 set	From private vendor in SI
Pumpkin seeds prepared for eating	1 set	From express air transport business in SP
Chocolate	1	From transport business in SP
	1	From private home in Zim
Cocoa and cinnamon	1	From private home in Zim
Mine residues	6	From diverse locations in the SI deposit
Cultivated soil	1	Adjacent to SI deposit
Water	1	Small watershed in SI
	1	From Atoyac river
	1	For field irrigation (between towns)
	1	From drinking water well in SI
	3	From drinking water well in SP
Sampling 6: February 5–9, 2007 (mostly samples from interviewees)		
Soil	5	On the road to, and in Golallo
Dried plants	4	On the road to, and in Golallo
Pumpkin seeds	1 set roasted	From private home in SP
	2 sets prepared	From private homes in SP and Zim
	2 sets fresh	From private homes in SP and Zim
Spices	1 set dried combo	From private home in SP
	2 sets fresh garlic	From private homes in SP and Zim
	2 sets salt	From private homes in SP and Zim
	1 salt–garlic–lime mix	Ground in “chirmolera” from Zim
	1 lime	From private home in Zim
	1 “epazote”	From private home in Zim
Beans	1 prepared (refried)	From private home in Zim
	1 fresh	From private home in SP
Chocolate	2	From private homes in SP
Tortilla (foodstuff)	1	From private home in SP
Mole (foodstuff)	1	From private home in SP

Table 1 (*continued*)

Type of sample	Number of samples	Source
Sampling 6: February 5-9, 2007 (mostly samples from interviewees)		
Ground meat	1 prepared (cooked)	From private home in Zim
Soil	1	From private field in SP
	4 ploughed	From private field in SI
	1	Adjacent to mine deposit in SI
	9 ploughed	Every 50 steps outward from deposit in SI
Plants	1 fresh	From private home in SP
	2 dry	From private field in SI
	2 fresh	From private field in SI
	6 dry	Outward from mine deposit in SI
	3 fresh	Outward from mine deposit in SI
Sampling 7: May 10–17, 2007		
Spices	1 Fresh oregano	From family X from SP
	1 Ground oregano	From family X from SP
	1 Fresh cumin	From family X from SP
	1 Ground cumin	From family X from SP
	1 Fresh garlic	From family Z from SP
	1 Ground garlic	From family Z from SP
Glazed pottery	1 used saucepan	From family Y from SP
	1 semi-new saucepan	From family X from SP
	1 new saucepan	Acquired in Oaxaca City
	1 used “comal”	From family Y from SP
	1 new “comal”	Acquired in San Mateo
	1 new “chirmolera”	Acquired in Oaxaca City
Clay	2	1 red, 1 brown from San Mateo

2002; Spiro and Stigliani, 2002), and thus continues to represent a widespread environmental hazard. As such, elimination of childhood lead poisoning has become one of the United States' National Health Objectives for 2010 (Oller and Bates, 2004).

Upon close inspection, there is no current activity in the Zimatlán area identified for specific production and use of leaded products. Oaxaca has an important mining history and 17 mining regions are defined statewide. One of the towns inside the Zimatlán District has the second most important mine processing plant of the state, which specialized in gold and silver ores, processing 200 T of ore per day. Lead occurs in these minerals as an impurity and thus gets discarded in the mine processing residues. The plant was closed in 1992 but tons of residues remain disposed in the open on adjacent fields to the former plant (COREMI, 1996).

The objective of this investigation was to examine sources of lead in the exported foodstuffs, focusing on the chapulines, which were so highly contaminated in the initial study testing, and to clarify the contamination route that has caused the lead poisoning outbreak in Seaside, California. One of the initial hypotheses that had not previously been studied was that wind-blown dust particles from mine residues rich in lead and other heavy metals were contaminating fields adjacent to the area where the residues are disposed of, and as a result soils, plant, and fauna would be contaminated with these dusts. This would be relevant for the chapulines which seasonally swarm to fields in the area and are then captured there for local consumption. A previous study in ecosystems developed on mine tailings in Wales and Ireland, identified grasshoppers and other ground-dwelling invertebrates as contaminated with lead, whose concentrations correlated well with those of grasses (Milton et al., 2002).

The Zimatlán District is a rural area where agriculture is a seasonal economic activity, found within the Western Central Valley of Oaxaca, Southwest of the Oaxaca City state capital. Chapulines are both collected and consumed locally, and they flourish during the rainy season, feeding on plant leaves and thus thriving in agricultural plantations such as maize, one of the main products of the area. However, some of the chapulines sold in the area during the dry season are from the Mexican state of Puebla, in the Central part of the country (Ramona Perez, personal communication).

Small lead particles deposited in soils (disregarding the source) may be re-suspended in air and thus may contribute to significant spreading of lead contamination throughout extensive areas (Harris and Davidson, 2005). This spreading was especially prevalent in the past from

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combustion of coal and of leaded gasoline (Kaste et al., 2003). For example, dietary sources of lead in Omaha and Nebraska have been identified as coming from dusts originating from mine ores in Idaho, Missouri, and Mexico (Gallon et al., 2005; Manton et al., 2005).

According to this environmental-contamination-focused hypothesis, deposition of wind-blown lead-rich particles that originate from the mine residues in Zimatlán may be responsible for contaminating not only chapulines in the adjacent fields, but also soils, plants and surface waters in these fields.

In the case of the scenario under investigation, quantitative airborne lead deposition on the external parts of chapulines and on maize leave surfaces would be sufficient to cause the lead poisoning observed upon human consumption of these species. In the present article we describe the work pursued and the challenges encountered in elucidating the source and pathway of the transnational lead poisoning-food contamination outbreak first identified in Seaside, California, which represents a case study of interdisciplinary collaborative work among environmental geochemists, epidemiologists, nurses and anthropologists.

2. Materials and methods

2.1. Study area

The Zimatlán District is one of the 30 political districts of the State of Oaxaca, in the South of Mexico, and is found in the Western part of the Central Oaxacan Valley, approximately 1500 m above mean sea level. This district is found within the mining district of “Taviche”, itself within the mining region of “Tlacolula”. The study was performed within the three principal towns of the district: Zimatlán de Álvarez (Zim) (municipal head), San Pablo Huixtepec (SP), and Santa Inés Yatzeche (SI). The current economic activities consist of services, small-scale industrial activities and commerce, and seasonal agriculture, but a large proportion of the income flux originates from the migrant workers living in the US.

2.2. Sampling

A total of seven different sampling trips were performed between 2004 and 2007. These, and the results from the lead analyses, are described separately in chronological order because the design scheme for each subsequent sampling was built on the results obtained from the immediately previous one, and thus the sampling sequence and frequency may be better understood if described sequentially. A total of 172 samples were analyzed. All soils sampled showed pH levels between 7.5 and 8.5. A summary list describing the samples from each session is shown in Table 1, and following is a short summary of the sampling approaches for each:

- ➢ Sampling 1: Community sampling of fresh chapulines from fields where they are collected for local consumption, soils both from agricultural and non-agricultural fields, water from Atoyac river, leaves from wild and cultivated plants, and miscellaneous foodstuff acquired from the Zimatlán weekly market, including cooked and seasoned (prepared) chapulines.
- ➢ Sampling 2: Community sampling of prepared chapulines and food coloring from the Zimatlán market, and several soil samples.
- ➢ Sampling 3: Community sampling of prepared chapulines, exclusively, from market in Zimatlán, and in Oaxaca City, which is outside the study area but included to provide preliminary information for other regions.
- ➢ Sampling 4: Complements previous sampling outside the study area.
- ➢ Sampling 5: Targeted sampling of prepared foodstuff samples (mostly prepared chapulines) from one of several of the express air transport businesses in San Pablo Huixtepec, called envios, from which food is sent to migrant's homes in California, from relative's homes in Oaxaca (Handley and Grieshop, 2007). Also, prepared chapulines from various sources, mine residues from the deposit in Santa Inés, and water samples from the field and from drinking sources were collected.
- ➢ Sampling 6: Targeted sampling of foods, and fresh soil and plant samples among Zimatlán relatives of lead poisoning case families. Also, soil and plant samples from the cultivated field adjacent to the deposit of mine residues. This was the most extensive sampling performed.
- ➢ Sampling 7: Targeted sampling of foods and cooking observations among Zimatlán relatives of lead poisoning case families. Relatives whose food samples yielded high lead levels were asked to prepare fresh foodstuff in real time. Several lead-glazed clay pots were acquired, both new and used.

2.3. Sample treatment

All fresh samples, especially of chapulines, were dried *in-situ* in local bread-baking ovens with uncontrolled temperature from a host family in Zimatlán, to avoid decomposition during transfer to analysis lab in Mexico City. Once in the lab, all samples, except the pottery, were dried at 80 °C, and ground in an agate mortar. All were digested in acid to determine total lead content and, in the case of soil and mine residues samples, the contents of other metals and trace elements as well.

2.4. Acid digestions

Following US-EPA method 3051 (US-EPA, 1994), an accurate mass of approximately 0.5 g for the inorganic samples, or of 0.3 g for the organic samples was weighed in an analytical balance, in triplicate and placed inside Teflon reactors designed for the microwave oven treatment. A volume of 10 mL of analytical-grade concentrated nitric acid (J.T. Baker) was added and placed in a CEM MARSX microwave oven. The oven was programmed with a 1200 W power, a maximum security pressure of 300 PSI, sequential temperature ramps to 130 °C in 4′30″, to 150 °C in 2′, and to 175 °C in 3′, maintaining this latter temperature for 4′30″. All organic samples were completely digested by this procedure. The refractory

Fig. 1. Lead-glazed clay pots used for cooking in the Zimatlán District, in Oaxaca, Mexico. (a) Glazed “chirmolera” used for grinding spices, such as garlic, as shown; (b) pumpkin seeds cooking in a glazed “cazuela”.

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residue in the case of inorganic samples was filtered through Whatman 40 ashless filter paper. All digested liquids were filled to the mark in 50-mL volumetric flasks with nanopure (≥17 mΩ/cm) water.

2.5. Pb(II) availability from glazed clay pots

The glazed clay pots acquired, both new and used, were: saucepans (“cazuelas”), “comales” (round dish used for heating tortillas, or roasting seeds), and “chirmoleras” (used for grinding spices, like a mortar and pestle) (Fig. 1). These were washed with nanopure water and left to dry at ambient temperature. A solution of 1 L of 0.02 M citric acid (J.T. Baker, analytical reagent) was added inside each pot (except in the “comales”) (Tunstall and Amarasiriwardena, 2002). These were magnetically stirred for 24 h at ambient temperature, covering them from dust with plastic. 10-mL aliquots were withdrawn at 20, 40, 60, 120 min, and 4, 8, 12, 20 and 24 h. Subsequently the pots were washed three times with 1 L nanopure water, and 10-mL aliquots were withdrawn each time for Pb(II) analysis, in order to confirm no remnant Pb(II) was left before the following experiment. The pots were subsequently washed with nanopure water and left to dry in air. A solution of 1 L of 4% (v/v) acetic acid (J.T. Baker, analytical reagent) was added inside each pot (except in the “comales”) (Seth et al., 1973; Gonzalez de Mejia and Craigmill, 1996; Ajmal et al., 1997), and the same treatment and withdrawal schedule as for the citric acid treatment was carried out.

2.6. Lead(II) analysis

Liquid samples either from digestions or from extractions were analyzed for Pb(II) using a VARIAN SpectrAA 110 atomic absorption spectrophotometer (AAS). The conditions used were: air-acetylene gas mixture and a slit opening of 1.0 nm. Three different wavelengths were chosen depending on the concentration level expected, according to:

Wavelength (nm)	Concentration interval of standards (mg/L)
217	0.25 to 10
283.4	0.5 to 20
261.4	7.5 to 200

The matrices for the standard curves were prepared by adding 10 mL of concentrated nitric acid for every 50-mL solution, and for the extraction experiments they were either 0.02 M citric acid or 4% acetic acid.

Other elements were analyzed from solution using a Thermo Electron IRIS INTREPID II XSP Duo Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES). The analysis was performed exclusively for those chapulines and soil samples that showed positive lead contents, from sampling sessions 2 and 3. The conditions set for every element are listed as follows:

Element	Wavelength (nm)	Measurement mode
Ag	328.068	Radial
As	189.042	Axial
Ba	455.403	Radial
Be	313.402	Radial
Cd	226.502	Axial
Co	228.616	Axial
Cr	267.716	Radial
Cu	324.754	Radial
Mn	257.610	Radial
Mo	202.030	Axial
Ni	231.604	Axial
Pb	220.353	Axial
Se	196.090	Axial
Tl	190.864	Axial
V	292.402	Radial
Zn	213.856	Axial
Al	308.215	Radial
Ca	317.933	Radial
Fe	259.940	Radial
Mg	279.079	Radial

2.7. Quality assurance

The laboratory where the analyses were performed (LAFQA) has been certified by the Mexican government for the analysis of 5 elements, including Pb, in water and soil matrices. All standard curves, at the three different wavelengths, yielded squared correlation coefficients above 0.998. For every 14 samples analyzed a blank was processed, and for every 28 samples, a 5 mg/L control sample and a Pb (II)-spiked sample were included. Control measurements with more than a 5% relative error prompted re-calibration of the standard curve before continuing the analysis. Pb(II) detection limits were determined from the calculated concentration derived from three times the standard deviation of the average absorbance signal from 10 measurements of the lowest calibration standard at each wavelength set. These were:

Atomic absorption wavelength (nm)	Solution detection limit (mg/L)	Solid detection limit (mg/kg)
217	0.0082	1.4
283.3	0.097	9.7
261.4	0.042	–

2.8. Interviews

Six relatives of lead-poisoned cases in Seaside, from different households in the study area, were contacted and interviewed by trained interviewers after providing written informed consent in Spanish (three from San Pablo, two from Zimatlán, and one from Santa Inés) during the sixth sampling trip, and after their relatives in California had contacted them about the interview and obtained permission. All necessary human subjects protocols were fulfilled to satisfy the University of California San Francisco Committee on Human Research for work with human subjects. The main goals of the interviews were to obtain information about people's eating and food-preparation habits, as well as to determine whether any other source of lead exposure might be present, such as through occupational exposures. Questions related to the general health status of the subjects, their consumption of certain foodstuff such as chapulines and its frequency, and their preparation methods, including the type of cookware used. During the interviews people were asked to prepare and provide some of the food samples (for example spices and pumpkin seeds) they described as prepared in clay pots, and were offered the results of the lead testing once the results were available.

3. Results and discussion

Table 2 shows the results of total lead analyses, divided by sampling trip, but only for samples that showed detectable levels of this element ($n=39$: 13 prepared chapulines and 8 other foodstuffs, 9 soils, 2 fresh plants, and 7 mine residues). A global analysis of the data in Table 2 shows that approximately 24% of all samples taken ($n=164$, excluding clay pots), and 30% of prepared chapulines sampled ($n=40$) showed some level of Pb content, whereas none of the fresh chapulines sampled ($n=5$ sets) showed detectable levels of Pb. Also, it is noteworthy from Table 2 that all mine residues sampled ($n=7$) showed very high levels of total Pb. Finally, 20% of non-targeted food samples from case families, at the end of the study, yielded detectable lead levels, suggesting more widespread exposures to lead may be occurring than previously believed.

3.1. First sampling

The fresh chapulines samples were taken from all different fields around Zimatlán where they are normally collected for local consumption (Aquino Family, personal communication). It is noteworthy that none of

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Table 2
Total detectable Pb concentrations in samples (some samples where Pb was not detectable (ND) are included for comparison)

Type of sample	Pb(II) concentration (mg/kg)	Relative std dev (%)
Sampling 1:		
Boiled chapulines	2.7	31.7
Prepared chapulines	12.5	7.4
Prepared chapulines	45.1	5.7
Soil South of Zimatlán	84.2	2.5
Soil South of Zimatlán	66	10.5
Fresh plants from South field	11.7	10.2
Fresh plants from South field	17.7	4.5
Sampling 2:		
Prepared chapulines	189	4.2
Prepared chapulines	70	7.1
Prepared chapulines	2,326	3.6
Prepared chapulines	1,701	1.1
Mine residues in SI	1,486	–
Sampling 3 and 4:		
Prepared chapulines from Oaxaca City	251	2.7
Prepared chapulines from Villas de Etla	44.6	1.3
Prepared chapulines from Ocotlán	177	3.9
Sampling 5:		
Prepared chapulines from SP	158	9.5
Prepared chapulines from SP	668	0.4
Prepared chapulines from Zim	216	4.6
Prepared pumpkin seeds from SP	592	4.2
Mine residues	1,428	1.6
Mine residues	1,235	1.7
Mine residues	3,099	1.0
Mine residues	1,622	0.9
Mine residues	562	4.6
Mine residues	3,253	0.9
Soil 10 m from mine residues	822	1.4
Sampling 6:		
Soil adjacent to mine residues	1211	1.6
Soil 50 m from mine residues	156	5.7
Soil 100 m from mine residues	42	11.9
Soil 150 m from mine residues	48	4.1
Soil 200 m from mine residues	60	3.3
Soil 250 m from mine residues	28	10.7
Mixture of spices	554	16.8
Mole	32	15.6
Roasted pumpkin seeds	1,009	4.1
Pumpkin seeds after preparing	584	4.4
Fresh pumpkin seeds before preparing	ND	–
Sampling 7:		
Fresh oregano before grinding	ND	–
Oregano after grinding	1,367	0.8
Fresh cumin	ND	–
Cumin after grinding	339	4.7
Fresh garlic before grinding	ND	–
Ground garlic	1,528	11.2

these samples contained detectable Pb, whereas all three samples of cooked and seasoned chapulines taken showed variable Pb levels (Table 2). However, in our previous work we did find evidence of lead in uncooked chapulines (Handley and Grieshop, 2007; Handley et al., 2007).

Two of the soils sampled in the South part of Zimatlán yielded positive Pb results (Table 2 – 66 and 84 mg Pb/kg). These levels are low and may represent natural background concentrations, as they are within the range of natural lead contents reported in soils (Siegel, 2002; Essington, 2004), and well within those in other regions of Mexico (with average and median values between 50 and 70 mg/kg, and maxima near 1000 mg/kg – Dr. Francisco Romero, Geology Institute, UNAM, Mexico, personal communication). In fact, natural lead levels in world soils may reach values as high as 1200 mg/kg (Aubert and Pinta, 1977), and as high as 700 mg/kg in US soils (Shacklette and Boerngen, 1984).

The two plant samples taken from the same area as the Pb-containing soils also showed detectable Pb levels (Table 2 – 12–18 mg/kg). The levels are even lower than those of soils, and performing an external 0.1 M HCl wash on the plant samples previous to the acid digestion decreased these total Pb contents by 17% and 80%, respectively (results not shown). This suggested that the Pb measured in the fresh plants must come from externally-attached soil particles, which, considering the alkaline soil pH values, discards the possibility of Pb having become bioavailable to plants, as it may occur in low-solubility solids such as Pb phosphates and carbonates.

The total Pb levels for the prepared chapulines samples in this first sampling were in the low range (3–45 mg/kg) of that found in the chapulines foodstuff samples taken from the migrants' homes in Seaside, California earlier (up to 2300 mg/kg – Handley and Grieshop, 2007; Handley et al., 2007). Although the low Pb levels found in the prepared chapulines would easily surpass the ingestion limits established by the Food and Drug Administration (Farley, 1998) of 6 to 75 µg Pb per day. However, the lack of matching magnitudes with those found earlier, and the very low Pb levels found in soils and plants, made the results of this first sampling non-conclusive.

3.2. Second sampling

The results of the second sampling confirmed cookware as a source of Pb contamination, since 40% of the cooked and seasoned chapulines sampled showed high levels of Pb (Table 2), of which 50% showed levels above 1700 mg/kg. These higher levels approximate those from the sampling of chapulines foodstuff in Seaside, California (Handley and Grieshop, 2007; Handley et al., 2007). Coincidentally, the mine residues collected during this sampling visit also showed Pb levels within the higher levels found in prepared chapulines (Table 2).

However, the hypothesis of Pb in prepared chapulines as originating from mine residues was considerably weakened from the ICP analyses performed of other heavy metals in residues and in chapulines. Mine residues, as would be expected, contained similar or considerably higher levels of other metals than those of lead, especially of Cu, Mn, and Zn (Table 3). In contrast, for Pb-containing prepared chapulines the opposite behavior was observed, as evidenced by the Pb/metal ratios $>>1$ in most cases (Table 3). Also, the low Cu, Mn, and Zn concentrations in chapulines showed very constant values, as compared to the high variability of Pb content in these (Table 3), suggesting that the Cu, Mn, and Zn levels correspond to normal micro-nutrient levels. Concentration comparisons of macro-nutrient elements such as Ca, Mg, and Fe is non-conclusive, as is that of Al, because they are present at high levels both in chapulines and in non-contaminated soils (Table 3).

3.3. Third and fourth sampling

The results from the third and fourth sampling suggest there is considerable contamination (16%) of foodstuffs in other areas of Oaxaca as well, and confirm what is observed within Zimatlán sampling, that there is significant variation in the order of magnitude of lead content in prepared chapulines. Although the very high Pb levels found in Zimatlán foodstuffs was not reproduced here.

3.4. Fifth sampling

Contacting the owner of one of the many express food transport businesses allowed in the fifth sampling to examine other commonly exported foods form Zimatlán to the Seaside area, for which both chapulines and a new contaminated food emerged: pumpkin seeds ($n = 1$).

Again, a considerable number chapulines samples were contaminated (15%), but the majority were not. Pb content of contaminated chapulines ranged from 160 mg/kg to 670 mg/kg (Table 2), but the one pumpkin seed sample set taken yielded Pb levels in the higher part of this range. Although the samples were collected in San Pablo, where the business is

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Table 3
Metal concentrations in prepared chapulines and soil samples from second sampling, using ICP-AES

	Prepared chapulines								Geological material			
	1		2		3		4		Mine residues		Non-leaded soil	
Metal	Conc mg/kg	Pb/ metal	Conc mg/kg	Pb/ metal	Conc mg/kg	Pb/ metal	Conc mg/kg	Pb/ metal	Conc mg/kg	Pb/ metal	Conc mg/kg	Pb/ metal
Pb	189	*1*	70	*1*	2326	*1*	1701	*1*	1486	*1*	ND	–
Al	58	*3.3*	28	*2.5*	69	*34*	60	*28*	**5898**	***0.25***	40,814	–
Ca	1763	*0.11*	1122	*0.06*	1584	*1.5*	1274	*1.3*	**5506**	*0.27*	13,991	–
Cu	25.8	*7.3*	26	*2.7*	23.91	*97*	35	*49*	**4497**	***0.33***	53	–
Fe	115	*1.6*	235	*0.30*	110	*21*	456	*3.7*	**19,079**	***0.08***	29,912	–
Mg	728	*0.26*	668	*0.10*	655	*3.6*	914	*1.9*	**2789**	*0.53*	10,343	–
Mn	7.82	*24*	7	*10*	13.54	*172*	8	*213*	**883**	***1.7***	877	–
Zn	107	*1.8*	87	*0.80*	90	*26*	113	*15*	**1480**	***1.0***	112	–

Standard deviations of measurements are not reported in order to facilitate readability of table. The relative standard deviations ranged as follows for: Pb: 1–7%, Al: 10–23%, Ca: 1–10%, Cu: 0.7–15%, Fe:3.5–31%, Mg: 1.5–7%, Mn: 1–29%, Zn: 3–11.5%. The detection limits, converted to mg/kg from the digestion and dilution procedure, were as follows for: Pb: 5.3, Al: 82, Ca: 2.28, Cu: 4.6, Fe: 150, Mg: 60, Mn: 4.9, Zn: 18. All Pb/metal ratios were expressed in italics to distinguish them from the actual metal concentrations.

located, the contaminated food samples originated both from families in San Pablo and in Santa Inés, in the study area. The one other contaminated sample of chapulines (216 mg/kg) originated from our contact family in Zimatlán (Table 2), and was prepared by them. Unfortunately they did not provide us with a fresh sample before preparation, but they did inform us that glazed cookware was used for the preparation.

The mine residues showed, as expected, very high Pb concentrations, which affected soils adjacent to the deposit, as may be observed in samplings 5 and 6 (Table 2). As the distance from the deposit increased from 50 m to 100 m Pb content reached background levels. This again weakens the hypothesis of wind-blown mine-residues dusts as source of the Pb contamination found.

3.5. Sixth and seventh sampling

Samplings 6 and 7 provided direct sampling of case families at the Zimatlán area, based on working with relatives of people in Seaside who had significantly elevated blood lead levels. The sampling and analysis, together with the interviews allowed us to detect a new foodstuff heavily contaminated with Pb, seasoning blends composed of garlic, salt, and lime, and oregano. The results of the interviews allowed us to establish that these spice mixtures were usually ground in a glazed clay pot called "chirmolera" (Fig. 1a), instead of the typical stone grinder, because the latter is very porous and retains a large proportion of the spices ground at any one time. Chapulines and pumpkin seeds are also prepared in glazed pots (Fig. 1b), but not ground, and they are both subsequently mixed with the previous seasoning preparation in a very similar manner and proportion.

At the time of the interviews, chapulín season was over, so we were only able to sample *in-situ* fresh spices before and after grinding, from three different families. The results from sampling 7 clearly show the influence of grinding in a "chirmolera", for three different spices that originally contained no detectable lead, reaching Pb levels from the hundreds to 1500 mg/kg (Table 2) after grinding. Pumpkin seeds were adequate surrogates of chapulines because of the very similar preparation procedure, and the results show that the mixing with the previous seasoning blend introduces concentrations of lead of the same magnitudes as those found for the contaminated samples in the previous sampling efforts (Table 2). The high lead content found in the pumpkin seed sample reported as only roasted in a non-glazed comal, is probably due to the sample actually being subsequently seasoned (from its "limy" taste).

Other foodstuffs that are usually prepared in glazed cookware are chocolate, beans and mole (a multi-component spicy sauce blend that usually includes chocolate). None of these samples in our study were found contaminated with Pb, except in one sample of "mole" (32 mg/kg – Table 2). Additional testing of more varied foodstuffs that are not exported should also be investigated in the Zimatlán community, but were not the focus of this investigation into the transnational food exportation associated with the original Monterey County outbreak.

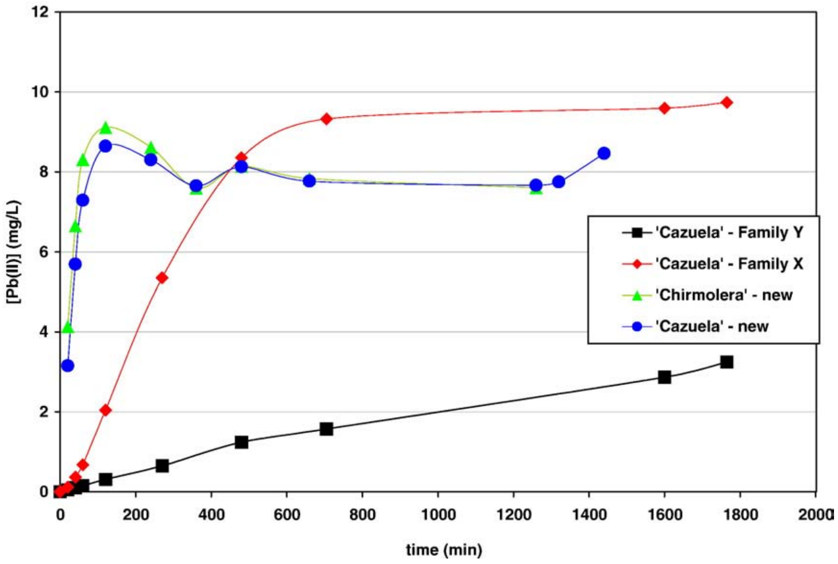

Fig. 2. Results from Pb(II) extraction experiments using 1 L of 0.02 M citric acid on different glazed clay pots.

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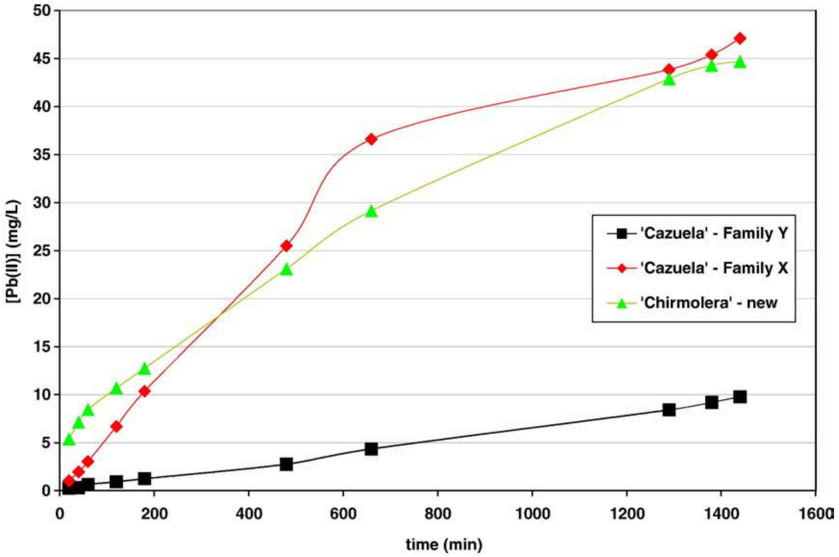

Fig. 3. Results from Pb(II) extraction experiments using 1 L of 4% acetic acid on different glazed clay pots.

3.6. Extraction of available lead from ceramic cookware

In-situ colorimetric tests for lead content were performed on the pots used by the families interviewed, by means of Lead Check SWABS sticks, which turn to pink and red upon presence of 1 µg or higher lead contents. All pots except the "comales" gave positive lead results. Results from the lead extraction tests performed in the laboratory for all pots except the "comales" are shown in Figs. 2 and 3.

The test with 0.02 M citric acid was meant to mimic the use of these pots for storage of lime or orange juice. Kinetics of lead dissolution was slow for one of the used pots (Fig. 2), which did not reach equilibrium in the 30-h experiment, but the three other pots tested (new and semi-new) showed much faster dissolution kinetics and reached definitive maxima. The new pots reached the maxima at approximately 3–4 h (Fig. 2), and in fact showed almost matching behavior despite their different design and cooking purpose, while the semi-new pot took about 12 h to reach a similar maximum value as the latter, of between 8 and 10 mg/L, corresponding to 0.04 to 0.05 mM Pb.

The ratio of total citrate to extracted Pb is thus of approximately 400 to 500, so the extraction maximum cannot be explained as a simple Pb-citrate complex stoichiometry. This is probably due to the fact that the available Pb is found in the glazing as some kind of Pb(II) oxide bound to different degrees to the alumino-silicate compound on the clay surface, as a result of the high temperatures of the glazing process. Therefore, high concentrations of complexing agents in acid medium are necessary to accomplish quantitative Pb extraction. The similar maxima results indicate that similar proportions of available Pb are extracted by citrate in the different pots, and given enough time, perhaps even the highly used pot would yield the same maximum. Apparently, high firing temperatures during fabrication and glazing of the clay pots favor a high retention of Pb on the clay surface, making it less available (Casson, 1997; Hamilton, 2001).

Extraction with 4% acetic acid, which may be related to use of pots for storage of "salsas" (spicy pepper-based sauces) (Gonzalez de Mejia and Craigmill, 1996), showed that lead may be available in much larger concentrations than shown with citric acid at a lower concentration, by factors of around 4 (Fig. 3). Similar differences in the dissolution kinetics behavior between pots may be observed as was the case with citric acid, but this time the semi-new pot of family X showed very similar behavior as the new pot. After 24 h, the concentration of Pb(II) extracted reached levels as high as 45 mg/L (0.22 mM) for the new and semi-new pots. However, maxima of Pb(II) extracted were not reached after 24 h, where the rate of Pb(II) release was still very high, and thus the maxima could reach much higher levels than those shown in Fig. 3.

This latter test points to the important fact that lead is highly available in the cookware used. If either mechanical and frictional energy or heat were to be added to the tests, as is the case with grinding and cooking, it is probable that the availability is drastically increased not just from dissolution of lead from the glaze by the combined action of acid and complexing ligand, but also from direct detachment of solid particles from the glazing that may directly deposit on the food being processed in the pot.

3.7. Summary of evidences

In the present work we confirmed that significant numbers of Zimatlán foods were highly contaminated with lead, and that there was significant inter- and intra-sample variation in sample lead content, which is consistent with the sporadic nature of lead poisoning

Table 4
Evidence-based decision framework to support or reject Pb-source hypotheses

Type of evidence	Pb-source hypotheses:	
	Soil, plant, and fauna contamination from wind-blown dusts originating from mine residues	Food preparation contamination from use of Pb-glazed cookware
Supporting evidence	High Pb contents in all mine residues. Large extension of open-air long-term abandoned mine residues, adjacent to agricultural fields.	High contents of available Pb in all glazed cookware tested. Foodstuff tested before processing in cookware showed appearance of high Pb contents only after processing. Low, constant levels of Cu, Zn, Mn in leaded chapulines, but highly variable Pb contents in these, while no content of the former in glazed cookware.
Non-supporting evidence	No extensive soil, plant, and water contamination identified, even in the vicinity of residues. Low, constant levels of Cu, Zn, and Mn in Pb-containing chapulines, while high, variable contents of these metals in residues.	The majority of prepared chapulines (70%) did not show Pb contents.

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cases in the migrated population in Seaside that receives imported foods from this region of Oaxaca.

Although we did find, as expected, significant levels of lead in mine tailings that are adjacent to fields of active crop production, we did not find evidence of extensive contamination of soils, plants and fauna transported via air from mine-residues dusts. Therefore this Pb source was discarded as explaining the foodstuff contamination observed. Table 4 shows a summary of supporting and non-supporting evidences for each of the two hypotheses proposed.

We also identified that among the few chapulines samples we collected outside the Zimatlán area, a considerable proportion (16%) was significantly contaminated with lead, even if they were not as highly contaminated as some of those from the case families in Zimatlán, suggesting a larger public health problem exists across this region of Oaxaca.

We observed through our work with the relatives of case families that there is more variation in the production of lead-glazed cookware than was previously thought to be centralized production in one particular town in the State of Oaxaca (Atzompa). There are many producers who sell pottery locally and may have loose ties or no ties with the Atzompa potters, and therefore may have more lead content in their cookware. In our work in Monterey County, we found that among the diverse group of migrants from Oaxaca, it was only those from the Zimatlán region that had evidence of lead poisoning, other Oaxacan migrants did not. We intend to look at the cooking and food preparation practices in Oaxaca in these more diverse communities to determine whether they may vary significantly, where for example they may not use “chirmoleras” for grinding their spices or may use pottery produced with less available lead in the glaze.

4. Conclusion

In the present work we identified that grinding of spice mixtures that were *not* heated, was a significant source of lead among the extended family members of case families from Seaside. High variations in the cooking practices that make use of lead-glazed cookware, especially “chirmoleras”, among the Zimatlán population may explain the variable lead content found in seasoned chapulines, where the majority of those sampled (70%) did not show detectable lead contents. Our findings support the likelihood that lead exposure is more extensive in other communities near the Zimatlan area, and that replacing specific cooking vessels with less lead-available products can provide substantial reductions in the incidence of lead poisoning among Oaxacans in Mexico and in the US.

Because a chirmolera is not found in all households in Zimatlán, and is widely used across the region of Oaxaca (Ramona Perez, personal communication), more work will need to be done to compare lead availability in cookware from the Zimatlán region, with similar cookware in other towns across the region. As well, additional testing of food preparation practices and cookware among families with relatives in Seaside that do not show lead poisoning, may help to identify which cooking materials yield low or no amounts of lead and can be substituted for those that yield the highest amounts of lead, as identified in our current work.

Appropriate ways to communicate these findings to the Zimatlán community are underway to: (1) encourage a decrease in use of cookware that is most able to transfer lead into foods, even without heating. The release of lead from lead-glazed “chirmoleras” for grinding spices may be avoided if lead is not used in glazing for cookware sold in the community. Recent collaborations between a pottery-producing community organization that does not use lead in the glazing may result in replacement of lead-containing products with those that are lead-free (Ramona Perez, personal communication), and as well, other cookware such as traditional stone-made “molcajetes”, or non-glazed clay utensil cookware can become suitable substitutes; (2) increase awareness of the lead risk from soil in close proximity to the contaminated mine tailings, which have been dispersed throughout the community and are not always located in areas where there was well marked indication of the tailings. We did not examine to what extent mine tailings have been transported to other areas by local residents, and were presumed to be clean soil fill (due to their resemblance to soil), and are currently being used in areas of agricultural production. We did find that the tailings themselves were highly contaminated and there are widespread views in the community that such tailings have been used for fill.

There is an on-going need to examine the lead content in the local cookware and investigations on the possibility of eliminating Pb availability in these glazed pots as a function of firing temperature.

Although one may infer that the relatives who prepare these foodstuffs in Mexico must also be chronically poisoned with lead, the extent of the lead poisoning in the community would need to be confirmed through blood lead screening with appropriate clinical follow-up for those with significant blood lead levels in concordance with international lead screening guidelines. Because the consumption of tortillas produced locally may provide high calcium doses in a highly bioavailable form (compared to the more industrialized product in California), and which may outcompete lead in crucial metabolic processes, it is possible that these sources could limit the extent of lead poisoning in this community.

The interdisciplinary experience obtained from work among environmental geochemists, epidemiologists, nurses and anthropologists in the present investigation has stimulated the continued collaboration between the groups to approach environmental problems from the physicochemical, epidemiological and the sociological angles, and to include methods from these diverse disciplines in order to find suitable solutions for this and other similar contamination scenarios.

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