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Heavy Metal Bioaccumulation in Lamb and Sheep Bred in Smelting and Mining Areas of S.W. Sardinia (Italy)

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It is widely known that anthropogenic activities strongly contributed to heavy metals contamination of environment. For this reason, distribution of heavy metals in soils and plants around urban and industrial point sources have been the object of extensive studies. Lesser attention has been given to accumulation of heavy metals in animals bred in these polluted environment, and relatively few works are concerned with the accumulation of heavy metals through the food chain.

To correctly evaluate environmental effects of heavy metals, the complete soil-plant-animal system should be considered. Therefore, a comprensive investigation on naturally and unnaturally contaminated environment, such as mining and industrial areas is particularly suitable to wholistically study a polluted environment.

The aim of this work is to improve the knowledge about distribution of heavy metals in two differently contaminated environments, such as a smelter-refinery and mine areas situated in S.W. Sardinia. The first sampling area is located near one of the most important Pb and Zn smelting-refineries of Europe, and the second near to the centre of a mine area including both abandoned and working mines. In the mine area, ores have been mined for Pb, Zn and Cd since the Roman age and soils and vegetation are heavily polluted, as reported in a preliminary investigation (Leita et al. 1990).

To this propose the target organs of sheep and lamb were considered for their heavy metals accumulation.

Samples of vegetation were collected throughout the vegetative period and analyzed for their Pb, Zn and Cd content. Winter forage and soil were also considered.

MATERIALS AND METHODS

An indigenous sheep, a sheep introduced for 1 year into the flock and a lamb born of local sheep were chosen from a sheep breeding in the sampling area (S) situated within 4 km South-East of the smelter - refinery, in an exposed site along the direction of

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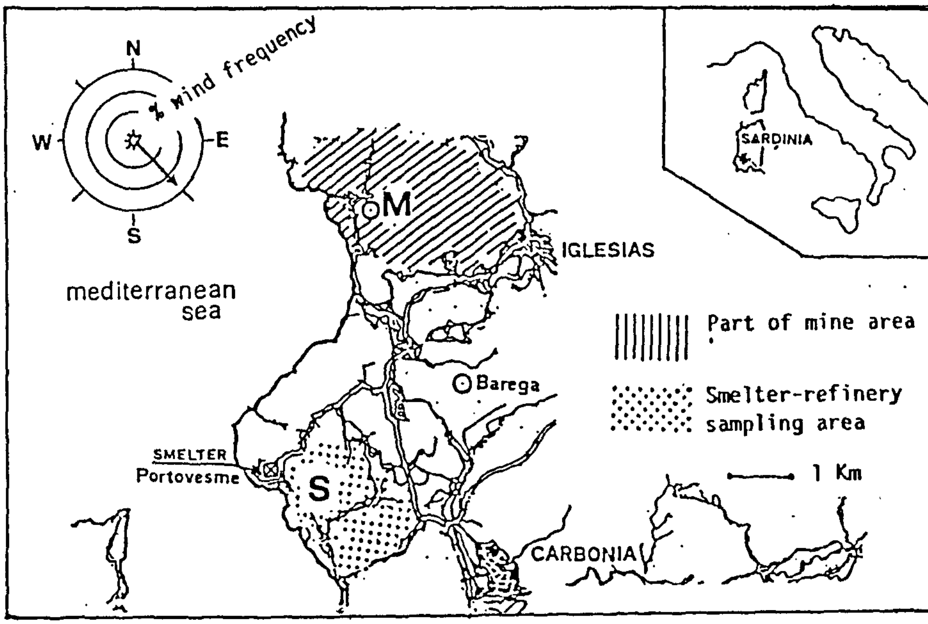

Figure 1. Topography of sampling areas.

prevailing winds (Figure 1).
In addition, an adult sheep reared in the sampling site (M) located in the mine area was taken. The three sheep and the lamb were sacrificed and samples of soft and hard tissues were collected on slaugther and frozen as soon as possible to reduce lysis and redistribution of heavy metals between tissues. Tissues were digested in concentrated HNO_3 - HCl acid (3:1 v/v) at a temperature of about 150 C until clear solutions were obtained. Mineralized solutions were then analyzed for Pb, Zn and Cd content by means of a Perkin-Elmer 2380 atomic absorption spectrophotometer with an air acetylene flame and deuterium arc background correction.
Samples of soil and leaves of the grassland components, (gramineae and *Scolymus hispanicus*), were taken from April to November in the pasture near to the sheep breeding areas S and M. In addition, samples of winter forage (hay, gramineae and leaves of *Vitis vinifera*) used to feed sheep of breeding, were collected near Barega, far from both mining and smelter-refinery areas, where the lithological matrix is completely different.
Samples of vegetation were oven dried at 105 C and the analyses of the total amount of heavy metals was carried out by digestion in a mixture (3:1) of concentrated nitric-hydrochloric acids. The mineralized solutions were centrifuged at 8500 g for 30 min. and the clear supernatant was analyzed for Pb, Zn and Cd content by atomic absorption spectrophotometer.
Soil samples were air dried and sieved to pass a 2 mm sieve.
Concentrations of heavy metals are expressed as micrograms per gram on fresh weight of tissues,or mL (in the case of whole blood). In the case of vegetation, the amount of heavy metals are

expressed on the basis of dry weight.

RESULTS AND DISCUSSION

Heavy metal concentration higher than the threshold of toxicity (0.3-0.5 $\mu g\ g^{-1}$) reported by Carson et al. (1973) was found in organs of all sheep and the lamb. As it can be seen in table 1, organs removed from the sheep bred in mining area showed the highest overall Pb content. Among the soft tissues, liver contained a Pb concentration of about 30 times higher than that found in hepatic tissue of the sheep and lamb reared near the smelter-rafinery. High amounts of Pb were observed in lung (10.2 $\mu g\ g^{-1}$), muscle (15.3 $\mu g\ g^{-1}$) and in an athrophic kidney (43.1 $\mu g\ g^{-1}$), which was found in the indigenous sheep from mine area only. Except in the case of the athrophic kidney, zinc accumulated particularly in liver, which reached 73.6 $\mu g\ g^{-1}$ in the organ removed from sheep bred in mining area.

The Zn content in the other soft tissues was higher in organs of sheep and of the lamb taken from breeding located in the smelting area, whereas Cd content was higher in soft tissues removed from sheep reared in mine area. Cadmium, in particular, accumulated in renal tissue of sheep and the lamb.

No evident differences were observed on accumulation of Pb, Zn and Cd in soft tissues of both indigenous and unindigenous sheep bred in the smelting area.

Accumulation of heavy metals in kidney and liver could be related to metal-binding-proteins (metallothioneins) observed in these organs, and concurring to detoxifying metabolism (Kagi e Shaffer 1988).

The heavy metals toxicological processes in animals, and the pathology of saturnism in particular, are closely related to the content of heavy metals in food as well as to animal age (Sharma 1982). The amount of heavy metals in soft tissues of the lamb fed on milk only was high, notwithstanding the young age. As the mammalian gland releases only small fractions of heavy metals, the pathology of subchronic saturnism, could have been of prenatal nature, rather than caused by milk. In fact, high amounts of Pb, Zn (49.3 $\mu g\ g^{-1}$; 113.3 $\mu g\ g^{-1}$ respectively) were found in udder of the sheep taken from mine sampling site, whereas only traces (0.02 $\mu g\ g^{-1}$) were found in the milk.

Contrary to the sheep bred in the smelting area, high contents of Pb, Zn and Cd were found in the hard tissues of the indigenous sheep reared in mining area. Outstanding values of heavy metals were found in the incisors, molars (up to 145 $\mu g\ g^{-1}$ of Pb and 197 ug g^{-1} of Zn) and high amount of Pb was recorded in the three parts of the femus (up to 62 $\mu g\ g^{-1}$) and in the ribs (68.2 ug g^{-1}). As reported by many authors, e.g. Edelstein et al. (1984), the accumulation of Pb in bones is closely related to the Ca metabolism because this metal may be substituted by Pb. This fact, together with the high concentration found in the whole blood (2.4

Table 1. Total concentration (μg g^{-1}) of heavy metals in soft and hard tissues of sheep and lamb

Tissue	Indigenous sheep from smelting area			Sheep introduced for 1 year into the flock			Indigenous lamb from smelting area			Indigenous sheep from mine area		
	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd
Whole blood	0.05	n.d.	<0.05*	0.05	2.6	<0.05*	0.1	2.8	<0.05*	2.4	2.8	n.d.
Cerebral cortex	0.8	11.2	0.1	0.2	12	<0.05*	0.4	10.6	<0.05*	2.3	0.8	0.4
Cerebellar cortex	0.8	10.9	0.1	0.1	9.8	<0.05*	n.d.	n.d.	n.d.	2.2	9.3	0.5
Kidney	2.5	42.3	7.9	3.1	24.6	1.8	1	15.6	0.4	5.2	9	3.5
Atrophic kidney										43.1	61.6	5.5
Liver	1.5	47.6	0.6	1.6	44.9	0.7	1.1	21.8	0.4	45	73.6	1.2
Spleen	0.4	16.5	<0.05*	0.1	18.6	<0.05*	0.2	17.5	<0.05*	4.5	11.3	1
Rumen	2.5	32.9	0.2	0.5	2.7	1.6	n.d.	n.d.	n.d.	2.6	3.8	0.2
Lung	0.2	16.3	0.2	0.1	15.5	0.1	0.2	16.4	<0.05*	10.2	11.3	0.7
Udder										49.3	11.3	5.2
Muscle	0.1	28.8	<0.05*	0.8	23.7	<0.05*	1	18	<0.05*	15.3	9.1	0.5
Femus distalis	2	19.6	<0.05*	0.3	27.1	<0.05*	5.5	29.1	<0.05*	62	123	1.9
Femus medius	4	18.5	0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	58.7	89	1.2
Femus proximalis	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.*	n.d.	n.d.	n.d.*	35.5	66	0.7
Rib	40.5	55	0.1	3.3	42.3	<0.05*	11.1	48	<0.05*	68.2	86.3	2.5
Incisor	1.2	42.9	0.1	1	49	<0.05*	n.d.	n.d.	n.d.	145	197	3.6
Molar	0.4	n.d.	n.d.	1.3	n.d.	n.d.	n.d.	n.d.	n.d.	79.5	160	2

* detectable limit of analytical procedure

μg g^{-1}) and the presence of a athrophic kidney suggest the occurrence of chronic heavy metals toxicosis (Cerrati 1974).
Samples of pasture (gramineae and *Scolymus hispanicus*) collected in smelting and mine sampling sites during the growing season showed high contents of heavy metals (Table 2). Total Pb, Zn and Cd contents even higher than the maximum threshold of concentration (20 μg g^{-1} of Pb; 200 μg g^{-1} of Zn and 0.8 μg g^{-1} of Cd) reported for uncontaminated environments by Allaway (1968) were found. However, heavy metal concentrations of similar or higher order of magnitude were usually found in vegetation grown in mine areas, where naturally contaminated soils were cultivated (Davies 1980; Leita et al 1988).

Table 2. Total heavy metal content in gramineae (gram.) and *Scolymus hispanicus (Scol. hisp.)* collected during the vegetative period in industrial and mine sampling areas

SAMPLING SITE	MONTH	LEAD		ZINC		CADMIUM	
		gram.	*Scol. hisp.*	gram.	*Scol. hisp.*	gram.	*Scol. hisp.*
		μg g^{-1}					
2 km South-East from smelting-refinery	April	213	201	219	255	6	7
	June	200	205	201	215	5	8
	August	113	208	255	320	81	13
	September	100	1353	226	623	8	19
	November	554	667	258	704	8	13
4 km South-East from smelting-refinery	April	118	105	240	151	8	3
	June	146	140	321	272	7	8
	August	178	670	214	270	7	16
	September	148	330	203	494	14	10
	November	256	687	253	284	12	39
Mine (M)	April	1231	1630	792	402	23	5
	June	595	973	972	680	18	10
	August	136	383	181	502	4	6
	September	1912	1785	607	573	31	50
	November	177	279	190	282	12	35

In a recent investigation on heavy metal distribution in soils and plants of mine sampling sites of S.W. Sardinia, Leita et al. (1990) found outstanding amounts of total Pb, Zn and Cd not only in soils and pasture, grazed by sheep, but also in leaves and fruits taken from trees.
Vegetation collected from the smelting area showed heavy metals concentration lower than samples collected in mining area.

Apart from zinc whose concentration was in most cases only slightly above the 200 µg g^{-1} threshold, the amount of lead and cadmium was high even at the beginning of the growing season. Afterwards, heavy metal accumulation was observed from summer to senescence was in vegetation collected in sampling sites near the smelter-refinery. Samples of vegetation growing near the smelter can contain elevated amounts of heavy metals (up to several thousand), as indicated Palmer and Kucera (1980). However, the concentration of Pb, Zn and Cd in winter forage was quite high, although the sampling site was far from mining and smelting areas (Table 3).

Table 3. Total heavy metal concentration (µg g^{-1}) in winter forage (hay, gramineae and leaves of *Vitis vinifera*

	LEAD		ZINC		CADMIUM	
	Mean	SD	Mean	SD	Mean	SD
Hay	109.2 +	34.3	73.4 +	40.6	15.4 +	1.8
Gramineae	135.8 +	89	684.4 +	323.2	8.6 +	5.5
Vitis vinifera (leaves)	94.5 +	49.9	361.3 +	41.7	3.1 +	0.4

According to Grupe and Kuntze (1988), the discrepancy between the values found in vegetation taken from mine and smelting areas could be due to the different lithological matrix and to the content of heavy metals present in the soil (reported in table 4), developed afterwards.

Table 4. Total concentration (µg g^{-1}) of Pb, Zn and Cd in soil sampled in smelting and mine areas.

SAMPLING SITE	LEAD	ZINC	CADMIUM
2 Km South from smelting-refinery	192	392	6
4 Km South from smelting refinery	147	453	5
Mine	36000	38500	148
Barega	398	288	3

Since the concentration of heavy metals in the animal body is fairly related to the amount ingested, the accumulation of these metals in the organs of sheep and lamb is fully justified by the concentration of Pb, Zn and Cd found in vegetation samples. Therefore, we can confirm that the animals breedings in a naturally contaminated environment may be particularly exposed to heavy metal accumulation hazards.

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