INCIDENCE AND REPERCUSSION OF LEAD PELLETS INTRODUCED INTO THE TERRESTRIAL ENVIRONMENT ON TERRESTRIAL GAME BIRDS

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1 INTRODUCTION

1.1 ENGAGEMENT AND AIM

The engagement was made by the Spanish Sectoral Federation of Weapons and Ammunition (FSA) and it is aimed at making available a technical and scientific document which assesses the possible incidence and repercussion of lead pellets introduced into the terrestrial environment as a result of hunting activities that use lead ammunition after the relevant planning and opportune work have been performed.

In order to carry out the work, the FSA entered into agreements with:

- Polytechnic University of Valencia (UPV): Juan Bautista Torregrosa Soler (Tenured Professor at the Polytechnic University of Valencia) and Andrés Ferrer Gisbert (Tenured Professor at the Polytechnic University of Valencia).
- University of Murcia (UMU): Diego Romero García (Tenured Professor at the University of Murcia).
- Juan Manuel Theureau de la Peña (Forest Engineer).
- Antonio de José Prada (Forest Engineer).

The engagement's other specifications state that:

- Said assessment will not be made on aquatic game birds or birds of prey;
- The study will be conducted on both sedentary and migratory granivore birds (which require grit to feed);
- The study will be conducted by taking field samples from different territories and/or conditions as far as hunting intensities or habitats are concerned.

This report sets out the final results found to make all the information thus obtained available.

1.2 RESEARCH PHASES

- Review of the bibliography. Done by Antonio de José Prada.
- Fieldwork, consisting of obtaining game specimens (around 25% of the samples were taken from Natura Network 2000 spaces). Done by Antonio de José Prada and Juan Manuel Theureau de la Peña.
- Primary data collection and sample taking, consisting of measuring several physical parameters of the game specimens and processing them to obtain samples from each specimen. Done by Antonio de José Prada and Juan Manuel Theureau de la Peña.
- Obtaining secondary values (crops), consisting of observing the contents of the different game specimens' gizzards. Done by Antonio de José Prada and Juan Manuel Theureau de la Peña.
- Obtaining secondary values (gizzards and intestines), consisting of observing the contents of the different game specimens' gizzards and intestines. Done at the University of Murcia by Diego Romero García.
- Obtaining secondary values (chemical analysis of biological samples). Done at the University of Murcia by Diego Romero García.

- Analysis and discussion of results. Drafting preliminary and final reports. Done by Antonio de José Prada, Juan Manuel Theureau de la Peña, Andrés Ferrer Gisbert, Juan Bautista Torregrosa Soler and Diego Romero García.

1.3 SIMILAR PRECEDING STUDIES

Scientific studies conducted on terrestrial birds and the possible effects lead pellets can have on their populations have been very scarce to date.

After performing a review on them, thirteen articles of interest on terrestrial-habitat birds were found. Said studies are set out below and are repeatedly cited in other scientific publications or references.

Articles published in Spain:

- Lead-Shot Exposure in Red-Legged Partridge (*Alectoris rufa*) on a Driven Shooting Estate. Environ. Sci. Technol. (2008).
- Lead Exposition by Gunshot Ingestion in Red-Legged Partridge (*Alectoris rufa*). Vet Human Toxicol. 46 (2004).

Articles published in Europe:

- Incidence of ingested lead gunshot in wild grey partridges (*Perdix perdix*) from the UK. Eur J Wildl Res (2005).
- Incidence of lead shot ingestion in red-legged partridges (*Alectoris rufa*) in Great Britain. Veterinary Record (2005).
- Lead exposure in ring-necked pheasants on shooting estates in Great Britain. Wildlife Society Bulletin 33 (2005).
- Lead Poisoning in Game from Denmark. Danish review of Game Biology Vol. 11 No. 2 (1979).
- Lead intoxication by ingestion of lead shot in racing pigeons (*Columba livia*). Vlaams Diergeneeskundig Tijdschrift 73 (2004).

Articles published in the United States:

- Fall Diet of Chukars (*Alectoris chukar*) in eastern Oregon and discovery of ingested lead pellets. Western North American Naturalist 63 (2003).
- Grit size preferences and confirmation of ingested lead pellets in chukars (*Alectoris chukar*). Western North American Naturalist 67 (2007).
- Widespread ingestion of lead pellets by wild chukars in Northwestern Utah. Wildlife Society Bulletin 39 (2015).
- Fall diet of a relict pheasant population in North Carolina. The Journal of the Elisha Mitchell Scientific Society 116 (2000).
- Toxic lead exposure in the urban rock dove. Journal of Wildlife Diseases 23 (1987).
- Availability and ingestion of lead shot by mourning doves (*Zenaida macroura*) in southeastern New Mexico. The Southwestern Naturalist 37 (1992).

2 TIME FRAMES OF THE RESEARCH WORK

The work began in March 2016 by choosing the multidisciplinary team to determine the methodology and the aims to be pursued.

The initial phase commenced with choosing the species and areas to conduct the sampling. Subsequently, an action protocol was set for taking field samples, along with to a protocol to determine which samples to take and the analyses to be performed on the specimens obtained over the course of the study.

The first game specimens were obtained in August 2016 (2016/17 hunting season). The batches and number of samples chosen for this report were completed in December 2017 (2017/18 hunting season).

The processing and the analyses of specimens overlapped during this time and were completed in August 2018.

3 FIELDWORK

Obtaining a sufficiently large number of game specimens to ensure the study was representative was planned by taking into account the list of potential bird species to be sampled, the way the birds were to be captured, the potential differences in the specimens' places of origin (by population density in the environment and hunting intensity) and the fieldwork team's actual sampling possibilities.

The quantifiable variables observed were as follows:

- Species
- Method of capture
- Territory
    - General characteristics
    - Environmental contamination
- Hunting intensity
- Season of capture

3.1 DEFINITION OF THE DIFFERENT VARIABLES

3.1.1 *SPECIES variable*

Species chosen

Game bird species having a terrestrial environment habitat which require grit in their digestive process, preferably granivores, were chosen from the range of possible species susceptible of being sampled.

Table 1. *Species chosen for the study*

	Sedentary	Summer migratory	Other (**)
Gallinaceans	*Alectoris rufa* *Alectoris barbara*	*Coturnix coturnix (*)*	-
Pigeons	*Columba livia (*)*	*Streptopelia turtur*	*Columba oenas* *Columba palumbus*

*Not strictly migratory. ** *Columba palumbus* has territories in which it is permanently present, present in the summer, and wintering populations with marked migration routes. *C. oenas* is mainly a wintering species in the Iberian Peninsula.

<u>*Number of species chosen per batch*</u>

A stratified sampling was conducted due to the size of the population. To achieve this, the management unit (hunting area or non-hunting area) was deemed as a stratum or segment, and the target species were captured on a random basis within the hunting unit (stratum).

It was considered that specimens from the same place would show a similar contamination profile in order to the determine the size of the sample. The availability of samples is usually a limiting factor in environmental contamination monitoring studies. Numerous studies are therefore conducted based on a small number of samples, especially when they deal with well-preserved tissues or organs. It is assumed in this study that 30 samples of the same species and place are enough to provide information on lead levels in tissues. The distribution of specimens obtained is given in Table 2 below:

Table 2. *Samples foreseen and obtained in the different sampling locations*

Area	Species	Type	Samples foreseen	Samples obtained	Batch
Castilla y León	*Alectoris rufa*	Wild	30	30	Complete
	Coturnix coturnix	Wild	30	31	Complete
	Columba palumbus	Half-closed season	30	30	Complete
	Columba palumbus	Wintering	30	13	Incomplete
	Columba livia	-	30	28	Incomplete
	Columba oenas	Wintering	30	30	Complete
Castilla-La Mancha	*Alectoris rufa*	Acclimated	90	97	Complete
Navarre Regional Authority	*Alectoris rufa*	Game farm	30	26	Incomplete
Canary Islands	*Alectoris barbara*	Wild	30	13	Incomplete
	Columba livia	-	-	1	*
Valencia Regional Authority	*Alectoris rufa*	Wild	60	66	Complete
	Streptopelia turtur	-	30	31	Complete
	Columba palumbus	Half-closed season	30	30	Complete
	Columba palumbus	-	-	4	*
	Columba livia	-	60	60	Complete
Madrid	*Columba palumbus*	-	30	30	Complete
	Columba livia	-	30	10	Incomplete
*Not initially considered in the study.			**570**	**530**	

Table 2 above does not include any specimens initially used to set protocols or any specimens replaced (22 samples from Museros and nine specimens that could not be digested in the sample's pre-processing before the chemical analysis for Pb was performed, making a total of 561 samples delivered to the University of Murcia's Toxicology Area).

The batch of wintering *Columba palumbus* from Castilla y León remained incomplete due to the difficulty encountered in obtaining game specimens as a result of an atypical migration in the 2016/17 season.

The batch of *Columba livia* from Castilla y León remained incomplete due to the lack of specimens captured in the 2016/17 and 2017/18 seasons.

The batch of *Alectoris rufa* from the game farm of Navarre remained incomplete because three specimens escaped during delivery and the sample from one specimen could not be taken. The game farm specimens were directly supplied to the collaborating veterinarian by the farmer for slaughter and were immediately frozen at -20° C.

The batch of *Alectoris barbara* from La Gomera remained incomplete because there was only one legal hunting day in the 2017/18 season due to a drought and the collaborating hunters were unavailable during said season.

The batch of *Columba livia* from Madrid remained incomplete because the collaborating hunters failed to provide enough specimens.

Despite this, we considered it opportune to process all these samples.

Foreseeing that some batches would not be completed with 30 specimens from the places previously established, some specimens were obtained from other areas This was the case of *Columba palumbus* in the Valencia Regional Authority (half-closed season and wintering, Sacañet Local Authority). The case of a single specimen of *Columba livia* in the Canary Islands was due to the fact that said specimen was delivered outside the sampling. It was, however, not discarded.

It should be noted that some batches exceeded 30 specimens. This was because we did not wish to subjectively discard specimens exceeding the 30-specimen limit when the last specimens of a batch were delivered at the same time by the hunters. This was the case of the batches of red-legged partridges *(Alectoris rufa)* acclimated in Ciudad Real: October (32), November (31) and December (34). This was also the case of the batches of common quails (*Coturnix coturnix*) from Zamora (31), European turtle-doves (*Streptopelia turtur*) from Alicante (31) and red-legged partridges (*Alectoris rufa*) from Alicante (36).

When the total batch of 30 specimens was not reached in a hunting season (2016/17), it was completed, or an effort was made to complete it in the following hunting season (2017/18).

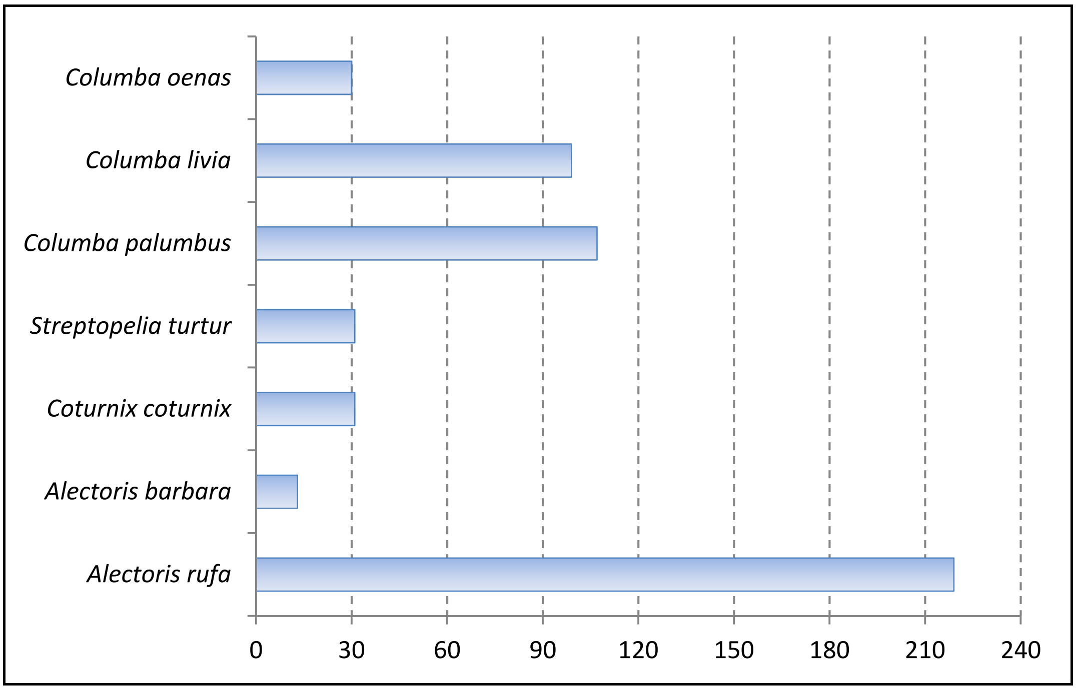
Total number of game specimens

The total number of game specimens on which a full study was finally conducted amounted to 530, which were distributed by species as follows:

- *Alectoris rufa* 219 specimens Photograph 1
- *Alectoris Barbara* 13 specimens Photograph 2
- *Coturnix coturnix* 31 specimens Photograph 3
- *Streptopelia turtur* 31 specimens Photograph 4
- *Columba palumbus* 107 specimens Photograph 5

- *Columba livia* 99 specimens Photograph 6
- *Columba oenas* 30 specimens Photograph 7

Figure 1. *Distribution of the number of game specimens by species*



Identification code designation

The code used to identify each of the game specimens captured is made up of several sub-codes:

AA-BB-CC-00

AA: species and type (PS: wild partridge, PA: acclimated partridge, PG: farm partridge, PM: barbary partridge, CS: wild quail, TE: European turtle-dove, PT: common woodpigeon, PB: rock dove, PZ: stock dove)

BB: province of capture (ZA: Zamora, A: Alicante, CR: Ciudad Real, GOM: Santa Cruz de Tenerife, M: Madrid, AV: Ávila, N: Navarre, V: Valencia, CS: Castellón). Certain batches were identified with a sub-code due to their particular features (FARM: game farm; APT: Manises Airport; PV: Port of Valencia).

CC: month captured (in Roman numerals)

00: sort number of the sample (in Arabic numerals)

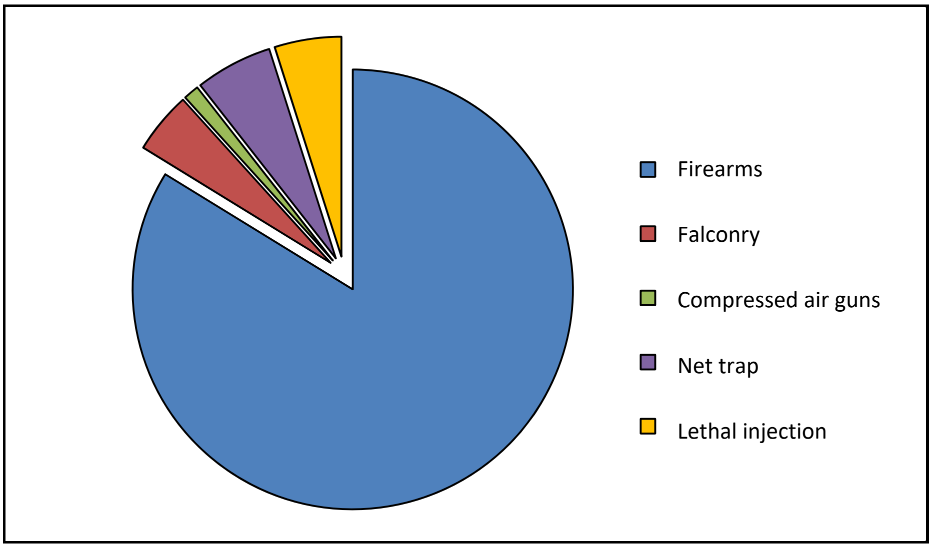
3.1.2 METHOD OF CAPTURE variable

The methods of capture of the 530 specimens sampled were as follows:

- With firearms 444 specimens
- Falconry 24 specimens
- With compressed air gun 6 specimens
- Net trap 30 specimens

- Lethal injection 26 specimens

***Figure 2.** Distribution of the number of game specimens by method of capture*



These methods were chosen from among all the possible ways of capturing birds, considering the need to obtain specimens in certain areas where the use of firearms is forbidden. It should be noted, however, that there are other ways to capture birds which were not used. Each of the methods used in the fieldwork performed are briefly described below:

- In the case of the specimens captured by means of firearms (shotgun), the ammunition used in all cases was lead pellets of differing calibres depending on the target species, ranging from number 10 pellets (Ø 1.9 mm) to number 6 pellets (Ø 2.7 mm). In order to obtain some game specimens, particularly partridges and quails, this method was complemented with the use hunting dogs to flush out and retrieve the game birds. The number of specimens captured with this method totalled 444 specimens.

  NOTE: The use of steel pellets was initially broached due to the great advantage such ammunition provides of not adding any kind of lead contamination to the specimen from the gunshot. However, due to the large number of samples needed (more than 500), agreements had to be reached with hunters so that they would deliver the specimens after having been supplied with the ammunition by the research team. Given the difficulties of reaching said agreements and the objections raised regarding the collaborators' safety, lead was eventually chosen as the only ammunition. Furthermore, practically all the studies performed to date have been conducted with few samples that were obtained using lead ammunition. It was therefore deemed of interest to carry on with this methodology to compare results, without ruling out a second part in which steel pellets could be used.

- Falcons were used to hunt the specimens (24 specimens) in the case of the captures made by means of falconry (Manises Airport).

- In the case of the captures made by means of compressed air guns (compressed air carbines at Manises Airport), 5.5 calibre lead ammunition was used (six specimens). It was initially foreseen that all the captures would be made by means of falconry.

- As regards the captures made with a net trap (Port of Valencia), the specimens were subsequently slaughtered by using gas in accordance with the control authorisation permit (30 specimens). Said permit was issued on 19 April 2016 under the reference BIO/FC/15661/ 21 03 2016.

- As far the game farm specimens are concerned, the specimens were transferred alive from the game farm and slaughtered according to the regulations set forth for such purpose (26 specimens).

3.1.3 *TERRITORY variable*

General characteristics

A brief summary is set out below of the main characteristics (approximate values) of each of the areas in six regional authorities where the specimens were captured:

Castilla y León:

The game specimens were obtained from the provinces of Ávila and Zamora.

Only one sample was obtained from Ávila (El Losar del Barco). This territory's characteristics are therefore not significant. To this it must be added that the sample was a wintering common woodpigeon (*Columba palumbus*).

The samples from the province of Zamora were obtained from three management units (game reserves). Two of them are adjacent and located within the municipal boundaries of Palacios del Pan and Andavías, more specifically the private game reserves registered under the references ZA-10303 (Palacios del Pan) and ZA-10304 (Andavías). The third unit is the "Lagunas de Villafáfila" Regional Game Reserve (RRCLV), more specifically the Villalba de la Lampreana barracks. The first two will be considered as a single stratum for the purpose of their characteristics, while the third will be considered as a single unit for the purpose of description.

Table 3. *General characteristics of the ZA-10303-ZA-10304 Stratum and the RRCLV Stratum*

	ZA-10303-ZA-10304	RRCLV
Surface area (ha)	4251	2829
Average rainfall (mm)	474.3	416.0
Average temperature (°C)	11.7	12.1
Warm period (months)	0	2
Cold or frosty period (months)	6	8
Dry or arid period (months)	3.5	4
Altitude (m)	720	680
Protected spaces	NO	YES
Land uses	80% agricultural – 20% forestry	95% agricultural – 5% forestry
Main agricultural crop	Dryland cereals	Dryland cereals

Castilla-La Mancha:

The game specimens were obtained from the province of Ciudad Real.

The samples from the province of Ciudad Real were obtained from three management units (game reserves). All of them are located within the same local authority, Torre de Juan Abad. Due to the proximity of the different management areas (CR-10420, CR-10517 and CR-10530), they will be considered as a single stratum for the purpose of the territory's characteristics.

Table 4. *General characteristics of the CR-10420-CR-10517-CR-10530 Stratum*

	CR-10420-CR-10517-CR-10530
Surface area (ha)	1544
Average rainfall (mm)	525.6
Average temperature (°C)	13.6
Warm period (months)	2
Cold or frosty period (months)	6
Dry or arid period (months)	4
Altitude (m)	820
Protected spaces	NO
Land uses	65% agricultural – 35% forestry
Main agricultural crops	Dryland cereals, grapevines and olive trees

Valencia Regional Authority:

The game specimens were obtained from the provinces of Alicante, Castellón and Valencia.

The samples obtained from the province of Alicante were taken from a single management unit (game reserve), more specifically the sporting game reserve A-10517 located within the municipal boundaries of Pilar de la Horadada.

Only four samples were obtained from the province of Castellón within the municipal boundaries of Sacañet (game reserve CS-10063). The characteristics of this territory, which is essentially dedicated to forestry, are therefore not significant. It should also be noted that the samples were common woodpigeons (*Columba palumbus*), two of which were obtained in the half-closed season and two were wintering.

The samples from the province of Valencia were obtained from two heavily anthropized areas, more specifically Valencia Airport within the municipal boundaries of Manises and Quart de Poblet, and the Port of Valencia within the municipal boundaries of Valencia.

Table 5. *General characteristics of the A-10517 Stratum, the Airport Stratum and the Port Stratum*

	A-10517	Valencia Airport	Port of Valencia
Surface area (ha)	432	-	-
Average rainfall (mm)	322.2	458.2	443.4
Average temperature (°C)	18.3	17.2	17.5
Warm period (months)	2	2	0
Cold or frosty period (months)	1	3	1
Dry or arid period (months)	6	4	4
Altitude (m)	60	73	2
Protected spaces	YES	NO	NO
Land uses	95% agricultural – 5% forestry	Unproductive	Unproductive
Main agricultural crop	Citrus fruits	Fallow lands and citrus fruits	-

Canary Islands:

The game specimens were obtained from the province of Santa Cruz de Tenerife.

The samples obtained from this province were taken from a single management unit (game reserve), more specifically the Insular Government of La Gomera’s controlled game area (ZCC CI) located within the municipal boundaries of Pilar de la Horadada.

Table 6. *General characteristics of the La Gomera ZCC CI Stratum*

	La Gomera ZCC CI
Surface area (ha)	6300
Average rainfall (mm)	164.8
Average temperature (°C)	19.7
Warm period (months)	0
Cold or frosty period (months)	0
Dry or arid period (months)	10
Altitude (m)	400
Protected spaces	YES
Land uses	2% agricultural – 98% forestry
Main agricultural crop	Tropical fruits and orchards

Madrid Regional Authority:

The game specimens were obtained from the province of Madrid.

The samples obtained from this province were taken from a single management unit (game reserve), more specifically the private game reserve registered under reference number M-10734 located within the municipal boundaries of Humanes de Madrid.

Table 7. *General characteristics of the M-10374 Stratum*

	M-10734
Surface area (ha)	500
Average rainfall (mm)	414.7
Average temperature (°C)	14.5
Warm period (months)	2
Cold or frosty period (months)	6
Dry or arid period (months)	4
Altitude (m)	680
Protected spaces	NO
Land uses	95% agricultural – 5% forestry
Main agricultural crop	Dryland cereals

Navarre Regional Authority:

The game specimens were obtained from the province of Navarre, more specifically from the La Patirroja, S.A. game farm located in the Mendigorria local authority. Given the specimens' origin, it is not necessary to describe the territory's characteristics.

Table 8 sums up the game specimens obtained from the six regional authorities in nine provinces and twelve local authorities, as well as in the different management units. Some of the latter are grouped together under the same stratum due to the conditions mentioned above.

Table 8. *Distribution of game specimens captured*

Regional Authority	Province	Local Authority	Management unit	No. of specimens	Species
Castilla y León	Ávila	El Losar del Barco	AV-10475	1	*Columba palumbus*
	Zamora	Palacios del Pan	ZA-10303	26	*Alectoris rufa*
				21	*Coturnix coturnix*
				42	*Columba palumbus*
				13	*Columba livia*
				30	*Columba oenas*
		Andavías	ZA-10304	4	*Alectoris rufa*
				10	*Coturnix coturnix*
		Villalba de la Lampreana	RRCLV	15	*Columba livia*
Castilla-La Mancha	Ciudad Real	Torre de Juan Abad	CR-10530	56	*Alectoris rufa*
			CR-10517	24	*Alectoris rufa*
			CR-10420	17	*Alectoris rufa*
Valencia Regional Authority	Alicante	Pilar de la Horadada	A-10517	36	*Alectoris rufa*
				31	*Streptopelia turtur*
				30	*Columba palumbus*
				30	*Columba livia*
	Castellón	Sacañet	CS-10063	4	*Columba palumbus*
	Valencia	Manises [1]	Airport	30	*Alectoris rufa*
		Valencia	Port	30	*Columba livia*
Canary Islands	Santa Cruz de Tenerife	San Sebastián de la Gomera	ZCC	13	*Alectoris barbara*
			ZCC	1	*Columba livia*
Madrid Regional Authority	Madrid	Humanes de Madrid	M-10734	30	*Columba palumbus*
				10	*Columba livia*
Navarre Regional Authority	Navarre	Mendigorria	Game farm	26	*Alectoris rufa*

[1] For this purpose, Manises represents the two local authorities within which Valencia Airport is located; namely Manises and Quart de Poblet.

The distribution of the total number of birds captured by local authorities and provinces is shown in the following graphs (Figures 3 and 4):

Figure 3. *Distribution of the number of game specimens by provinces*

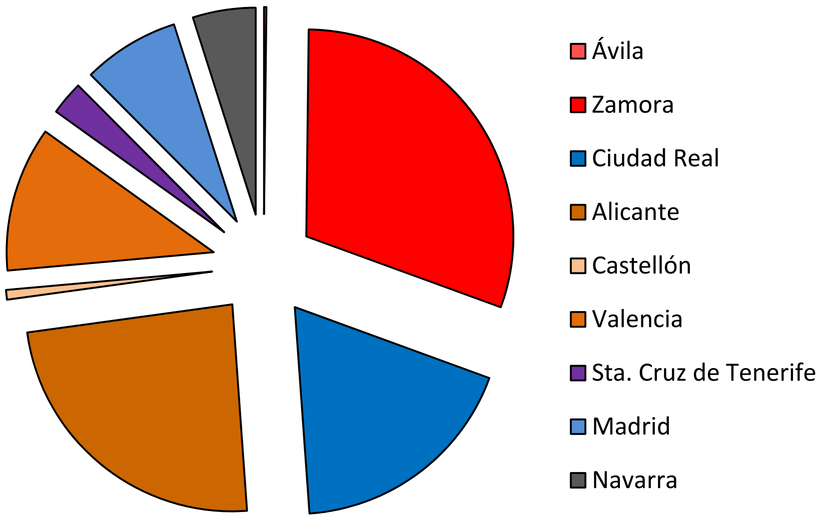
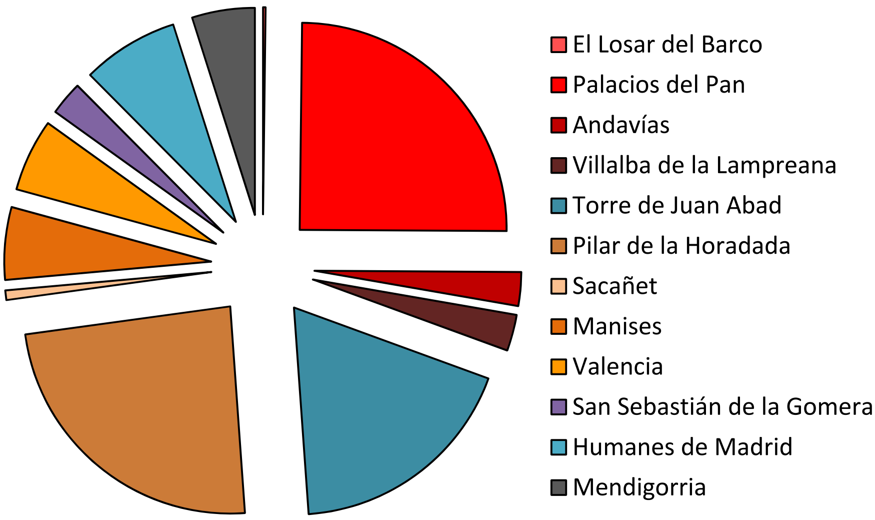


Figure 4. *Distribution of the number of game specimens by local authorities*



Environmental contamination

We considered as a variable the sampling area's proximity to heavily anthropized spaces —where cumulative environmental contamination may affect the chemical or biological values of the living being populations to be found in said areas— based on the Geochemical Atlas of Spain (Geological and Mining Institute of Spain dependent on the Government of Spain's Ministry of Science, Innovation and Universities).

More specifically, in the case which concerns us here, the following values for lead (Pb) were found in the soil samples of the different management units sampled, which were associated to a local authority (Table 9).

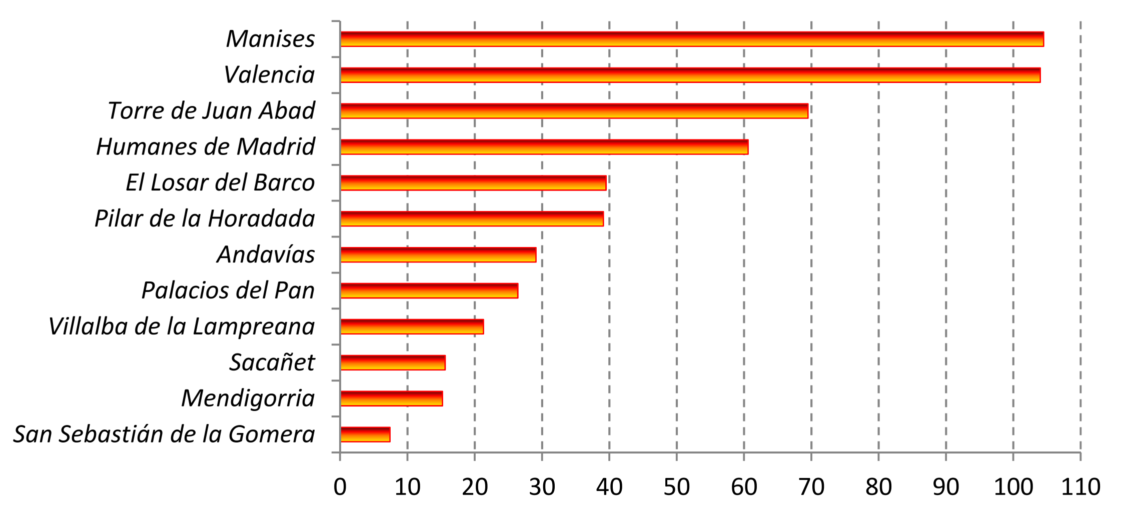
Table 9. *Parts per million of Pb in soil samples*

Regional Authority	Province	Local Authority	Management unit	Pb (ppm)
Castilla y León	Ávila	El Losar del Barco	AV-10475	39.5
	Zamora	Palacios del Pan	ZA-10303	20.7 - 32.0
		Andavías	ZA-10304	29.1
		Villalba de la Lampreana [1]	RRCLV	20.1 - 22.5
Castilla-La Mancha	Ciudad Real	Torre de Juan Abad	CR-10530	69.3 - 69.7
			CR-10517	
			CR-10420	
Valencia Regional Authority	Alicante	Pilar de la Horadada	A-10517	39.1
	Castellón	Sacañet [2]	CS-10063	15.6
	Valencia	Manises	Airport	85.3 - 124.0
		Valencia	Port	104
Canary Islands	Santa Cruz de Tenerife	San Sebastián de la Gomera	ZCC	4.8 - 9.9
Madrid Regional Authority	Madrid	Humanes de Madrid	M-10734	60.6
Navarre Regional Authority	Navarre	Mendigorria [3]	Game farm	12.5 - 17.9

[1] The local authority considered is Villafáfila due to the lack of data for Villalba de la Lampreana. [2] The local authority considered is Bejis due to the lack of data for Sacañet. [3] The local authority considered is Artajona due to the lack of data for Mendigorria.

The following graph (Figure 5) shows from least to most the concentrations found in soil samples by local authorities, as contained in the Geochemical Map of Spain.

Figure 5. *Distribution of lead concentrations (ppm) in soil by local authorities*



3.1.4 *HUNTING INTENSITY variable*

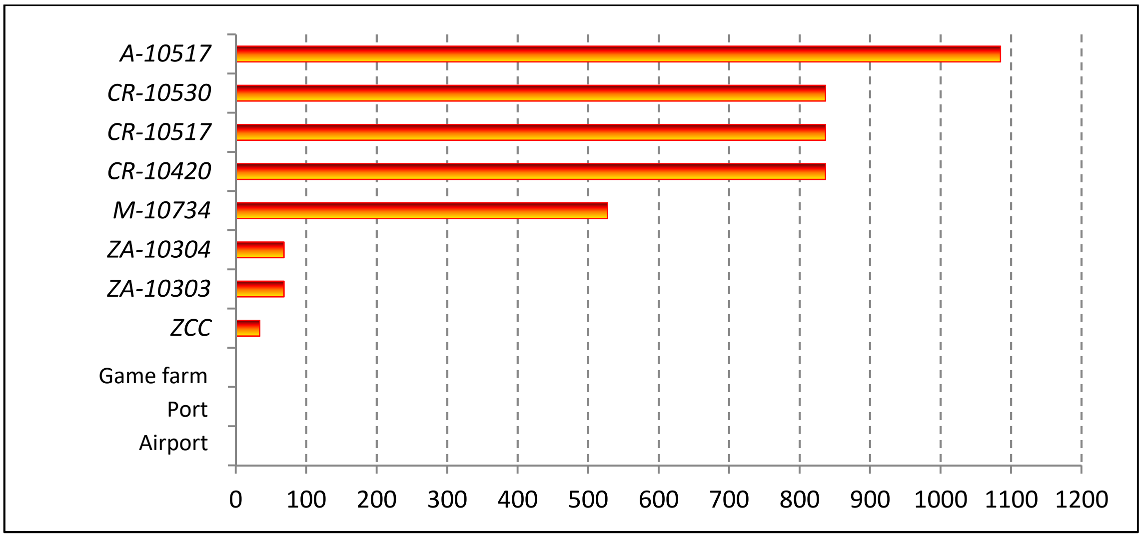
The number of gunshots per hectare and thus the weight of lead pellets which end up in the terrestrial environment can be estimated on the basis of the captures declared by the owners or users of the different management units where hunting activities are carried out (game reserves). The following have thus been estimated per management unit taking the most unfavourable scenario into consideration, pellet cartridges weighing 34 gr.

Table 10. *Intensity of gunshots per hectare and season in management units*

Management unit	Gunshots/ha and season	gr of Pb/ha and season
AV-10475	n.a.	n.a.
ZA-10303	2.01	68.34
ZA-10304	2.01	68.34
RRCLV	n.a.	n.a.
CR-10530	24.61	836.74
CR-10517	24.61	836.74
CR-10420	24.61	836.74
A-10517	31.91	1084.94
CS-10063	n.a.	n.a.
Airport	0.00	0.00
Port	0.00	0.00
La Gomera ZCC	< 1.00	< 34.00
M-10734	15.51	527.34
Game farm	0.00	0.00

The graph below (Figure 6) shows from least to most the estimated amounts of lead in grams per hectare and hunting season which are supposedly introduced into the environment.

Figure 6. *Weight of lead from cartridges in gr/ha and season*



3.1.5 *SEASON OF CAPTURE variable*

Under the hypotheses that the number of pellets within the reach of birds increases from the beginning (October) to the end (January) of the season, a decision was taken to plan the capture of red-legged partridge specimens (mainly acclimated partridges) from Ciudad Real, making an effort to take samples in October, November and December. The season begins on 8 October and ends on 8 February in the management units chosen in this area.

Said variable was only taken into consideration for acclimated partridges within this area of Ciudad Real. The batches were eventually constituted in the following way:

Table 11. *Batches of acclimated partridge from Ciudad Real based on the SEASON OF CAPTURE variable*

<table>
<tr><td rowspan="2">Management unit</td><td>October</td><td>November</td><td colspan="2">December</td></tr>
<tr><td>2016</td><td>2016</td><td>2016</td><td>2017</td></tr>
<tr><td>CR-10530</td><td>32</td><td>0</td><td>15</td><td>9</td></tr>
<tr><td>CR-10517</td><td>0</td><td>16</td><td>3</td><td>5</td></tr>
<tr><td>CR-10420</td><td>0</td><td>15</td><td>0</td><td>2</td></tr>
<tr><td>TOTAL</td><td>32</td><td>31</td><td colspan="2">34</td></tr>
</table>

The three reserves in Cuidad Real are subject to the same kind of management and are located within the same local authority, Torre de Juan Abad. These reserves are identified in the results report as PA1-CR and PA2-CR (CR-10530, start and end of season), PA3-CR (CR-10517) and PA4-CR (CR-10420).

3.2 SUMMARY OF THE VARIABLES

Table 12 contains a summary of the different variables determined for this study:

Table 12. *Summary of the variables considered associated to codes and number of samples*

Species	Type	Capture	Management unit	Contamination	Hunting intensity	Season	Code	N
Alectoris rufa	Wild	Hunted	ZA-10303-4	20.7 - 32.0	Low	Season	PS-ZA	36 [1]
	Acclimated	Hunted	CR-10530	69.3 - 69.7	High	Start of S.	PA1-CR	34 [2]
	Acclimated	Hunted	CR-10517	69.3 - 69.7	High	Start of S.	PA3-CR	24
	Acclimated	Hunted	CR-10420	69.3 - 69.7	High	Start of S.	PA4-CR	17
	Acclimated	Hunted	CR-10530	69.3 - 69.7	High	End of S.	PA2-CR	24
	Wild [α]	Hunted	A-10517	39.1	Very high	Decoy	PS-A	36
	Wild	Falconry	Airport	85.3 -124.0	None	-	PS-APT	30
	Farm	-	Game farm	12.5 - 17.9	None	-	PG-FARM	27 [3]
Alectoris barbara	Wild	Hunted	La Gomera ZCC	4.8 - 9.9	Very low	Season	PM-GOM	13
Coturnix coturnix	Wild	Hunted	ZA-10303-4	20.7 - 32.0	Low	Half-closed	CS-ZA	31
Columba palumbus	Wild	Hunted	AV-10475	39.5	Low	Migration	PT-AV	1
	Wild	Hunted	ZA-10303	20.7 - 32.0	Low	Half-closed	PT-ZA	30
	Wild	Hunted	ZA-10303	20.7 - 32.0	Low	Migration	PT-ZA	12
	Wild	Hunted	A-10517	39.1	Very high	Half-closed	PT-A	30
	Wild	Hunted	CS-10063	15.6	Low	Half-closed	PT-CS	2
	Wild	Hunted	CS-10063	15.6	Low	Migration	PT-CS	2
	Wild	Hunted	M-10734	60.6	High	Half-closed	PT-M	30
Columba livia	Wild	Hunted	ZA-10303	20.7 - 32.0	Low	Season	PB-ZA	13
	Wild	Hunted	RRCLV	20.1 - 22.5	Low	Season	PB-ZA	15
	Wild	Hunted	A-10517	39.1	Very high	Half-closed	PB-A	30
	Wild	Hunted	M-10734	60.6	High	Half-closed	PB-M	10
	Wild	Hunted	La Gomera ZCC	4.8 - 9.9	Very low	Season	PB-GOM	1
	Wild	Net	Port	104	None	-	PB-PV	30
Columba oenas	Wild	Hunted	ZA-10303	20.7 - 32.0	Low	Migration	PZ-ZA	30
Streptopelia turtur	Wild	Hunted	A-10517	39.1	Very high	Half-closed	TE-A	31
Several	Wild	Hunted	Museros	-	-	-	-	22 [4]

[1] Six samples of this batch could not be digested. [2] Two samples of this batch could not be digested. [3] One sample of this batch could not be digested. [4] This batch was not taken into account for the report. [α] These were initially considered wild partridges, but some farm specimens have appeared.

3.3 ACTION PROTOCOL TO OBTAIN SPECIMENS

3.3.1 Obtaining specimens in the field

The batches of specimens were obtained from captures made under differing conditions, taking advantage of:

- the legal hunting season at reserves (specimens shot with a shotgun);
- bird control permits at airports (falconry and others) or ports (net trap).

To do so, the team responsible for getting the specimens first obtained (depending on the programme) the relevant permits or commitments from the different game reserves, groups of hunters or control companies.

All collaborating hunters were given the relevant instructions beforehand. The percentage of samples obtained by the technicians in charge of the sampling amounted to 62.6% (332 game specimens), while 37.4% (198 game specimens) were obtained by collaborating hunters.

3.3.2 Shipment of specimens

In all cases, one of the people responsible for the fieldwork either went to the place of capture to pick up recently hunted specimens or to the game reserves or control sites to pick up specimens that had previously been frozen at -20° C in freezers belonging to the hunters themselves or to the authorised control company.

The specimens then were shipped in portable refrigerators on the same day. If the shipment was made on the following day, it was done in portable refrigerators after the specimens had been cooled in refrigerators at the places of capture.

The shipment of the specimens (previously frozen by collaborators) to the freezers (expressly located in the workplaces made available in Zamora, Valencia and San Sebastián de la Gomera) was carried out in portable refrigerators through direct routes. In the event of lengthier distances and shipment times (Alicante and Zamora), a WAECO Coolfun portable refrigerator was used for the shipment.

Aside from exceptional cases in which the specimens were processed fresh, the specimens were frozen at the destination in separate bags, depending on the place of capture and date they were hunted. As mentioned above, the place and date of capture were recorded on the bag label.

4 PRIMARY DATA AND SAMPLE TAKING

4.1 MATERIALS USED

The materials used for this action are set out below (Photograph 8):

- Gloves
- Self-closure bags
- Indelible marker pen
- Scissors
- Tweezers
- Scalpel
- Conical microtubes (*Eppendorf* 1.5 ml)

- Sample containers (50 and 100 ml)
- Scales (accuracy of 1 gr)
- Refrigerator
- Freezer
- Ice packs (freezer packs)

4.2 PROTOCOL FOLLOWED

The specimens were processed on a workbench with a nearby sink (Photograph 9) according to the protocol set out below setting out the steps to be followed and the instructions given by the veterinarians Diego Romero García and Miguel Ángel Sánchez Isarrias:

- **Defrosting** the specimens to be processed in a refrigerator or portable refrigerator. The defrosting should last until mobility of the joints is ensured, though slightly hampered to prevent total defrosting and thus the mixing and alteration of fluids.
- **Prior labelling of self-closure bags and containers used to store samples**: a bag for the wing and head, a container for the gizzard and digestive tract and *Eppendorf* tubes for the liver and kidney samples. The labelling was done with indelible ink markers on the white strips of the bags, containers or microtubes to identify the contents. The coding set out above in Section 3.1.1 (SPECIES variable. Identification code designation) was used for the labelling.
- **Weighing the specimen (grams)**. If any part of the specimen was missing (leg, head or wing) due to the damage sustained by the specimen and only if it was captured by falconry, its total weight was estimated by determining the weight of the missing member and recording it in observations.
- **General visual examination** of the specimen to record any observations that might be relevant (i.e. lack of head, double spurs in partridges, specimens damaged by gunshot, etc.)
- **Examination of primary remiges' moulting stage**. It was recorded if the moulting was complete or which feather of the remiges was in the process of moulting. To determine which primary remiges were moulting or which was the last one to be shed, the number of primary remiges was counted (if one had been recently shed) and the wing's internal feathers covering the shaft were pulled out. After counting the feathers, the moulting remiges were recorded (Photograph 10). In case of any doubt, the left wing's remiges were pulled out to examine the shafts' insertion into the wing. If it was observed that one was more tender, that feather was recorded as the last one to undergo moulting. If any feather had recently been shed without any signs of regrowth, said feather was recorded as the moulting remiges.
- **Examination of feather wear in farm-bred red-legged partridges acclimated** to the site. Partridges acclimated to the wild before the hunting season generally show very characteristic damage to the feathers. It can even be clearly seen which remiges have moulted in the wild and which have moulted at the farm. The marks on primary and secondary remiges of farm partridges were recorded as abundant, a few, scarce or none. If the marks were clearly visible, the first remiges which had moulted in the wild were recorded.

- **The specimen's age**. After the aforementioned examinations were done, we then proceeded to assign the specimen's age, differentiating between young (0+) and adult (>1+), without prejudice to any observations related to age due to double spurs or any other sign which allows an age above 2+ to be attributed.
- **Wing sample (complete wing)**. The right wing was cut off with scissors, including the humerus (muscle, bone and feathers), without prejudice to taking the left wing as a sample due to damage to the right wing (i.e. as a result of the method of capture). The wing sample was then put into the labelled bag.
- **Head sample (complete head, including encephalon)**. The head was cut off along with the first vertebrae with scissors. The head sample was then put into the labelled bag.

The feathers of the belly, chest and throat were then **plucked** for the following steps.

- **Crop sample (complete crop)**. Manual extraction of the crop with the aid of small scissors and tweezers. The crop's extraction must be done carefully to avoid damaging its tissue (Photograph 11). The crop's exterior was examined to find any orifices or breakages, recording any that were found. If the crop was empty or almost empty, its interior was examined and the presence of any pellets inside was recorded (if any were found, the crops were kept in *Eppendorf* microtubes), and it was discarded. If the crop contained food, it was weighed and kept for subsequent analysis in a sample container labelled with the appropriate code. These containers containing crops were frozen immediately.
- **Access to internal organs**. An incision and cut were made with scissors in the belly's soft area just below the keel, which was prolonged with other cuts beside the muscles leading from the chest to the wings. Holding the legs, the breast was then swung towards the head, thereby leaving the internal organs visible.
- **Liver sample**. With aid of some scissors and tweezers, a sample was taken from the lower right-hand lobe of the liver, which was then placed into a labelled *Eppendorf* microtube. Said microtube was kept in a bag along with the bag containing the wing and head samples. If the side of the liver chosen for sampling was seriously damaged as a result of the method of capture, the sample was taken from the left-hand side and recorded in observations.
- **Gizzard and intestinal tract sample (complete organs)**. A cut was made where the proventriculus joins the gizzard, which was then tugged to extract the entire intestinal tract right up to the cloaca, where it was cut (including the cloaca). All this was weighed and then the gizzard and intestinal tract sample was placed into a labelled sample container.
- **Recording the sex.** After examining the gonads (Photograph 12), the specimen's sex was recorded (male or female).
- **Kidney sample (complete organ)**. The left kidney was extracted from its cavity with scissors and tweezers. The organ was carefully separated from the cavity with the tip of some tweezers until the ligaments were reached, which were then cut with scissors. If the left kidney was damaged as a result of the method of capture, it was recorded, and the right kidney was taken as a sample. The kidney was then placed in a labelled *Eppendorf* tube, which was placed in a self-closure bag along with the other microtube containing the liver sample, the container with the gizzard and intestinal tract sample and the bag containing the wing and head.

- **Conservation of the bag containing all the samples taken from the specimen.** The properly identified bag was kept in a freezer at -20° C, along with others in the same drawer or bag.
- **Taking photographs**. The relevant photographs were taken during all these steps with a time and date stamp, which might be of importance to provide data or document observations.
- **Recording observations.** Any observation about the specimens that might reveal significant differences as compared to other specimens was recorded.
- **Shipment of samples to the laboratory.** All the samples taken from the specimens, which had been processed, properly identified and grouped together by batches or sub-batches, were finally shipped by road from Valencia or Zamora to the University of Murcia by the people responsible for the work team. The samples were removed from the freezers and shipped from Valencia (a trip of 2 h and 15 min) in portable refrigerators. From Zamora (a trip of 6 h), they were transported in WAECO Coolfun refrigerator freezers, which generate cold through an electrical connection to the vehicle. Only in the case of the samples from of the Canary Islands was there an intermediate shipment in a portable refrigerator from the area's freezer to the freezer in Valencia.

All the samples not used in this study were kept in storage (-20° C) for future studies (if necessary) at the facilities of the University of Murcia's Toxicology Area.

4.3 SUMMARY OF PRIMARY DATA RESULTS

The data set out below were recorded for each specimen and subsequently compiled on a spreadsheet:

- The specimen's code
- Origin (usually the reference number of the reserve or specific area, if it was not a perfectly identifiable hunting management unit)
- Date of capture
- Date the primary data was obtained and sample taking
- Date of the samples' shipment to the University of Murcia.
- Species
- Nature (in this section a differentiation is made about whether the bird's origin is from wild, acclimated or game farm populations)
- Environment (in this section a first impression is recorded about the environment's population density, differentiating between rural and peri-urban environments)
- Age (differentiating between young or adult)
- The specimens' sex
- Remiges (this deals with the stage of the primary remiges' moulting — complete or remiges undergoing moulting and, if so, their number)
- Signs of damage to remiges done at the farm in the case of acclimated partridges (recorded in observations)
- The specimen's weight
- The crop's weight (except for crops which were empty or contained very little food)

- Weight of the of the gizzard and the intestinal tract (including the cloaca)
- Presence, if any, of possible perforations caused by pellets in the crop
- Any other observations not indicated in the sections above

The summary by stratum is given in Table 13.

Table 13. *Hunted specimens' primary data*

Species	Management unit	O	N	A	S	WT
Alectoris rufa	ZA-10303	W	26	19/7	16/10	433.7
Alectoris rufa	ZA-10304	W	4	3/1	1/3	402.5
Alectoris rufa	CR-10530	A	56	25/31	30/26	430.6
Alectoris rufa	CR-10517	A	24	15/9	11/13	474.3
Alectoris rufa	CR-10420	A	17	11/6	11/6	448.7
Alectoris rufa	A-10517	W[1]	36	19/17	21/15	449.6
Alectoris rufa	Airport	A	30	16/14	12/18	390.5
Alectoris rufa	Game farm	G	26	0/26	11/15	410.0
Alectoris barbara	ZCC	W	13	3/10	7/6	485.3
Coturnix coturnix	ZA-10303	W	21	3/18	7/14	100.4
Coturnix coturnix	ZA-10304	W	10	8/2	6/4	100.9
Columba palumbus	AV-10475	W	1	0/1	0/1	428.0
Columba palumbus	ZA-10303	W	42	4/38	24/18	424.8
Columba palumbus	A-10517	W	30	30/0	16/14	398.4
Columba palumbus	CS-10063	W	4	2/2	-/-	374.6
Columba palumbus	M-10734	W	30	8/22	22/8	405.6
Columba livia	ZA-10303	W	13	3/10	9/4	287.31
Columba livia	RRCLV	W	15	1/14	8/7	309.5
Columba livia	A-10517	W	30	19/11	17/13	288.0
Columba livia	Port	W	30	11/19	13/9	309.1
Columba livia	ZCC	W	1	-/-	-/-	286.0
Columba livia	M-10734	W	10	0/10	5/5	297.3
Columba oenas	ZA-10303	W	30	7/23	15/15	278.0
Streptopelia turtur	A-10517	W	31	23/8	15/16	139.1

O: origin (W-wild; A-acclimated; G-game farm); N: number of game specimens sampled; A: age (young/adults); S: sex (males/females); WT: average weight (in grams). [1] Specimens which had been released into the wild were observed (according to the owner, specimens were released into the wild at the adjacent reserve before the decoy hunting season).

5 CROPS' SECONDARY VALUES

5.1 PROTOCOL TO EXAMINE CROPS

The steps followed to obtain the values for crops were as follows:

- Extraction of the sample container's contents and checking the recorded weight and for any possible perforations or breakages recorded.
- Opening the crop with scissors and tweezers.
- Taking a photograph of the contents, including the code assigned thereto (Photographs 13, 14 and 15).
- Recording any identifiable food.
- Searching for pellets and recording the number thereof, if any.

- Attributing the pellet's origin: in the case of obvious breakages with an entry orifice but no exit orifice or of pellets embedded in the feathers, it was assigned as a gunshot pellet.
- Slight drying of the crop's contents and putting them back into the container, only in the event of a possible interest (subsequent identification of the crop's contents, to conserve the pellets in it or to merely conserve them in a microtube).

5.2 SUMMARY OF SECONDARY VALUES — CROPS

The findings on the presence of lead pellets in the crop by species are set out below. In addition, their origin was determined on an individualised basis. In case of any doubt, it was deemed that the pellet was suspected of having been ingested. "N" stands for the number of specimens and "n" for the number of pellets in the following tables:

Red-legged partridge (*Alectoris rufa*)

Table 14. *Results for the contents of red-legged partridge crops*

		Number of red-legged partridge crops		
Management unit	N	Empty	With contents	With presence of pellets
ZA-10303	26	20	6	0
ZA-10304	4	3	1	0
CR-10530	56	24	32	4
CR-10517	24	10	14	1
CR-10420	17	11	16	1
A-10517	36	17	19	5
Airport	30	9	21	0
Game farm	26	26	0	0
TOTAL	219	120	109	11

Table 15. *Number of pellets by specimen and origin in red-legged partridge crops*

			Number and origin of pellets	
Management unit	Specimen	n	Gunshot	Ingestion suspected
CR-10530	PA1-CR-X-21	1	1	0
CR-10530	PA2-CR-XII-6	3	1	2
CR-10530	PA2-CR-XII-8	4	0	4
CR-10530	PA2-CR-XII-9	1	0	1
CR-10517	PA3-CR-XI-16	1	1	0
CR-10420	PA4-CR-XI-11	1	1	0
A-10517	PS-A-II-10	1	1	0
A-10517	PS-A-II-24	1	1	0
A-10517	PS-A-II-29	2	1	1
A-10517	PS-A-II-30	1	1	0
A-10517	PS-A-II-36	1	1	0
TOTAL	11	17	9	8

Barbary partridge (*Alectoris barbara*)

Table 16. *Results for the contents of Barbary partridge crops*

		Number of Barbary partridge crops		
Management unit	N	Empty	With contents	With presence of pellets
ZCC	13	6	7	0

Quail (*Coturnix coturnix*)

Table 17. *Results for the contents of quail crops*

		Number of quail crops		
Management unit	N	Empty	With contents	With presence of pellets
ZA-10303	21	9	12	3
ZA-10304	10	2	8	1
TOTAL	31	11	20	4

Table 18. *Number of pellets by specimen and origin in quail crops*

			Number and origin of pellets	
Management unit	Specimen	n	Gunshot	Ingestion suspected
ZA-10303	CS-ZA-IX-01	1	1	0
ZA-10303	CS-ZA-IX-13	1	1	0
ZA-10303	CS-ZA-IX-19	1	1	0
ZA-10304	CS-ZA-VIII-25	1	1	0
TOTAL	4	4	4	0

Common woodpigeon (*Columba palumbus*)

Table 19. *Results for the contents of common woodpigeon crops*

		Number of common woodpigeon crops		
Management unit	N	Empty	With contents	With presence of pellets
AV-10475	1	1	0	0
ZA-10303	42	28	14	1
A-10517	30	16	14	0
CS-10063	4	4	0	0
M-10734	30	24	6	1
TOTAL	107	73	34	2

Table 20. *Number of pellets by specimen and origin in common woodpigeon crops*

			Number and origin of pellets	
Management unit	Specimen	n	Gunshot	Ingestion suspected
ZA-10303	PT-ZA-VIII-09	4	0	4
M-10734	PT-M-VIII-22	2	0	2
TOTAL	2	6	0	6

Rock dove (*Columba livia*)

Table 21. *Results for the contents of rock dove crops*

		Number of rock dove crops		
Management unit	N	Empty	With contents	With presence of pellets
ZA-10303	13	9	4	1
RRCLV	15	4	11	1
A-10517	30	21	9	0
Port	30	0	30	0
ZCC	1	1	0	0
M-10734	10	9	1	0
TOTAL	99	44	55	2

Table 21b. *Number of pellets by specimen and origin in rock dove crops*

			Number and origin of pellets	
Management unit	Specimen	n	Gunshot	Ingestion suspected
ZA-10303	PB-ZA-XII-13	1	1	0
RRCLV	PB-ZA-X-26	1	1	0
TOTAL	2	2	2	0

Stock dove (*Columba oenas*)

Table 22. *Results for the contents of stock dove crops*

		Number of stock dove crops		
Management unit	N	Empty	With contents	With presence of pellets
ZA-10303	30	28	2	1

Table 23. *Number of pellets by specimen and origin in stock dove crops*

			Number and origin of pellets	
Management unit	Specimen	n	Gunshot	Ingestion suspected
ZA-10303	PZ-ZA-XI-5	1	1	0

European turtle-dove (*Streptopelia turtur*)

Table 24. *Results for the contents of European turtle-dove crops*

		Number of European turtle-dove crops		
Management unit	N	Empty	With contents	With presence of pellets
A-10517	31	24	6	0

Pellets were only found in 19 crops of the total of the 530 game specimens processed. Sixteen of these had their origin in the method of capture (gunshot), as opposed to six whose possible origin can be attributed to ingestion (the doubtful specimens having several pellets and an orifice, which amounted to two, were included in the percentage for this type). Table 25 below was drawn up based on this data.

Table 25. *Results for crop contents of the species sampled*

			Origin of pellets	
Species	N	With pellet	Gunshot	No gunshot
Alectoris rufa	219	11	9	4 (1.8%)
Alectoris barbara	13	0	0	0
Coturnix coturnix	31	4	4	0
Columba palumbus	107	2	0	2 (1.9%)
Columba livia	99	2	2	0
Columba oenas	30	1	1	0
Streptopelia turtur	31	0	0	0
TOTAL	530	20	14	6 (1.1%)
TOTAL (WITHOUT FARM)	504	20	14	6 (1.2%)

As can be observed, there are groups in which no specimen was found to have pellets in the crop (*A. Barbara* and *S. turtur*), as well as others that do indeed have pellets in the crop, but which were not attributed to ingestion (*C. coturnix*, *C. livia* and *C. oenas*).

Table 26. *Specimens suspected of having ingested pellets*

Management unit	Specimen
CR-10530	PA2-CR-XII-6
CR-10530	PA2-CR-XII-8
CR-10530	PA2-CR-XII-9
A-10517	PS-A-II-29
ZA-10303	PT-ZA-VIII-09
M-10734	PT-M-VIII-22
TOTAL	6

Only 1.2 % of the specimens suspected of having been in contact with lead pellets in their habitat (504 specimens) had pellets in their crop that can be attributed to ingestion (including the doubtful cases). Three of the six cases involved partridges which had been repopulated and hunted in December at a game reserve located in Ciudad Real with high-intensity hunting activities. A detailed study of these specimens can be found in Section 7.

6 SECONDARY VALUE PROTOCOLS — GIZZARDS AND INTESTINES — AND ANALYSES

6.1 PROTOCOL TO EXAMINE GIZZARDS AND INTESTINES

The steps followed to obtain the gizzard and intestine values were as follows. To begin with, X-rays were taken of all the birds' digestive apparatus (from the joint of the proventriculus to the gizzard right up to the cloaca), which were kept frozen throughout the process. To do so, the samples were placed on X-ray plates at the facilities of the University of Murcia's Veterinary Clinical Hospital (Photograph 16). Once the X-rays had been taken, the samples were stored once again in the same containers at -20° C.

The digestive apparatus' (gizzards and intestines) processing to investigate the presence of lead pellets was carried out by following the same steps on all the samples. Photographs were taken of every step performed on each of the samples. The sequence was initiated by allowing the sample to defrost for its subsequent handling. A photograph of the sample was taken just after it was taken out from the container (Photograph 17). The gizzard and intestine were then separated and photographed individually (Photographs 18 and 19). Any X-ray of the sample which indicated the possible existence of lead pellets (Photograph 20) was displayed on a computer screen throughout the entire process of studying the digestive apparatus. The intestine and gizzard were searched separately for lead pellets. The entire structure of the intestine was palpated if the presence of any pellets was suspected as a result of said palpation or possible confirmation by X-ray. A search was then made for any possible orifices which would indicate the entry of a gunshot pellet. If there was no such orifice, or this is not clear, the pellet was extracted by making an incision on the intestine right up to its lumen.

The gizzard's entire surface was explored to search for a possible orifice or for any evidence of the entry of ammunition (for instance, the dragging of feathers). It was then opened by making an incision with a scalpel. The gizzard's contents were subsequently photographed (Photograph 21) and extracted. To achieve this, all the contents were poured onto a filter paper, which was in turn placed on a metal mesh (Photograph 22), so that all the contents could be washed with distilled water without losing any solid parts (in suspension). The contents were then left to dry overnight (Photograph 23). The gizzard's contents were subsequently stored at ambient temperature until they were viewed with a 40x magnifying glass (Photographs 24 and 25). The gizzards were likewise photographed with no contents to record their state and the mucosa's colour (Photograph 26). Finally, the gizzards and intestines were weighed, identified and stored separately in a frozen state (-20°C) (Photograph 27).

6.2 PROTOCOL FOR THE CHEMICAL ANALYSIS OF TISSUES

The samples were received at the laboratory of the University of Murcia's Toxicology area in a frozen state (-20°C). Once they were identified and recorded, the samples were kept frozen.

A total of 552 samples were available for the lead analysis. Although 22 samples from Museros, Valencia were received, their results were discarded from the study, since they were not hunting species and only served to evaluate the protocol used. The analytical data to be finally considered in the study amounted to 530 samples.

Prior to conducting the analysis, 0.1 to 0.3 grams of each of the samples' liver were separated and stored in a frozen state in an *Eppendorf* microtube with a new code. These samples were kept as a "control" to be used in the event of it being necessary to perform a new chemical Pb analysis, though in some cases it could not be obtained.

The samples were processed and analysed following this protocol: prior to the chemical analysis the samples were digested with 4 ml of HNO_3 (PA-ISO 69%, Suprapure, Merck) and 1 ml of H_2O_2 (33%, Suprapure, Merck) in Teflon tubes. They were then subjected to a temperature of 220° C during 20 to 30 minutes in a UltraClave-Milestone microwave oven. The cooled and digested samples were subsequently mixed with 10 ml of Milli-Q water. The same protocol was followed for the digestion of samples at the UPV.

The 552 samples were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES). Two readings were taken for each sample, and concentration values were obtained from the mean of both readings. To check for possible contaminants, one blank sample for every eleven samples was also analysed. Multi-element calibration standards were prepared with specific concentrations of Pb, taking as a reference UNE-EN ISO 11885 for the determination of elements by ICP-OES. Furthermore, intermediate patterns of Pb were prepared. The device's calibration was established per batch, with a minimum of three points for every single batch. Each run started out with the calibration standards, continued with samples and intermediate patterns, and the series was finished with intermediate patterns

(10% variation coefficient). The wavelength used was 220.353 nm and the uncertainty percentage was 6.14%.

The above-mentioned protocol has been used in recent research work on different species performed by this team and published in several internationally renowned academic journals (Cortés-Gómez et al., 2014; Mulero et al., 2016; Ráez-Bravo et al., 2016; García-Navarro et al., 2017; Cortés-Gómez et al., 2018a, 2018b; 2018c).

7 SECONDARY VALUE AND ANALYSES RESULTS

As has already been mentioned above, a decision was taken, given the study's characteristics, not to take into consideration the results obtained from the samples taken from Museros which were analysed (n=22), since they were not hunting species. Hence, the number of samples to be finally considered amounted to 530. As can be seen in Table 12, the species from which the largest number of samples was taken was the red-legged partridge (228 specimens, 43.0%), followed by the common woodpigeon (107 specimens, 20.2%) and the rock dove (99 specimens, 18.7%). According to the areas of capture, the number of samples analysed by areas was as follows:

Table 27. *Number of samples by province*

	Zamora	Alicante	Madrid	C. Real	Sta. Cruz de Tf.	Valencia	Castellón	Navarre	Ávila
No. of samples	161	127	40	97	14	60	4	26	1

- Zamora: low hunting intensity (red-legged partridge, quail, common woodpigeon, stock dove, rock dove).
- Alicante: very high hunting intensity (red-legged partridge, common woodpigeon, rock dove, European turtle-dove).
- Madrid: high hunting intensity, peri-urban area (common woodpigeon, rock dove).
- Ciudad Real: high hunting intensity; repopulated areas; acclimated animals (red-legged partridge).
- La Gomera: low hunting intensity (Barbary partridge).
- Valencia: no hunting intensity; port and airport (red-legged partridge, rock dove).
- Castellón: low hunting intensity (common woodpigeon).
- Navarre: no hunting intensity; control (game farm) (red-legged partridge).
- Ávila: low hunting intensity (common woodpigeon).

The number of samples analysed, the number of samples in which Pb was detected and the percentage of these are shown for each group in Table 28. Without taking into account the common woodpigeon samples collected in Castellón (PT-CS), as there were only four specimens, or the ones from PT-AV-MIGR (one specimen), it can be seen that the group having the lowest percentage of samples with Pb above the detection limit was PT-ZA-MIG; in other words, the common woodpigeons hunted in Zamora which had migrated (9/12, 75%). This group was followed by the group of rock doves from Alicante (PB-A, 24/30, 80%), the group of rock doves from Zamora (PB-ZA, 24/28, 85.7%) and the group of red-legged partridges from Ciudad Real at the end of the season (PA2 CR, 21/24, 87.5%). The remaining groups were above the figure of 90% of samples with lead concentrations above the detection limit.

The results of the chemical analysis for lead are shown in Table 29. Any liver samples in which lead had not been detected because its concentration was below the equipment's detection limit (0.001 µg/g) were assigned a concentration level equivalent to half of said limit. This criterion was applied to 30 samples (5.7% of the total). We have included two new pieces of data which are of interest in Table 29b: maximum concentration without considering values above 0.65 mg/kg (Maximum 2), and number of specimens having a concentration of between 0.2 and 0.65 mg/kg, together with data on hunting intensity, the environment and nature.

Table 28. *Number of samples analysed, number of samples in which Pb was detected and percentage of samples with Pb concentration above the detection limit (DL).*

Code	Species	Origin	No. of samples analysed	No. of samples in which Pb was detected	% of samples with a Pb concentration above the DL
PT-ZA	Common woodpigeon	Zamora	30	30	100.0
PT-A	Common woodpigeon	Alicante	30	27	90.0
PT-M	Common woodpigeon	Madrid	30	30	100.0
PA1-CR	Red-legged partridge	Ciudad Real	32	31	96.9
PZ-FARM	Red-legged partridge	Navarre	26	26	100.0
PS-ZA	Red-legged partridge	Zamora	30	28	93.3
CS-ZA	Quail	Zamora	31	31	100.0
PB-A	Rock dove	Alicante	30	24	80.0
PZ-ZA	Stock dove	Zamora	30	28	93.3
PB-M	Rock dove	Madrid	10	10	100.0
PS-APT	Red-legged partridge	Valencia	30	28	93.3
PM-GOM	Barbary partridge	La Gomera	13	13	100.0
PB-GOM	Rock dove	La Gomera	1	1	100.0
PA3-CR	Red-legged partridge	Ciudad Real	24	24	100.0
PA4-CR	Red-legged partridge	Ciudad Real	17	17	100.0
TE-A	European turtle-dove	Alicante	31	30	96.8
PT-ZA-MIGR	Common woodpigeon	Zamora	12	9	75.0
PT-AV-MIGR	Common woodpigeon	Ávila	1	0	0.0
PB-ZA	Rock dove	Zamora	28	24	85.7
PT-CS	Common woodpigeon	Castellón	4	3	75.0
PB-PV	Rock dove	Valencia	30	29	96.7
PS-A	Red-legged partridge	Alicante	36	36	100.0
PA2-CR	Red-legged partridge	Ciudad Real	24	21	87.5
Total	-	-	530	500	94.3

***Tables 29 and 29b.** Concentration of lead in the liver of game birds belonging to different groups*

GROUP	Species	Location	n	Mean	Minimum	Maximum	>0.65 µg/g; No. (%)	>0.74 µg/g; No. (%)	>1 µg/g; No. (%)	>1.5 µg/g; No. (%)	>2 µg/g; No. (%)	>6 µg/g; No. (%)	>10 µg/g; No. (%)
PT-ZA	Common woodpigeon	Zamora	30	0.087	0.034	31.253	1 (3.3)	1 (3.3)	1 (3.3)	1 (3.3)	1 (3.3)	1 (3.3)	1 (3.3)
PT-A	Common woodpigeon	Alicante	30	0.094	*n.a.*	0.395	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PT-M	Common woodpigeon	Madrid	30	0.124	0.047	35.567	2 (6.7)	2 (6.7)	2 (6.7)	2 (6.7)	2 (6.7)	2 (6.7)	1 (3.3)
PA1-CR	Partridge	Ciudad Real	32	0.077	*n.a.*	5.946	1 (3.1)	1 (3.1)	1 (3.1)	1 (3.1)	1 (3.1)	0 (0)	0 (0)
P-FARM	Partridge	Navarre	26	0.074	0.006	0.213	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PS-ZA	Partridge	Zamora	30	0.026	*n.a.*	0.439	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CS-ZA	Quail	Zamora	31	0.099	0.028	6.270	2 (6.5)	2 (6.5)	2 (6.5)	2 (6.5)	2 (6.5)	1 (3.2)	0 (0)
PB-A	Rock dove	Alicante	30	0.043	*n.a.*	0.142	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PZ-ZA	Stock dove	Zamora*	30	0.048	*n.a.*	0.151	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PB-M	Rock dove	Madrid	10	0.201	0.022	1.101	1 (10)	1 (10)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)
PS-APT	Partridge	Valencia	30	0.045	*n.a.*	0.434	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PM-GOM	Barbary partridge	La Gomera	13	0.038	0.002	8.766	3 (23.1)	2 (15.4)	2 (15.4)	2 (15.4)	2 (15.4)	2 (15.4)	0 (0)
PB-GOM	Rock dove	La Gomera	1	0.165	-	-	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PA3-CR	Partridge	Ciudad Real	24	0.047	0.011	10.379	2 (8.3)	2 (8.3)	2 (8.3)	2 (8.3)	2 (8.3)	1 (4.2)	1 (4.2)
PA4-CR	Partridge	Ciudad Real	17	0.035	0.008	4.329	2 (11.8)	2 (11.8)	2 (11.8)	2 (11.8)	2 (11.8)	0 (0)	0 (0)
TE-A	European turtle-dove	Alicante	31	0.040	*n.a.*	0.846	1 (3.2)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PT-ZA-MIGR	Common woodpigeon	Zamora*	12	0.025	*n.a.*	2.788	1 (8.3)	1 (8.3)	1 (8.3)	1 (8.3)	1 (8.3)	0 (0)	0 (0)
PT-AV-MIGR	Common woodpigeon	Ávila*	1	*n.a.*	*n.a.*	*n.a.*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PB-ZA	Rock dove	Zamora	28	0.033	*n.a.*	0.226	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PT-CS	Common woodpigeon	Castellón*	4	0.004	*n.a.*	0.081	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PB-PV	Rock dove	Valencia	30	0.086	*n.a.*	1.171	2 (2.67)	2 (6.7)	1 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)
PS-A	Partridge	Alicante	36	0.067	0.007	14.187	2 (5.6)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.8)	2.8 (0)
PA2-CR	Partridge	Ciudad Real	24	0.094	*n.a.*	7.885	4 (16.7)	4 (16.7)	4 (16.7)	3 (12.5)	3 (12.5)	1 (4.2)	0 (0)
TOTAL	-	-	530	0.064	*n.a.*	35.567	24 (4.5)	22 (4.2)	20 (3.8)	17 (3.2)	17 (3.2)	9 (1.7)	4 (0.8)

ORIGIN	Species	Location	n total	Mean	Maximum	n<0.65	Maximum 2*	n birds 0.2-0.65	n birds >0.65	Hunt. int.	Nature	Environment
PT-ZA	Common woodpigeon	Zamora	30	0.087	31.253	29	0.309	3	1	Low	Wild	Rural
PT-A	Common woodpigeon	Alicante	30	0.094	0.395	30	0.395	5	0	Very high	Wild	Rural
PT-M	Common woodpigeon	Madrid	30	0.124	35.567	28	0.494	5	2	High	Wild	Peri-urban
PA1-CR	Red-legged partridge	Ciudad Real	32	0.077	5.946	31	0.341	3	1	High	Acclimated	Rural
P-FARM	Red-legged partridge	Navarre	26	0.074	0.213	26	0.213	2	0	None	Farm	Rural
PS-ZA	Red-legged partridge	Zamora	30	0.026	0.439	30	0.439	2	0	Low	Wild	Rural
CS-ZA	Quail	Zamora	31	0.099	6.270	29	0.369	4	2	Low	Wild	Rural
PB-A	Rock dove	Alicante	30	0.043	0.142	30	0.142	0	0	Very high	Wild	Rural
PZ-ZA	Stock dove	Zamora	30	0.048	0.151	30	0.151	0	0	Low	Wild	Rural
PB-M	Rock dove	Madrid	10	0.201	1.101	9	0.585	4	1	High	Wild	Peri-urban
PS-APT	Red-legged partridge	Valencia	30	0.045	0.434	30	0.434	2	0	None	Wild	Urban
PM-GOM	Barbary partridge	La Gomera	13	0.038	8.766	10	0.255	1	3	Low	Wild	Rural
PB-GOM	Rock dove	La Gomera	1	0.165	0.165	1	0.165	0	0	Low	Wild	Rural
PA3-CR	Red-legged partridge	Ciudad Real	24	0.047	10.379	22	0.095	0	2	High	Acclimated	Rural
PA4-CR	Red-legged partridge	Ciudad Real	17	0.035	4.329	15	0.069	0	2	High	Acclimated	Rural
TE-A	European turtle-dove	Alicante	31	0.040	0.846	30	0.418	2	1	Very high	Wild	Rural
PT-ZA-MIGR	Common woodpigeon	Zamora	12	0.025	2.788	11	0.097	0	1	Low	Wild	Rural
PT-AV-MIGR	Common woodpigeon	Ávila	1	0.001	0.001	1	0.001	0	0	Low	Wild	Rural
PB-ZA	Rock dove	Zamora	28	0.033	0.226	28	0.226	1	0	Low	Wild	Rural
PT-CS	Common woodpigeon	Castellón	4	0.004	0.081	4	0.081	0	0	High	Wild	Rural
PB-PV	Rock dove	Valencia	30	0.086	1.171	28	0.335	3	2	None	Wild	Urban
PS-A	Red-legged partridge	Alicante	36	0.067	14.187	34	0.335	2	2	Very high	Wild	Rural
PA2-CR	Red-legged partridge	Ciudad Real	24	0.094	7.885	20	0.229	1	4	High	Acclimated	Rural
Total	-	-	530	0.064	35.567	506	0.585	40	24	-	-	-

*Maximum without taking values >0.65 mg/kg into account

In order to assess these results, we considered the criteria set out by several authors:

- Beyer et al. (2013): Pb concentrations in tissues, especially in liver tissues, are probably the most reliable measure to assess said metal's possible toxic effects.
- Beyer et al. (2013): the criteria used to assess Pb concentrations in the liver of other species should be similar to the ones developed for water birds.
- Friend (1985) and the USFWS (1986) state that high exposure to Pb using liver samples is situated at 2 mg/kg. However, the estimation of this threshold was developed for water birds (Friend 1985, USFWS 1986, Pain 1996).
- Franson and Pain (2011): the "subclinical" effects of lead intoxication appear when hepatic lead concentration (of wet weight) is situated between 2 and 6 mg/kg; clinical poisoning occurs when hepatic lead concentration is from 6 to 10 mg/kg; and the animal's life is endangered when these concentrations exceed 10 mg/kg.
- Guitar et al. (1994): clinical poisoning occurs when hepatic lead concentration (wet weight) exceeds 1.5 mg/kg (water birds).
- Bingham et al. (2015) suggest that the threshold value between the background level and high exposure to Pb is 1 mg/kg (wet weight) in *Alectoris Chukar*.
- Ferrandis et al. (2008): between 0.58 and 2.17 mg/kg of Pb in the liver (of dry weight) in partridges that have not ingested Pb through pellets on the ground. Assuming that there is 34% of dry matter in the liver (according to our study), these concentrations are respectively equivalent to 0.20 and 0.74 mg/kg.
- According to Berny et al. (2015), the environmental exposure threshold is situated at 0.65 mg/kg.

Nonetheless, several studies state the hepatic lead levels from which an abnormal exposure to lead (>1.5 mg/kg of wet weight) or severe lead poisoning (>6 mg/kg of wet weight) can be considered to have taken place in water birds. A review of said data can be obtained at https://www.mapama.gob.es. (Mateo et al., 1994). According to the sources consulted by said authors, the renal lead concentration which indicates severe exposure in water birds is 5 mg/kg and above.

Table 29 sets out the number and percentage of samples (of each group) having hepatic lead concentration above 0.65, 0.74, 1, 1.5, 2, 6 and 10 mg/kg. Table 29b shows how the maximum Pb concentration in most groups is greatly diminished and even reaches levels below 0.1 mg/kg (PA3-CR, PA4-CR, PT-ZA-MIGR, PT-CS) when any values above 0.65 mg/kg are discarded.

As can be seen in Table 29, between 95.5% and 96.8% of the population under study turned out to have a base hepatic Pb concentration or a concentration below the level considered for environmental exposure based on the range found in the bibliography (0.65 to 2.0 mg/kg). When farm specimens (n=26) are discarded, said percentage amounts to between 95.2% and 96.6%. It could therefore be considered on an *a priori* basis that these birds have not ingested Pb pellets from the environment. It should be noted, however, that the margin would actually be between 0.65 and 1.5 mg/kg, as we have not found any specimens with values between 1.5 and 2.0 mg/kg.

We will now address the results by also taking into account the results of the analyses, the radiological study's observations and the results from the dissections performed on the digestive apparatus (gizzard and intestine).

Table 30 sets out the results obtained from the macroscopic study performed on the gizzards and intestines of the 530 game birds. A total of 125 pellets were found in them. Fifty-seven specimens were found to have pellets in the lumen of their gizzard (78 pellets) and 13 were found to have pellets in the lumen of their intestine (18 pellets). In addition, we also found 21 pellets embedded in the gizzard's muscle layers and serosa (19 specimens). Some of these specimens had pellets in several of these places (gizzard and intestine lumen and gizzard layers). Eight pellets were found between intestinal loops (five specimens). The number of specimens suspected of having ingested ammunition because a pellet had been found in the lumen of the gizzard and/or intestine and no orifice found in the organs' external surface amounted to 23.

Table 30. *Macroscopic observations of the surface and interior the gizzard and intestine. Lead pellets in the different groups' digestive apparatus*

	Specimens with orifice in gizzard	Specimens with orifice in intestine	Specimens suspected of having ingested pellets*	Specimens with pellets in gizzard	Gizzard: No. of pellets in lumen/No. of specimens with pellets in lumen	Gizzard: No. of embedded pellets/No. of specimens with embedded pellets	Specimens with pellets in intestine	No. of pellets in intestine	Pellets between intestinal loops	Total No. of pellets
P-Farm	0	0	0	0	0/0	0/0	0	0	0	0 (Control)
Cs-ZA	10	0	0	6	5/5	1/1	0	0	1/1	7
PT-ZA	4	2	4	5	3/3	3/3	5	5	1/1	12
PA1-CR	5	0	0	5	6/4	1/1	0	0	0	7
PT-M	4	0	1	4	1/1	3/3	1	1	0	5
PT-A	6	0	3	2	2/2	1/1	2	2	1/1	6
PS-ZA-XI	7	1	1	7	5/5	3/2	1	1	0	9
PB-A	1	1	1	1	1/1	0/0	0	0	1	2
TE-A	9	0	0	2	2/2	0/0	0	0	0	2
PT-ZA-MIGR	3	0	0	2	3/2	0/0	0	0	0	3
PT-AV-MIGR	0	0	0	0	0/0	0/0	0	0	0	0
PB-ZA	4	0	1	3	2/2	2/1	1	3	0	7
PZ-ZA	6	0	1	6	5/5	1/1	0	0	0	6
PB-M	0	0	0	0	0/0	0/0	0	0	0	0
PM-GOM	2	0	1	2	3/2	0/0	0	0	0	3
PB-GOM	0	0	0	0	0/0	0/0	0	0	0	0
PA3-CR	4	0	2	5	13/3	2/2	0	0	0	15
PA4-CR	0	0	1	1	1/1	0/0	0	0	0	1
PS-APT	0	0	0	0	0	0	0	0	0	0
PS-A	16	0	2	13	14/11	3/3	1	1	4/3	22
PB-PV	1	0	0	1	1/1	0/0	0	0	0	1
PT-CS	1	0	0	0	0	0	0	0	0	0
PA2-CR	6	0	5	8	11/7	1/1	2	5	0	17

* Suspected because no entry orifice was found and there were pellets in the gizzard or intestine.

In this regard, we firstly considered as specimens suspected of having ingested lead pellets any in which we did not find ammunition entry orifices, but which do have pellets in the crop, gizzard and/or intestine. In the intestine it was very difficult to detect the orifices, but we have considered them all, although perhaps some of those considered as "suspects" were not. According to this criterion, 28 specimens are suspected of having ingested pellets (5.3% of the total population under analysis; 5.6% if we consider all the specimens apart from farm animals, n=504). Obviously, none of these specimens came from urban environments (Port of Valencia and Valencia Airport) or from a farm. It should be taken into account that this criterion is based on the direct observation of orifices. Though some can be clearly seen, others may be difficult to observe.

We therefore considered it appropriate to initially include as “suspected of having ingested pellets” any specimens in which a Pb pellet has been found in the crop, gizzard or intestine and in which no ammunition entry orifice was found, though there were doubts about some cases (two cases with pellets in the crop). The outcome of these observations is set out in Table 31. As can be seen, only six specimens were found to have high hepatic lead concentrations (four red-legged partridges from Ciudad Real, one red-legged partridge from Alicante and one common woodpigeon from Zamora). The rest of the cases were below 0.299 mg/kg, apart from one case having 0.701 mg/kg (PS-A-23). Of the four specimens from Ciudad Real, two had a pellet in the gizzard (PA4-CR-2 and PA1-CR-10), while the other two had 11 (PA3-CR-14) and 4 pellets (PA2-CR-5) respectively. The rock dove from Zamora was the only specimen to have pellets in the all three organs.

To obtain further information on the state of said specimens, the kidneys of any specimens having a hepatic Pb concentration above 0.65 mg/kg were analysed, except for specimen PA1-CR-10, since we lacked a sample of said tissue because it had been destroyed by a gunshot. The kidney is considered, along with the liver, as the organ with the highest concentration of lead after severe exposure to this metal. The results of the analyses performed on these five specimens are set out in Table 32.

As can be seen in this table, almost all specimens considered suspicious for finding pellets in intestine and no orifices, did not have high Pb concentration in liver, so the term "suspect" should not be considered in these cases.

Table 31. *Specimens considered as "suspected of having ingested pellets" (absence of visible orifice in organs and with pellets in any of them). Location of pellets and hepatic Pb concentration*

	Pellets in gizzard	Pellets in intestine	Pellets in crop	Pb concentration (mg/kg, wet weight)
PT-ZA-26		1		0.299
PT-ZA-7		1		0.049
PT-ZA-16	1			0.082
PT-ZA-20		1		0.176
PT-ZA-9			4	31,253
PT-A-11		1		0.119
PT-A-27	1			<0,001
PS-ZA-7	1			0.023
PB-ZA-26	1*	3	1**	0.135
PZ-ZA-7	1			0.151
PM-GOM-5	2			0.255
PA3-CR-2	1			0.017
PA3-CR-14	11			10,379
PA4-CR-2	1			4.329
PS-A-30	1		1**	0.104
PS-A-23		1		0.701
PA2-CR-3	1			0.064
PA2-CR-10	1			4.986
PA2-CR-7	2			0.132
PA2-CR-8		1	4	0,005
PA2-CR-9	1**		1	0.130
PA2-CR-5		4		7.885
PT-M-VII-22			2	0.166
PT-A-5		1		0.029
PT-M-12		1		0.061
PB-A-18	1			0.036
PS-A-29			1***	0.083
PA2-6			1***	0.192

*Apparently a different calibre from the rest. **The pellet's entry is not suspected, as there is an entry orifice in said organ. ***Doubt about entry not being due to gunshot.

Table 32. *Hepatic and renal Pb concentration in specimens suspected of having ingested lead with high hepatic Pb concentration. Colour of the gizzard's mucosa. Presence of cage marks on feathers*

	Hepatic Pb concentration (mg/kg, wet weight)	Renal Pb concentration (mg/kg, wet weight)	Colour of the gizzard	Cage marks
PA3-CR-14	10,379	28.037	Dark green	Yes
PA4-CR-2	4.329	26.768	Dark green	Yes
PA2-CR-10	4.986	-	Pink	Yes
PA2-CR-5	7.885	41.842	Dirty Green	Yes
PT-ZA-9	31,253	0.081	Green	No
PS-A-23	0.701	289,737	Green	No

In the case of the specimen identified as **PA3-CR-14** (female weighing 431 grams, complete remiges and with a few cage marks), the number of pellets found in the gizzard (11), along with the appearance of the gizzard's internal surface (dark green, Photograph 28), allow us to affirm that it had clearly ingested pellets from the environment. In this case, the weights of the pellets ranged between 0.049 and 0.097 g, with an average of 0.075 g (total weight 0.828 g). This data, along with the apparently normal state of the intestine, seem to suggest a fairly recent ingestion of pellets. Furthermore, the renal concentration of lead detected in this specimen amounted to 28.037 mg/kg, higher than the concentration detected in the liver (10.379 mg/kg). The game reserve where this specimen was hunted is repopulated with farm partridges in September, and this specimen was probably a farm animal (acclimated for repopulation). The cage marks may indicate that the specimen had only been in the area for a few weeks because it was hunted on 13 November 2016 (delivered to the University of Murcia on 12 December 2016). The ingestion of pellets may have therefore been very recent.

The specimen identified with the code **PA4-CR-2** had a very dark green mucosa surface (Photograph 29) and came from a reserve having similar characteristics to the one described above. It was of a young male weighing 458 g and had complete remiges and cage marks on the feathers. It was hunted on 19 November 2016 and had a pellet in the gizzard. The renal concentration of lead was quite similar to the one in the previous case (26.768 mg/kg), and its renal concentration was also lower (4.329 mg/kg). It is another specimen which is also likely to have ingested pellets from the environment.

The specimen identified with the code **PA2-CR-10** corresponds to a young male weighing 495.8 g, with complete remiges and cage marks. It was hunted on 28 December 2016. This specimen had a pellet in the gizzard. However, the appearance of the gizzard's surface (Photograph 30) did not suggest recent ingestion, as its colour was not greenish. This fact contrasts with the hepatic Pb concentration found (4.986 mg/kg), which suggest, along with the existence of said pellet, that the gizzard's surface had been altered. We could not get a better indication of the animal's state, since a liver sample was unavailable. There was no complete intestine sample in this case either, since the specimen had suffered severe gunshot impact damage. An analysis of lead content in the bones could provide us with further information. Nevertheless, it is also suspected of having ingested pellets from the environment, since it was an acclimated animal.

The specimen identified with the code **PA2-CR-5** (young female weighing 400.3 g, with complete remiges and many cage marks, hunted at the end of season on 28 December 2016 and with four pellets in the gizzard) came from a game reserve having the same characteristics as the ones above. The weight of the pellets ranged from 0.034 to 0.049 g, with an average of 0.040 g and a total weight of 0.161 g. The surface of the gizzard suggested it was likely the specimen had also ingested pellets from the environment (Photograph 31). A higher renal Pb concentration (41.842 mg/kg) than a hepatic Pb concentration (7.885 mg/kg) was also detected. Given the higher concentration detected in the kidney (41.842 mg/kg) and the time it was captured (at the end of season), we could be dealing here with an exposure of some days or weeks at most.

The specimen identified with the code **PS-A-23** was a young female weighting 417 g, which was captured at end of season (9 February 2017) as a decoy. In this case, the gizzard's internal surface (Photograph 32) did not show a dark green colour, which was a characteristic of the aforementioned cases. Moreover, its low hepatic Pb concentration (0.701 mg/kg) evidently contrasts with its high renal Pb concentration (289.737 mg/kg). Though it is true that a pellet was found in the intestine without any apparent entry orifice, the high renal concentration detected seems strange and could be due to fragments embedded in the renal tissue, as some authors have suggested (Kreager et al., 2008). As in the case of specimen PA2-CR-10, it would be convenient to perform a Pb analysis on other tissues. This specimen's results should be treated with caution.

Lastly, the common woodpigeon from Zamora No. 9 (**PT-ZA-9**) was a male specimen weighing 437 g. The surface of its gizzard was green (Photograph 33) and it had four pellets in the crop and none in the gizzard or intestine. The presence of these pellets in the crop could suggest they had been recently ingested, but some aspects are not altogether clear. The hepatic concentration was quite high (31.253 mg/kg), while the renal concentration was very low (0.081 mg/kg). Furthermore, the gizzard's mucosa did not have a dirty green appearance, which is indicative of lead absorption at this level. As has already been mentioned, some authors have described the existence of high tissue concentrations of Pb as a result of small fragments remaining embedded in tissues (Kreager et al., 2008). Others, however, have suggested that the Pb concentrations in the soft tissues of birds which have ingested metal Pb tend to be very biased or have different atypical values (Dieter 1979; Beyer et al. 1998). In this case, the concentration no was not as high as in the previous case. It should be remembered, however, that hepatic Pb lead concentrations above 10 mg/kg pose a risk to the animal's life (Franson and Pain, 2011). Other tissues in this specimen should therefore be investigated to clear up these possible hypotheses and affirm that this specimen has suffered lead poisoning. Nevertheless, the presence of those four pellets in the crop may indeed suggest they had been ingested.

Having analysed the cases suspected of having ingested pellets because they were detected in the digestive apparatus without observing any entry orifices and with a high hepatic Pb concentration, we will now move on to analyse the rest of the cases (18), in which the hepatic lead concentration was found to be above 0.65 mg/kg, regardless of the macroscopic findings of the organs' dissection. Once the cases above are discarded, the remaining cases are set out in Table 33. In this part of the study we will consider all the specimens, regardless of the place of capture. It therefore includes the specimens captured in the port and airport of Valencia.

Table 33. *Specimens having a lead concentration above 0.65 mg/kg (without including the cases set out in Table 32). The specimen's weight is indicated in grams. G=gizzard; I=intestine; C=crop. Pb concentration in mg/kg of wet weight*

Identification	Species	Origin	Age	Sex	Weight	Remiges	Pellets	Orifice(s)	Hepatic Pb concentration	Date hunted and observations
PM-GOM-12	Rock dove	LA GOMERA	young	male	479	complete	0	0	0.691	16/10/2016
PT-M-7	common woodpigeon	MADRID	adult	male	479.0	6	0	0	7.105	15/8/2016
PT-M-10	common woodpigeon	MADRID	adult	femal e	392.0	5	0	0	35.567	15/8/2016
PA1-CR-14	red-legged partridge	C REAL	young	femal e	338	8	0	0	5.946	15/10/2016
CS-ZA-7	quail	ZAMORA	young	femal e	92.0	5	1 (G)	1	2.695	4/9/2016
CS-ZA-18	quail	ZAMORA	young	male	80.0	5	1 (G)	1	6,270	18/8/2016
PB-M-9	rock dove	MADRID	adult	male	332.0	5	0	0	1.101	15/8/2016
PM-GOM-11	Barbary partridge	LA GOMERA	adult	male	507.0	10	0	0	8.300	16/10/2016
PM-GOM-2	Barbary partridge	LA GOMERA	indet.	male	520.0	complete	0	0	8.766	16/10/2016
PA3-CR-4	red-legged partridge	C REAL	young	femal e	390.3	complete	0	0	3.084	17/12/2017 Cage marks
PA4-CR-1	red-legged partridge	C REAL	young	male	489.0	complete	0	0	2.404	19/11/2016 Cage marks
TE-A-22	European turtle-dove	ALICANTE	young	male	136.4	3	0	0	0.846	4/9/2016
PT-ZA-1-MIG	common woodpigeon	ZAMORA	young	male	478.0	7	0	1	2.788	13/11/2016
PB-PV-28	rock dove	VALENCIA	young	male	329.0	1	0	0	0.766	16/12/2016
PB-PV-4	rock dove	VALENCIA	adult	femal e	355.0	complete	0	0	1.171	16/12/2016
PS-A-II-26	red-legged partridge	ALICANTE	adult	male	493.0	10	0	0	14.187	9/2/2017
PA2-CR-13	red-legged partridge	C REAL	indet.	male	486.3	complete	0	0	1.230	28/12/2016 Cage marks
PA2-CR-2	red-legged partridge	C REAL	young	femal e	396.8	complete	0	0	3.985	28/12/2016 Cage marks

As can be seen, there are certain cases (TE-A-22, PB-M-9, PT-M-7 and PT-M-10) in which the hepatic lead concentration was above the limit set in our study, which can be high (PT-M-7) or very high (PT-M-10), though the gizzard's appearance was not greenish or blackish (Photographs 34, 35, 39 and 44), an indication of exposure through pellets ingested from the environment. In addition, the specimens of these groups having low Pb concentrations had a gizzard with a similar appearance. One of these samples (**PT-M-10**) was the one which was found to have the highest hepatic concentration of lead (35.567 mg/kg), while its renal concentration was only 0.162 mg/kg (Table 34). Here again we find a case where the animal should be fairly ill according to Franson and Pain (2011). If we assume a very high recent exposure to pellets, the gizzard's surface should be greatly altered (greenish-blackish), which is simply not the case. Similarly, the intestine's external appearance did not seem abnormal, which is why exposure resulting from the ingestion of Pb pellets can be doubted on an *a priori* basis. The case of **PT-M-7** was similar (low renal lead concentration of 0.186 mg/kg but a hepatic concentration above 6 mg/kg) and without the gizzard appearing dark green. Environmental exposure other than through Pb pellets might therefore be surmised. The specimen identified with the code **PB-M-9** was found to have a high concentration of renal lead (46.034 mg/kg) and a low concentration of hepatic lead (1.101 mg/kg). This case would therefore be arguable (at least it cannot be emphatically stated) that there had been exposure through the ingestion of pellets from the environment. In the case of the specimen identified with the code **TE-A-22** (Photograph 44), a dirty green colour was likewise not observed on the gizzard's internal surface. It can therefore be surmised that the hepatic lead concentration (0.846 mg/kg) was not due to the ingestion of lead pellets either.

Another two samples having a low likelihood that the hepatic lead concentration found was a result of the ingestions of pellets are identified with the codes **PB-PV-28** (young specimen) and **PB-PV-4** (adult specimen) (Photographs 46 and 47). Apart from the hepatic concentration of lead not being high (0.766 and 1.171 mg/kg), the internal surface of the gizzard did not appear to be dirty green or black, its colour being similar to that of the rest of its group. These specimens came from an urban environment with a large expansion area. Renal lead was analysed in only one of the cases (PB-PV-4), which amounted to 3.211 mg/kg (Table 34) in this case.

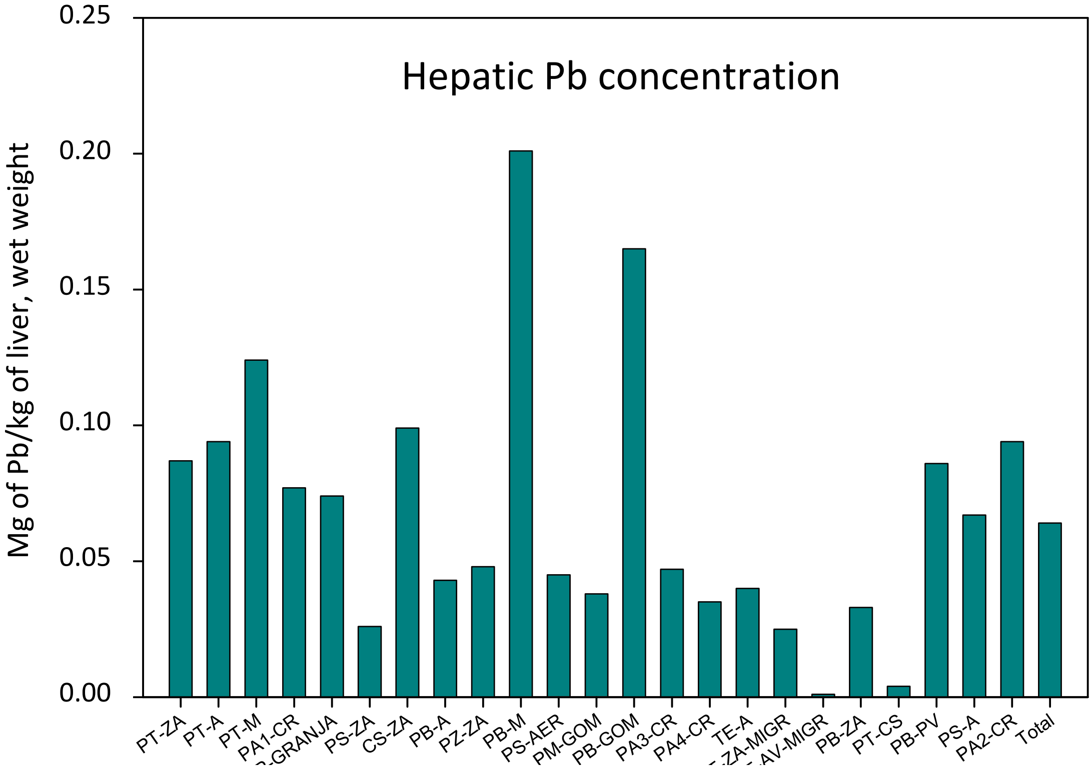
Table 34. *Concentration of hepatic and renal lead of the specimens included in Table 33. Colour of the gizzard*

IDENTIFICATION	Concentration of hepatic Pb	Concentration of renal Pb	Colour of gizzard
PM-GOM-12	0.691	Not determined	Dirty dark green
PT-M-7	7.105	0.186	Light green, not dirty
PT-M-10	35.567	0.162	Light green, not dirty
PA1-CR-14	5.946	0.576	Somewhat dirty dark green
CS-ZA-7	2.695	2.022	Dirty dark green
CS-ZA-18	6,270	131.085	Dirty dark green
PB-M-9	1.101	46.034	Light green, not dirty
PM-GOM-11	8.300	No sample	Dirty Green
PB-GOM-2	8.766	0.044	Dirty Green
PA3-CR-4	3.084	Not determined	Dirty Green
PA4-CR-1	2.404	19.608	Dirty Green
TE-A-22	0.846	Not determined	Light green, not dirty
PT-ZA-1-MIG	2.788	9.483	Green, not dirty
PB-PV-28	0.766	Not determined	Dark green, not dirty
PB-PV-4	1.171	3.211	Light green, not dirty
PS-A-II-26	14.187	10.208	Dirty Green
PA2-CR-13	1.230	6.968	Black
PA2-CR-2	3.985	5.209	Black

No pellets were found in the gizzard or intestine in any of these six cases (Table 33).

As has been mentioned, there are several specimens hunted in Madrid whose ingestion of pellets of Pb is, to say the least, debatable. At this point it should be pointed out that the group having the highest concentration of lead in the liver (mean) was the group of rock doves from Madrid, PB-M (0.201 mg/kg), followed by the common woodpigeons from Madrid, PT-M (0.124 mg/kg), two groups which come from a peri-urban environment near the city of Madrid. All the samples in both groups were found to have a lead concentration above the detection limit. If we discard the specimens with concentrations above 0.65 mg/kg (one in PB-M and two in PT-M), we can observe that they are still the groups with the highest mean (0.131 mg/kg for the PB-M group and 0.117 mg/kg for the PT-M group). The rest of groups had a mean below 0.100 mg/kg. The PB-GOM group was not taken into consideration in this case, since it was comprised of only a single specimen.

Figure 7. *Mean hepatic Pb concentrations by group*

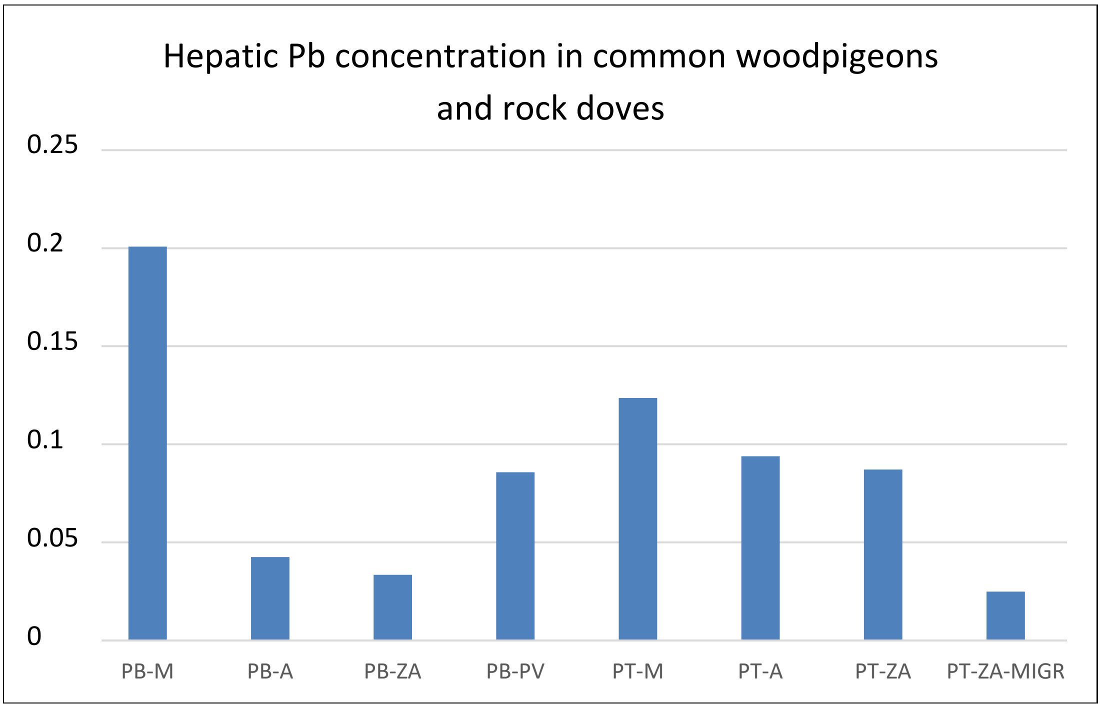


If we compare the groups of common woodpigeons from Madrid with the ones from Zamora and Alicante (Mann-Whitney U-test, $p<0.05$), we can see that the differences between groups PT-M and PT-ZA, and PT-M and PT-A are statistically significant, despite belonging to the same species. On the other hand, there were no statistically significant differences between groups PT-ZA and PT-A. Although the Madrid area is considered to be an area of high hunting intensity (though less so than Alicante), it can be affirmed that the common woodpigeons from Madrid have a higher hepatic concentration of lead, which is probably associated to their origin (a peri-urban environment). In this regard, the data obtained from the Geochemical Atlas of Spain (Geological and Mining Institute of Spain) given in the first part of this report should be taken into account. Said data reveals that the lead concentration in soil samples taken from the Madrid area (Humanes de Madrid), where these birds were hunted, were among the highest (60.6 mg/kg) and much higher than the ones from Zamora (20.1 to 32.0 mg/kg) and Alicante (39.1 mg/kg).

As far as the common woodpigeon is concerned, it should be pointed out that all the observations with a presence of pellets but without any orifices in the gizzard and/or intestine were below the threshold of 0.65 mg/kg and that the only observation that actually exceeded said limit did not have any pellets in the gizzard.

In the case of the rock dove, when the concentration of lead in the PB-M group was statistically compared with the rest of the groups (PB-A, PB-ZA and PB-PV), statistically significant differences were found with the groups from Alicante and Zamora, while there were no differences between groups PB-A and PB-ZA (despite being areas with different hunting intensities, very high and low). Statistical differences were indeed found between PB-ZA and PB-PV (Zamora is a low-intensity hunting area and Valencia a peri-urban area with no hunting), and between PB-A and PB-PV (Alicante, as has already been mentioned, is a very high-intensity hunting area and the Port of Valencia a peri-urban area with no hunting). Nonetheless, if a log transformation is applied to Pb concentration and a parametric statistical test (ANOVA) is performed, it can be seen that there are no significant differences in the Pb concentrations of Madrid and the Port of Valencia, thereby mirroring the aforementioned Mann-Whitney U-test results for the rest. It should also be noted that the information obtained from the Geochemical Atlas of Spain also states that the area around the Port of Valencia has a high concentration of lead in the soil (104 mg/kg), a figure which is even higher than in Madrid. Thus, there also seems to be a higher Pb concentration which is associated to peri-urban environments as far as this species is concerned. Regarding presence of pellets without any apparent orifice on the surface of the gizzard and/or intestine, it should be noted that only two pellets were observed in the crop. Hence, there seems to be no relationship at all between the presence of pellets in rock doves and whether or not the 0.65 mg/kg threshold of Pb in the liver is exceeded.

Figure 8. *Hepatic Pb concentrations in common woodpigeons and rock doves*



Focusing now on common woodpigeons, the specimen identified with the code PT-ZA-9 (Photograph 33) was found to have the second highest hepatic lead concentration (31.253 mg/kg), though the renal concentration was very low (0.081 mg/kg) and the intestine's appearance was normal, though somewhat greenish in some sections. As has already been indicated above, other of this specimen's tissues should be analysed to clear up the possible hypotheses (recent exposure vs fragments embedded in the tissue).

In the case of the common woodpigeon from Zamora which had migrated (**PT-ZA-1-MIGR**), we can see that the gizzard's surface did not have a blackish appearance, though it was green (Photograph 45) like in the rest of its group's specimens. The renal lead concentration was also high (9.483 mg/kg), while the hepatic concentration was not excessively high (2.788 mg/kg). An ingestion of pellets from the environment in the not so recent past or, alternatively, exposure through a cause other than pellets can therefore be surmised. Pellets were not found in this specimen's gizzard.

In the case of the red-legged partridges from Alicante (PS-A), we found one (**PS-A-26**, Photograph 48) in which the hepatic lead concentration was high, thus posing a serious risk for its health (14.187 mg/kg). The state of the gizzard's surface and the renal concentrations found in this specimen (10.208 mg/kg, Table 34) could suggest recent exposure to lead, though no pellets in this specimen's digestive apparatus or any signs of lead poisoning were found.

The five remaining cases of red-legged partridges with a hepatic lead concentration above 0.65 mg/kg were specimens hunted in games reserves located in Ciudad Real. The specimens identified with the codes PA1-CR-14, PA2-CR-13 and PA2-CR-2 belonged to the same game reserve, the difference being that the first was hunted at the beginning of the season (15/10/2016) while the other two were hunted at the end of the season (28/12/2016). No cage marks were discernible on the first (PA1-CR-14), while some could be seen on the other two. As can be seen in Photographs 36, 55 and 56, the internal surface of the gizzard had a blackish colour in **PA2-CR-13** and **PA2-CR-2**, which together with the renal lead concentration found (6.968 and 5.209 mg/kg), could suggest a likely ingestion of pellets from the environment. This was more doubtful in the case of the specimen identified with the code **PA1-CR-14**, since the renal lead concentration was not high (0.576 mg/kg) in spite of the dirty green colour of the gizzard's internal surface. Nevertheless, it was not considered as suspected of having ingested pellets.

Lastly, some cases found in the PA3 and PA4 game reserves in Cuidad Real (PA3-CR-4 and PA4-CR-1) had hepatic Pb levels above the threshold value of 0.65 mg/kg. The specimen identified with the code **PA4-CR-1** (Photograph 43) had a high renal lead concentration (19.608 mg/kg), along with cage marks and a green intestine, but not a green gizzard. We have no renal lead data for specimen **PA3-CR-4** (Photograph 42), though the dark colour of the gizzard's mucosa and the intestine were more than evident. There were cage marks on feathers in both these cases. A probable ingestion of pellets from the environment can likewise be surmised.

When the results for the groups of red-legged partridges are compared by areas (Figure 9), we can see that the group having the highest hepatic lead concentration was identified with the code PA2-CR; that is to say, a game reserve where hunting was carried out at the end of the season. When this group is compared to the rest, we can observe that there were no statistically significant differences with any of said groups (Mann-Whitney U-test, $p<0.05$). Neither were any statistically significant differences observed when a log transformation and parametric analysis (ANOVA) were performed. In this regard, it can be stated that the lead concentration in soil samples (Geochemical Atlas of Spain) followed this ranking: Valencia Airport>Ciudad Real>Alicante>Zamora>Navarre.

Curiously enough, the group which was found to have the highest hepatic Pb concentration was the farm (Navarre), which had significant differences with the partridges from Zamora. The partridges from Alicante had more Pb than the ones from Zamora (statistically significant), and the ones from Ciudad Real more than the ones in Zamora (also statistically significant). According to the Chi Square Test, there is an association between location and Pb values above 0.65 mg/kg.

We performed statistical tests on this species based on different variables, including environment (rural and urban), nature (wild/farm/acclimated) and hunting intensity (very high-Alicante, high-Ciudad Real, low-Zamora). In this regard, it was observed that there were no significant differences between environments (rural vs urban). Neither was an association observed between belonging to an environment and having a Pb value above 0.65 mg/kg (Chi Square).

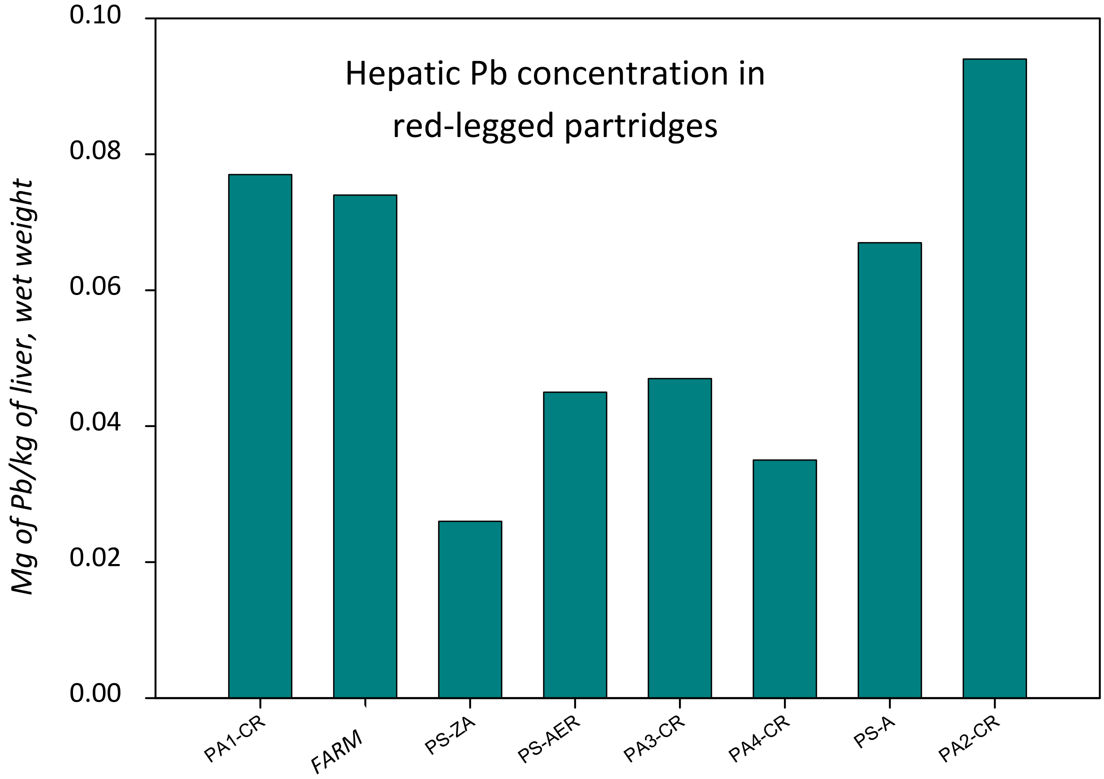
The ANOVA analysis according to the "nature" variable (wild/farm/acclimatized) did not show any significant differences between groups either. However, there was a significant statistical difference between having values above 0.65 mg/kg and the nature variable (most of the specimens above >0.65 mg/kg were acclimated animals). There were eleven red-legged partridges with Pb values >0.65 mg/kg, nine of which were acclimated birds.

If we consider locations to compare if there are any differences due to hunting intensity, it was verified that the highest values appear in Alicante (very high hunting intensity). The ANOVA test confirmed that there were indeed significant differences due to hunting intensity: low with high, and very high with low (very high=0.067; high=0.061; low=0.026 mg/kg). Nevertheless, the concentrations at places where there was no hunting (farm + airport) were similar to the levels found at high and very high-intensity areas (0.061 mg/kg), which suggests it is advisable to delve deeper into this question.

In the case of the Barbary partridges (PM-GOM), we found two specimens (**PM-GOM-11** and **PM-GOM-2**) with gizzard surfaces of a blackish colour (Photographs 40 and 41) and a fairly similar hepatic lead concentration (Table 33). A renal lead analysis could only be performed on one of the two specimens and a low lead concentration was detected (0.044 mg/kg, Table 34). Though no pellets were found in the gizzard or the intestine in either of these two cases, a possible fairly recent ingestion of lead pellets could be inferred in spite of the fact that the appearance of the gizzard's surface was similar to that of the rest of the group. The low renal concentration in one of the specimens and the heavy gunshot damage sustained by the other (PM-GOM-11) make it difficult to confirm this. The third case of this species with a hepatic concentration above 0.65 mg/kg (**PM-GOM-12**) was a specimen with a value at the threshold (0,691 mg/kg) and it likewise had a blackish gizzard surface (Photograph 51). As in the cases described above, there are doubts about this specimen's ingestion of pellets, to which the value at the threshold is added.

Finally, two of the quails hunted in Zamora (**CS-ZA-7** and **CS-ZA-18**), were found to have a green to blackish gizzard surface (Photographs 37 and 38), which were, however, similar to the other specimens of the group. A pellet was found in the crop of both specimens and orifices were also found. The renal lead analysis revealed a very high concentration in the case of the animal identified with the code CS-ZA-18 (131.085 mg/kg) and a much lower concentration in specimen CS-ZA-7 (2.022 mg/kg). A possible ingestion of pellets from the environment could also be suggested for both these cases, though in the case of CS-ZA-18 it would be placed into doubt by the high concentration in the liver without the existence of any evident clinical signs.

Figure 9. *Hepatic Pb concentration in red-legged partridges*



Based on all of the above, there seems to be some evidence of specimens with a high Pb concentration resulting from a possible ingestion of Pb pellets by twelve specimens: PA3-CR-14, PA4-CR-2, PA2-CR-5, PS-A-26, PA2-CR-13, PA2-CR-2, PA4-CR-1, PA3-CR-4, CS-ZA-7, PT-ZA-MIG-1 and PA1-CR-14. Specimen PM-GOM-12 was included in this group, even though the concentration detected was at the limit of the threshold set. The summary of these specimens is as follows:

	Hepatic Pb	Renal Pb	Pellets	Orifice	Gizzard
PA3-CR-14	10,379	28.037	11 (gizzard)	No	Compatible
PA4-CR-2	4.329	26.768	1 (gizzard)	No	Compatible
PA2-CR-5	7.885	41.842	4 (intestine)	No	Compatible
PS-A-26	14,187	10,208	0	-	Compatible
PA2-CR-13	1,230	6,968	0	-	Compatible
PA2-CR-2	3,985	5.209	0	-	Compatible
PA4-CR-1	2.404	19,608	0	-	Compatible
PA3-CR-4	3,084	n.a.	0	-	Compatible
CS-ZA-7	2.695	2.022	1 (gizzard)	Yes	Compatible
PT-ZA-MIG-1	2.788	9,483	0	-	Doubtful to compatible
PA1-CR-14	5.946	0.576	0	-	Doubtful to compatible
PM-GOM-12	0.691	n.a.	0	-	Compatible

This accounts for 2.4% of the total population, without including farm partridges.

There were three specimens whose concentration was situated between the two threshold concentration values set for hepatic Pb (0.65 to 2.0 mg/kg). This fact, along with the appearance of gizzard's internal surface and the renal Pb concentration within the limits considered in one case, means that they may be considered as incompatible cases (TE-A-22, PB-PV-28 and PB-PV-4).

	Hepatic Pb	Renal Pb	Pellets	Orifice	Gizzard
TE-A-22	0.846	n.a.	0	-	Incompatible
PB-PV-28	0.766	n.a.	0	-	Incompatible
PB-PV-4	1,171	3,211	0	-	Incompatible

This accounts for 0.6% of the total population, without including farm partridges

The results for another five specimens should be treated with caution, since the Pb concentration in one of the organs was very low while in the concentration detected the other was excessively high (PS-A-23, PT-ZA-9, PT-M-10 and PB-M-9). A fifth specimen could be added to this group (CS-ZA-18), though in this case the Pb concentration in the other tissue was indeed compatible with exposure to pellets. There was no evidence in these cases due to the gizzard's colour. If these specimens' tissue concentrations are correct and there are no embedded ammunition fragments, we should have found clinical signs of poisoning, which was not the case.

	Hepatic Pb	Renal Pb	Pellets	Orifice	Gizzard
PS-A-23	0.701	289,737	1 (intestine)	No	Incompatible
PT-ZA-9	31,253	0.081	4 (crop)	No	Incompatible
PT-M-10	35,567	0.162	0	-	Incompatible
PB-M-9	1,101	46.034	0	-	Incompatible
CS-ZA-18	6,270	131,085	1 (gizzard)	Yes	Compatible

This accounts for 1.0% of the total population, without including farm partridges.

In the case of PT-M-7 and PM-GOM-2, exposure to lead from another source could be surmised, since hepatic concentration was high, though the renal concentration was low and the gizzard's appearance normal.

	Hepatic Pb	Renal Pb	Pellets	Orifice	Gizzard
PT-M-7	7.105	0.186	0	-	Incompatible
PM-GOM-2	8.766	0.044	0	-	Compatible

This accounts for 0.4% of the total population, without including farm partridges.

Lastly, though the hepatic Pb concentration was high in the remaining two specimens (PA2-CR-10 and PM-GOM-11), the specimens were heavily damaged by the ammunition's impact and the gizzard's colour was not indicative of exposure to pellets. Hence, this data should also be treated with caution.

	Hepatic Pb	Renal Pb	Pellets	Orifice	Gizzard
PA2-CR-10	4.986	n.a.	1 (gizzard)	No	Incompatible
PM-GOM-11	8.300	n.a.	0	-	Compatible

This accounts for 0.4% of the total population, without including farm partridges.

To sum up, 15 specimens may be suspected of having ingested pellets from the environment, which would account for 3.0% of all the specimens under study (without taking farm specimens into account). If we were to include the specimens whose data we have stated should be treated with caution, said percentage would rise to 3.8% (19 specimens).

SYNOPSIS

To sum up, it was observed that the average value (mean) of all the groups was very low. The mean value for the 530 specimens analysed was 0.064 mg/kg (same value if the 26 farm bird specimens are discarded).

The mean hepatic Pb concentration exceeded 0.1 mg/kg only in the common woodpigeon (0.124 ppm) and rock dove (0.201 ppm) populations from Madrid. The rock dove from La Gomera (0.161 ppm) was not taken into consideration, since it only consisted of a single specimen.

Between 95.5% and 96.8% of the population under study turned out to have a base hepatic Pb concentration or a concentration below the level considered for environmental exposure based on the range found in the bibliography (0.65 to 2.0 mg/kg). When farm specimens (n=26) are discarded, said percentage amounts to between 95.2% and 96.6%. Hence, it could be considered on an *a priori* basis that these birds have not ingested Pb pellets from the environment. It should be noted that the aforementioned percentages actually refer to the range between 0.65 and 1.5 mg/kg, as we did not find any specimens having a hepatic Pb concentration between 1.5 and 2.0 mg/kg.

87.9% of the specimens had a hepatic Pb concentration below 0.2 mg/kg.

Of the specimens which were suspected of having ingested pellets from the environment either because pellets were found in their digestive apparatus without finding any apparent evidence of orifices (28 specimens) or because hepatic lead concentrations above 0.65 mg/kg were found (24 specimens), we can surmise that between 15 to 19 specimens (3.0 to 3.8%, discarding farm partridges) had probably ingested pellets from the environment with an evident repercussion on tissues. Of the 24 specimens with concentrations above 0.65 mg/kg, eight of them clearly had cage marks. These were therefore specimens which had been freed into the wild at game reserves during the hunting season. Nine of these birds were hunted in Cuidad Real.

In the specific case of red-legged partridges (n = 219), it could be deemed that high-intensity hunting might have a positive effect on the appearance of specimens which can be considered to have ingested pellets, which suggests it is advisable to delve deeper into this question.

As regards the rock dove (n=99), there seems to be a relationship between hepatic Pb concentration and the environment, which is related to this species' greater concentration in urban environments. As far as the common woodpigeon (n=107) is concerned, there also appears to be a relationship between the urban environment and a higher hepatic Pb concentration.

We should not consider the criterion of the presence of orifices in intestine, since it is difficult to find them in this organ, so it is possible to identify false suspects.

The need to obtain a large number of samples for the research and the need to avoid slaughtering animals only for said purpose led us to choosing animals from normal hunting activities; that is to say, birds killed with a shotgun using lead ammunition, fully aware of the difficulties this might cause as a result of the existence of lead metal on the samples.

CONCLUSIONS

This research puts forward important conclusions about the average Pb concentration in terrestrial-habitat game birds' liver, an organ that is a good indicator of exposure to Pb. This study's general conclusions are as follows:

1. The hepatic Pb concentration in the population under study is low, thereby indicating it is a good state as far as this contaminant is concerned. The mean concentration found was ten times lower than the environmental exposure concentration threshold described as the most restrictive in the bibliography.

2. The results of the analyses performed as well as the observations made on the tissues of some specimens suggest that an environmental exposure to other sources of Pb may have perhaps occurred in some specimens, particularly the ones from urban environments. Future studies could corroborate or refute this hypothesis.

3. There are doubts about the possible existence of samples with Pb fragments embedded in their tissues, especially given the high concentrations found in some specimens coinciding with an absence of any clinical signs. New studies on other tissues may clear up this doubt.

4. Though it is difficult to know exactly which specimens have ingested Pb in the form of pellets and ignoring the doubts set out in Conclusions 2 and 3, it seems likely that this kind of ingestion leading to an evident repercussion on tissues has only taken place in a small group of birds (3.0% to 3.8%).

5. The ecological, social and industrial importance of Pb ammunition's impact on terrestrial-habitat game birds makes it advisable to monitor the game bird populations studied and to conduct new research in the future to attain more in-depth knowledge about this problem.

RECOMMENDATIONS

Some lines of work for future studies are set out below:

- Pellet density studies in habitats of terrestrial game bird species (partridges, pigeons, turtledoves, etc.).
- Analysis of other tissue samples to corroborate or refute the hypotheses and doubts broached in this study.
- Analysis of stable isotopes in an effort to differentiate between Pb from ammunition and from natural or other kinds of contamination.
- Experimental studies involving game birds to study the kinetics of Pb deposition in tissues.
- Analysis of samples from game birds killed with lead-free ammunition.
- Assessment of the hazard posed to consumers by game birds through an analysis of Pb in these bird's muscles.

8 DATE AND SIGNATURES

This report was prepared at the request of the Spanish Sectoral Federation of Weapons and Ammunition (FSA).

Date: 25 January 2019.

Authors: Mr Juan Manuel Theureau de la Peña, Mr Antonio de José Prada, Mr Juan Bautista Torregrosa Soler, Mr Andrés Ferrer Gisbert and Mr Diego Romero García.

9 PHOTOGRAPHS

Photograph 1: *Alectoris rufa*

Photograph 2: *Coturnix coturnix*

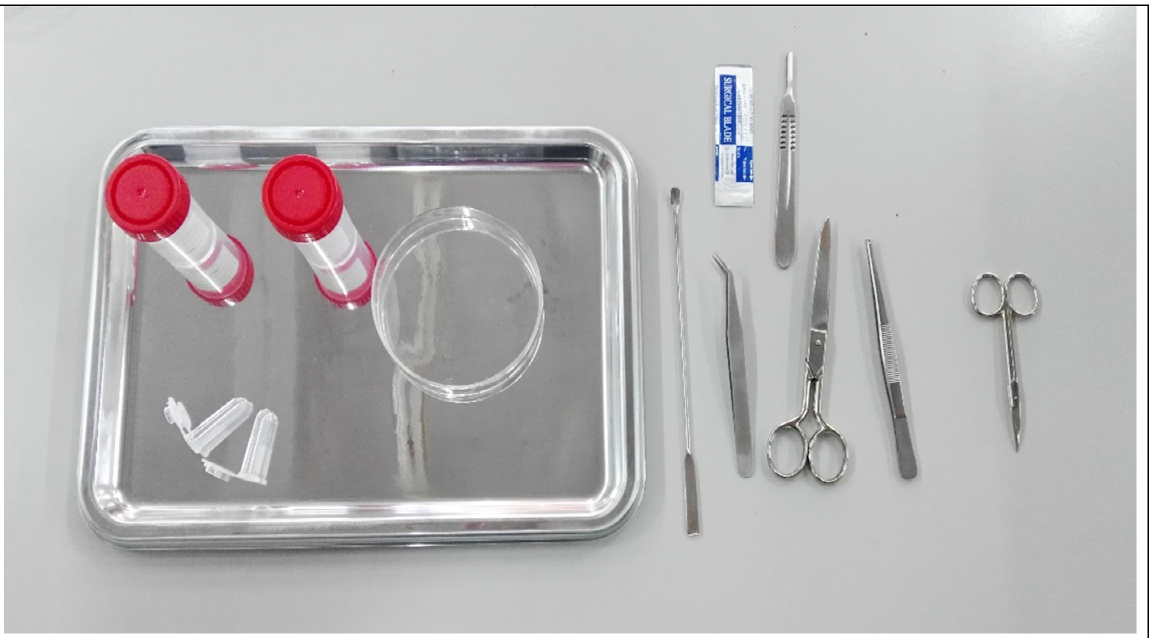
Photograph 3: *Alectoris barbara*

Photograph 4: *Streptopelia turtur*

Photograph 5: *Columba palumbus*

Photograph 6: *Columba livia*

Photograph 7: *Columba oenas*

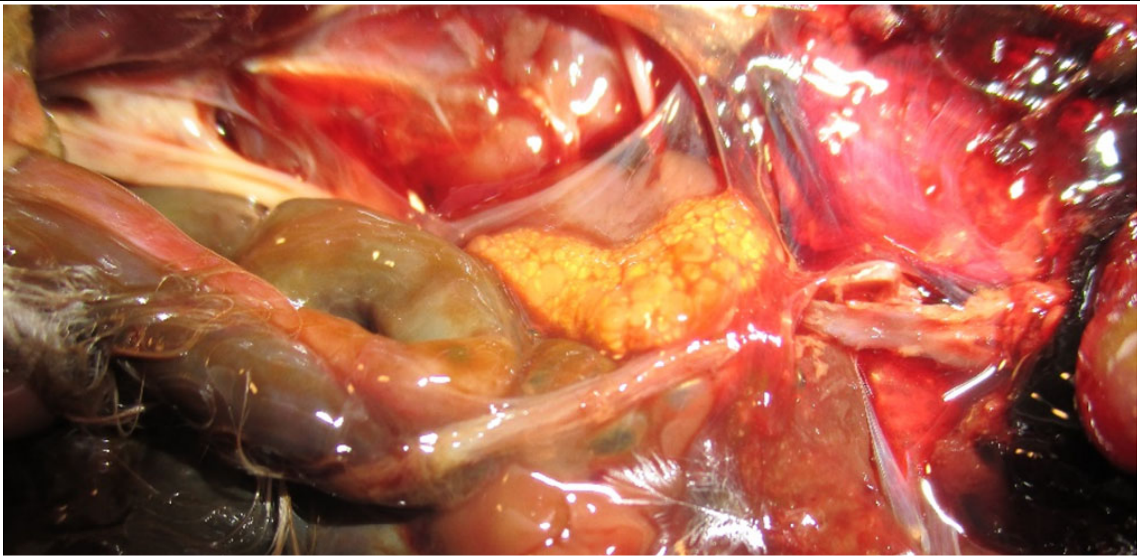


Photograph 8: Materials used in sample taking


Photograph 9: Workbench next to sink

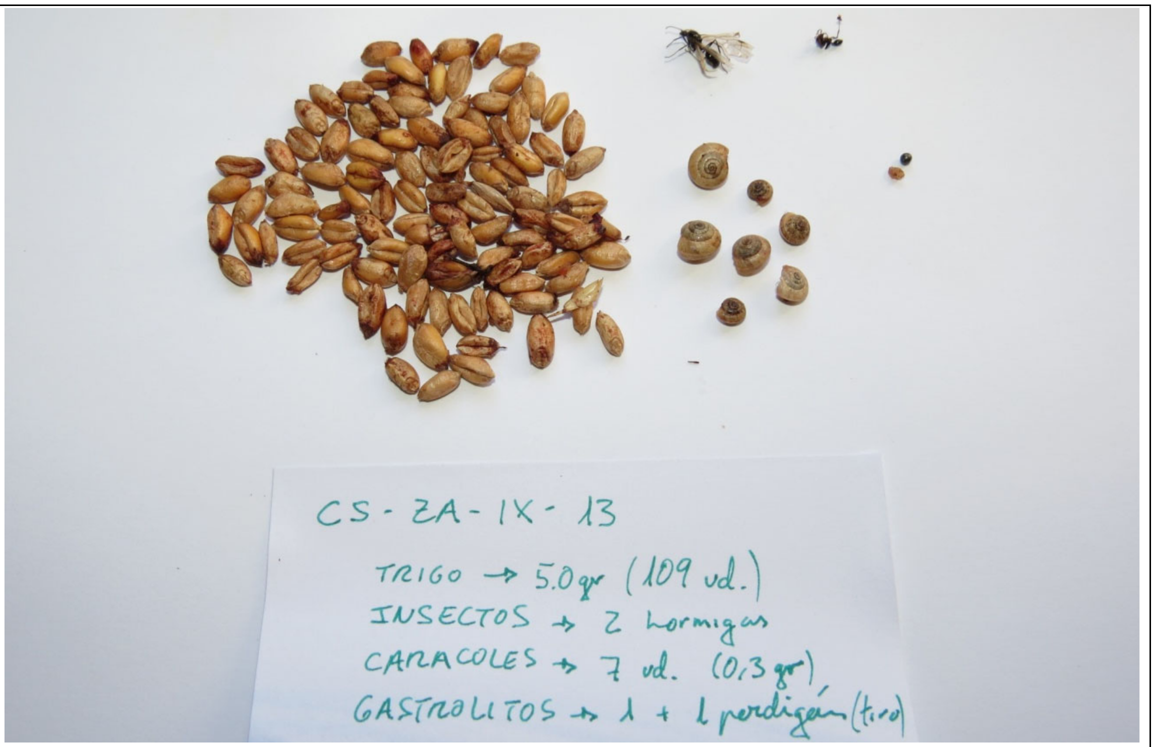

Photograph 10: Moulting state in primary remiges


Photograph 11: Crop sample


Photograph 12: Examination of gonads

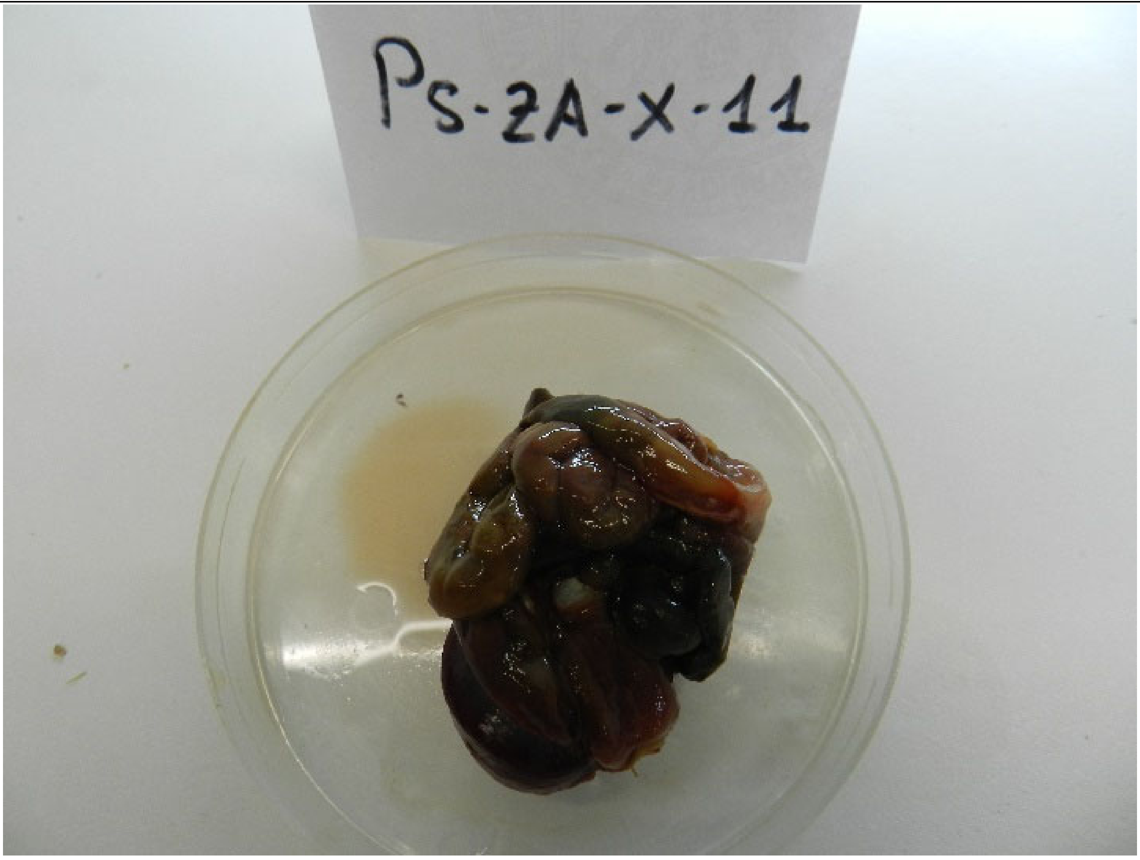

Photograph 13: Crop contents of PB-ZA-XII-15

Photograph 14: Crop contents of PT-A-IX-3

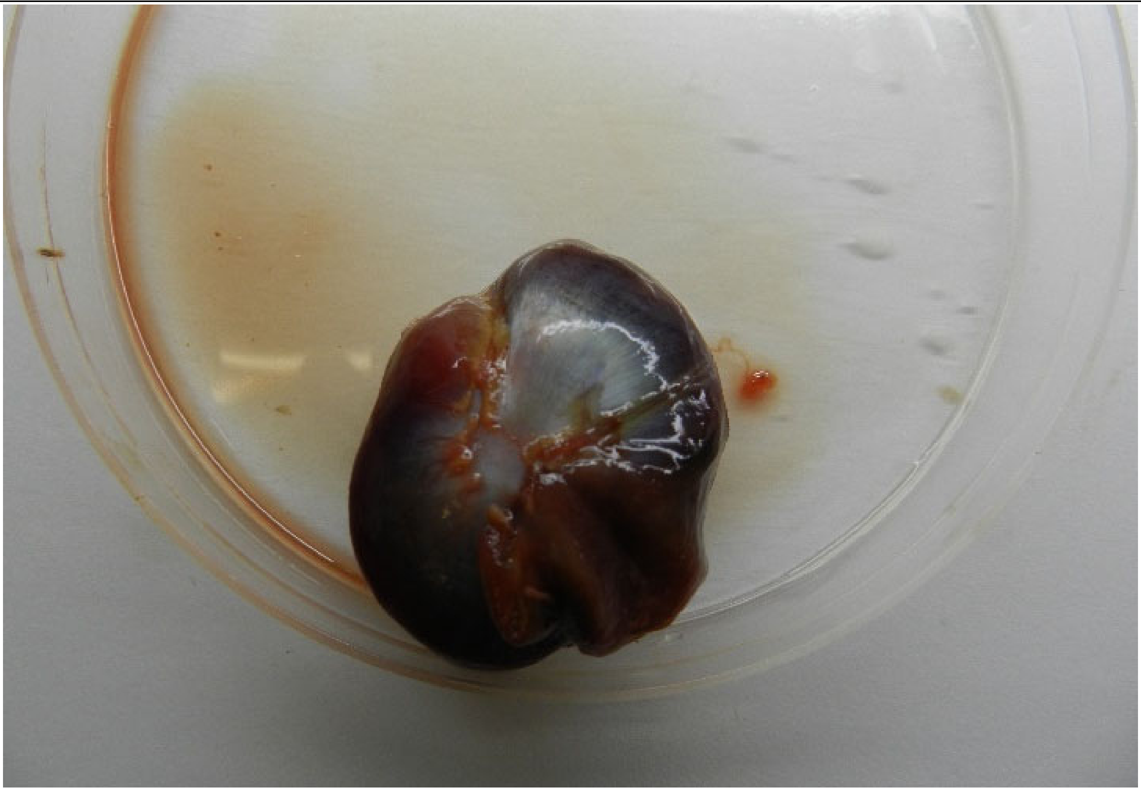


Photograph 15: Crop contents of CS-ZA-IX-13

Photograph 16: X-ray of digestive apparatus

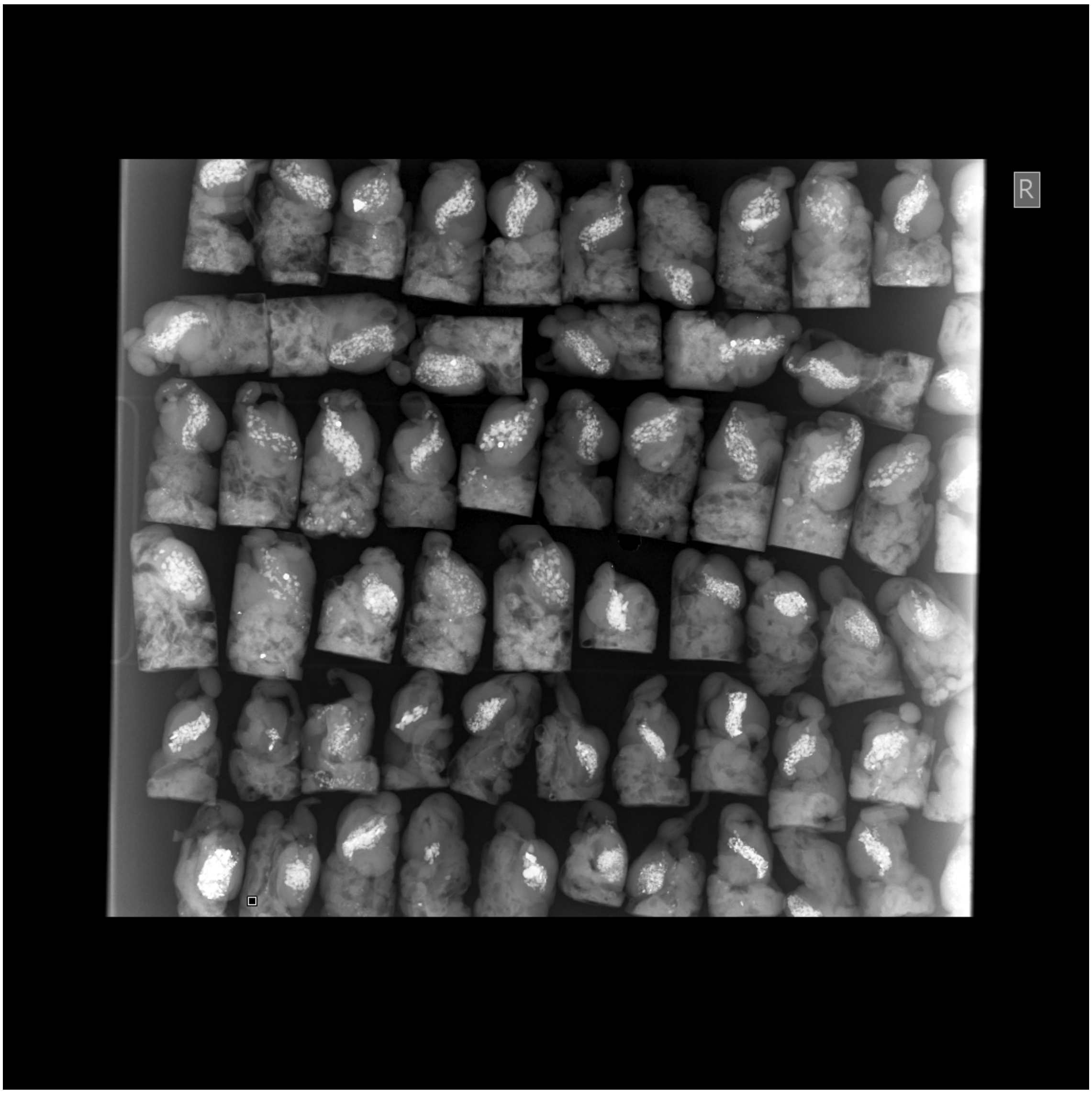


Photograph 17: Compete digestive apparatus

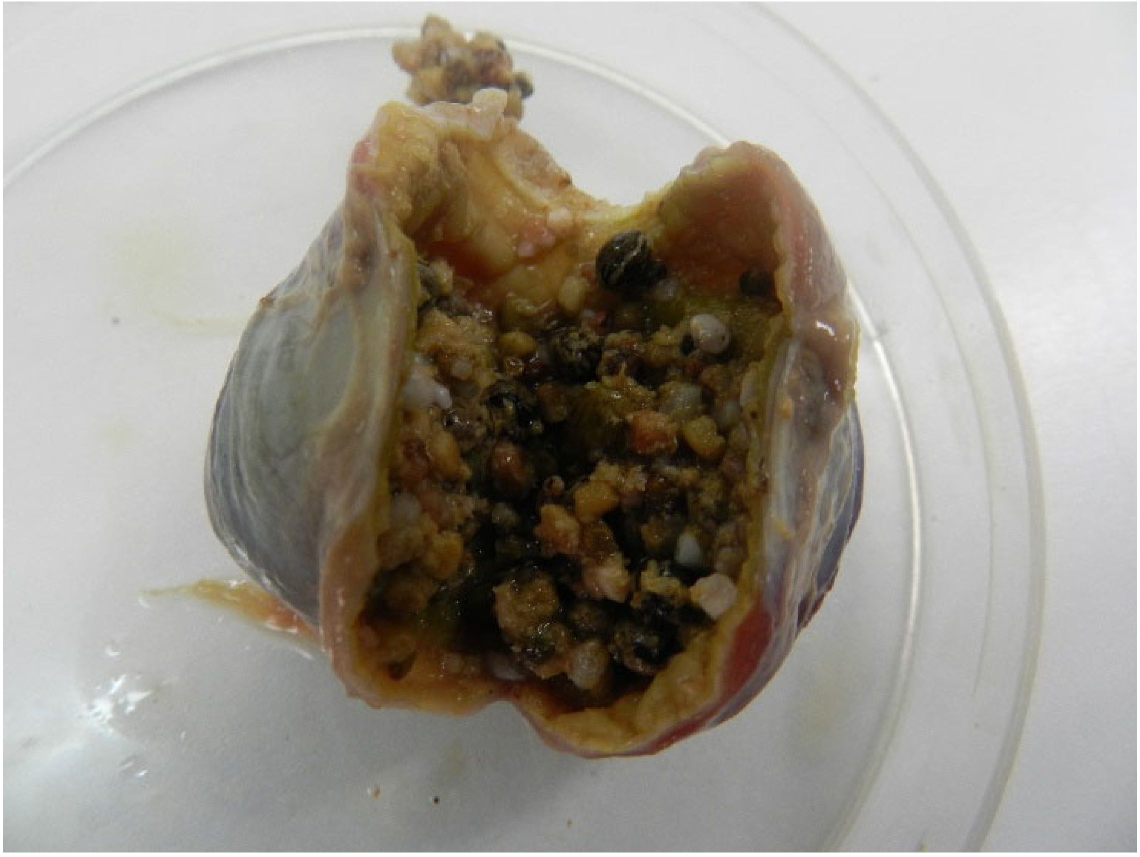


Photograph 18: Gizzard

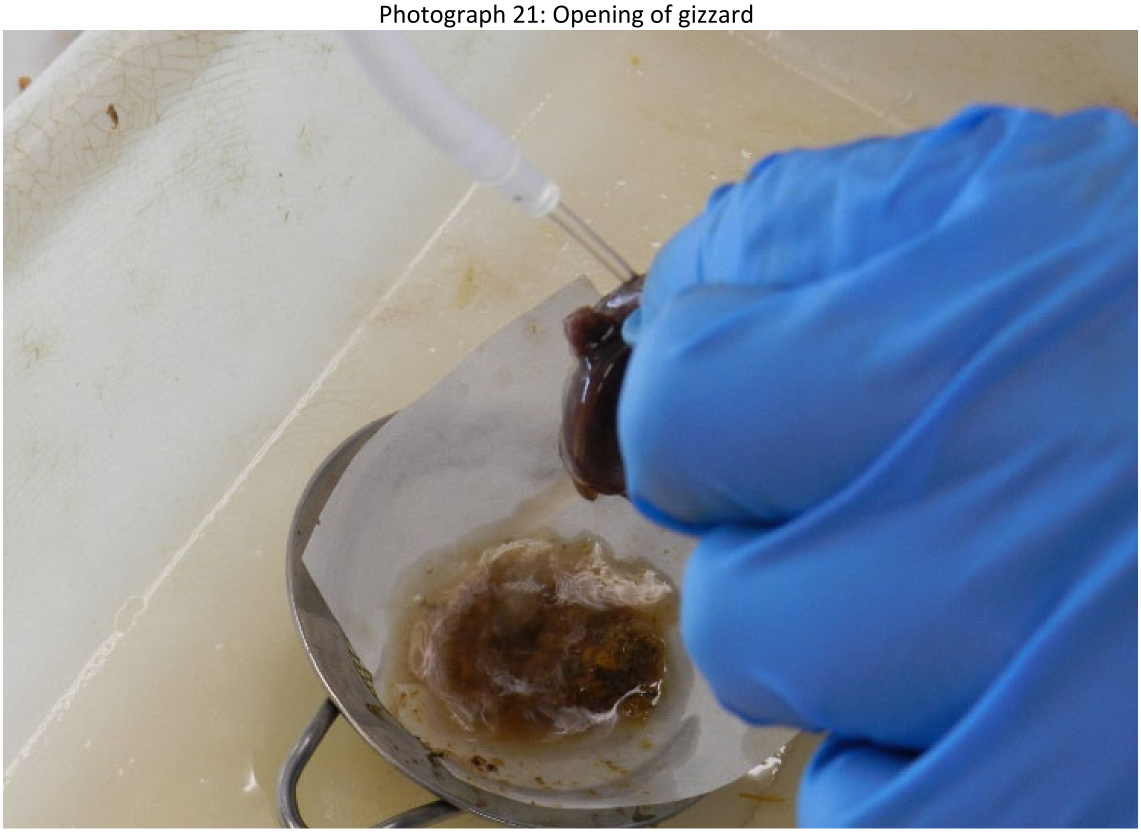
Photograph 19: Intestine



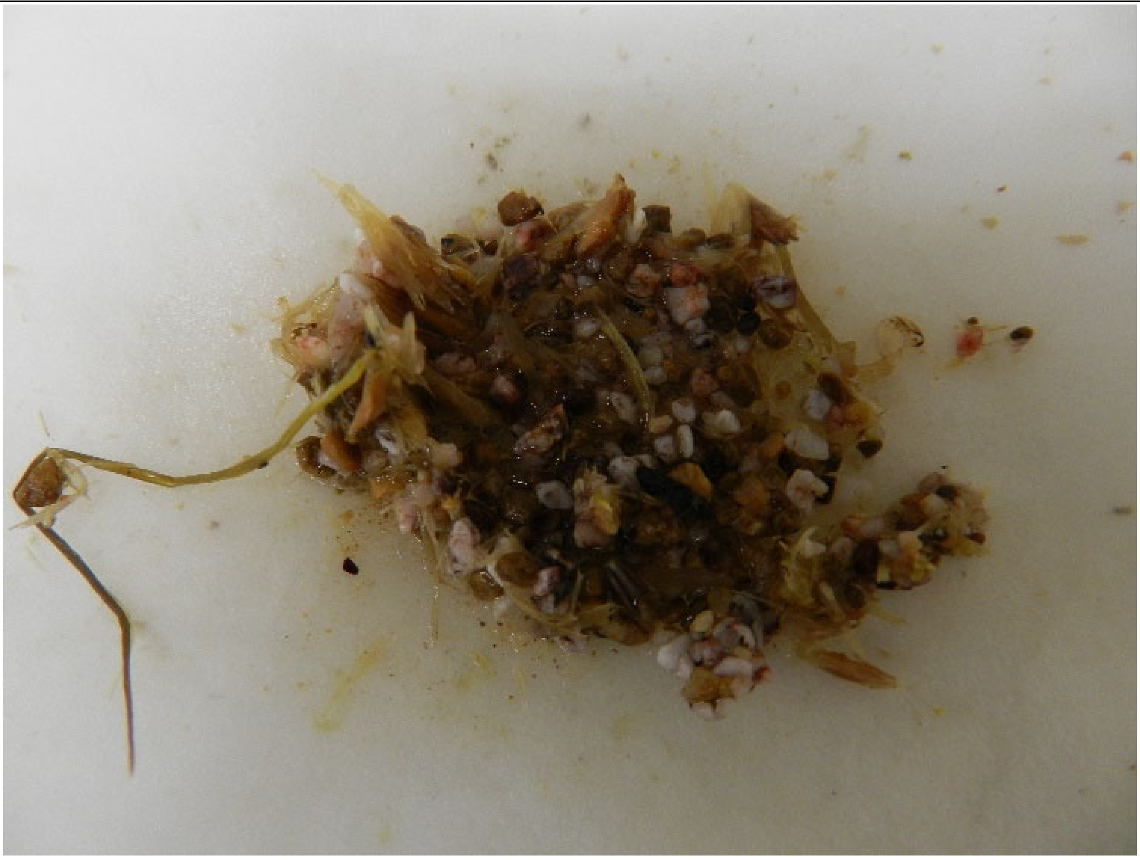
Photograph 20: Radiographic image



Photograph 21: Opening of gizzard



Photograph 22: Washing the gizzard's contents



Photograph 23: Gizzard's contents once washed

Photograph 24: Observing the gizzard's contents with a magnifying glass

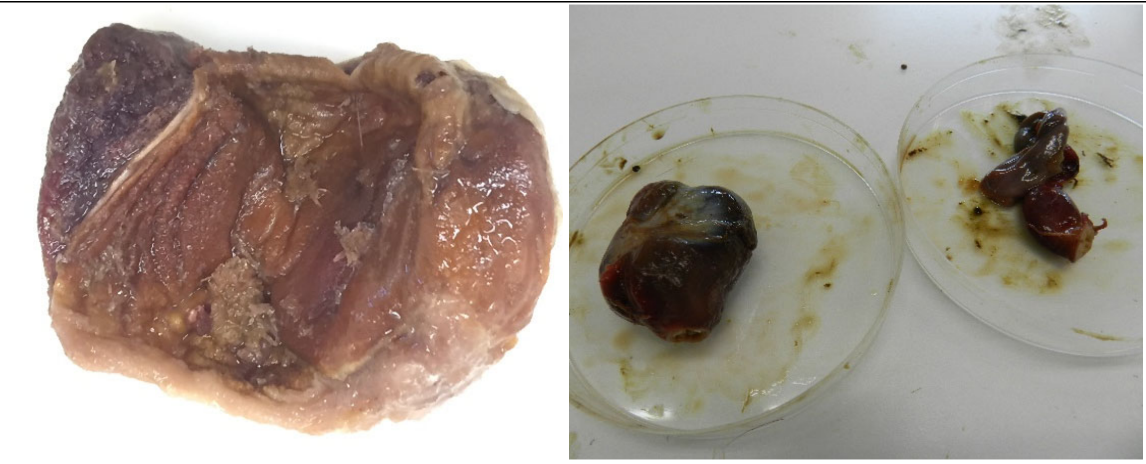
Photograph 25: Gizzard's contents washed and dry

Photograph 26: Mucosa of the gizzard

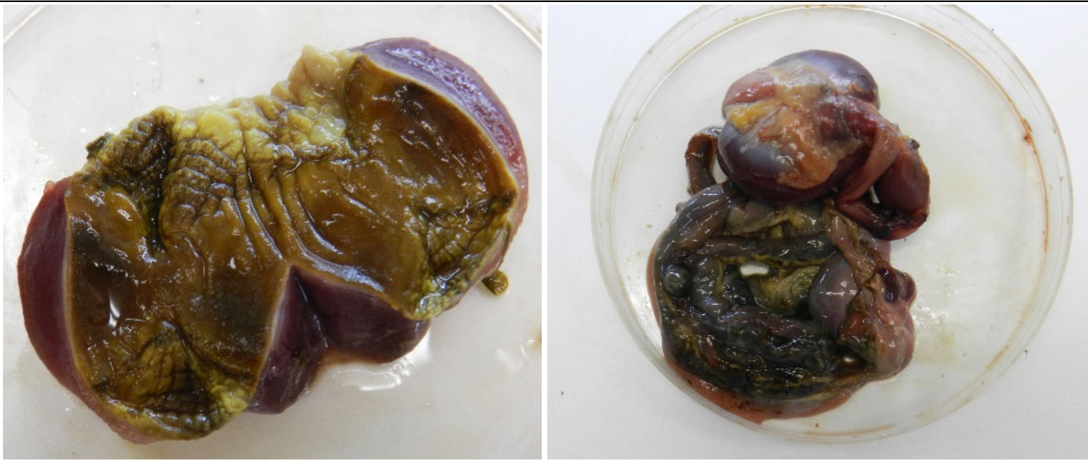
Photograph 27: Storage of gizzard and intestine

Photograph 28: Specimen PA3-CR-14. Internal surface of the gizzard, and external appearance of gizzard and intestine

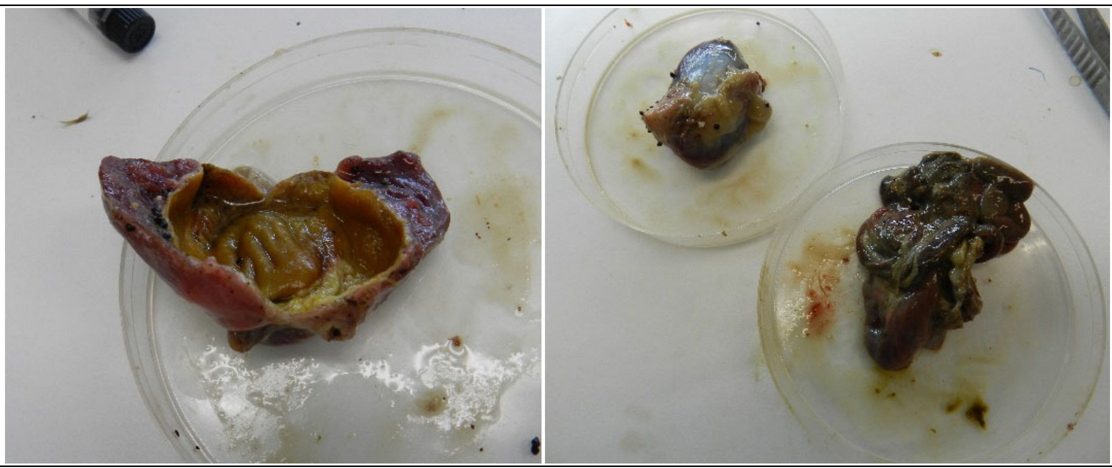
Photograph 29: Specimen PA4-CR-2. Internal surface of the gizzard, and external appearance of gizzard and intestine



Photograph 30: Specimen PA2-CR-10. Internal surface of the gizzard, and external appearance of gizzard and intestine

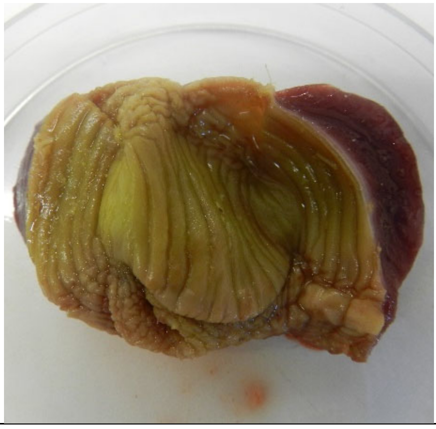


Photograph 31: Specimen PA2-CR-5. Internal surface of the gizzard, and external appearance of gizzard and intestine



Photograph 32: Specimen PS-A-23. Internal surface of the gizzard, and external appearance of gizzard and intestine

PT-ZA-9	Internal surface of the gizzard	External appearance of the intestine
Photograph 33.		

PT-M-7	Internal surface of the gizzard	External appearance of the intestine
Photograph 34.		

<table>
<tr><td>PT-M-10</td><td>Internal surface of the gizzard</td><td>External appearance of the intestine</td></tr>
<tr><td>Photograph 35.</td><td></td><td></td></tr>
</table>

<table>
<tr><td>PA1-CR-14</td><td>Internal surface of the gizzard</td><td>External appearance of the intestine</td></tr>
<tr><td>Photograph 36.</td><td></td><td></td></tr>
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<table>
<tr><td>CS-ZA-7</td><td>Internal surface of the gizzard</td><td>External appearance of the intestine</td></tr>
<tr><td>Photograph 37.</td><td></td><td></td></tr>
</table>

CS-ZA-18	Internal surface of the gizzard	External appearance of the intestine
Photograph 38.		

PB-M-9	Internal surface of the gizzard	External appearance of the intestine
Photograph 39.		

PM-GOM-11	Internal surface of the gizzard	External appearance of the intestine
Photograph 40.		Unavailable (destroyed by gunshot)

PM-GOM-2	Internal surface of the gizzard	External appearance of the intestine
Photograph 41.		

PA3-CR-4	Internal surface of the gizzard	External appearance of the intestine
Photograph 42.		

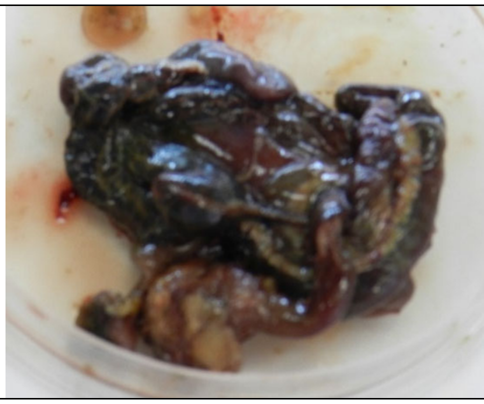
PA4-CR-1	Internal surface of the gizzard	External appearance of the intestine
Photograph 43.		

TE-A-22	Internal surface of the gizzard	External appearance of the intestine
Photograph 44.		

PT-ZA-1-MIG	Internal surface of the gizzard	External appearance of the intestine
Photograph 45.		

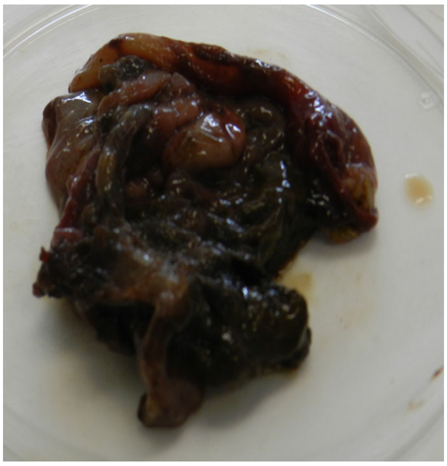
PB-PV-28	Internal surface of the gizzard	External appearance of the intestine
Photograph 46.		

PB-PV-4	Internal surface of the gizzard	External appearance of the intestine
Photograph 47.		

PS-A-26	Internal surface of the gizzard	External appearance of the intestine
Photograph 48.		

PA2-CR-13	Internal surface of the gizzard	External appearance of the intestine
Photograph 49.		

PA2-CR-2	Internal surface of the gizzard	External appearance of the intestine
Photograph 50.		

PM-12	Internal surface of the gizzard	External appearance of the intestine
Photograph 51.		

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