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An Integrated Health Risk Assessment Approach to the Study of Mining Sites Contaminated With Arsenic and Lead

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ABSTRACT

In order to test the value of an integrated approach for the analysis of health risks at contaminated sites, an integrated health risk assessment in a mining area was performed following 3 steps: 1) Environmental monitoring of surface soil, 2) assessment of exposure to metals in children and native rodents, and 3) DNA damage evaluation (comet assay) in children and rodents. These aspects also were studied in less exposed populations. Our results in humans showed that children living in the most polluted area (Villa de la Paz, Mexico) had higher lead blood concentrations (geometric mean of 13.8 μg/dL) and urinary arsenic levels (geometric mean of 52.1 μg/g creatinine) compared to children living in a control area (Matehuala, Mexico; blood lead of 7.3 μg/dL; urinary arsenic of 16.8 μg/g creatinine). Furthermore, the exposed children also had increased DNA damage (tail moment mean in Villa de la Paz of 4.8 vs 3.9 in Matehuala; $p < 0.05$). Results in rodents were identical. Animals captured in the polluted area had higher levels of arsenic (geometric mean of 1.3 μg/g in liver and 1.8 μg/g in kidney), lead (0.2 μg/g in liver and 0.9 μg/g in kidney), and cadmium (0.8 μg/g in liver and 2.2 μg/g in kidney), and increased DNA damage (tail moment mean of 18.2) when compared to control animals (arsenic in liver of 0.08 μg/g and kidney of 0.1 μg/g; lead in liver of 0.06 μg/g and kidney of 0.3 μg/g; cadmium in liver of 0.06 μg/g and kidney of 0.6 μg/g; and tail moment of 14.2). With the data in children and rodents, the weight-of-evidence for health risks (in this case DNA damage) associated with metal exposure in Villa de la Paz was strengthened. Therefore, a remediation program was easier to justify, and a feasibility study at this site is under way.

Keywords: Metals Comet assay Integrated risk assessment

INTRODUCTION

The term integration has many meanings, and several opportunities exist within risk assessment for integration. In this context, the International Program of Chemical Safety (IPCS) has defined the concept of integrated risk assessment as "a science-based approach that combines the processes of risk estimation for humans, biota, and natural resources in one assessment" (IPCS 2001). Two fundamental reasons for integrated risk assessment are 1) to improve the quality and efficiency of assessments through the exchange of information between human health and environmental risk assessors, and 2) to provide more coherent inputs in the decision-making process (IPCS 2001). With respect to the latter, human health and ecological risk assessors often provide decision makers with inconsistent input, which results in contradictory impressions of the nature of risks. This result from differences in approach should be eliminated in an integrated approach (IPCS 2001).

In this context, we designed an integrated health risk–assessment approach for mining areas, because mining, 1 of the most important sources of environmental toxicants, contributes significantly to the national economy of 158 countries (UNEP 2000a). This design includes an environmental assessment together with the study of the exposure to heavy metals and the analysis of DNA damage in both children and local fauna (rodents).

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Millions of people are exposed to metals in mining areas. For example, it has been estimated that miners represent approximately 1% of the global workforce or about 30 million workers (Joyce 1998). To this total we have to add 11 to 13 million people for whom artisanal mining represents their livelihood (UNEP 2000b). Occupational health risks in the mining industry have been studied extensively (Fisher 1998); however, little is known about the health risks of children exposed to metals in mining areas. Most studies concerning children living in mining or smelter sites are limited to exposure assessments (Díaz-Barriga et al. 1993, 1997; Hwang et al. 1997; Murgueytio et al. 1998; Yáñez et al. 2003). Few of them have described biological effects in the exposed children (Counter 1997; Calderon et al. 2001; Yáñez et al. 2003). If we assume that around 40 million individuals are working in the mining industry, then, millions of children (including the children of the miners) may be exposed directly to the environmental impacts associated with the mining industry. Thus, it is clear that more studies urgently are needed in regard to children's health in mining areas.

With regard to rodents, heavy metal toxicity has been studied extensively at the experimental level (ATSDR 2005a, 2005b); furthermore, the toxicity of simple and complex mixtures has been determined in rodents, showing higher effects than those found with their individual components (ATSDR 2004). Therefore, it is not unusual that, in the assessment of sites contaminated with metals, some protocol have included native small mammals as representatives of

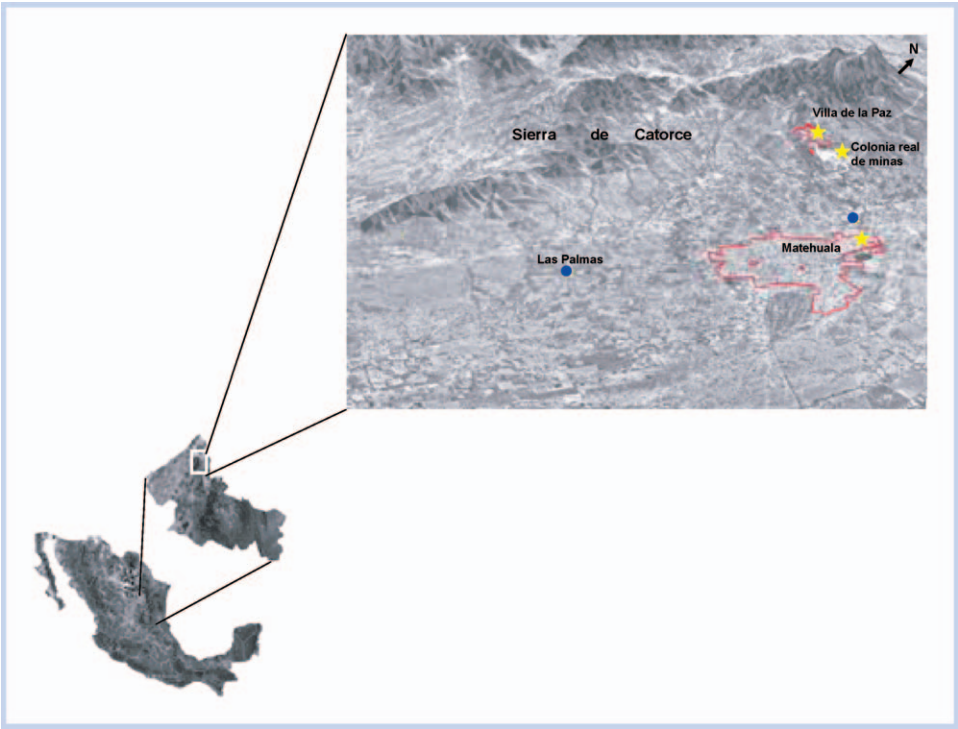

Figure 1. On the map are shown the sampling sites for children (stars) and rodents (circles).

wildlife (Talmage and Walton 1991; Erry et al. 2000; Milton et al. 2003).

Considering the genotoxic effects of lead and arsenic, in our integrated approach, DNA damage was chosen as the toxicological endpoint (Valverde et al. 2000, 2002). In mining areas, others have studied DNA damage (although separately) in wild rodents (Husby et al. 1999; Da Silva et al. 2000; Festa et al. 2003) and children exposed to metals (Yáñez et al. 2003).

Taking into account all the above points and in order to test the value of an integrated approach for the analysis of health risks in contaminated sites, in the present study we performed an integrated health risk assessment in a mining area following 3 steps: 1) Environmental monitoring of surface soil, 2) assessment of exposure to metals in children and native rodents, and 3) DNA damage evaluation in those children and rodents. These aspects also were studied in less exposed populations.

MATERIALS AND METHODS

Study areas

Villa de la Paz is a mining site where different ore deposits have been mined over the last 400 y. A preliminary analysis of the metal concentration in the tailings located in Villa de la Paz (Rodríguez et al. 1998) has reported arsenic (9,647 mg/kg), manganese (1,650 mg/kg), zinc (1,350 mg/kg), copper (1,180 mg/kg), lead (690 mg/kg), nickel (150 mg/kg), and cadmium (17 mg/kg). Studies of soil samples reported concentrations of 19 to 17,384 mg/kg As, 15 to 7,200 mg/kg Cu, 31 to 3,450 mg/kg Pb, and 26 to 6,270 mg/kg Zn (Razo et al. 2004). Meanwhile, the concentrations in dry stream sediment samples were found to vary 29 to 28,600 mg/kg As, 50 to 2,160 mg/kg Pb, 71 to 2,190 mg/kg Cu, and 98 to 5,940 mg/kg Zn (Razo et al. 2004). The results suggest that arsenic and heavy metal dispersion from their pollution sources (historical and active tailing impoundments, waste rock dumps, and historical slag piles), mainly is associated in this site with 1) fluvial transportation of mine waste through streams that cross the area in a west–east direction and 2) aeolian transportation of mineral particles in a southwest–northeast direction. The site impacted by arsenic and heavy metals has an area of 105 km^2. In the urban area of Villa de la Paz, the mining facility contains a raw mineral breaker and a mining waste disposal site (mining tailings; Figure 1). The exposure of children to arsenic and lead in Villa de la Paz has been reported previously (Yáñez et al. 2003).

Environmental monitoring

We took soil samples from areas repeatedly used by children (schools and recreational areas) and at those sites where rodents were collected. For areas used by children, a systematic sampling within a 400-m grid was undertaken at 3 sites: The urban area of Villa de la Paz; the community of Real de Minas, Mexico (closest urban community to the mining waste disposal site); and Matehuala (control community). For areas where rodents were collected, sampling within a 2.0-m grid was defined for 2 sites: Villa de la Paz (an area next to the urban site) and a control area against prevailing winds (Figure 1). Samples of surface soil (1–5 cm in depth) were obtained with a stainless steel scoop on an approximately 1-m^2 surface area and stored in polyethylene bags.

Selection of children

The studies involving humans were conducted in accordance with national and institutional health guidelines for the protection of human subjects. In Villa de la Paz, Real de Minas, and Matehuala, children attending schools in the area were selected at random from among those who met the inclusion criteria. Healthy children (as stated by the parents)

aged 4 to 11 y who had at least 3 y of residence in their particular area were considered for the study. Sixty children were selected for the study. All of them decided to participate in the study. The socioeconomic index of Villa de la Paz is 0.76, and the index of Matehuala is 1.2 (CONAPO 2000). Both locations have been classified as communities with a low level of margination (CONAPO 2000). The parameters considered in the construction of this index were academic level, housing conditions, and income (CONAPO 2000). All parents filled out an exposure questionnaire modified from a questionnaire previously used in Mexico (Carrizales et al. 2006). Among the major nonenvironmental determinants of lead exposure, "mother cooks in lead-glazed pottery," "hand-to-mouth activities," and "child bites colored pencils" were assessed through this questionnaire. Blood was obtained by venous puncture using lead-free tubes containing ethylenediaminetetra-acetic acid (EDTA) as anticoagulant. First void urine samples were collected, stored in plastic bottles, and kept frozen until analysis.

Collection of rodents

The studies involving experimental animals were conducted in accordance with national and institutional guidelines for the protection of animal subjects. Sherman traps for live capture were used during 2 consecutive nights; 40 traps were placed in a grid of 5×10 m. Ten rodents were captured in Villa de la Paz; in the control area, 11 animals were collected. However, in the present study we only examined species of the Heteromyidae family, because this granivorous family is the most abundant in the region, and the species (*Chaetodipus nelsoni* Merriam 1894 and *Dipodomys merriami* Mearns 1890) have similar ethologic habitats. The captured animals were weighed, and sex was determined before sending them to the laboratory where they were sacrificed by decapitation 1 d later; blood samples, liver, and kidneys were obtained.

Analytical methods

Soil samples were oven dried at 30 °C for 1 to 2 d. The <600-μM fraction was separated with a 28-mesh Tyler Series sieve. Soil samples were acid digested (25% HNO_3) for 30 min under 80 psi pressure using a chemical electronic mechanic MDS-2000 microwave extraction system. After digestion, the extracted solution was filtered through Whatman filter paper with 11-μM pore diameter. Arsenic was analyzed by flame atomic absorption spectrometry using a Perkin Elmer Analyst 100 atomic absorption spectrometer coupled to a Perkin Elmer FIAS 100 hydride generation system (Norwalk, CT, USA). Analysis for lead, cadmium, copper, and zinc in soil were carried out by flame atomic absorption spectrometry using a Varian Spectra AA 220 atomic absorption spectrometer (Mulgrave, Victoria, Australia). Analysis of primary standard reference material in each run was conducted as an internal quality control. For soil, NIST-SRM 2710 and 2711 (Montana soil) were used with recoveries of 97% at 98%. The linear quantification procedure had the following coefficients of variation: Cadmium 5.6% to 11.2% (range of 1–10 μg/kg), arsenic 3.1% to 4.8% (range of 1–80 μg/kg), and lead 2.1% to 2.8% (range of 1–80 μg/kg).

For children, an aliquot of urine (5.0 mL) was wet digested with nitric and perchloric acids according to Cox (1980). Arsenic was analyzed by atomic absorption spectrometry using a Perkin Elmer Analyst 100 atomic absorption spectrometer coupled to a Perkin Elmer FIAS 100 hydride generation system. As quality control, NIST-SRM 2670 urine standard reference materials were obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) with recoveries of 95%. The urinary arsenic concentration was standardized to urinary creatinine. Urinary creatinine was determined by the Jaffe colorimetric method (Brown 1986). Blood lead levels were determined with a matrix modifier (diammonium hydrogenphosphate-Triton X-100 in the presence of 0.2% nitric acid) according to Subramanian (1987), and the samples were analyzed with a Perkin-Elmer 3110 atomic absorption spectrophotometer using a graphite furnace. At the time of the study, our laboratory was participating in the blood lead proficiency-testing program of the Centers for Disease Control.

With regard to rodents, the animals were sacrificed by decapitation; rodent whole blood was obtained using lead-free tubes containing EDTA as anticoagulant. The liver and kidneys immediately were removed and, without delay, perfused with 10 mM Tris pH 7.2. Tissue samples of liver and kidney were placed in acid-washed glass test tubes and solubilized with a mixture of nitric and perchloric acids for at least 9 h. Arsenic was determined by atomic absorption spectrometry, using a Perkin Elmer Analyst 100 spectrometer coupled to a Perkin Elmer FIAS 100 HGS. Lead and cadmium analysis were performed using atomic absorption spectrophotometry with a Perkin Elmer model 3110 coupled to a graphite furnace. As quality control, blind random samples of National Bureau of Standards bovine liver 1577a, standard reference material, were analyzed; the percent recovery values were better than 95% for arsenic and lead.

Comet assay

Single cell gel electrophoresis was performed as described by Singh et al. (1988). A whole blood sample (obtained at the same time as the samples used for the exposure assessment to metals) was suspended in a layer of 0.5% low–melting point agarose (37 °C) and placed on a precoated slide with a layer of 0.5% regular agarose. After the agarose solidified, the cells were placed in a lysis solution (consisting of 10 mM Tris-HCl, 2.5 M NaCl, and 0.1 M Na_2EDTA, pH 10), to which 10% dimethylsulfoxide and 1% Triton X-100 were added just before use. The solution was chilled prior to use and the lysis duration was a maximum of 24 h at 4 °C. Slides were incubated in an alkaline buffer (300 mM NaOH and 10 mM Na_2EDTA pH > 13) for 20 min. After alkali incubation, the electrophoresis was performed in the same buffer (pH > 13) for 20 min at 25 V and 300 mA. All procedures were performed under very dim, indirect light and conducted at a temperature of 4 °C. After electrophoresis, slides were washed gently with 0.4 M Tris-HCl buffer (pH 7.5) and then dehydrated in ethanol. The slides were stained with ethidium bromide (20 μL of a 20-μg/mL solution), and a cover glass was placed over the gel. The basal level of DNA damage in leukocytes was analyzed in 100 cells (50 randomly selected cell nuclei by duplication) using an epifluorescent microscope (Nikon Eclipse E400). The comet image magnification was 200×. Olive tail moment [(tail mean − head mean) · tail %DNA/100] was measured by image analysis (Komet, version 4; Kinetic Imaging Ltd., Liverpool, UK). Cell viability was determined by trypan blue dye exclusion and was always >95%. All slides were coded independently before analysis (they were scored without knowledge of the code). For

Table 1. Concentrations of arsenic, lead, cadmium, copper, and zinc in surface soil in areas where children were sampled (mg/kg)[a]

	Zone	*n*	G mean	SE	Min.	Max.	%>100 mg/kg
Arsenic	Villa de la Paz	42	1,932	759	133	27,945	100
	Real de Minas[b]	14	709	215	144	3073	100
	Matehuala[b]	41	437	364	40	9,822	83
	Zone	*N*	**G mean**	**SE**	**Min.**	**Max.**	%>400 mg/kg
Lead	Villa de la Paz	42	932	507	45	16,800	74
	Real de Minas[b]	14	261	231	135	3,450	14
	Matehuala[bc]	41	400	86	63	2,214	56
	Zone	*N*	**G mean**	**SE**	**Min.**	**Max.**	
Cadmium	Villa de la Paz	42	28.4	4.4	4.0	125.4	—
	Real de Minas[b]	14	7.4	0.9	5.0	16.0	—
	Matehuala[b]	41	12.2	2.6	0.2	68.0	—
	Zone	*N*	**G mean**	**SE**	**Min.**	**Max.**	
Copper	Villa de la Paz	42	826	375	107	10,920	—
	Real de Minas[b]	14	268	100	87	1,288	—
	Matehuala[b]	41	296	87	50	3,264	—
	Zone	*N*	**G mean**	**SE**	**Min.**	**Max.**	
Zinc	Villa de la Paz	42	1,565	254	236	6,080	—
	Real de Minas[b]	14	344	40	230	760	—
	Matehuala[b]	41	438	124	103	3,180	—

[a] G mean = Geometric mean; SE = Standard error; Min. = minimum; Max. = maximum.
[b] Different from Villa de la Paz ($p < 0.05$).
[c] Different from Real de Minas ($p < 0.05$).

rodents, we followed the same method used for humans, except that 20 µL of blood was used.

Statistical analysis

For humans, differences between mean values of metal concentrations in soil samples, blood lead levels (PbB), urinary arsenic concentrations (AsU), and olive tail moment, were assessed by one-way analysis of variance, followed by the least significant difference test for comparison among groups. All values were log transformed to stabilize the variance and to better achieve a normal distribution. With rodents, differences between mean values of metal concentration in soil samples, metals in rodent tissues, and olive tail moment were assessed by the Student's *t* test for independent groups. Only values of rodent tissues were log transformed to stabilize the variance and to better achieve a normal distribution. In both cases, the level of statistical significance was $p < 0.05$. All analyses were conducted using STATISTICA version 6.0 (STATISTICA 1993).

RESULTS

Table 1 shows that the area of Villa de la Paz had arsenic ($p < 0.05$), lead ($p < 0.05$), cadmium ($p < 0.05$), copper ($p < 0.05$), and zinc ($p < 0.05$) levels in surface soil higher than the control area of Matehuala. With regard to the area of Real de Minas, Mexico, located next to the tailing deposit, the only difference when compared to the results in Matehuala were the levels of lead, which were lower ($p < 0.05$). It is important to mention that the concentrations of 400 mg/kg for lead and 100 mg/kg for arsenic in soil are intervention guideline levels recommended by the US Environmental Protection Agency (USEPA 1990, 2001).

Taking into account the findings in soil, exposure to metals was assessed in children using biomarkers such as urinary arsenic and lead in blood (Table 2). The percentage of exposed children (urinary arsenic > 50 µg/g creatinine) was higher in Villa de la Paz; however, regarding mean urinary arsenic, a statistical difference was observed only with Matehuala (Table 2). Considering lead in blood concentration (Table 2), children living in Villa de la Paz had higher levels than did the other 2 communities, and children in Real de Minas had higher concentrations than did the control area of Matehuala; all the differences were significant. Reference levels of 10 µg/dL for blood lead and of 50 µg/g creatinine for urinary arsenic are guidelines set by the US Centers for Disease Control (1991; Belson et al. 2005).

Finally, DNA damage was not different between children living in Villa de la Paz or in Real de Minas, but in these areas it was significantly higher than the damage found in children living in Matehuala (Table 3). It is important to note that baseline DNA damage in the normal population had a tail moment lower than 4.0 (Bajpayee et al. 2002).

Table 2. Urinary arsenic (μg/g) and lead in blood (μg/dL) concentrations in children[a]

	Zone	*n*	G mean	SE	Min.	Max.	%>50 μg/gc
Arsenic	Villa de la Paz	20	52.1	7.5	21.4	129	55
	Real de Minas	28	39.5	6.1	13.2	166	30
	Matehuala[b]	12	16.8	1.6	10.8	26	0
	Zone	***N***	**G mean**	**SE**	**Min.**	**Max.**	**%>10 μg/dl**
Lead	Villa de la Paz	20	13.8	1.0	5.1	23.5	95
	Real de Minas[b]	28	9.9	0.7	3.7	20.8	50
	Matehuala[bc]	12	7.3	1.5	1.8	22.2	25

[a] G mean = Geometric mean; SE = Standard error; Min. = minimum; Max. = maximum. For urinary arsenic, micrograms of arsenic per gram of creatinine (μg/g).
[b] Different from Villa de la Paz ($p < 0.05$).
[c] Different from Real de Minas ($p < 0.05$).

All the above results showed that Villa de la Paz is contaminated with metals and that children living there were the most exposed to arsenic and lead and were among those with a greater DNA damage. However, a clear correlation with soil contamination was not found; thus, we decided to assess the exposure to metals and the same DNA damage effect in rodents, because these animals are exposed heavily to soil. Furthermore, we took advantage of this model, and the concentration of metals also was analyzed in different tissues.

Rodents were collected in the area of Villa de la Paz (exposed area) and in a control area as shown in Figure 1. In the area of Villa de la Paz, arsenic, lead, cadmium, copper, and zinc levels in soil were higher and statistically different ($p < 0.05$) when compared to the levels at the control area (Table 4). Furthermore, concentrations of lead, arsenic, and cadmium found in kidney and liver in rodents collected in Villa de la Paz (exposed animals) were significantly higher than those in rodents collected in the control area (Figure 2). With regard to DNA damage, this was also higher in rodents from Villa de la Paz ($p < 0.001$; Figure 3).

DISCUSSION

In this work we have shown that stronger conclusions can be obtained in the assessment of contaminated sites by studying humans and local fauna simultaneously. In Villa de la Paz, a heavily polluted mining site (as shown by the soil concentrations of arsenic, lead, cadmium, copper, and zinc), an increased exposure to metals, and an increased DNA

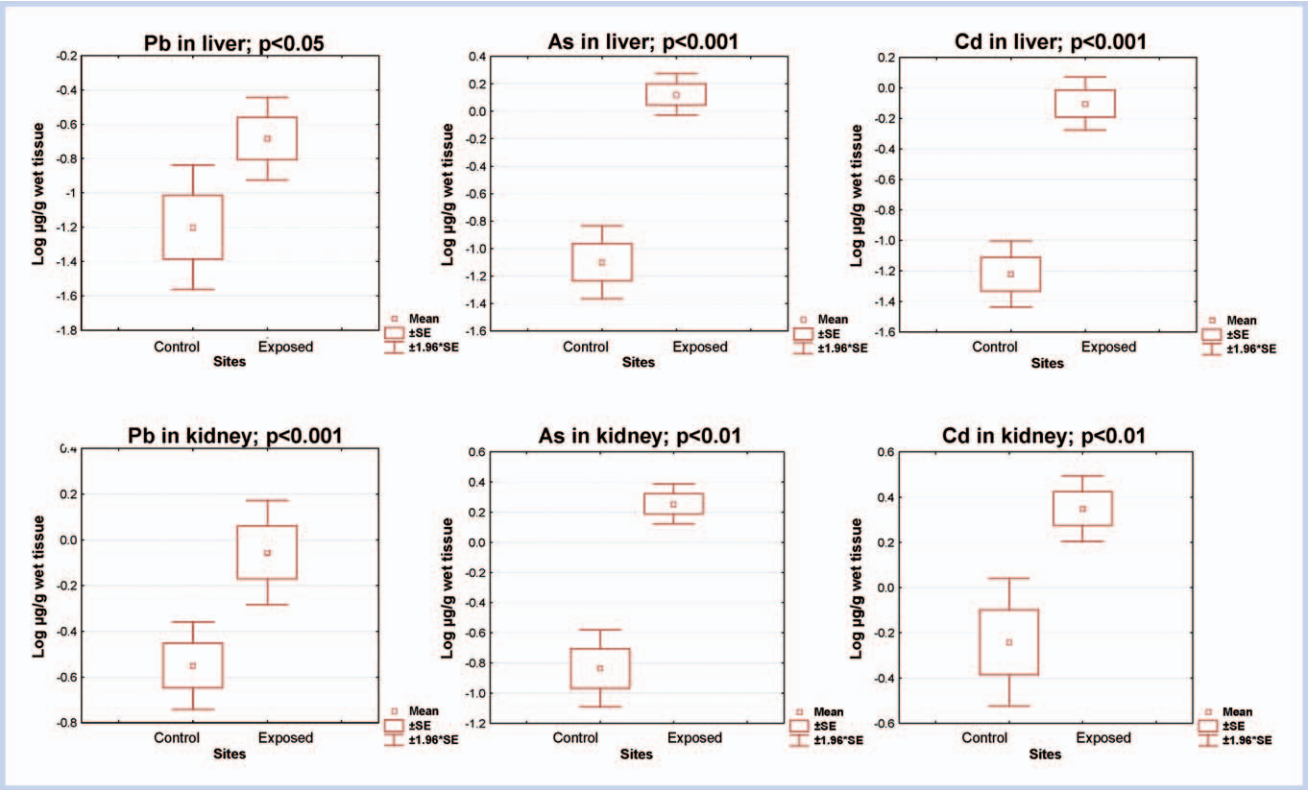

Figure 2. Concentration of lead, arsenic, and cadmium (μg/g wet tissue) in liver and kidney of rodents. Control site (Matehuala), exposed site (Villa de La Paz).

Table 3. Tail moment in comet cells of children[a]

Zone	*n*	G mean	SE	Min.	Max.	%>4
Villa de la Paz	20	4.8	0.3	2.6	8.9	75
Real de Minas	28	5.4	0.2	3.0	8.0	89
Matehuala[b]	12	3.9	0.2	3.1	5.9	42

[a] DNA damage was measured using the comet assay as described in *Methods* section. G mean = Geometric mean; SE = Standard error; Min. = minimum; Max. = maximum. %>4 = Percentage of children with a tail moment higher than 4.
[b] Different from Villa de la Paz ($p < 0.05$).

damage was observed in both children and rodents, as compared to control areas.

The results in children are similar to previous studies of our group, in which we have found a direct correlation between DNA damage and urinary arsenic (Yáñez et al. 2003). Furthermore, 3 studies have been published recently describing arsenic-induced DNA damage, either in vitro (Gómez et al. 2005) or in humans (Basu et al. 2005; Palus et al. 2005). Taking into account these antecedents, it is important to note that, although environmental levels (metal concentrations in soil) were different between Villa de la Paz and Real de Minas, the risk scenario was similar in these areas (urinary arsenic and tail moment in children were not statistically different in these communities and both were above the control area of Matehuala).

With regard to the results obtained in rodents, our results using the comet assay are comparable to previous reported findings in rodents captured in mining areas (Erry et al. 2000; Milton et al. 2003). However, our study differed from those previous reports in that we also determined metal concentrations both in tissues and in environmental samples (soil). Therefore, we were able to show a correlation with increased DNA damage in the most exposed animals.

Although arsenic-induced genotoxicity has been reported, it is important to take into account that other genotoxic metals were found at high concentrations in surface soil in the area of Villa de la Paz, such as lead (Valverde et al. 2002), cadmium (Valverde et al. 2000; Devi et al. 2001), copper

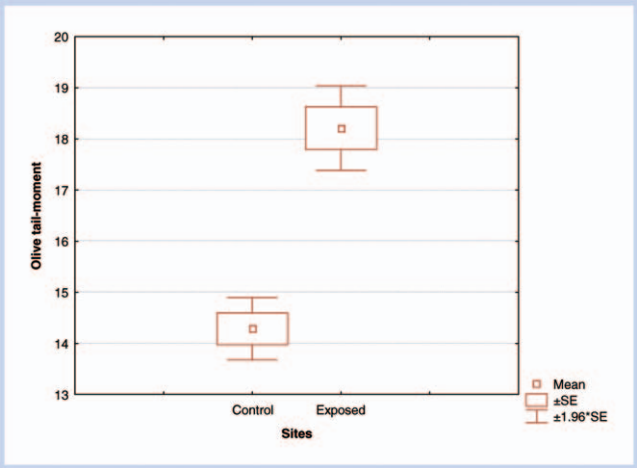

Figure 3. Olive tail moments in blood cells from rodents collected in the control and exposed areas ($p < 0.001$).

(Banu et al. 2001), and zinc (Banu et al. 2004). Therefore, more studies are needed in order to identify the toxicant(s) involved in the damage of DNA observed in children and rodents. For example, whereas the genotoxic species for copper and zinc are sulfates, in Villa de la Paz we found increased levels of oxides.

Results in children and rodents indicate a common pathway (i.e., soil ingestion). For children, soil ingestion is 1 of the most important pathways of exposure (USEPA 2002); whereas for rodents, soil/dust ingestion can be an accidental pathway, because the particles may be present on food items. Thus, considering that the objective of risk assessment is to support decision-making by determining risks of adverse effects in human and ecological receivers, the best risk reduction program in Villa de la Paz would be soil remediation.

Remediation programs in developing countries are difficult to establish due to social and economic limitations. Thus, justifications in terms of public health issues and/or preservation of natural resources (including biota protection) are the conclusions needed in any assessment of a polluted site. In this context, uncertainties have to be reduced as much as

Table 4. Metal levels in surface soil at sites where rodents were collected (mg/kg)[a]

Zone	Metal	*n*	G mean	SE	Min.	Max.
Arsenic	Villa de la Paz	15	6,230.7	1,099.9	813.5	16,450.0
	Control[b]	15	21.7	1.3	19.1	37.5
Lead	Villa de la Paz	15	1,192.3	70.1	528.0	1,704.0
	Control[b]	15	23.8	1.2	16.0	30.4
Cadmium	Villa de la Paz	42	28.4	4.4	4.0	125.4
	Control[b]	3	0.8	0.1	0.7	0.9
Copper	Villa de la Paz	15	703.7	226.3	297.5	1,047.5
	Control[b]	15	25.6	1.6	23.8	28.8
Zinc	Villa de la Paz	15	3,513.5	1,501.1	617.5	6,025.0
	Control[b]	15	87.3	43.9	58.8	192.5

[a] G mean = Gometric mean; SE = Standard error; Min. = minimum; Max. = maximum.
[b] Different from Villa de la Paz ($p < 0.05$).

possible. Therefore, an integrated approach can be an interesting tool for developing countries. In the case of Villa de la Paz, the data in children and rodents strengthened the weight of evidence about the health risks (in this case DNA damage) associated with exposure to contaminated soil. A remediation program thereby was easier to justify, and a feasibility study at this site is under way.

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