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Environmental health aspects of lead

Die gesundheitlichen Aspekte der Umweltverschmutzung durch Blei

Les problèmes sanitaires posés par le plomb présent dans l'environnement

ENVIRONMENTAL LEAD CONTAMINATION OF MEZA VALLEY
- STUDY ON LEAD EXPOSURE OF POPULATION -

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Summary:

The lead-zinc mines "Mežica" are exploited for 300 years. In 1896 in Žerjav a modern smelting plant was constructed producing a considerable lead contamination of the topographically closed, long valley of river Meža. Therefore some thousands of inhabitants are long-life exposed to lead for many generations.

From 1968 a broad ecological and epidemiological study has been developed on professionally non-exposed population, mostly women and children. For detailed study 286 persons has been selected, together with 52 controls. Medical, biochemical, toxicological, hematological and genetic investigations has been performed.

Many tests for exposure evaluation are applied: determination of ALA, Pb, copro, and uro, PBG in the urine and ALA-D in the blood. Determination of ALA represents the most suitable exposure test in this case.

To establish the integral exposure lead mobilization by CaEDTA injection was performed in 209 persons. It was established that lead body burden in exposed is up to 10 times higher than in non-exposed. Hematologic findings (Hb,Er,RTC,RSC) showed moderate changes in RTC and RSC.

In 15 randomly selected persons, 13 showed some kind of chromosome aberrations in the lymphocytes of blood, which seems to be characteristic for lead effects.

Physical findings did not differ from the control except high percentage of hypertension.

Applying mentioned methods on exposed population in the valley it was not possible to reveal any expressed clinical signs of toxic effects of lead. The most impressive changes are established on the enzyme system of porphyrin biosynthesis, chromosome aberrations and lead body burden.

The lead-zinc mines "Mežica" are in exploatation from 1665, located in the mountains of Slovenia (Yugoslavia) around the valley of river Meža. In this topographycally closed long valley the first smelter was built in Žerjav (picture 1) in 1746. This smelter was modernized in 1896 producing now about 22 000 t. of lead and 11 000 t. of zinc annually/1/. Two chimneys of the smelter are emitting more than 200 t. of lead aerosols annually producing a heavy contamination of the environment in the valley. All settlements (Mežica,Žerjav,Rudarjevo,Črna,Pristava) are located in this narrow valley along the river Meža. Geographic and topographic situation is favourable for spreading of lead aerosols in north or south-west direction, depending on the wind direction. Around settlements and on hills the farmer's houses are located on various height.

At end of 1967, together with the local experts, we started a broad ecologic and epidemiologic study on environmental lead contamination of Meža valley. First reports on lead in air, water /2/, soil /3,4/, plants /3,5/, food /6/ revealed enormous environmental lead contamination of Meža valley in comparison with a control area, about 40 km. away. As an illustration we are presenting lead contamination of the soil in the valley /4/ on picture 2.

Taking into consideration the results obtained by ecologic study it was necessary to perform an epidemiologic study on population exposed to lead for many generations (from 1896) and for whole life. The aim of the epidemiologic study was:

1/ to establish if the population is exposed to lead
2/ to establish the level of the exposure in relation to the geographic location to the source of contamination
3/ to establish the integral lead exposure (lead body burden) from the birth
4/ to establish by medical, biochemical and toxicologic methods the possible effects of lead exposure.

Methods and material.

To establish the degree of lead exposure in 1968 a determination of ALA in the urine of 912 inhabitants, professionally non-exposed to lead, was performed /2/. In 559 inhabitants (61.3 %) ALA values were over 10 mg/l. In such a way the increased lead exposure was evident. From 559 inhabitants 286 persons has been randomly selected for further detailed investigation. Basic data on age and sex of this group is presented on Table 1.

On this group following investigations has been performed:

in urine: determination of ALA, copro- and uroporphyrin, porphobilinogen, lead
in blood: determination of ALA-dehydrase (ALA-D), lead, haemoglobin, erythrocytes, retyculocytes, red stippled cells.

The lead body burden was determinated by analysis of lead excretion after CA-EDTA injection in the urine.

The same procedures has been performed on 52 persons from the control group selected in the non-contaminated area, about 40 km. far. Lead body burden determination was not performed in the control group.

To establish the morbidity a great attention was paid to the personal and family anamnesis. A detailed questionaire contained all data on all complaints, duration of exposure, way of life, proveniency of the food (contaminated or non-contaminated area) etc. For children a heteroamnesis from parents has been taken.

Unfortunately, it was not possible to perform all mentioned analyses in the 100 % of the selected group due to many reasons: sampling and analyses has been performed on the spot, mostly in improvised ambulances and laboratories connected with many technical difficulties. Some persons did not accept venepunction or brought inadequate urine samle. Also we did not perform venepunction in children till 8 years. In spite of great difficulties in the field a high percentage of results was obtained taking into consideration such conditions.

Results.

The results of ALA determination in 912 inhabitants performed in 1968 have been published /2/. In that time ALA values were between 0.7-44 mg/l and mean value were over 10 mg/l in all settlements. On graph 1 the mean values (with standard deviation) of the selected group from 1968 are presented. We mentioned that in this group only persons with ALA values over 10 mg/l has been selected.

Next year this analysis was repeated on 273 persons (95,4 %). On graph 2 the values from 1968 are compered with that from 1969. A significant decrease of ALA values are evident due to the fact that in January 1969 a "Lurgi" filter was installed on one of the two chimneys, emitting lead aerosols. But individual values are different from case to case, even in the members of the same family. Taking into consideration values obtained in various parts of Žerjav (zone I,II,III) it is evident that statistically significant difference exists between I and III (t-2,13, $p < 0.05$) which could be explained by topographic differences to the source of pollution.

On Table 2 mean ALA values 1968/69 are presented in relation to sex and age of examined persons.

The activity of ALA-D in the blood was determined in 220 persons (88 %) in 1969. The decreased number of analysis is due to the mentioned fact that we avoided the venepuncture in children under 8 years. On Table 3 obtained results of ALA-D determination are presented. The decreased activity of ALA-D (under 70 units/ccm er.) is evident in 200 persons, or 90.9 % of examined group. In the control group only 6.6 % (from 44 persons) showed such decrease. Therefore, the difference between the exposed and control

group is very significant (t - 12.2). Detailed examination of results reveals that relatively the highest percentage of the decrease could be found in children (8-15 years old) -64 (95.5 %). In women 90 % are showing the decrease and 86.7 % of men. On the other hand, these differences are not statistically significant.

Taking into consideration the groups from various settlements, it is of interest to establish that 100 % of farmers are showing decrease of ALA-D activity, 96.7 % of inhabitants examined in Črna, 92.5 % examined in Žerjav, and only 71.7 % from Polena. These data are presented on Graph 3, as mean values for groups in various settlements.

From these data it is evident that in all areas mean ALA-D values are decreased absolutely (under 70 units/ccm er.) and also in relation to the control group (mean ALA-D: 87.6 units/ccm er.). The mean ALA-D values are from 26.1 units/ccm er. for children to 31.1 units/ccm er. for men.

Coproporphyrin was determined in 255 persons (92.6 % from the observed group). The coproporphyrin values until 0.2 mg/l are accepted as normal. The obtained results are presented on Table 4. It is evident that increased copro-values are evident in 18 cases (6.7 %) and no one case was observed in the control group. The increased values are more frequent in men (12.7 %) and less frequent in women (3.6 %). But, the level of copro-values veries from 0.204 mg/l to 2.280 mg/l.

The spontaneous excretion of lead in the urine is normal in most cases (until 0.100 mg/l). Only in 6 adults and 6 children this excretion was slightly increased.

The uroporphyrin and porphobilinogen values will be published elsewhere.

Red stippled cells has been determined in 100 % of examined persons and results are presented on Table 5. We accepted appearance of 100 RSC, as upper normal limit. In 8.3 % of the exposed group this value was increased and in no one case of the control. This increase was in the limits from 150-1250, mostly 150-400 RSC. The frequency of the increase is highest in men (10.9 %), especially in Žerjav.

Obtained results are unexpected low, probably due to the decrease of lead concentration in air after installation of the filter (January 1969). This is suggested by similar decrease of ALA values too.

Retyculocytes has been determined in 100 % of examined persons, accepting 1 RTC on 100 erythrocytes as upper normal limit. Obtained results are presented on Table 6, showing increased values in 34.9 % of exposed group and 5.7 % of the control group. This difference is statistically significant (t-4.17).

The highest frequency of RTC increase appears in children: 55.5 % in pre-school and 38 % in pupils. The frequency in women is 34.2 % and in men 18.1 %. The statistical evaluation is showing significant difference in RTC increase frequency between pre-school children and women (t- - 2.29), between women and men (t - 2.19).

Hemoglobin has been determined in 100 % of examined persons and results are presented on Table 7. By statistical evaluation it was established that hemoglobin was not decreased in adults of the exposed group in relation to the control group. The exposed pre-school children showed the lowest values, but in the control group a similar subgroup does not exist, to be compared. This group is characterized also by highest frequency of RTC and RSC, asking for a detailed study on a similar control group, which represents our next task. In pupils the meen Hb value is 11.5 gr % which represents a statistically significant increase in relation to the pre-school children (t-3.50,p 0.001)

Statistical evaluation of the blood red cells (Table 8) did not show a decrease in adults of the exposed group. The lowest mean values of erythrocytes are evident in pre-school children (3,912 000 er).

Analyzing the anamnestic data a great number of different subjective complaints has been recorded in both groups. Table 9 is containing data about subjective complaints appearing in higher percentage in the exposed group, in relation to the control group. These data are related to the adults only, because data from children are not reliable. Comparing the exposed and control group, statistically significant increase of following complaints is evident: pains in bones, muscles and joints, parestesion in arms and intensive tiredness.

Physical findings did not show any statistically significant differences between the exposed and control group, except for the arterial blood pressure. This phenomenon was observed in 34.7 % cases of exposed and 18.8 % of the control group. Ethyology of this observation is not clarified yet. It is hard to ascribe it to the effects of lead, because it is not observed in professional chronic lead exposure.

The changes in ALA values (1968/69) are clearly showing the dependence on meteoreologic, topographic and technologic factors. That means ALA determination represents a good exposure test for the actual exposure. From our point of view the integral lead exposure from the birth is highly important. To evaluate the integral exposure we performed determination of lead in urine before (spontaneous) and after Ca-Na$_2$-EDTA injection (lead mobilization). The second data represent pretty reliable index of lead body burden due to integral exposure (7,8).

CaEDTA injection is administered only to persons who voluntarly accepted this treatment, and in whom any contraindication were absent. To adults 1 gr. CaEDTA and to children (8-15 y.) has been administered. Results of lead mobilization in 209 persons are presented on Table 9. The spontaneous lead excretion until 0.1 mg/l is accepted as normal. Only 8.5 % persons showed higher values (reaching 0.190 mg/l), mostly children. As "normal lead mobilization yield" /7/ we accepted values until 0.5 mg/l. From Table 9 it is evident that 78.4 % of examined persons showed higher value, mostly from 0.5-2 mg/l, reaching even 4.2 mg/l.

In 15 randomly selected inhabitants determination of chromosome aberrations was performed in the lymphocytes of peripheral blood and positive results obtained in 13 cases. Chromosome aberrations found in mentioned cases could be classified roughly in 3 groups:
- chromosomes showing various degree of unclear forms
- mistostatic effects of colchycine type
- chromosomes showing various degree of adhesions.

Sporadicalla tetraploidy was observed, but quantitative analysis was not performed.

Discussion.

Applying a set of exposure test /ALA-D,ALA,coproporphyrin,uroporphyrin,PBG/ an increased lead exposure was established in inhabitants of Meža valley.

For this particular level of lead exposure determination of ALA-D in the blood and ALA in urine represent the suitable exposure tests,from the technical, practical and analytical point of view determination of ALA in urine represent more suitable test.

The highest ALA values are established in Žerjav, as expected. But, relatively increased values obtained in settlements pretty far from the source of the contamination, are stressing the importance of meteorologic factors too (wind direction/. For example, Mežica and Črna are separated by hills from the smelter. Obviously the smokes transported by the wind are contaminating the whole valley in both directions. Due to this, even in Žerjav 3 subareas /I,II,III/ showing different levels of exposure, can be differentiated. These data are supported by data obtained by determination of Pb in soil /picture 2/ /4/.

In subareas ALA values are mostly pretty uniform with sporadically higher values, probably due to differences in individual exposure. Such differences are observed even in members of same family.

Determination of ALA-D activity in the blood represents a very sensitive test of low lead exposure, which are parallel to data obtained by ALA determination.

The decrease of the ALA-D activity represents a consequence of a direct action of lead on this enzyme, and the increase of ALA represents a consequence of lead action on porphyrin biosynthesis. Both methods are useful for evaluation of lead exposure, but it is difficult to conclude the meaning of this phenomenon as a toxic effect of lead.

Coproporphyrin determination is not sensitive enough for this level of lead exposure. Determination of uroporphyrin is discussed in an other paper. But, it is of interest to mention that uroporphyrin increase is more frequent in control group.

Determination of lead in blood could not be used for exposure evaluation, because all obtained values were in the normal limits /until 80 micrograms per 100 ccm blood/.

Taking into consideration such a relatively low lead exposure hematological changes are not expressed.

These changes are most expressed in children, especially in subgroup from 2-7 years. These data are suggesting that young organism is more susceptible to lead effects. We will pay a special attention to this problem including a good control group too.

Subjective complaints in population are very similar to that observed in professionally exposed workers, which could be explained by existence of increased lead body burden.

Physical findings did not show any signs of lead toxic effects.

Mentioned exposure tests are giving a good idea about actual lead exposure, influenced by topographic and meteorologic factors. Taking into consideration that almost all inhabitants are exposed to lead from birth /and for many generations/ we were interested to find out the lead body burden /integral exposure/ and possible genetic effects. Here we will discuss our efforts to determine lead body burden first.

Teisinger et al. /9/ established on rabbits that in cases of chronic lead exposure about 7.9 % of deposited lead is excreted in the urine during first 24 h. after one CaEDTA injection. If we assume that animal data could be extrapolated on human beings, it is possible to suppose that in chronically exposed inhabitants about 8 % of lead body burden will be excreted in first 24 h. after one CaEDTA injection.

The lead mobilization by CaEDTA was performed on 209 inhabitants. The "spontaneous lead excretion" was increased over 0.100 mg as normal value, only 8.5 % /from 0.100 to 0.200 mg/l/.

As criteria for mobilization yield of non-exposed persons we accepted the most rigorous criteria of 0.5 mg/l. Even in that case 78.4 % of examined persons showed mobilization yield from 0.5 to 4.2 mg/l. This allow us to suppose that inhabitants of Meža valley contain lead body burden from normal /4-5 mg/ to about 10 times higher /in skeleton/ /7/.

In connection with this, results of our genetic investigations are very interesting. In 13 /from 15/ examined persons we established one, two or all three chromosome aberration types, which seems to be specific for genetic effects of lead. These data will be published elsewhere and we hope continue this investigation to establish duration and level of lead exposure causing this phenomenon.

Determination of activity of transaminases, aldolase and pseudocholinesterase in the blood showed results in normal limits.

Our opinion is that changes in ALA-D activity, porphyrin biosynthesis and chromosome aberrations represent the most impressive effects of lead on population of Meža valley exposed to higher level of lead from 1894.

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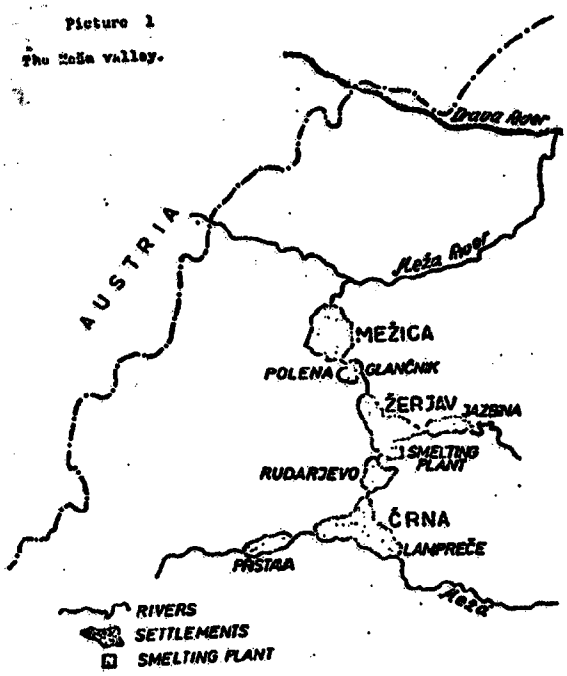
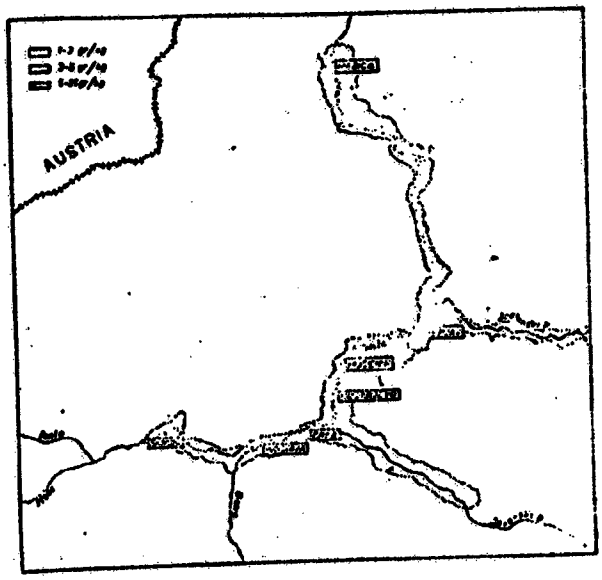
Picture 1

The Meža valley.



Picture 2

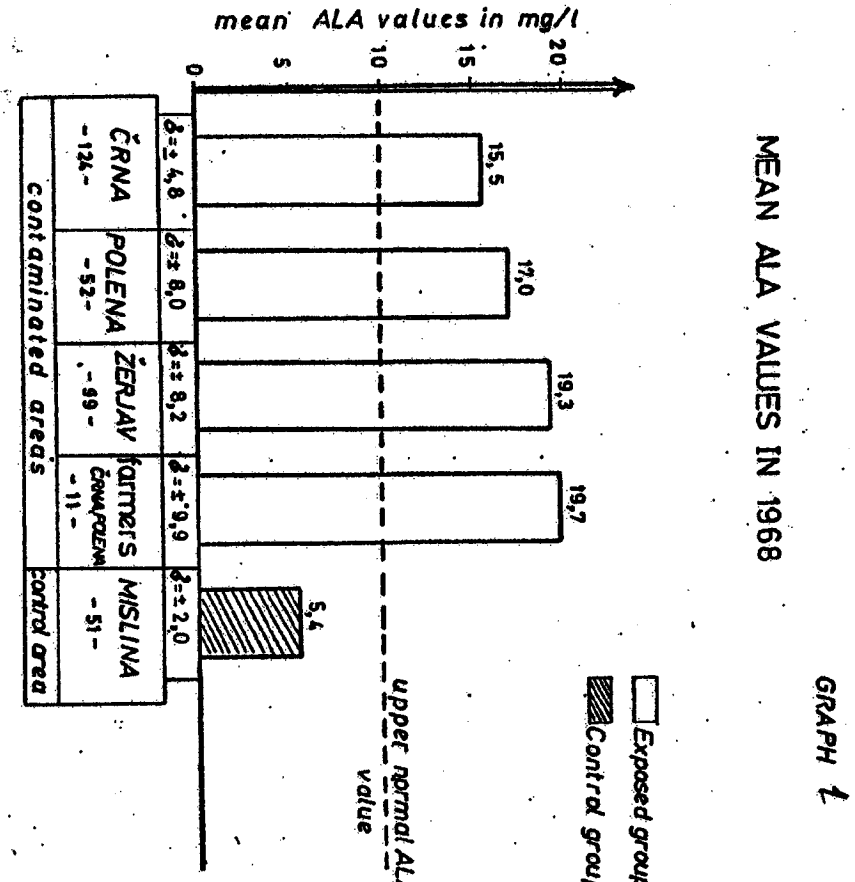
Lead content of soil in Meža valley



DATA ON SEX AND AGE OF EXAMINED PERSONS IN VARIOUS SETTLEMENTS

Tab.1

	sex			age						Total
	women	men	children	2-7y	8-15y	16-20y	21-40y	41-60y	over61y	
Žerjav	42	18	39	9	30	6	23	21	10	99
Polena	16	11	25	6	19	11	6	5	5	52
Črna	49	25	50	19	31	6	27	22	19	124
Farmers	4	1	6	2	4	1	1	1	2	11
Total	111	55	120	36	84	24	57	49	36	286
Mislinja (control group)	22	9	21	1	20	1	14	14	2	52



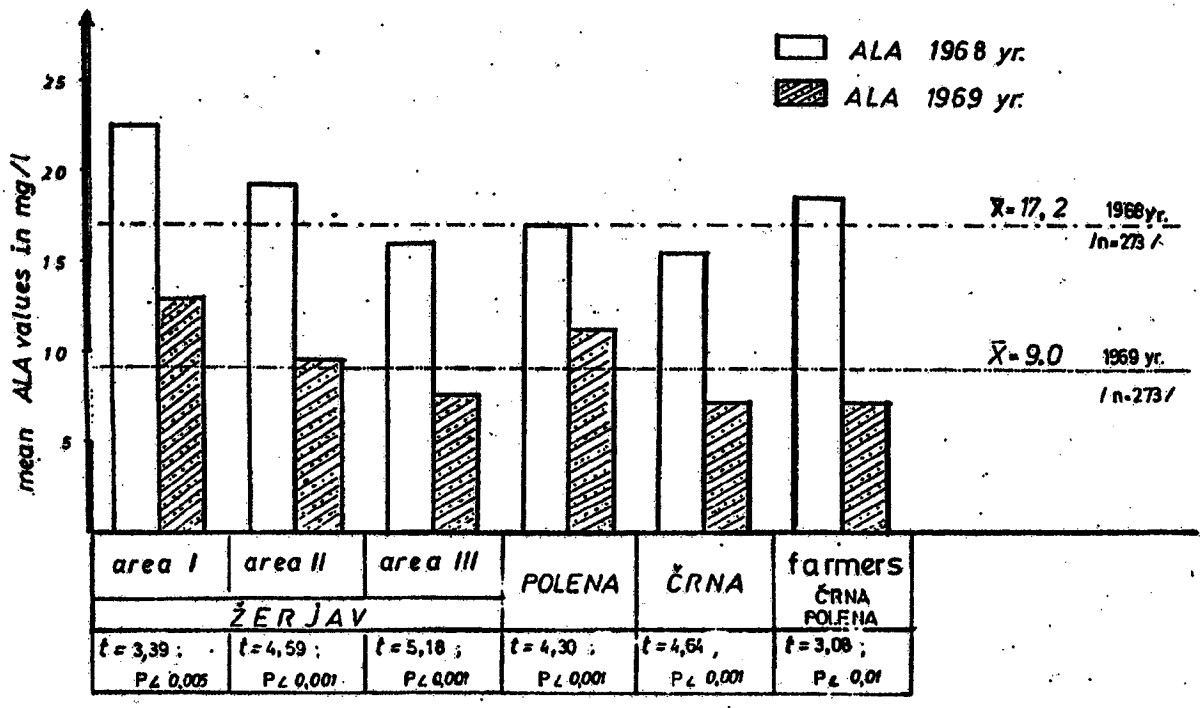
MEAN ALA VALUES IN 1968

GRAPH 1

GRAPH 2

MEAN ALA VALUES IN 1968 AND 1969

WITH STATISTICAL EVALUATION



Tab.2

COMPARISON OF ALA VALUES FROM 1968 and 1969 IN ŽERJAV

		No examined	mean value $\bar{x}$ in mg/l		standard deviation (σ)		T (test) 1968/69
			ALA 1968	ALA 1969	ALA 68	ALA 69	
children	pre-school (2-7y)	8	21.7	9.4	5.9	12.8	t = 2.46 PL 0.05
children	pupils (8-15y)	31	18.7	12.1	7.4	15.0	t = 2.20 PL 0.05
adults	men	12	23.9	9.9	7.1	7.8	t = 4.62 PL 0.001
adults	women	42	18.5	9.0	9.0	4.8	t = 5.94 PL 0.001

Tab.3

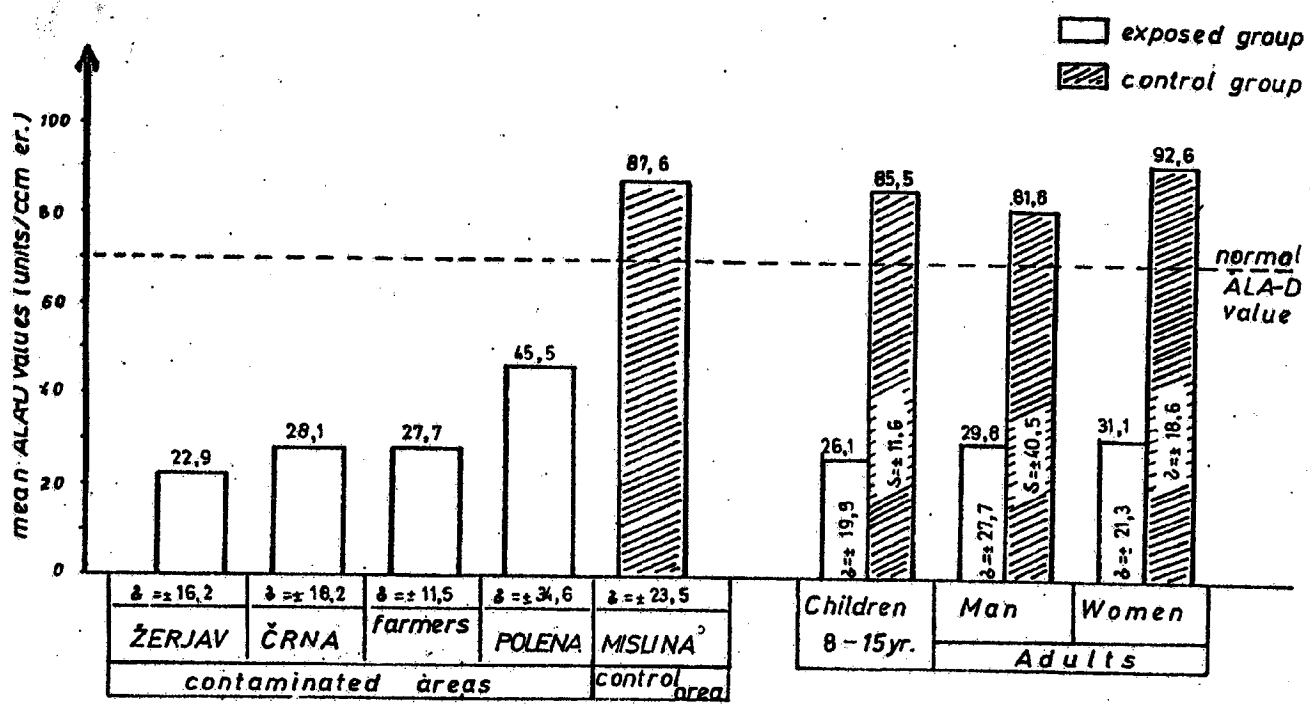
DATA ON DECREASED ALA-D VALUES IN EXPOSED AND CONTROL GROUP

		Exposed group										control	
		Žerjav		Polena		Črna		Farmers		Total		Mislinja	
		No	Decr. ALA-D	No	Decr. ALA-D	No	Decr. ALA-D	No	Decr. ALA-D	No	Decr. ALA-D	No	Decr. ALA-D
Children 8-15y	No	25	25	15	12	24	24	3	3	67	64	17	-
	%		100.0		80.0		100.0		100.0		95.5		-
Men	No	17	17	11	7	24	21	1	1	53	46	9	2
	%		100.0		63.6		87.5		100.0		86.7		22.2
Women	No	39	33	13	9	44	44	4	4	100	90	18	1
	%		84.6		69.2		100.0		100.0		90.0		5.5
Total	No	81	75	39	28	92	89	8	8	220	200	44	3
			92.5		71.7		96.7		100.0		90.9		6.6

*DECREASED VALUES OF ALA-D: UNDER 70 UNITS/ccm ERYTHROCYTES

GRAPH 3

MEAN ALA-D VALUES
FOR CHILDREN AND ADULTS IN VARIOUS AREAS



Tab.4

INCREASED COPROPORPHYRIN VALUES IN EXPOSED AND CONTROL GROUP

		Žerjav			Polena			Črna			Farmers			Total			Control		
		No examined	increased		No examined	increased		No examined	increased		No examined	increased		No examined	increased		No examined	increased	
			No	%		No	%		No	%		No	%		No	%		No	%
Children	2-7y	9	2	22,9	4	-	-	15	-	-	1	-	-	29	2	6,8	1	-	-
	8-15y	30	4	13,3	17	1	5,8	30	1	3,3	2	-	-	79	6	7,5	19	-	-
Adults	Men	12	1	8,3	11	1	9,0	23	4	17,3	1	-	-	47	6	12,7	9	-	-
	Women	42	3	7,1	16	1	6,2	49	-	-	3	-	-	110	4	3,6	21	-	-
Total		93	10	10,7	48	3	6,2	117	5	4,2	7	-	-	265	18	6,7	50	-	-

* INCREASED COPRO: OVER 0,200mg/l

INCREASE OF STIPPLED BLOOD CELLS IN EXPOSED AND CONTROL GROUP

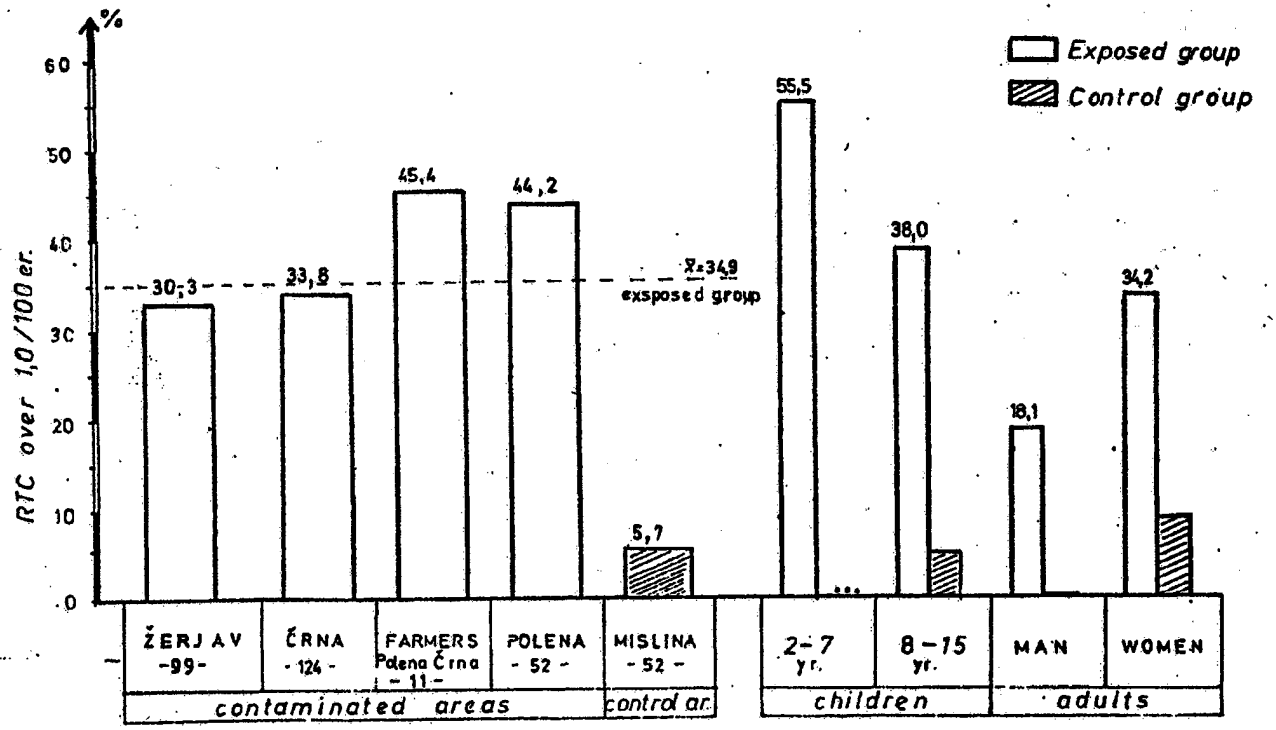
Tab. 5

		Žerjav			Polena			Črna			Farmers			Total			Control		
		No exam.	RSC increased		No exam.	RSC increased		No exam.	RSC increased		No exam.	RSC increased		No exam.	RSC increased		No exam.	RSC increased	
			No	%		No	%		No	%		No	%		No	%		No	%
Children	2-7y	9	3	33.3	6	-	-	19	-	-	2	-	-	36	3	8.3	1	-	-
	8-15y	30	3	10.0	19	1	5.2	31	-	-	4	1	25.0	84	5	5.9	20	-	-
Adults	Men	18	4	22.2	11	-	-	25	1	4.0	1	1	100.0	55	6	10.9	9	-	-
	Women	42	7	16.6	16	1	6.2	49	2	4.0	4	-	-	111	10	9.0	22	-	-
Total		99	17	17.1	52	2	3.8	124	3	2.4	11	2	18.1	286	24	8.3	52	-	-

* INCREASED SBC VALUES: OVER 100/MILLION Er.

GRAPH 4

THE FREQUENCY OF INCREASED RTC VALUES



Tab. 6

HAEMOGLOBIN VALUES IN EXPOSED AND CONTROL GROUP IN gr%

		EXPOSED										Control	
		Žerjav		Polena		Črna		Farmers		Total		Mislinja	
		No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$
Children	2-7y	9	10.7 ± 0.7	6	11.0 ± 1.4	19	10.8 ± 1.2	2	11.2 ± 0.2	36	10.8 ± 1.0	-	-
	8-15y	30	11.6 ± 1.0	19	11.8 ± 1.0	31	11.4 ± 1.4	4	11.9 ± 1.3	84	11.5 ± 1.2	20	11.9 ± 2.2
Adults	Men	18	13.2 ± 1.3	11	12.7 ± 1.4	25	13.1 ± 1.7	1.	12.0 ± 0.0	55	13.0 ± 1.5	9	11.4 ± 1.2
	Women	42	11.9 ± 1.0	16	12.4 ± 1.0	49	11.8 ± 0.9	4	11.4 ± 1.3	111	11.9 ± 0.9	22	12.0 ± 0.7

* $\bar{X}$-mean value ; G-standard deviation

Tab. 7

DATA ON ERYTHROCYTES IN THAUSANDS

		EXPOSED										Control	
		Žerjav		Polena		Črna		Farmers		Total		Mislinja	
		No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$	No	$\bar{x} \pm G$
Children	2-7y	9	3900 ±217	6	3840 ±342	19	3950 ±245	2	3825 ± 25	36	3912 ±242	-	-
	8-15y	30	4056 ±390	19	4062 ±433	31	4063 ±248	4	3945 ±271	84	4054 ±342	20	4125 ±186
Adults	Men	18	4402 ±493	11	4334 ±448	25	4692 ±355	1	3900 ± 0	55	4511 ±412	9	4046 ±841
	Women	42	4140 ±347	16	4110 ±234	49	4094 ±355	4	4000 ±158	111	4110 ±327	22	4021 ±320

$\bar{X}$-mean value; G-standard deviation

SUBJECTIV COMPLAINTS IN ADULTS

Tab.8

	No		%		Significaci
	Exposed	Control	Exposed	Control	
Intensive tiredness	95	7	45.2	22.5	t=2.39
Quick tiredness at work	77	8	36.6	25.8	t=1.19
Pains in bones	99	1	47.1	3.2	t=4.67
Pains in joints	82	6	39.0	19.3	t=2.14
Pains in muscles	34	2	16.1	6.4	t=1.43
Weaknes in extremities	60	6	28.5	19.3	t=1.08
Parrestesions in arms	67	2	31.9	6.4	t=2.93
Cramps in muscles	57	5	27.1	16.1	t=1.32

LEAD EXCRETION IN THE URINE AFTER $Ca\text{-}Na_2$ -EDTA INJECTION (IN MICROGRAME/L)

Tab.9

		Lead excretion in micrograms/l					
		til 500	501--1000	1001--2000	2001--4000	over -4000	Total
Children 6 -15 y	No	1	7	14	1	-	23
	%	4.3	30.4	60.8	4.3	-	100.0
Adults Men	No	5	13	19	8	1	46
	%	10.8	28.2	41.3	17.3	2.1	100.0
Adults Women	No	39	45	39	15	2	14
	%	27.8	32.1	27.8	10.7	1.4	100.0
Total	No	45	65	72	24	3	209
	%	21.5	31.1	34.4	11.4	1.4	100.0