

Analyzing dried blood spots to monitor lead exposure in Calabria, the Southernmost region of Peninsular Italy

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ABSTRACT

Lead exposure in inhabitants of Calabria, the southernmost region of peninsular Italy, was monitored. Calabria has an area of 15,000 km² and two million inhabitants. Lead concentrations in anonymized residual dried blood spots (DBSs) from neonatal screening tests were measured using graphite furnace atomic absorption spectrophotometry (GF-AAS). DBSs were collected in the capitals of the Calabrian provinces (Catanzaro, Reggio Calabria, Cosenza, Crotona, and Vibo Valentia), Calabrian towns on the Tyrrhenian and Ionian coasts, and in the Sila and Serre-Aspromonte mountain areas. The median lead concentrations in the blood samples were 3.93 µg/dL in the Sila area, 3.32 µg/dL in the Tyrrhenian area, 2.91 µg/dL in Vibo Valentia, 2.76 µg/dL in the Ionian area, 2.69 µg/dL in Cosenza, 2.65 µg/dL in Catanzaro, 2.64 µg/dL in Crotona, 1.69 µg/dL in Reggio Calabria, and 1.52 µg/dL in the Aspromonte area. The lead concentrations were significantly higher ($P < 0.05$) in the Sila area than in the other locations except for the Tyrrhenian area ($P = 0.229$). The difference between the median lead concentrations in liquid whole blood samples from inhabitants of the Sila area (3.4 µg/dL) and Reggio Calabria (2.2 µg/dL) was statistically significant ($P = 0.002$), and the very different lead concentrations that were found in drinking water from the Sila (0.115 µg/L) and Serre-Aspromonte areas (0.016 µg/L) were consistent with the lead concentrations in the DBSs and the geochemical distribution of lead in soils in the region. The results of this study confirmed that analyzing residual DBSs from neonatal screening tests by GF-AAS is a useful way of monitoring lead exposure in a region.

Keywords: Lead, Environmental exposure, Dried blood spot, Calabria region.

1. Introduction

Filter paper was first used as a simple support for the collection and transportation of blood samples in the early 1980s, to monitor lead exposure in a remote Himalayan population (Piomelli et al, 1980). Analyzing lead in residual dried blood spots (DBSs) from neonatal screening tests to determine the exposure of children to lead has been proposed because these DBSs are a convenient source of blood samples, and because analyzing lead in these samples would allow the exposure of different human populations to lead in the environment to be assessed (Morrow et al, 1994).

Monitoring the exposure of humans to lead using DBSs requires a simple, rapid, precise, and accurate analytical procedure. Proficiency testing programs have confirmed the accuracy of methods in which lead is analyzed in whole blood collected on filter paper (Stanton & Maney, 1999). However, criticisms have been made of the use of DBSs as an alternative to determining lead in liquid blood when screening lead in children, mainly because low lead

recoveries have been found when filter papers containing DBSs have been extracted, because poor reproducibility can be caused by differences in hematocrit levels, and because contamination can occur during the collection process (Moyer et al, 1999; Verebey, 2000; Moyer et al, 2000). However, measuring lead concentrations in DBSs that have been stored for 4 weeks was shown to be appropriate for determining lead concentrations in blood sampled from a large number of Chinese children (Shen et al, 2003). Analytical techniques in which lead can be determined in solid samples have been shown to be effective for measuring lead in DBSs by GF-AAS (Resano et al, 2007), inductively coupled plasma-mass spectrometry (Chaudhuri et al, 2008; Funk et al, 2013), and laser ablation inductively coupled plasma-time of flight mass spectrometry have been found to be appropriate techniques for such measurements (Cizdziel, 2007).

We have developed for the quantitative liquid extraction of lead from old DBSs stored at room temperature (Di Martino et al, 2004). This stimulated us to measure lead, using GF-AAS, in DBSs left over from the neonatal screening test, to allow us to monitor the exposure of humans to lead in the environment. The mothers of the subjects of the study lived in five provincial capitals and four different areas in Calabria, which is the southernmost region in the Italian peninsula, covering 15,000 km² and having about two million inhabitants.

The highest lead concentrations were unexpectedly found in DBSs from subjects from a mountainous and heavily forested area that has clean air, and these high concentrations were confirmed by determining lead in liquid whole blood and drinking water from that area. The high lead concentrations were associated with high lead concentrations in the soil in the area, which were taken from a geochemical map of the region.

2. Materials and methods

2.1 Dried blood spots

Lead concentrations were measured in anonymized DBS samples that were left over from neonatal screening tests and had been stored at room temperature for 3 months or more. The blood samples were taken between 48 h and 6 days after birth, following neonatal screening guidelines. The DBSs came from newborns whose mothers were resident in the Calabrian provincial capitals of Reggio Calabria (RC), Catanzaro (CZ), Cosenza (CS), Crotona (KR), and Vibo Valentia (VV), and from small towns or villages in four areas, Sila (Sa) and Serre-Aspromonte (S-Aa) (which are both mountainous areas in the interior of Calabria) and Tyrrhenian (Ta) and Ionian (Ia) coastal areas (on the slopes leading from the hills and mountains to the Tyrrhenian and Ionian seas, respectively) (Figure 1).

A total of 130 DBSs from each area were tested. The sample size was chosen so that significant differences between the median lead concentrations in the blanks and samples could be detected with a confidence level of more than 0.9. The mean age of the mothers was 31 years, which matches the mean age of mothers in Italy as a whole. Lead from the DBSs was extracted and measured according to our previous published procedure (Di Martino et al, 2004).

The lead concentrations in the ES blanks and DBSs were calculated from the equation for the straight line calibration plot ($y = a + bx$, where y is the absorbance, x is the concentration, b is the slope, and a is the intercept). To determine the lead concentration in the blood, the difference between the lead concentration in the DBS extract and its blank was multiplied by

the dilution factor (150 μ L divided by the volume of blood absorbed by the 6.3 mm disk). The volume of blood absorbed by the 6.3 mm disk was measured using a procedure that has been described previously; the accuracy of this procedure has been verified by comparing the lead concentrations in whole blood measured in the form of the liquid and as a dried spot sample (Di Martino et al, 2004). The lead concentrations in the blanks and samples that were above the limit of detection (LOD) were considered, and the median lead concentration was determined from these values for each group (town, area, or city).



Figure 1: Map of Calabria towns (●) and area (-) were the mothers of the screened newborn resided

The precision and accuracy of each analytical run was assessed by performing duplicate analyses of a known reference control (Serorm Trace Elements and Whole Blood; Nycomed Pharma AS, Oslo, Norway) diluted tenfold with ES at the start and end of each run and after every 10 samples. The expected concentration range for lead in the control was 0.31–0.39 μ g/dL.

2.2 Measuring lead concentrations in liquid whole blood

The lead concentrations were measured in anonymized liquid whole blood (LWB) samples taken from 130 adult residents of RC and another 130 adult residents of Sa. These samples were leftover EDTA-treated blood samples that were originally taken for hematological tests performed by laboratories in public hospitals. The mean age of the subjects was 50 ± 10 years and the female: male ratio was 2:1 for both groups of subjects. The number of LWB samples taken was chosen to match the number of DBSs that were used (from the same areas as the mothers of the newborns lived in during the gestation period) so that the LWB and DBS populations matched as closely as possible. It took about 3 months to collect the liquid blood samples from the sparsely populated Sa area because of the small number of patients from

this area that attended the public hospitals. The LWB samples from RC were collected from the largest public hospital (Ospedali Riuniti Melacrino-Morelli) in 1 day. The blood samples were collected in lead-free plastic tubes and stored at $-80\text{ }^{\circ}\text{C}$ until they were analyzed.

The lead concentrations in the LWB samples were determined using the standard addition method using a Varian Spectra AA-220Z atomic absorption spectrometer equipped with a Zeeman background corrector and an auto-sampler. A $100\text{-}\mu\text{L}$ aliquot of a whole blood sample was added to $900\text{ }\mu\text{L}$ of 0.05% (v/v) aqueous Triton X100 and the lead concentration in that mixture was determined. An aqueous solution of 0.05 mg/L Pb in 0.1% (v/v) HNO_3 and 0.05% (v/v) aqueous Triton X100 was prepared from a 1.010 g/L lead standard solution (Aldrich) for use preparing the calibration plot. The LOD and the limit of quantitation (LOQ) were $0.22\text{ }\mu\text{g/dL}$ and $0.75\text{ }\mu\text{g/dL}$, respectively.

2.3 Measuring lead in drinking water

Drinking water samples (500 mL) were collected in lead-free polypropylene bottles after the bottles had been flushed with the running water for 3 min. Samples were collected from 18 villages and towns in the Sa area (45 samples) and the S-Aa area (34 samples). When several samples were collected from the same town or village, the mean concentration was reported. The lead concentrations in the drinking water samples were analyzed using a Varian Spectra AA-220Z spectrometer. Although GF-AAS is a very sensitive analytical technique, determining low concentrations of lead in a water sample requires a pre-concentration step in which a cationic exchange resin is used (Facundo et al, 1997). We used the Chelex-100 chelating resin to pre-concentrate the drinking water samples. Before use, 10 g of the Chelex-100 resin was washed four times with 10 mL of 5 M HNO_3 and three times with 10 mL of double-glass-distilled water, then it was equilibrated in 100 mL of $1\text{ M CH}_3\text{COONH}_4$ buffer at $\text{pH } 5.2$. A 2-mL aliquot of the resin suspension was centrifuged for 1 min at 1600 rpm in a 15-mL polypropylene tube, then the supernatant was removed and a 10-mL aliquot of the drinking water sample was added and the tube was centrifuged. The supernatant was removed and another 10-mL aliquot of the drinking water sample was added, and this process was repeated until five aliquots had been extracted. The lead was recovered from the resin by adding 0.6 mL of 2 M HNO_3 , vortexing the mixture for a few seconds, centrifuging the tube, and removing the supernatant.

Two separate recovery measurements were made to determine the capacity of the resin. A 10-mL aliquot of an aqueous solution containing either 10 ng or 2.5 ng of lead was added to 2 g of the resin suspension (as used to pre-concentrate the drinking water samples). When the resin was washed with 0.6 mL of 2 M HNO_3 , 8.1 ng of lead was recovered from the 10 ng test and 2.6 ng of lead was recovered from the 2.5 ng test. The capacity of the Chelex-100 was higher than the amount of lead in 50 mL of any of the drinking water samples that were analyzed.

Any lead eluted with 0.6 mL of 2 M HNO_3 after the resin had been treated with double-glass-distilled water was assumed to come from contamination in the eluting solution and was subtracted from the lead concentrations found in the pre-concentrated drinking water samples and the aqueous standard solutions. Standard solutions for building the calibration plot were prepared by diluting a 1.010 g/L Pb aqueous stock standard solution (Aldrich). The LOD and LOQ were $0.006\text{ }\mu\text{g/L}$ and $0.02\text{ }\mu\text{g/L}$, respectively. The Chelex-100 resin was purchased from Bio-Rad Laboratories (Richmond, CA, USA). All of the reagents were of analytical grade. Solutions were prepared using double-glass-distilled water.

2.4 Statistical analysis and calculations

The linear regression of the daily calibration plot was used to determine the correlation coefficient (R) and the LOD was defined as $3S_{yx}/b$, where S_{yx} is the standard error of the estimate and b is the slope of the calibration plot (Q2B ICH, 1996).

Once the analytical runs for each group were completed, the Mann–Whitney rank sum test, which was performed if the normality (Student’s t-test) failed was used to determine the statistical significance (P) of the difference between the median absorbance values found for the DBSs and their respective blanks. The same test was used to determine the P of the difference between the median lead concentrations in the blanks (background) and the DBS extracts.

The Kruskal–Wallis one-way analysis of variance ranks test was used to determine if the differences between the median lead concentrations found for the sample groups were statistically significant. The Dunn’s test was then used to compare the concentrations found in the samples from all of the groups at the same time.

The LODs and LOQs for the drinking water and LWB samples were defined as $x_m + 3sd$ and $x_m + 10sd$, respectively, where x_m is the mean and sd is the standard deviation of the lead concentrations measured in 20 aliquots of double-glass-distilled water after the appropriate pre-concentration procedure had been performed. The mean and the standard deviation of the lead concentrations in the drinking water samples were calculated because the concentrations were normally distributed according to the Student’s t-test.

The SigmaStat 3.1 program (Systat Software, Inc., San Jose, CA, USA) was used to perform the linear regressions and normality tests and to determine the linear coefficients, errors in the estimates, sample size and descriptive statistics. The lead concentrations were calculated from the calibration line equations using Microsoft Excel.

3. Results

3.1 Statistical differences between the lead concentrations in the blanks and samples

Table 1 shows (a) the median lead absorbance (at 283.3 nm) values for the DBS sample and matching blank extracts, and (b) the statistical significances of the differences between the median values, which were based on the Mann–Whitney rank sum test (which was performed because the normality test failed). The difference between the median absorbance values found for the blanks and the samples was statistically significant ($P < 0.001$) for all of the groups.

Table 1: Median absorbance values (at 283.3 nm) of the blank and dried blood spot (DBS) samples, and the statistical significances of the differences

Sample location	Blanks	Samples (DBSs)	Statistical significance (P)
Sila (Sa)	0.023	0.048	<0.001
Vibo Valentia (VV)	0.027	0.044	<0.001
Tyrrhenian Area (Ta)	0.033	0.051	<0.001
Ionian Area (Ia)	0.021	0.035	<0.001

Cosenza (CS)	0.040	0.054	<0.001
Catanzaro (CZ)	0.032	0.045	<0.001
Crotone (KR)	0.056	0.068	<0.001
Serre-Aspromonte (S-Aa)	0.021	0.032	<0.001
Reggio Calabria (RC)	0.031	0.042	<0.001

A blank was an extract of a disk punched from near the DBS. The same extraction solution was used for the blanks as was used for the DBSs. The sample size for each group of samples and blanks was 130. Table 2 shows the lowest and highest LODs found for the analytical runs of each group of 130 DBSs, the percentage of lead concentrations that were higher than the LOD (U.S EPA, 2002)), the median lead concentrations in the sample and matching blank extracts, and the statistical significances of the differences between the median lead concentrations in the sample and blank extracts. The mean R value (and standard deviation) for all of the calibration plots was 0.995 ± 0.00624 , and the LODs were in the range 0.06–0.27 $\mu\text{g/dL}$ for all of the analytical runs.

Table 2: Median lead concentrations in the dried blood spot (DBS) and blank samples, the limits of detection (LODs), and the percentage of the samples that contained a detectable lead concentration

Sample location	Blank and DBS samples	LOD min–max $\mu\text{g/dL}$	% ^a	Median value $\mu\text{g/dL}$
Sila (Sa)	blank DBS	0.06–0.20	70	0.32
			94	0.59
ViboValentia (VV)	blank DBS	0.13–0.27	92	0.30
			100	0.51
Tyrrhenian Area (Ta)	blank DBS	0.09–0.19	93	0.37
			99	0.60
Ionian Area (Ia)	blank DBS	0.08–0.19	82	0.27
			94	0.43
Cosenza (CS)	blank DBS	0.14–0.24	98	0.45
			100	0.62
Catanzaro (CZ)	blank DBS	0.17–0.20	99	0.34
			100	0.48
Crotone (KR)	blank DBS	0.12–0.25	99	0.65
			100	0.78
Serre Aspromonte (S-Aa)	blank DBS	0.08–0.16	82	0.27
			95	0.40
Reggio Calabria (RC)	blank DBS	0.16–0.20	84	0.35
			97	0.44

a = percentage of blank and DBS samples with detectable lead concentrations ($\geq\text{LOD}$). The differences between the medians were statistical significant for each group according to the Mann–Whitney rank sum test.

3.2 Lead concentrations in the DBSs

Table 3 shows the median and the 25th and 75th percentiles of the lead concentration distribution in the DBSs from each group. The highest median lead concentrations were measured in samples from newborns with mothers who lived in Sa (3.93 $\mu\text{g/dL}$) and Ta (3.32 $\mu\text{g/dL}$). The Dunn’s test indicated that the median lead concentration in the DBSs from

newborns from Sa was significantly different ($P < 0.05$) from the median in the DBSs from newborns from the other areas except for from Ta. The Mann–Whitney test indicated that the difference between the median lead concentrations in the DBSs from Sa and Ta was not statistically significant ($P=0.229$). According to Dunn’s test, the median lead concentrations in the DBSs from KR, CZ, VV, CS, and Ia were in the range of 2.64–2.91 $\mu\text{g/dL}$ and were not statistically different, but the median lead concentrations in the DBSs from RC (1.69 $\mu\text{g/dL}$) and S-Aa (1.52 $\mu\text{g/dL}$) were statistically significantly lower.

Table 3: Lead concentration distribution in the dried blood spot samples from different towns and areas

Location	25th	Median value ($\mu\text{g/dL}$)	75th	P
Sila (Sa)	2.09	3.93	7.04	<0.05
ViboValentia (VV)	1.02	2.91	4.68	<0.05
Tyrrhenian Area (Ta)	1.89	3.32	5.91	0.229*
Ionian Area (Ia)	1.32	2.76	4.68	<0.05
Cosenza (CS)	1.52	2.69	4.81	<0.05
Catanzaro (CZ)	1.41	2.65	3.96	<0.05
Crotone (KR)	1.48	2.64	4.45	<0.05
Serre Aspromonte (S-Aa)	0.75	1.52	3.02	<0.05
Reggio Calabria (RC)	0.84	1.69	3.18	<0.05

P = the statistical significance of the difference between the median value for the Sila area and the other locations; The Dunn’s test indicated that the median lead concentration in the blood samples from newborns from the Sila area was statistically significantly different ($P < 0.05$) from the median lead concentrations in the blood samples from the other areas except for the Tyrrhenian area; *according to the Mann-Whitney rank sum test.

3.3 Lead concentrations in liquid whole blood and drinking water

The high lead concentrations found in the DBSs from newborns whose mothers lived in Sa (a mountainous area with a low population density and very clean air because of the forests in the area and a lack of industry) needed to be confirmed by measuring lead concentrations in LWB and drinking water samples. Table 4 shows the results of these measurements.

Table 4: Lead concentrations in liquid whole blood samples (median values) and drinking water samples (mean values)

Locations	No. of liquid blood samples	Median value ($\mu\text{g/dL}$)	P_{1-2}	No. water samples	Mean values ($\mu\text{g/L}$)
1- Sila (Sa)	130	3.41	<0.003	45	0.115
2-Reggio Calabria (RC)	130	2.18	--	---	---
3-Serre–Aspromonte (S-Aa)	--	--	--	34	0.016

The limits of detection and quantification for lead in liquid whole blood were 0.22 $\mu\text{g/dL}$ and 0.75 $\mu\text{g/dL}$, respectively, and the limits of detection and quantification for lead in drinking water were $5.67 \times 10^{-3} \mu\text{g/L}$ and $18.9 \times 10^{-3} \mu\text{g/L}$, respectively. The lead concentrations in the LWB samples from Sa were compared with the lead concentrations in the LWB samples from RC (from where the DBSs with the lowest median lead concentrations, 1.69 $\mu\text{g/dL}$,

were obtained). The lead concentrations were higher in the LWB samples from Sa than in the LWB samples from RC, and the median lead concentrations were 3.41 $\mu\text{g/dL}$ in Sa and 2.18 $\mu\text{g/L}$ in RC. The Mann–Whitney test indicated that the difference between the median concentrations was larger than would be expected by chance ($P=0.002$), although the ratio between the medians was lower for the LWB samples than for the DBSs.

To identify a possible cause for higher lead concentrations being present in human blood from Sa than from other areas, lead concentrations in drinking water from Sa and S-Aa (where the lowest lead concentrations in the DBSs were found) were measured. The mean lead concentration in the drinking water from Sa was 0.115 $\mu\text{g/L}$, which was considerably higher than the LOQ (0.019 $\mu\text{g/L}$), and the mean lead concentration in the drinking water from S-Aa was 0.016 $\mu\text{g/L}$, which was between the LOD (0.006 $\mu\text{g/L}$) and the LOQ (0.02 $\mu\text{g/L}$).

4. Discussion

The results shown in Table 1 were important to the continuation of the study, because they indicated that the background lead concentrations had no effect on the measurements of the lead concentration in the DBSs. The difference between the median absorbance values for the samples and the matching blanks was statistically significant for all of the groups. We concluded that the paper itself, the disk punching process, and the blood collection process had not adversely contributed to the lead concentrations measured in the DBSs. The R values for all of the analytical runs were in the range of 0.990–0.998, indicating that the calibration plots were strongly linear over the selected concentration range.

The LODs were in the range of 0.06–0.27 $\mu\text{g/dL}$, which we considered to be acceptable. The variability in the LOD over time in each GF-AAS analytical run depended on many factors, as it does in gas chromatography-mass spectroscopy methods (Lawson, 1994). The LOD in GF-AAS measurements could be affected by the furnace status, slit width, lamp current, electron multiplier voltage, and the sample introduction method. Similar LODs to those found in this study have been reported previously: 0.1 $\mu\text{g/dL}$ (Louis et al, 2003) and 0.09 $\mu\text{g/dL}$ (Castelli et al, 2005); LODs in the range 0.05–0.83 $\mu\text{g/dL}$ were found in a multi-center study involving 12 laboratories (Olichon et al, 2007). Similar LODs were also found in a pilot study using inductively coupled plasma mass spectrometry (Chaudhuri et al, 2009), and other results found in that study were also similar to the results of our study (for example, the lead concentrations in filter papers were in the range of 0.32–0.82 $\mu\text{g/dL}$, which is similar to the range found in our study, 0.27–0.65 $\mu\text{g/dL}$).

In our study, the median blank concentration (0.65 $\mu\text{g/dL}$) and the median sample concentration (0.78 $\mu\text{g/dL}$) were higher for the DBSs from KR than for the DBSs from the other areas, but the medians were statistically significantly different for the DBSs from all of the areas. This further confirms that the background lead concentration did not affect the lead concentrations measured in the DBSs.

The relatively high lead concentrations found in the blank samples for the DBSs from KR were not due to difference between lots of paper, because these were the same for all groups. Therefore, it appears to be more likely that the relatively high lead concentrations found in the blank samples for the DBSs from KR probably were by the paper being exposed to relatively high lead concentrations in the environment where they were used or stored in. It probably that the contamination of samples was because of exposure to relatively high lead

concentrations in the environment according to an epidemiological study of respiratory pathology (Frega et al, 2000). The higher lead concentrations in the DBSs from Sa (3.93 µg/dL) than from RC (1.69 µg/dL) were mirrored in higher lead concentrations being found in the LWB samples from those areas (3.41 µg/dL and 2.18 µg/dL for Sa and RC, respectively). The relatively high lead concentrations in the DBSs from Sa were also mirrored by there being higher lead concentrations in drinking water from Sa (0.115µg/L) than from S-Aa (0.0165 µg/L).

The lead concentrations were lower in the DBSs from S-Aa than from the other areas. The median lead concentrations were 3.93 µg/dL in the neonatal DBSs and 3.41 µg/dL in the adult LWBs from Sa, and these concentrations were similar to lead concentrations that have been found in blood samples from 103 children and adults living in the foothills of the Himalayas (Piomelli et al, 1980). The authors of that study indicated that the relatively high lead concentrations found in the blood samples were caused by the residents of that area being exposed to a natural source of lead.

It is also worth mentioning that the lowest median lead concentrations in our study, which were 1.69 µg/dL in the DBSs and 2.18 µg/dL in the LWBs from RC, were almost identical to the mean lead concentrations found in umbilical cords (1.69 µg/dL) and maternal blood (2.37 µg/dL) in a survey conducted in Istanbul (Furman et al, 2001). The authors of that survey concluded that a decrease in the lead concentrations in the blood samples compared with the concentrations, that had been found in previous years was caused by the decrease in the use of lead in gasoline.

We suggest that the soil or other types of geographically specific exposure probably play a role in controlling lead concentrations in blood in Sa. In our study, the lead concentrations in the DBSs from the other towns were in range 2.64–2.91 µg/dL, and these were significantly higher ($P < 0.001$) than in the DBSs from RC (1.69 µg/dL). Lead has not been added to gasoline in Italy since 1 January 2001, after being gradually removed since 1994 (EC/Directive 98/70/EC). Lead concentrations (2.53 ± 0.114 µg/dL) in blood from healthy adult women aged under 45 years from the urban area of Rome, (Alimonti et al, 2005) were comparable to the median lead concentration in the DBSs from Calabria, except for from RC, found in our study. A median lead concentration of 1.62 µg/L was found in a Brazilian study of blood from women in the process of delivering a child (Rudge et al, 2011), and this was comparable to the lead concentrations found in the DBSs from RC in our study.

The relatively high number of DBSs from S-Aa (a mountainous area) that had lead concentrations below the LOD was associated with the low lead concentrations in drinking water in that area. Drinking water may be an important source of lead to humans. The low lead concentrations in the DBSs from RC and S-Aa and the high lead concentrations in the DBSs from Ta and Sa were probably caused by the different lead concentrations in the soil in those areas, soil being another important source of human exposure to lead (Mielke et al, 1989). This hypothesis was strengthened by data from the geochemical map of lead concentrations in stream sediments in Calabria (De Vivo et al, 1988).

The geochemical map is shown in Figure 2, with different colors representing different lead concentrations in the soil. The small towns in the Ta and Sa areas from which the DBSs with the highest lead concentrations ($3.32 \mu\text{g/dL}$ in Ta and $3.93 \mu\text{g/dL}$ in Sa, the difference not being statistically significant) came are in areas where the lead concentrations in the soil are higher, at 23–356 ppm (ppm=mg/kg), than the median value for Calabria. In contrast, the blood samples from both newborns and adults from RC contained lower lead concentrations than the samples from Sa contained, and RC is in an area where lead concentrations in soils are the lowest in Calabria (0–15 ppm).

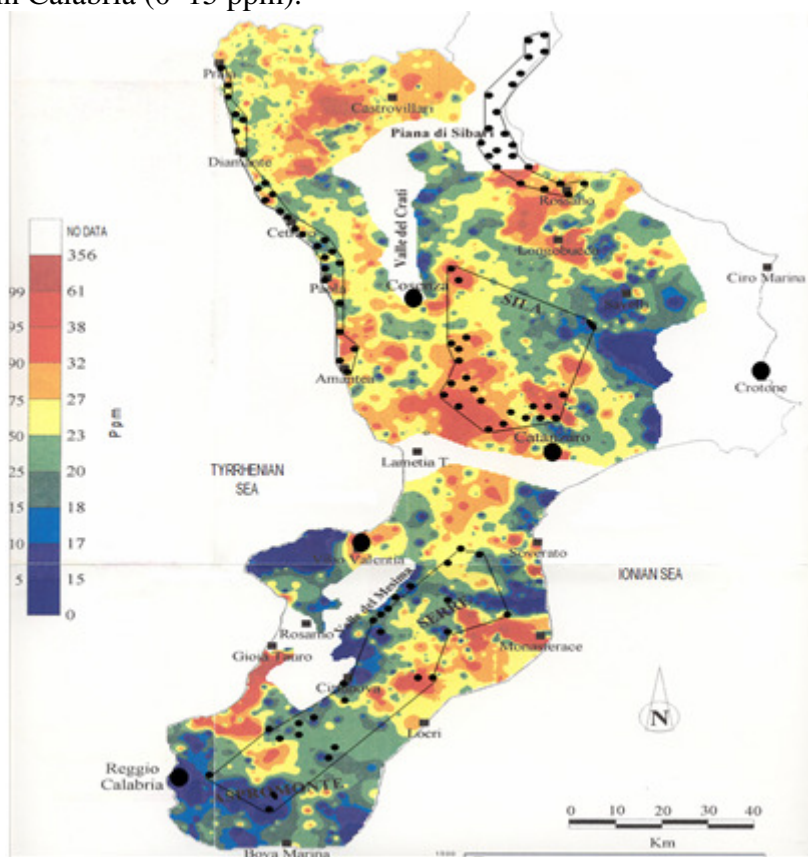


Figure 2: Geological map of lead distribution soil (adopted from De Vivo et al., 1998)
Towns (●) and area (-) were the mothers of the screened newborn resided

The lead concentrations being lower in the DBSs from S-Aa than from the other areas and the low lead concentrations in the drinking water samples from S-Aa are consistent with the fact that most towns and villages in S-Aa have lead concentrations of 0–23 ppm in soils, and these concentrations are lower than the median for Calabria. Seventeen of the DBSs came from four small towns in S-Aa that have lead concentrations in soils in the range of 23–38 ppm, while the remaining 86.9% of the DBSs from S-Aa were from 23 towns or villages with lead concentrations in soils in the range of 0–23 ppm. Lead was not measured in drinking water samples from the four towns in S-Aa that had relatively high lead concentrations in soils. The DBSs from these four towns contained lead concentrations that were at the higher end of the distribution that was found in the study. The intermediate lead concentrations found in the DBSs from CZ, VV, and CS were consistent with these being areas where the lead concentrations in soils are in the range of 23–38 ppm. No geochemical data were available for KR and Ia.

Lead concentrations of 0.1 – $9.92 \mu\text{g/kg}$ have been found in whole bovine milk from 40 dairy farms in unspecified areas in Calabria (Licata et al, 2004). The authors of that study were

surprised to find such high (but not dangerous) lead concentrations in milk from Calabria, because there is relatively little industrial activity in this region. The authors ignored the presence of lead in the soil, and assumed that the relatively high lead concentrations were probably caused by pesticide use, contaminated fodder, transhumance along roads, and climatic factors, such as wind blowing contaminated particles into the area. We believe that the dairy farms with the high lead concentrations in milk were probably in the Ta or Sa areas because these areas have relatively high lead concentrations in soils and because we found relatively high lead concentrations in the DBSs from these areas.

It is worth noting that the highest lead concentrations in the blood samples from Sa were very much lower than the critical value for lead poisoning ($> 10 \mu\text{g/dL}$) in children (CDC DHHS, 1991) and the highest lead concentrations in the drinking water samples ($0.115 \mu\text{g/L}$) were much lower than the median lead concentration ($4.8 \mu\text{g/L}$) that was found in drinking water over a 1-week sampling period in Ontario, Canada (DNHW, 1992).

5. Conclusions

The lead concentrations measured in the DBSs showed that the exposure of residents of urban areas in Calabria to lead is comparable to the exposure of populations outside Calabria (e.g., in Istanbul, Rome, and Brazilian towns). The relatively high lead concentrations found in the blood samples from inhabitants of the mountainous Sa area were similar to the concentrations found in blood samples from the inhabitants of an area in the foothills of the Himalayas. The high lead concentrations in the blood samples from the inhabitants of Sa and in drinking water samples from Sa (which is a sparsely populated, heavily wooded, and mountainous area) were associated with relatively high lead concentrations (according to a geochemical map) in soils in that area. Additionally, this study shows that residual DBSs from neonatal screening programs are a useful source of samples for surveys of lead exposure in human populations, with liquid extracts of the samples being analyzed by GF-AAS.

6. References

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