

Lead contamination: preliminary results of the ongoing research projects



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Raptors are subject to saturnism
due to ingestion of shot animals
by hunters and poachers

Hunting: lead fragments in meat, bones and viscera

The most vulnerable species are scavengers (vultures, kites, Corvidae, buzzards, eagles and hawks).

The risk involves also species that hunt live preys.

Lead is ingested because raptors swallow large pieces of food, also containing cartilage and bone fragments, regardless of hard parts or artificial components present.

5 evident cases of **SATURNISM** in 7 years

- 1) Doraja 24th of December 2005
- 2) Ikarus 19th of December 2008
- 3) Nicola 23rd of January 2012
- 4) Lousa 1st October 2012
- 5) Glocknerlady 3rd of November 2012

In general symptoms related to Saturnism are difficult to detect in moribund or dead raptors



PROBLEMS

- Lead intoxication is one of the major risks for BV population increasing
- Lead bullets are widely used in Alps and Pyrenees
- Often raptors carcasses are partially analysed or not at all
- To find the real causes of disease or death, autopsy, X-Ray and a complete analyses on internal organs (kidney, liver), blood, feathers and bones are strictly necessary.
- Actual bibliography reports only few casualties of intoxicated birds, often in small areas and in a short period of time.
- Lack of general view about this problem (Alpine range)
- Lack of specified rules in hunting management to avoid this risk

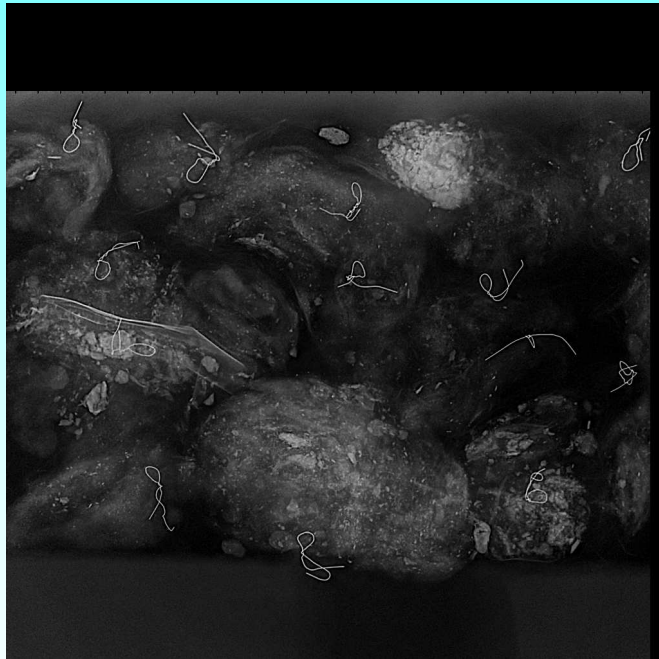
Main goals

- ✓ collect randomly data from **different countries** with **standard** methods
 - ✓ collect **more data on the mortality** of umbrella species to increase the sample size analyzed
 - ✓ determine if wild scavenger raptors have experienced a **lead exposure** event and, if so, analyze **exposure patterns**
 - ✓ **reduce the risk of intoxication** and improve strategies to contrast it
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- ✓ starting **disseminative actions** at a large scale
 - ✓ introduce **new rules** to address hunting management and political decisions, in order to progressively **ban use of lead** in ammunitions

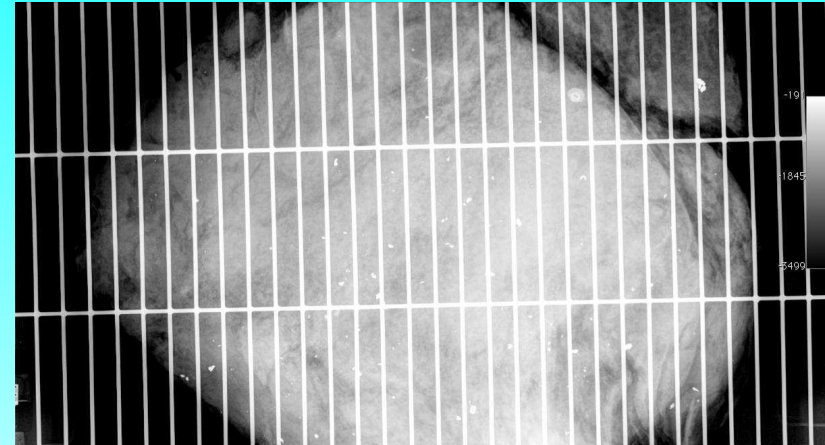
3 research projects

different approaches to improve the knowledge on lead problem for raptors

- **1) Lead detection and quantification, in viscera of shot ungulates** to assess the real risk of saturnism



- **3) Standard lead analyses of all carcasses of 4 target species:**
Golden Eagle *Aquila chrysaetos*, Bearded Vulture *Gypaetus barbatus*, Griffon Vulture *Gyps fulvus* and Raven *Corvus corax*.



- **2) Pellets analyses of large diurnal raptors**, to obtain more data on the diet quality in breeding and wintering period **and RX analyses to detect lead**



Research n. 1

Lead detection in the viscera of shot ungulates

200 viscera of shot ungulates analyzed

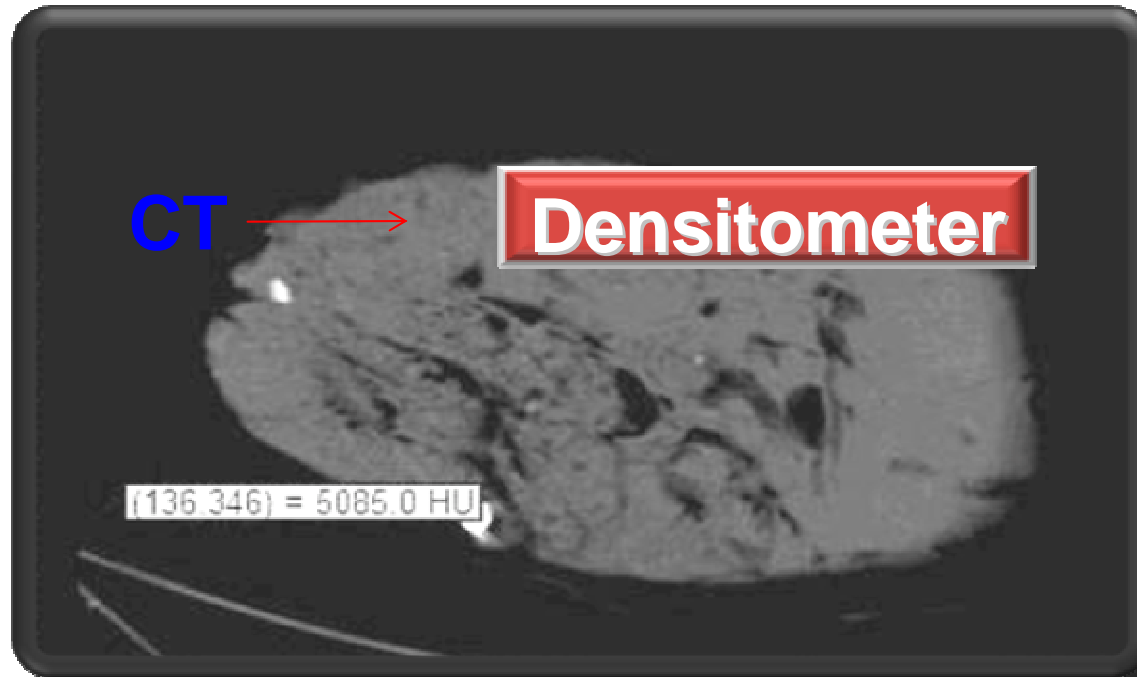
(2009-2012)



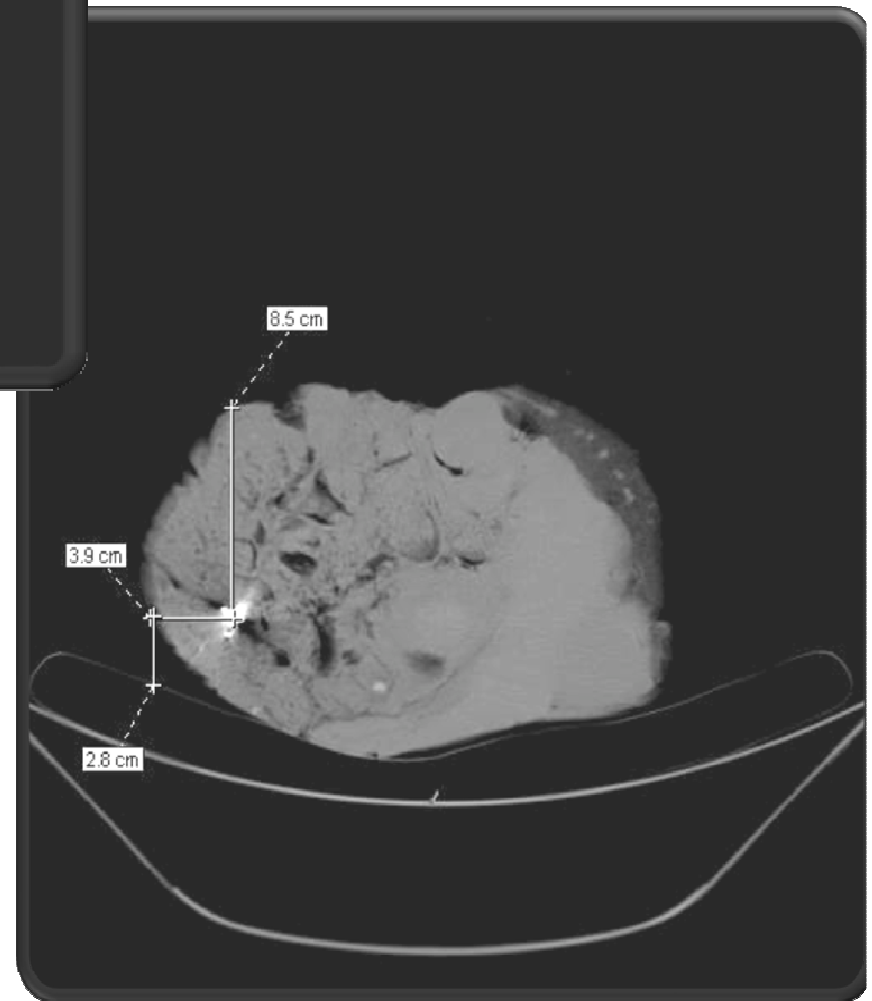
Provincia di Sondrio and Stelvio National Park



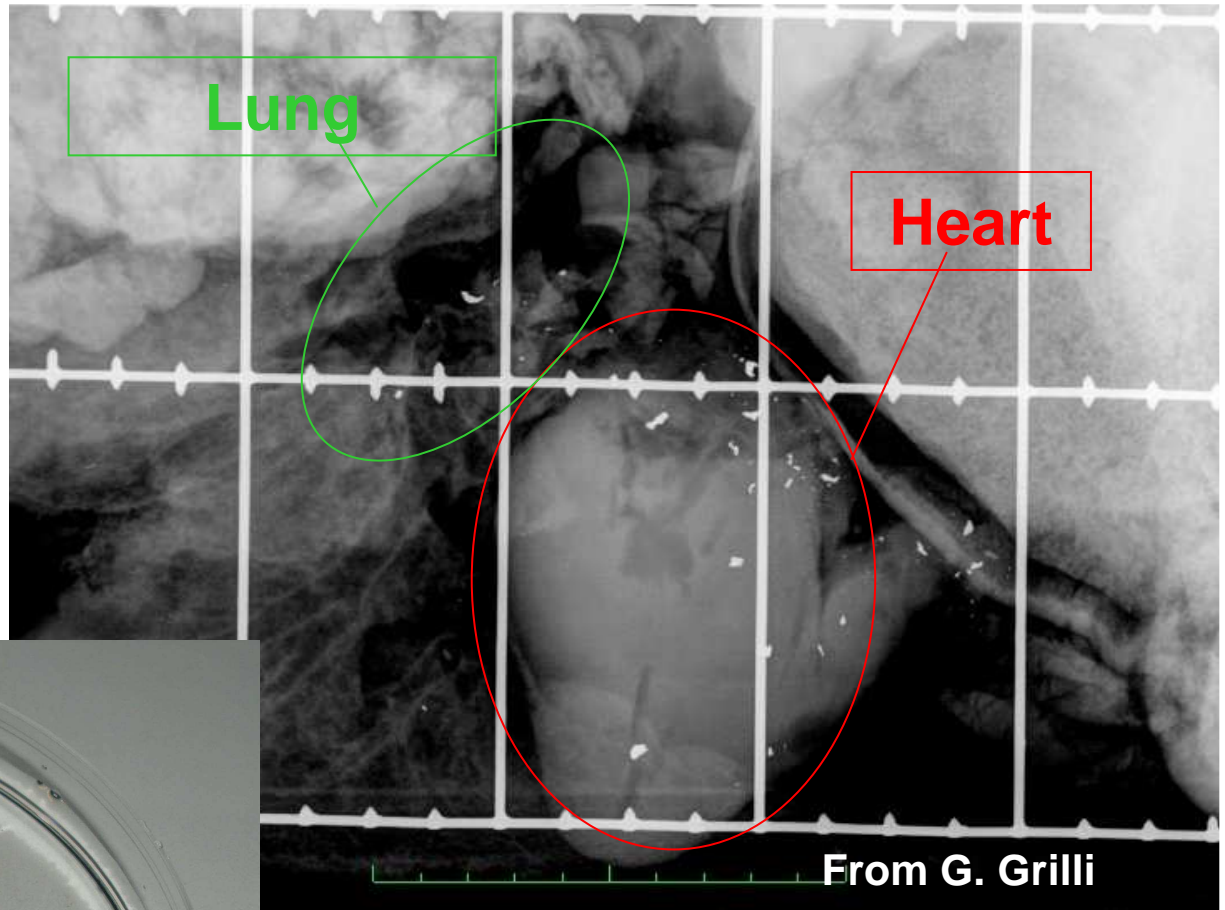
CT INVESTIGATION of FROZEN VISCERA



Use of Computed Tomography
to distinguish
the density of the different tissues



Computed Radiography: lead fragments in heart and lung

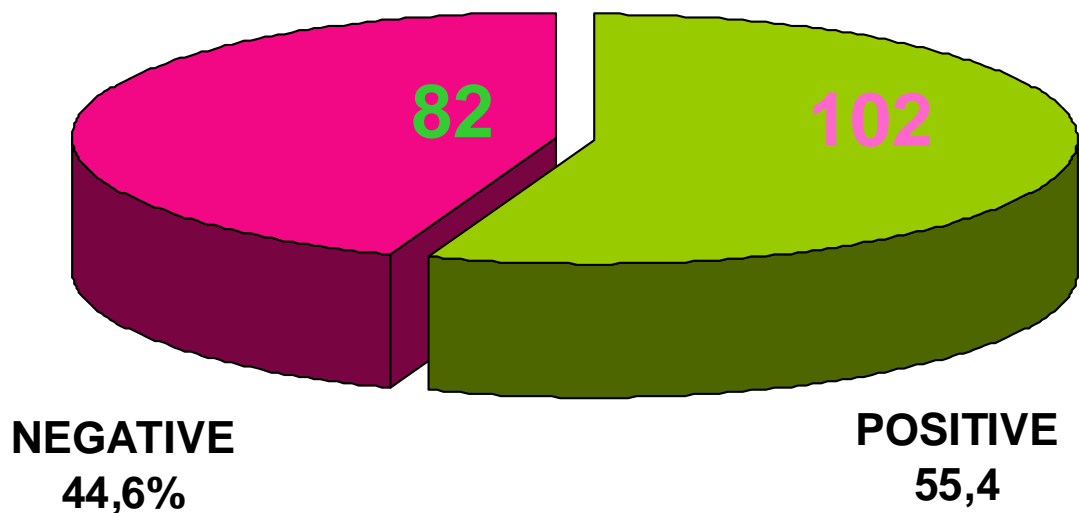


Fragments of lead and copper of a bullet

Total analyzed: 200 viscera

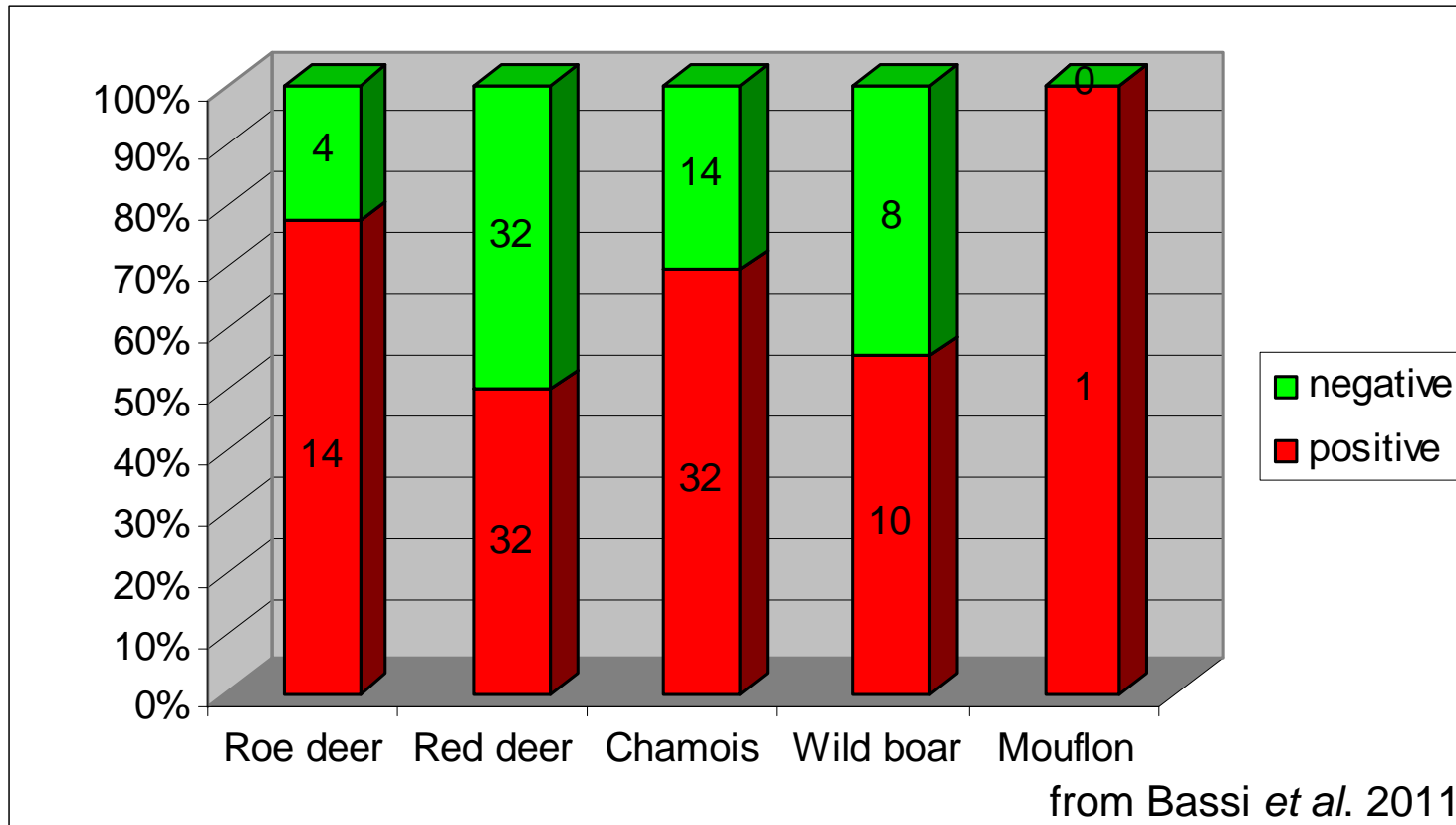
Roe deer: 22 Chamois: 54 Mouflon: 1
Wild boar: 20 Red deer: 103

Results: % of viscera containing lead (N= 184)



Stelvio NP & Sondrio Province *unpublished data*

Lead in the viscera (in % and Number)



LEAD % very high in

Roe deer (77.7%), Chamois (69.6%),

Wild boar (55.6%) and Red deer (46.5%)

Research n. 2

Lead detection in the pellets of large diurnal raptors

To evaluate the food quality in breeding period we analysed with X-Ray technique the pellets from 13 Golden Eagle and 3 Bearded Vulture nests (2005-2012)

LEAD INGESTION has been found in many species of raptors, through the autopsy of subjects found dead or by pellets analysis (Fisher *et al.*, 2006).

The frequency of ingestion increases when the hunting is high.

70% eagle pellets in Norway were found to have lead (Pain 1992).

RX of a pellet 2h post recovery



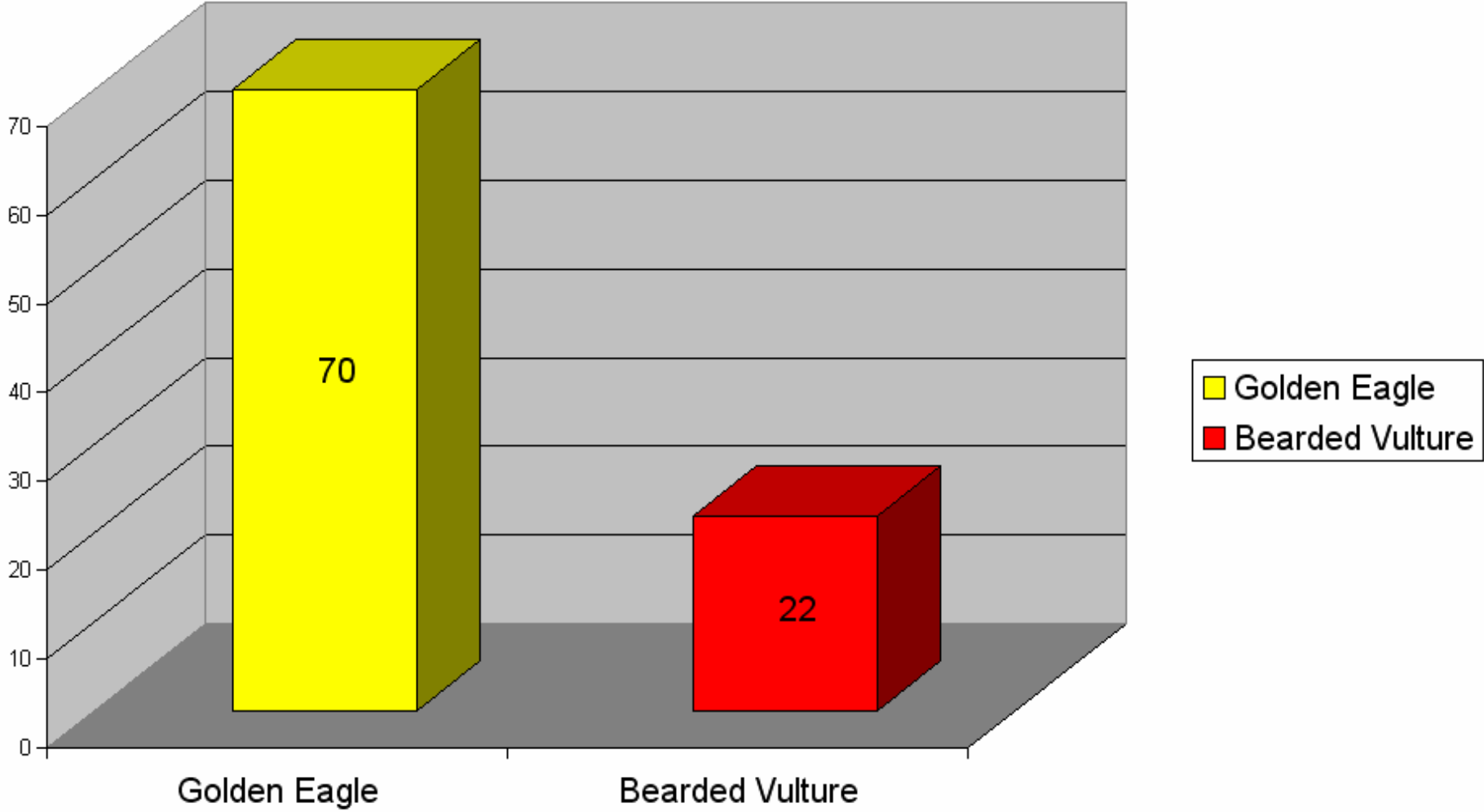
Gressmann

AUSTRIA December 2005 - 'Doraja'





N° Pellets RX Analyzed



PELLETS ANALYSIS

No lead presence in the BV and GE pellets collected in the nests (breeding period)

It will be important to analyse also winter pellets (extra breeding period)



In Marsh Harrier *Circus aeruginosus* in Camargue, during the hunting period, number of pellets containing lead shot increases and lead concentrations in the blood become appreciably higher (Pain *et al.* 1997).

**Number and % of pellets of Marsh Harrier containing lead shot
(from Pain *et al.*, 1997).**

Date of Recovery	N° pellets	N° lead shot found				Tot %
		1	2	3	>3	
Winter 1993-94	72	14	1	2	1	25.4
May-June 1994	71	1	0	0	0	1.4
Winter 1994-95	116	16	8	2	0	15.6
Winter *1991-92	200	20	3	0	0	11.5

* Dec 1991-Feb 1992 data from Pain and Amiard-Triquet 1993). Table modified from Andreotti & Bprghesi 2012

Research n. 3

Carcasse analyses of large diurnal raptors

Since 2010 Stelvio NP and Sondrio Province carry on detailed necropsies of scavenger species (raptors and Raven), analyzing in particular:

- a. Internal **ORGANS**
- b. 2 cm of Long **BONES** (for long term storage of Lead)
- c. **Feathers**



Research n. 3

Carcasse analyses of large diurnal raptors

**To have a carcasse X-Ray is ESSENTIAL to understand
the real origin of the intoxication**

(to distinguish *embedding cases from ingestion lead fragment*)

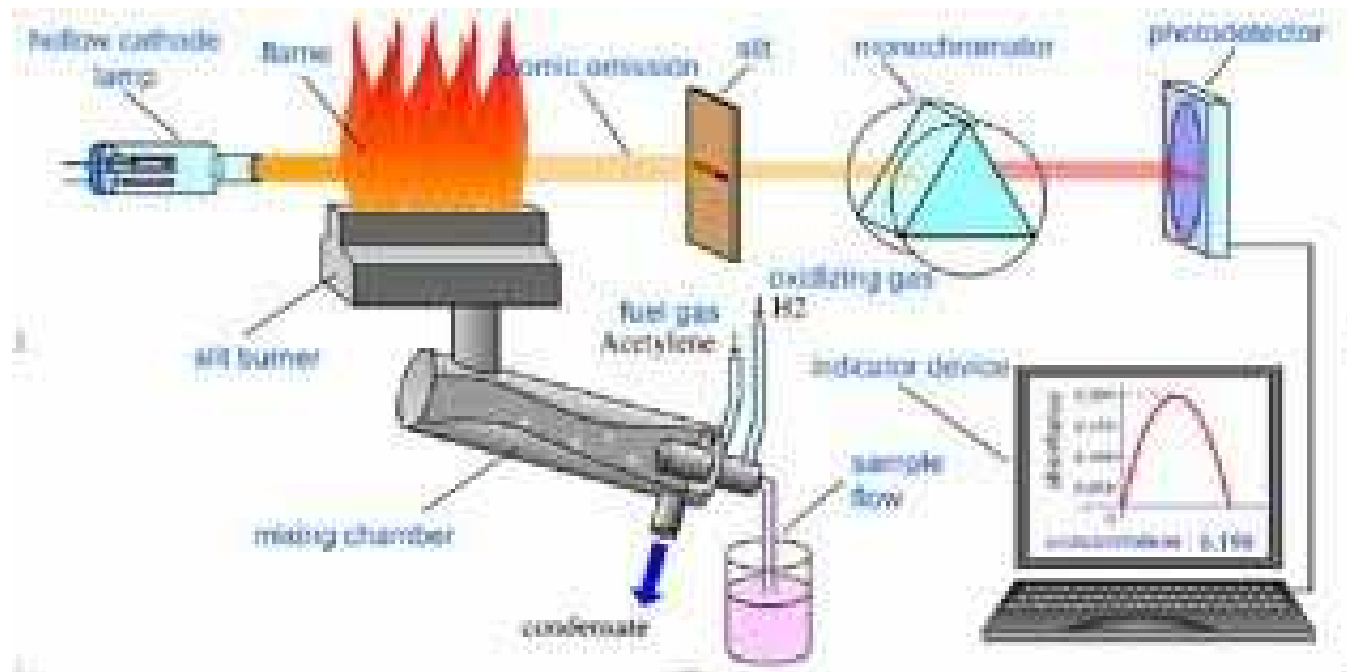


Methods

LABORATORY ANALYSIS

The samples are subjected to mineralization by wet with concentrated nitric acid, hydrogen peroxide and microwave. The mineralized thus obtained are then diluted with demineralized water and analyzed by graphite furnace atomic Absorption spectrophotometry (AAS-GF).

Pb concentrations in tissues were expressed as mg/kg of tissues dry weight basis.



Methods

Specificity: In the case of the search of lead specificity is guaranteed by the use of single-element lamp, with a specific wavelength (λ 283,3 nm) and by the possibility of correcting the bottom with the Zeeman effect.

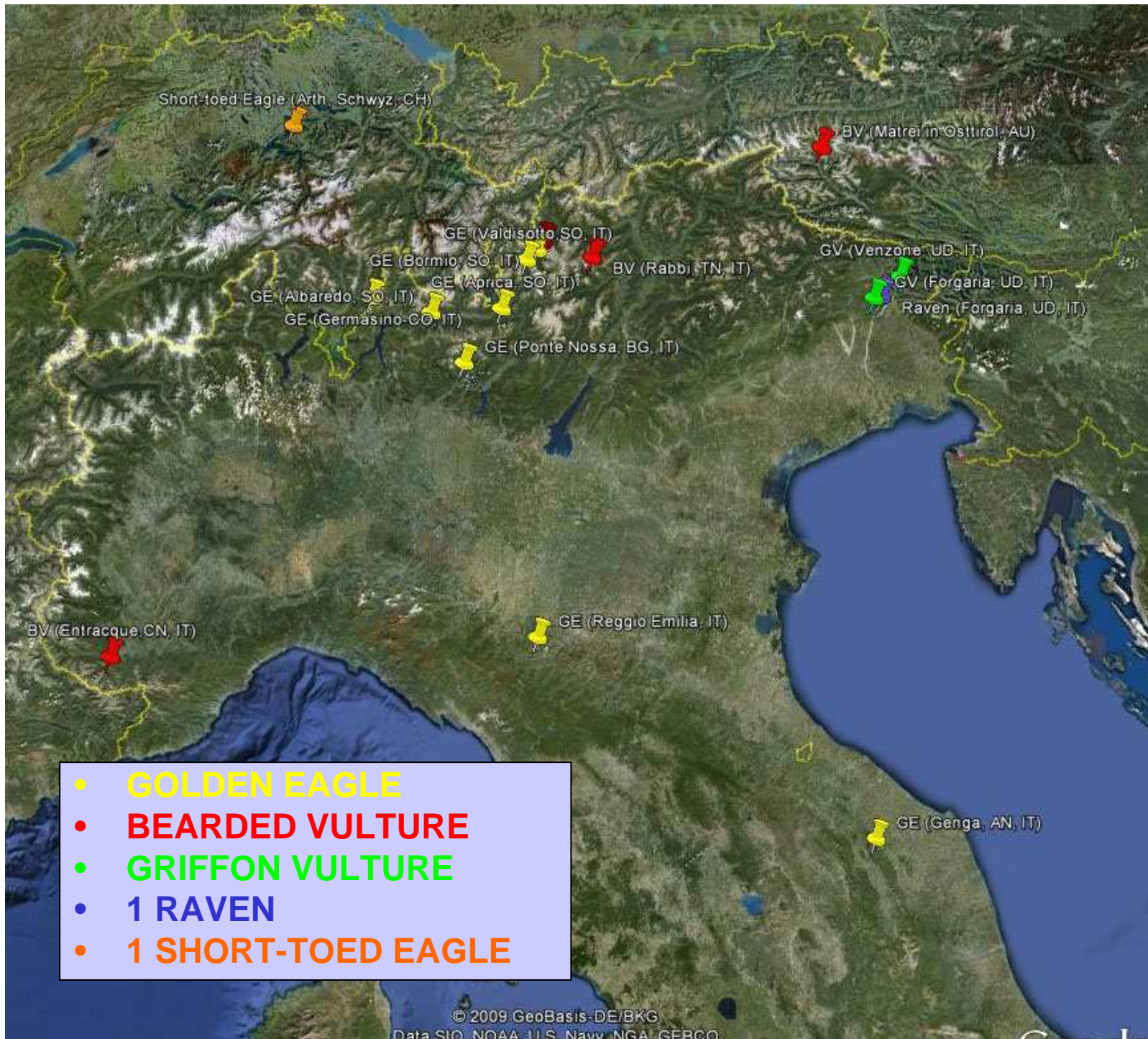
Limit of quantification (LOQ):

For lead, the calculated value is 0.02 mg/kg

Accuracy and precision: the evidence for the calculation of precision and accuracy were performed by analyzing n ° 6 dies certified for lead (lyophilized bovine liver BCR 185, BCR lyophilized pig kidney 186). The value R% found for the liver is 114 and for the kidney 104.

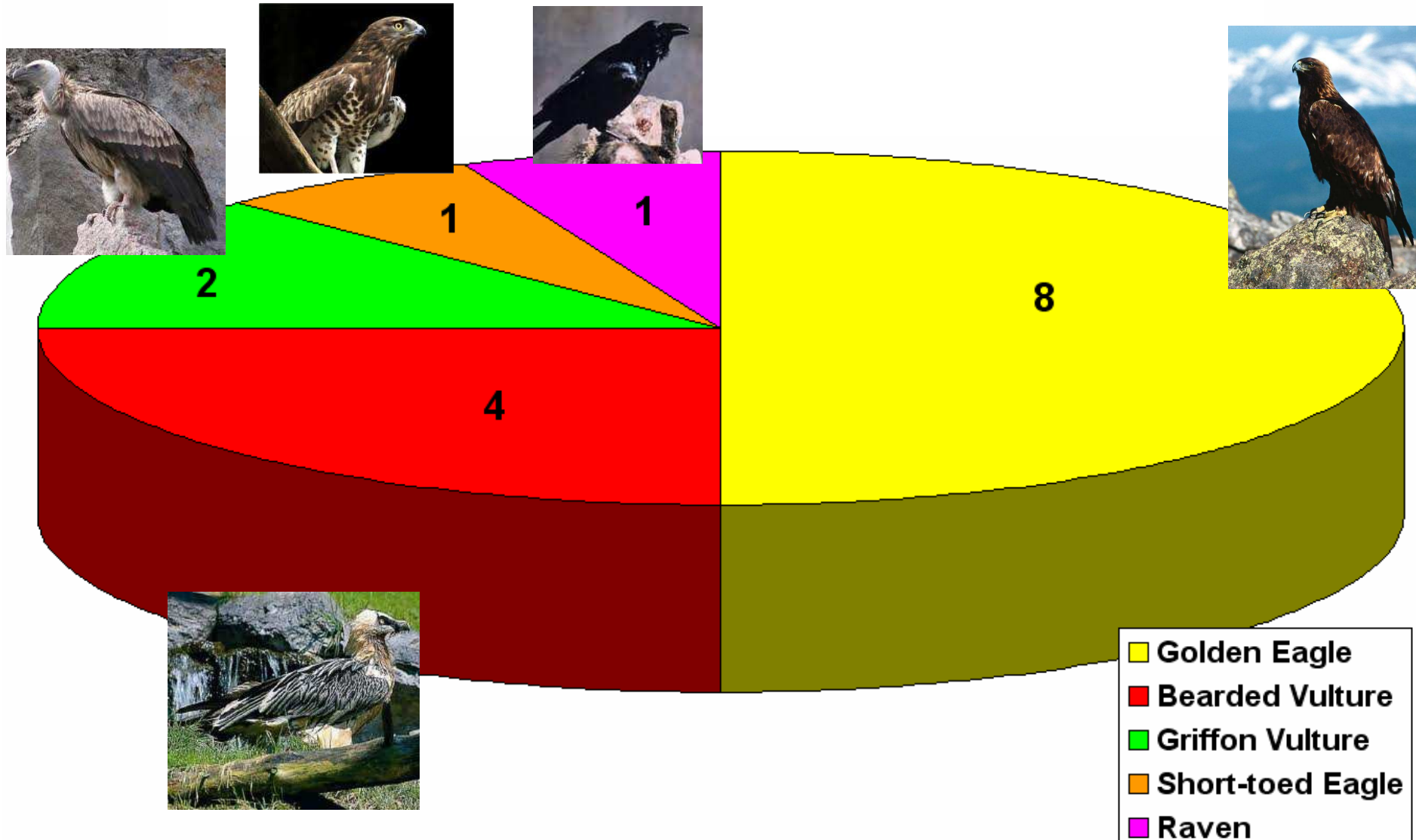


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- **GOLDEN EAGLE**
- **BEARDED VULTURE**
- **GRIFFON VULTURE**
- **1 RAVEN**
- **1 SHORT-TOED EAGLE**

Species distribution of analyzed carcasses (N= 16)



Golden Eagle from I (N= 8)

	Bone	Liver	Kidney
Mean	8,1	7,7	1,6
SD	4,34	13,03	2,49
Median	2,60	0,22	0,24

Bassi et al. unpublished data

In Germany, Switzerland and Austria

Mean Liver: 9,69 Kidney: 3,087 (N= 7) Kenntner et al. 2007

Bearded Vulture from I, CH, A (N= 4)

Age/ Name	Sex	Date of recovery	Bone	Liver	Kidney	Pb ingested	Pb incapsulated
Imm (1,5 y) <i>Ikarus</i>	M	17/Dec/2008	58,90	0,64	1,24	n.a.	no
Subad (5 y) <i>Blangiar</i>	M	09/Jul/2008	1,38	n.a.	n.a.	no	n.a.
Adult (>7 y) <i>Regina Livigno</i>	F	May/2010	1,17	0,14	n.a.	no	No
Adult (>7 y) <i>Nicola</i>	F	23/Jan/2012	6,23	29,6	49,6	no	yes
		Mean	16,9	10,1	25,4		
		SD	28,08	16,87	34,20		
		Median	3,81	0,64	25,42		

Bassi et al. unpublished data

Bearded Vulture from I, CH, A (N= 4)

Pyrenean population (Margalida *et al.* in press) :

Mean Liver 0,97 (N= 30)

Mean bone 2,83 (N= 54)

Griffon Vulture from I (N= 2)

Age	Sex	Date of recovery	Bone	Liver	Kidney	Pb ingested	Pb incapsulated
Juv 1 y	F	12/Feb/2012	13,3	19	7,34	yes	Yes
Adu 10 y	-	01/Apr/2012	2,36	1,21	0,246	no	no
		Mean	7,8	10,1	3,79		
		SD	7,74	12,58	5,02		
		Median	7,83	10,11	3,79		

Bassi et al. unpublished data

Other species (**Raven** & **Short-toed Eagle**)

Age	Sex	Date of recovery	Bone	Liver	Kidney	Pb ingested	Pb encapsulated
Juv (1 y)	F	Mar-Apr 2012	1,17	0,265	-	n.a.	n.a.
Juv (1 y)	M	11/May/2010	0,89	-	-	no	no

Bassi et al. unpublished data



Collecting data from carcasses of Golden Eagle, BV and other scavengers following a standard protocol

Proposal of collaboration to IBM partners

Stelvio NP & Sondrio Province will cover the costs for lead analyses up to a maximum of 1000€/year with Brescia IZS collaboration



Registration Data Sheet

Survey on Lead intoxication in scavenger birds

Provincia di Sondrio
Servizio Caccia, Pesca e
Strutture Agrarie

Referent: Postal address:
E-mail: Telephone n°:
Date of recovery: Locality: Altitude:
Coordinates (WGS84): lat. (decimal): longit. (decimal):

RECOVERY DATA: individual status: injured/flightless dead animal part of carcasse
Species: Golden Eagle Bearded Vulture Griffon Vulture Egyptian Vulture Black Vulture Raven
 Other
Sex: Male Female Unknown Marked/Ringed Bird No Yes Name of the individual
Age: Chick Juvenile (1 year) Immature Subadult Adult Unknown Years of age:

ANALYSES DONE
Photographic detailed documentation (*original position of the animal in the recovery site*) Yes No
Necropsy: Yes No Cause of death/recovery:
..... X-Ray Analyses: done not done
If X-Ray done: Lead ingested Yes No Lead incapsulated: Yes (*specify where*) No
Pellet collected: Yes No Pellet X-Ray analyses Yes No Lead presence in pellets: Yes No
Vomit collected: Yes No Notes on vomit collection:

SAMPLES DELIVERED (*Please send all samples at one time; samples of internal organs must be in waterproof cases*)
Blood collected from vein (*only if injured*) or from hearth (*only if just dead*) Yes No
Bone collected (*must be at least 2 cm lenght*) Yes No If no, specify why:
 Thigh-bone Ribs Tarsus Other (*specify*):
Internal organs collected (*at least 5 g of tissues*) Yes No If no, specify why:
 Liver Kidney Brain
Feathers collected Yes No
 Primary feather (*feather number if possible P __*) Secondary (*feather number if possible S __*) Tail feather

Other informations:

The analyses are free of charge and the results will be returned to the sender as soon as available.

Send all the samples along with the filled sheet to: dott. Alessandro Bianchi - Istituto Zooprofilattico sez. di Sondrio - via Bormio, 30 - Sondrio 23100 - Italy

e-mail address for further infos: **Enrico Bassi:** rxbas@tin.it **Alessandro Bianchi:** alessandro.bianchi@izsler.it



ISPRA
Istituto Superiore per la Protezione
e la Ricerca Ambientale



MINISTERO DELL'AMBIENTE
E DELLA TUTELA DEL TERRITORIO E DEL MARE

Il piombo nelle munizioni da caccia: problematiche e possibili soluzioni



RAPPORTI

Results and discussion

<http://www.isprambiente.gov.it/files/pubblicazioni/rapporti/Rapporti158.pdf>



**New hunting rules introduced in Sondrio province (2012):
no lead bullets OR total viscera
removal from hunting place**

**AND ALSO New hunting rules
regarding lead bullets in other
provinces/regions**

Lead Level in Bone	hunting period	other period	n.r.	total
N°ind Pb 0-2 mg/kg	1	6		7
N°ind Pb 2-5 mg/kg		2	2	4
N°ind Pb 5-10 mg/kg	1	1		2
N°ind Pb 10-60 mg/kg	3		1	4
total recovery	5	8	3	16
N°ind Pb >5 mg/kg (%)	80	11,1	33,3	

64.2% of the GE, BV and GV collected randomly shows lead level in the bone >2 mg/kg N= 14.

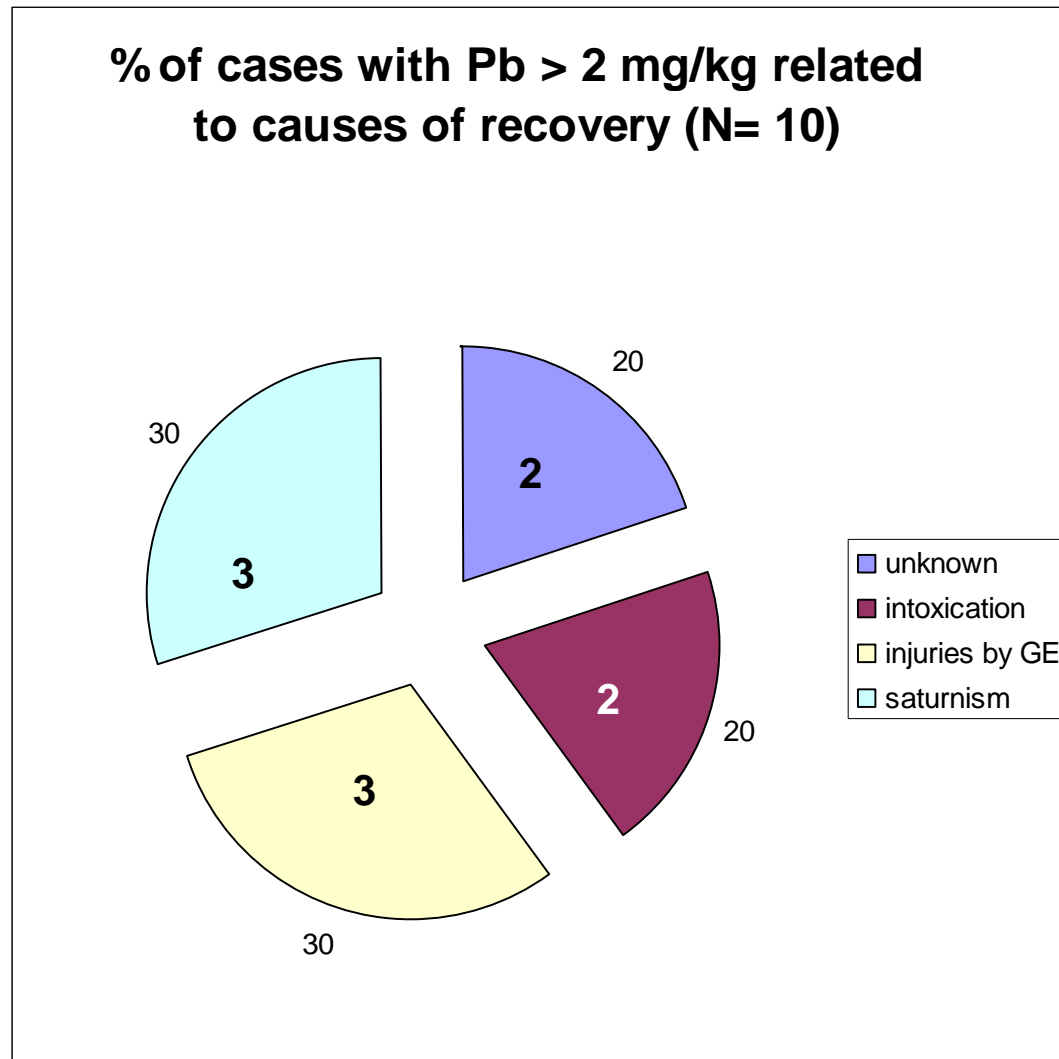
In Pyrenees (BV, EV, GV, RK): 38.7% N= 109 (Razin 2012.)

The 25% of carcasses (N= 4) have a lead level >2 mg/kg without embedded shots in the body.

Saturnism directly provoked by lead ingestion

100% of the embedded carcasses show lead level > 6 mg/kg in the bone

Points of Discussion



**Lead exposure would be ignored in the 70% of the recoveries
in absence of specific analyses of bone!**

Points of Discussion

Bones are good indicators for long term bioaccumulation

Saturnism cases could happen also in extra hunting period

(often associated with embedding shot cases)

X-ray (also partial on some part of carcasse) and complete autopsy

(when is possible) are always necessary.

It's very important to collect always also the remains of carcasse!

Collection and X- Ray analyses of the fall/winter pellets is a possible tool to improve the status of the knowledge about the problem

Proposal of a common protochol and standard method

Starting disseminative actions towards hunters and public

Introduction of new rules to address hunting management

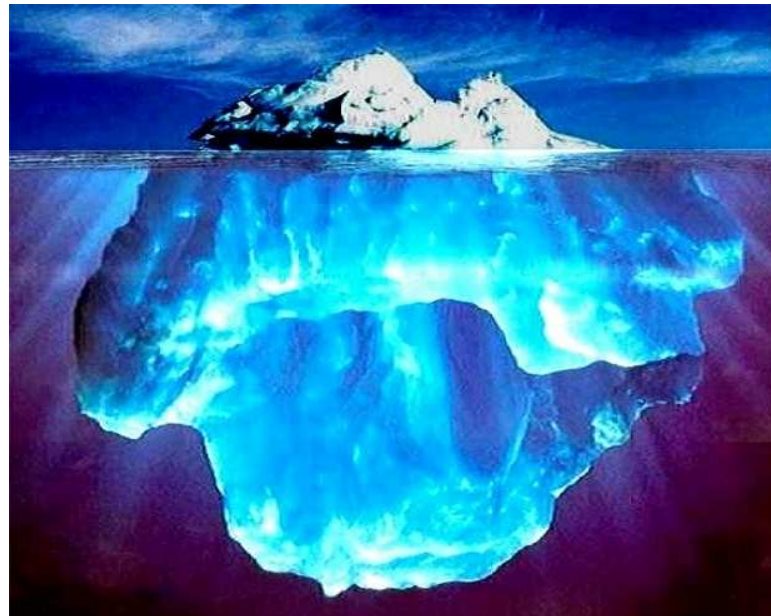
(ban use of lead in ammunitions) is URGENT

Stelvio NP asks your collaboration to recover other samples

and is available to cover the cost for the analyses

Lead poisoning from ingestion of lead shot or non-lethal shooting injuries kills eagles and may be more widespread than suspected (Craig Harmata & Restani 1995).

Doraja, Ikarus, Nicola, Lousa and other anonymous raptors are very probably only the top of an iceberg



THANKS TO

**Provincia di Sondrio
Parco Nazionale dello Stelvio
Fondazione Cariplo**

Ettore Mozzetti, Alessandro Andreotti, Compensori Alpini di Caccia della Provincia di Sondrio, Polizia Provinciale della Provincia di Sondrio, IZS della Lombardia e dell'Emilia Romagna sezione di Sondrio e di Brescia (Lab. Chimica degli Alimenti di Brescia), Hans Frey (RFZ), Marta Gambarin, Fulvio Genero, Alessandro Gugiatti, Luca Giraud, David Jenny, Michael Knollseisen, Luca Pedrotti, Stefano Pesaro, Martine Razin, Roberto Tinarelli, Felix (Felice) Weber ed Ettore Zanon.

For viscera analyses:

Veterinary Univ. of Milan

**Guido Grilli, Mauro Di Giandomenico, Simone Borgonovo,
Viviana Ferrazzi e Melania Moioli**