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Trace Elements in Soil and Coniferous Needles

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Monitoring of various regions to heavy metal pollution is very often carried out by measuring metals in different biological matrices - animals, moss, lichens or soil. Some of these matrices concentrate certain element from the environment in a higher degree enabling easier analytical measurement. It has been generally accepted that concentration of metal in such biological material reflects environmental contamination in certain area. Coniferous tree needles were also occasionally used in searching for a possible and reliable pollution indicator (Rautio et al, 1998a; 1998b). In differently polluted area of FR Germany needles were collected and analyzed for the environmental specimen bank (Wagner et al., 1985). In Croatia, no systematic monitoring study has been carried out to establish the status of soil trace metal content in different areas. Studies have been done on soil heavy metal contamination of some rural and urban parts of Zagreb area (Namjesnik et al., 1992) and of soil in some agricultural (Cosić et al, 1994) and lowland forests (Komlenovic et al., 1994) areas. For 1991 at the beginning of the war in Croatia an ammunition stockpile of 6,000 tones was blown up in the Gorski kotar region the question was raised whether the surrounding was contaminated with heavy metals. One study (Palinkaš et al., 1994) was done in the same region six months after the blow. High concentrations of cadmium and mercury in soil were found at the center of the explosion. There are no studies described in the literature impact of war on environmental contamination with heavy metals. In one study from Finland lead and cadmium were measured in soil and found to be very high near a shooting range area coming from bullets and pellets (Tanskanen, 1991).

The aim of this study was to assess possible environmental pollution by lead, cadmium, copper, zinc and mercury in the Gorski kotar and in a control region few years after the explosion. Since the region is covered by forests and arable lands lead, cadmium, zinc and copper were measured in soil and coniferous needles. Mercury was measured only in soil. A reliable pollution indicator was sought by correlating metals in soil and needles.

MATERIALS AND METHODS

Three years after the ammunition stockpile explosion soil samples were collected at different distances from the center of explosion (Figure 1). Sampling was also done in a “control” nonpolluted region (Zagreb surrounding), situated 80-100 km north from the “exposed” region (Gorski kotar). Both regions are far from any industrial or heavy traffic pollution, covered by forests and arable lands. Soil samples were collected from 22 locations in exposed and 39 locations in the control region. Samples taken from the upper 10 cm of soil were dried, ground in a mortar and sieved to 0.6 cm screen. Five grams of dry sample was weighed, extracted by shaking with 25 mL of HNO_3 (20%) left to stand overnight without shaking, filtered and adjusted to 50 mL with distilled water (10% HNO_3 final solution). In 18 soil samples from each region lead, cadmium, copper and zinc were measured by flame atomic absorption spectrometry (F AAS) with D_2 background correction (Varian, AA-375, Australia). Analysis of total mercury (organic and inorganic) in soil was carried out using the same extraction procedure as for other elements.

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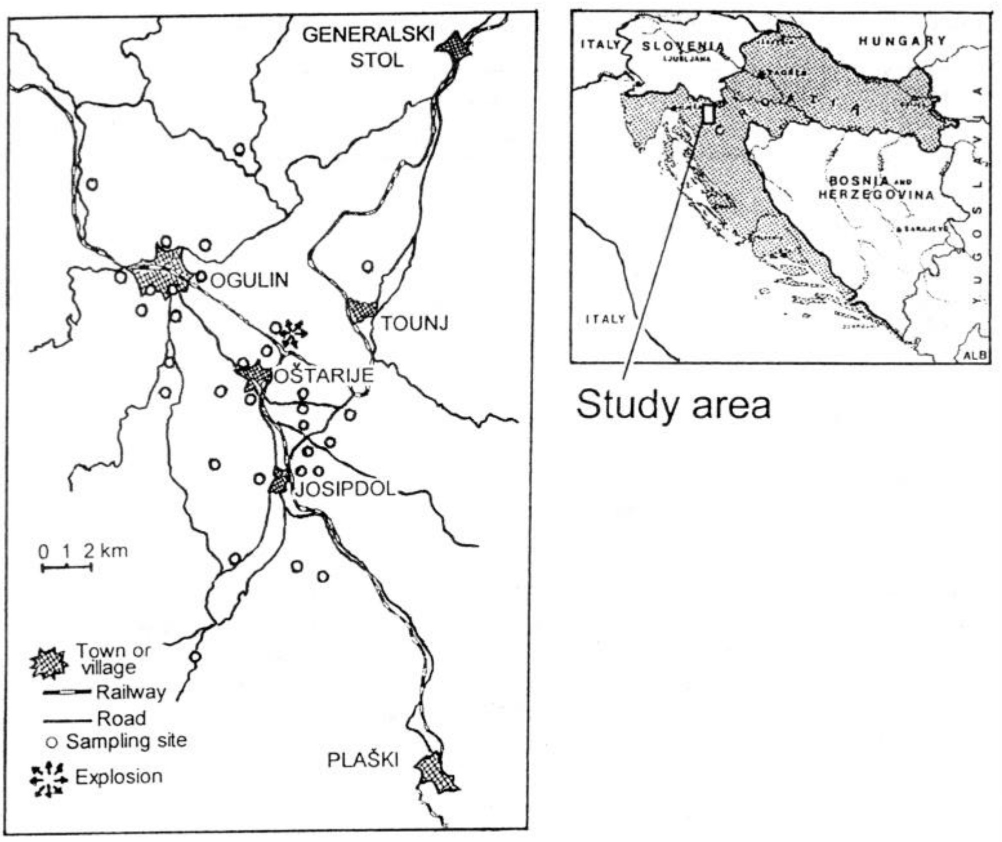

Figure 1. Study area with sampling sites within Croatia.

Cold vapor atomic absorption spectrometry (CV AAS) method (Mercury Monitor LDC, UK) after reduction of mercury with stannous chloride was applied according to modified Farant's method (Farant et al., 1981; Prester et al.,1993). Soil pH was determined by adding 10 mL of deionized water to about 1 g of dried sieved sample.

At some locations in both regions, branches from coniferous threes (*Coniferae Pinaceae*) were also collected for cadmium and lead analysis. New needles (latest year) were separated from the old ones (several years old) and treated separately. One to two g of needles were weighed without prior washing in quartz crucibles, dried at 105 °C, weighed again and ashed in a muffle furnace overnight at 450 °C. After ashing samples were dissolved and adjusted to 10 mL in 10% nitric acid. Cadmium and lead were analyzed by electrothermal atomic absorption spectrometry (ET AAS) with D_2 background correction on Varian model SpectrAA300 (Australia) according to method described earlier (Blanuša and Breški, 1991).

To verify methods applied certified standard reference materials (SRM) were measured: Bovine Liver SRM 1577b and San Joaquin Soil SRM 2709 from NIST (USA). Preparation of reference materials for AAS measurements was identical as for environmental samples. Lead and cadmium in bovine liver was analyzed by ET AAS and copper and zinc by F AAS. In soil reference material lead, copper and zinc were measured by F AAS, cadmium by ET AAS and mercury by CV AAS.

Table 1. Validation of the methods of trace elements analysis (mg/kg dry weight) by AAS.

Certif. material	Lead		Cadmium		Copper		Zinc		Mercury	
	Obt.	Ref.	Obt.	Ref.	Obt.	Ref.	Obt.	Ref.	Obt.	Ref.
[a] Bovine Liver	0.13± 0.002 (3)	0.13± 0.004	0.55± 0.016 (4)	0.50± 0.03	178 ± 2 (8)	160 ± 8	138 ± 4 (8)	127± 16	-	-
[b] San Joaquin Soil	13.4± 0.7 (4)	18.9± 10.5	0.39± 0.01 (3)	0.38± 0.01	19.4± 0.6 (4)	34.6± 0.7	49.5± 2.6 (4)	106± 3	1.44 ± 0.32 (7)	1.40 ± 0.08

Results are presented as mean ± SD with number of replicates in parenthesis.
Obt. - Obtained values; Ref. - Certified reference values
[a] After dry ashing at 450 °C lead and cadmium in Bovine Liver were analyzed by ET AAS and copper and zinc by F AAS.
[b] After nitric acid extraction lead, copper and zinc in San Joaquin Soil were analyzed by F AAS, cadmium by ET AAS and mercury by CV AAS.

Differences between soil elemental concentrations in two regions was calculated by Student's t-test. Differences between new and old needles and between two regions were calculated by Duncan's multiple range test. Calculation was carried out for each element separately. Region and needles age was defined as independent and concentration of element as the dependent variable. Levels of $P<0.05$ were taken as statistically significant. General ANOVA/MANOVA Statistica for Windows release 4.0, Statsoft, Inc. 1993 package was used for statistical evaluation of data.

RESULTS AND DISCUSSION

Detection limit of analytical methods was estimated for lead 1.2 and cadmium 0.02 µg/L by ET AAS, for copper 12 and zinc 9 µg/L by F AAS, and for mercury 0.4 µg/L by CV AAS. Soil pH determined in the control region was between 4.9 and 6.7 (mean 5.4), while exposed region was between 5.9 and 8.8 (mean 6.9). Results of internal quality control measurements of accuracy are presented in Table 1. Recoveries of lead, cadmium, copper and zinc in Bovine Liver are within 98 and 111%, respectively. Cadmium and mercury value obtained in SRM San Joaquin Soil show that almost 100% of the element is extracted by nitric acid procedure. Lead, copper and zinc are extracted partly i.e. 71, 56 and 47%, respectively.

Most trace elements in soil (Table 2) in the exposed area were significantly higher than in control area Lead, cadmium, copper and zinc were from 1.5 to 2.4 times higher in exposed area. Mercury was, however, significantly lower by a factor of 2.

Concentrations of lead and cadmium in coniferous needles were much lower than in soil (Table 3). Only about 0.54% of lead and 3-21% of cadmium found in soil (by nitric acid extraction procedure) was incorporated into needles. Zinc and copper concentrations in soil and needles are of the same order of magnitude.

Lead, cadmium, copper and zinc in both regions and needles age were subjected to Duncan's multiple range test separately for each element. Only needles age was significant as main effect in the case of lead and copper (Table 3). Lead in old needles was much higher than in new ones (at $P<0.001$) and copper reversely significantly lower (at $P<0.05$). There was no significant main effect of region or needles trace element abundances.

Table 2. Lead, cadmium, copper, zinc and mercury concentrations (mg/kg dry weight) in soil.

	Lead	Cadmium	Copper	Zinc	Mercury
Control	28.2±2.4 (18)	0.375±0.018 (18)	10.9±1.92 (18)	31.5±3.82 (18)	0.11±0.01 (39)
Exposed	56.8±4.1* (18)	0.911±0.071* (18)	18.3±2.25** (18)	46.4±5.50* (18)	0.06±0.01*** (22)

Results are presented as arithmetic mean ± SE (No.of samples).
*P<=0.05; **P<0.02; ***P<0.001 compared to control by Student's t-test

Table 3. Lead, cadmium, copper, and zinc concentrations (mg/kg dry weight) in coniferous needles

		Lead[a]	Cadmium	Copper[a]	Zinc
Control	**New** (5)	0.15±0.047	0.029±0.009	5.50±0.83	42.1±4.9
	Old (4)	1.04±0.192*	0.033±0.021	2.61±0.26*	45.8±14.8
Exposed	**New** (11)	0.23±0.07	0.19±0.07	6.46±0.63	53.8±2.9
	Old (15)	1.12±0.125**	0.028±0.004	2.67±0.13**	47.2±6.9

Results are presented as arithmetic mean ± SE. Number of samples in parenthesis. Statistically significant differences by Duncan's multiple range test: * at level P<0.05, ** at level P<0.01. “Main effect of needles age irrespective of region was significant at level P<0.01.

Correlations between concentration of each element in soil (irrespective of region) and new grown needles did not show any significant correlation. However, coefficient of correlation between trace elements in soil and old needles were found positive and statistically significant for lead and zinc (Figure 2 and 3).

Samples of soil collected in control and region exposed to ammunition stockpile explosion were both mostly covered by forests with no heavy traffic or any industrial contamination. Therefore, it is not expected to find any heavy metal contamination caused by immediate human activities except long range contamination or eventually the stockpile explosion. However, data of metals in soil before the blow in the same region are not available. Although values of lead, cadmium, copper and zinc were significantly higher in the exposed region it is difficult to conclude that it was the consequence of the blow. Palinkaš et al. (1989; 1994) reported background soil concentrations in the same Croatian regions for lead, cadmium, zinc, copper of 20, 0.39, 35, 21 (mg/kg) and for mercury 88 (µg/kg), respectively. Similar data were reported for lead, zinc and copper (40, 42 and 8 mg/kg) in forests of East Slavonia (Komlenović et al, 1994). These values are mostly between our control and exposed region values. Six months after the accident in the

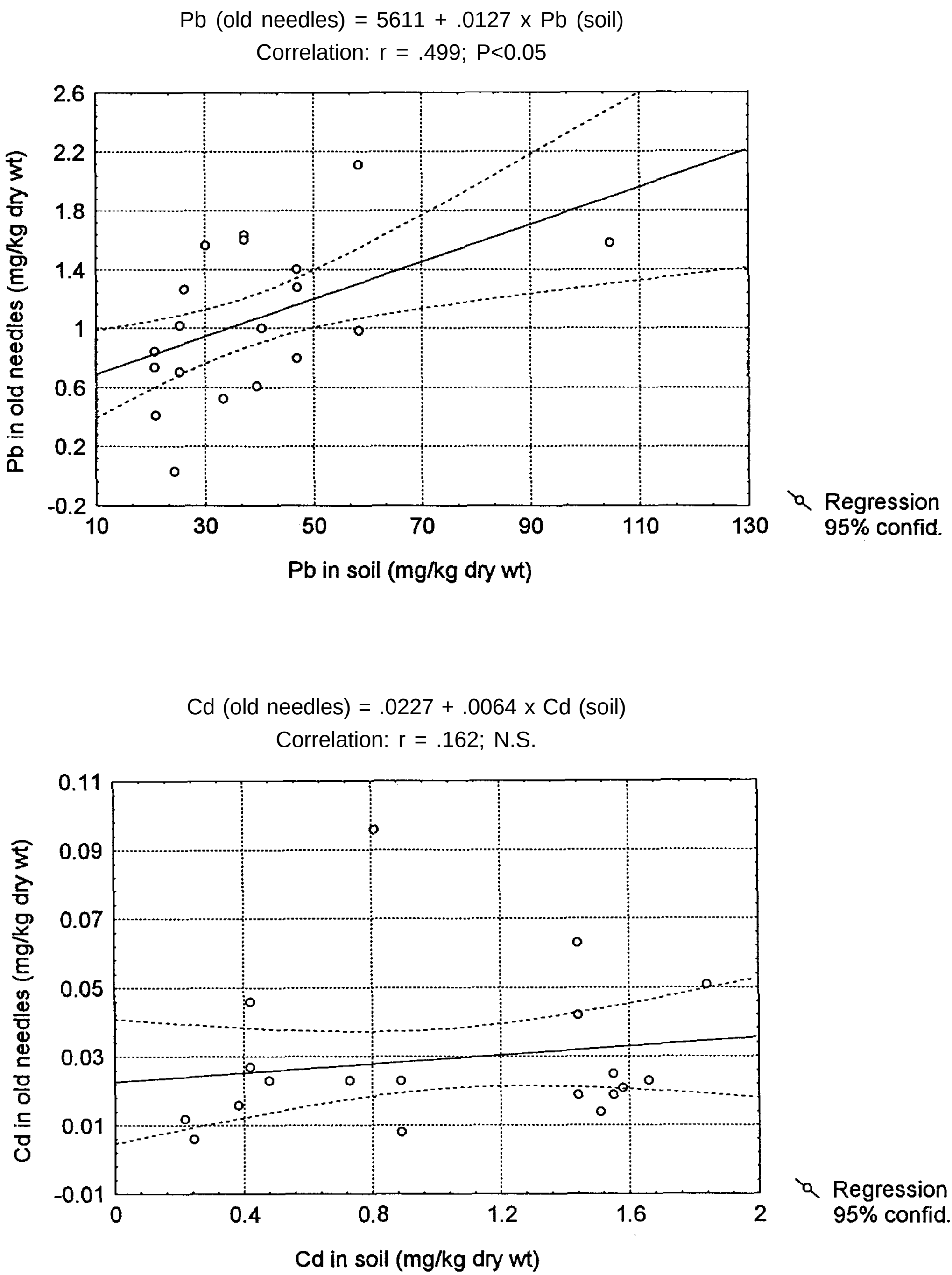

Figure 2. Correlations of trace element (lead and cadmium) in soil vs. trace element in old coniferous needles.

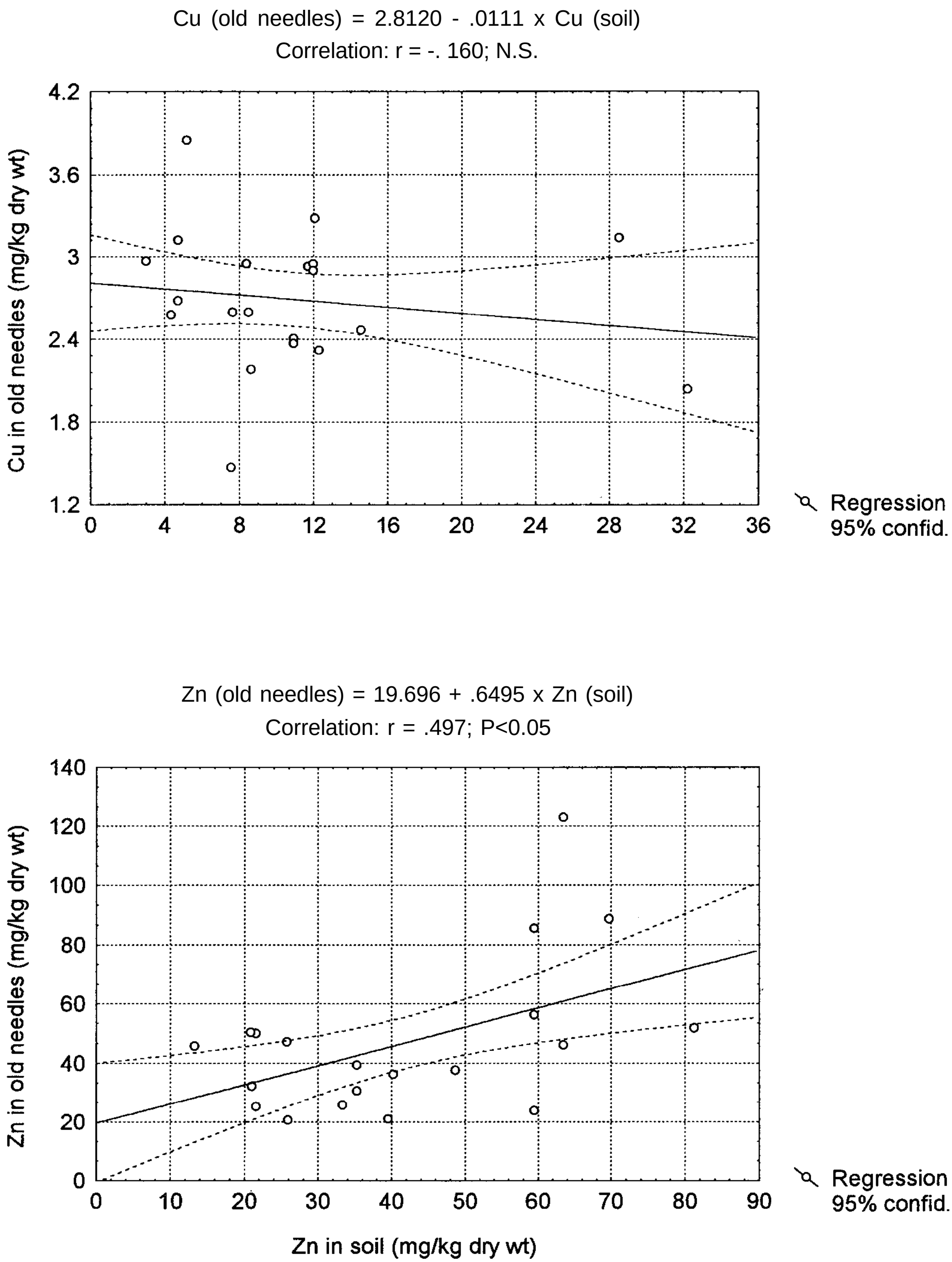

Figure 3. Correlations of trace element (copper and zinc) in soil vs. trace element in old coniferous needles.

Gorski kotar region Palinkaš and coworkers reported (1994) high values of cadmium and mercury near the center of the explosion. Such high values were not observed near the center of the explosion in our study carried out several years after the accident. The reason might be the rainfall which removes deposited materials.

The observed difference between two regions in concentrations of lead, cadmium, copper and zinc, being higher in exposed region, might be the consequence of the blow, but most probably the reason is difference in mineral composition of the soil between two regions. Mercury concentration, however, is lower in soil of the exposed region. Since mercury is widespread in the environment it is difficult to distinguish between contaminated and background levels of mercury. It depends mostly on quantity of humus, clay and pH of the soil (Palinkaš et al., 1989). In this study pH was measured in both regions and it was found lower in the control region, Lower pH and higher organic matter more firmly bind mercury. This might be the reason of higher mercury in the control region.

Of four elements measured both in soil and coniferous needles, two elements are essential (zinc and copper) and two toxic (lead and cadmium). The concentrations of zinc and copper were approximately the same order of magnitude in both soil and needles. Zinc concentrations were about 30 - 50 mg/kg dry weight in both media and no difference between new and old needles was found. Copper was lower in needles than in soil for a factor of 3 and significantly lower in old than in new needles. Lead and cadmium, as toxic elements are taken up only partly from soil (only few percents) and transported into needles, where they accumulate with age (Zoettl, 1985). In the present study lead accumulated for a factor of 5-7. This accumulation was statistically significant. Similar findings were reported by other authors (Zoettl, 1985; Wagner et al., 1985). Cadmium however in this study did not accumulate with needles age. There is also a possibility that a part of the element, particular lead, is deposited on the surface of the needles as a result of atmospheric deposition, since our method of analysis does not include washing of needles before analysis. No statistical difference was found between two regions in any of measured element in needles.

When correlation between soil and needles was done irrespective of region, significant correlations were obtained between soil and old needles for lead and zinc. There was no significant correlation between soil and new needles in any element. This indicates accumulation of lead and zinc and redistribution of cadmium and copper with age in coniferous needles. Significant and positive correlation between soil and old needles for lead and zinc would indicate that old needles might be an indicator of environmental contamination.

In conclusion, we could not find any significant contamination by heavy metals in the region exposed to ammunition stockpile explosion, except of differences to control region which are probably due to different mineral composition and pH of the soil. Only old coniferous needles reflect to a certain level the concentration of lead and zinc in soil.

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