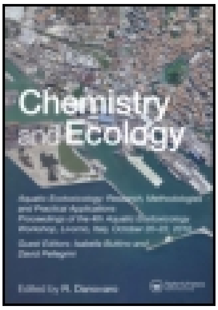

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Ecotoxicological study on lead shot from hunting in the Padule di Fucecchio marsh (Tuscany, Italy)

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Ingestion of lead shot has been recognised as an important cause of death in waterbirds for over a century. Chemical dissolution (oxidation) of lead shot in aquatic environments can make lead more available, distributing it throughout the aquatic ecosystem. The extent of the phenomenon has rarely been investigated in Italy and no systematic information is available for Italian wetlands. We investigated lead shot density and lead concentrations in sediment of the Padule di Fucecchio (Tuscany), one of the largest inland marshes in Italy (2000 ha), in order to evaluate the impact of lead shot from hunting. Moreover, to assess lead accumulation in organisms, red swamp crayfish, *Procambarus clarkii* (Girard 1852), was chosen as a bioindicator. The results showed a high lead shot density (0–311 shot $\cdot$ m^{-2}). Although lead concentrations and lead shot density were slightly less in protected areas than in hunting areas, contamination was widespread throughout the marsh, with a mean lead concentration of 115.6 mg $\cdot$ kg^{-1} d.w.. Crayfish were subject to heavy uptake of lead, with high concentrations in gut contents (43.84 ± 47.48 mg $\cdot$ kg^{-1} d.w.) and hepatopancreas (3.217 ± 4.850 mg $\cdot$ kg^{-1} d.w.). A detailed map of lead contamination was plotted using sediment data. Thematic mapping is a valuable support for environmental remediation and reserve management and assessment of lead contamination concomitant with the banning of lead shot in the area provides a useful database for long-term monitoring of the effectiveness of the ban.

Keywords: lead; shot; hunting; geographical information system; Padule di Fucecchio; crayfish

1. Introduction

Lead shot fired from fixed hunting posts falls and accumulates in a limited area, unlike shot fired by hunters on foot. Shot that misses its target persists in the environment, accumulates in sediments and may reach very high densities (millions per hectare). According to recent estimates, 2400–3000 t$\cdot$year^{-1} of lead accumulates in wetlands of the European Union, 148 t of which is in Italy [1]. More generally, it is estimated that hunters in Italy shoot up to 25,000 t of lead or $\sim$700 million cartridges every year [2].

Lead shot in wetlands is often ingested by waterbirds which confuse it with grit needed for grinding in the gizzard, or possibly with seeds [3–5]. Ingested shot is retained in the gizzard, releasing lead that is absorbed into the blood stream [6]. Ingestion of lead shot has been indicated as the main cause of heavy metal poisoning in waterfowl and has been recognised as a major cause of bird deaths for over a century [7–10].

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Although often incorrectly regarded as inert, once dispersed in the environment, lead shot undergoes slow transformation, with total disintegration taking 30–300 years [11,12]. Lead shot and fishing sinkers can be reduced to smaller particles and transformed into various molecular species that diffuse more readily in the environment, increasing the availability of the metal [11]. Exposure to water and air corrodes lead, forming a whitish grey or brown crust consisting principally of cerrusite ($PbCO_3$) and hydrocerrusite ($Pb(CO_3)_2(OH)_2$) and subordinately anglesite ($PbSO_4$) [12–14]. These compounds disperse in the environment.

Aerobic and acid conditions, combined with physical factors such as disturbance of sediment, further increase the breakdown of lead shot. The mobility of elemental lead and degradation compounds is influenced by rainfall, vegetation cover, soil acidity and the quantity of organic matter in sediment [11,15]. Hunting in wetlands is not only responsible for direct killing of many species of birds, but may be considered the main cause of lead poisoning in water birds and a source of lead contamination in the wetland ecosystem.

Although the consequences of the release of large quantities of Pb into wetland ecosystems have long been known [7–10,16–23], the extent of the phenomenon has rarely been investigated in Italy in terms of the concentrations of lead or density of shot in sediment, indeed no systematic information regarding Italian wetlands is available in the literature. Several studies have revealed higher levels of lead poisoning in waterfowl in Mediterranean countries than elsewhere in the world [16,17,19–25], but reports from Italy are limited to cases of poisoned waterbirds [24–34].

The African–Eurasian Migratory Waterbird Agreement (AEWA 1995) envisaged a ban on lead shot for hunting in wetlands and its replacement with shot made of steel or other approved materials, or chromium-plated shot. The use of lead shot for hunting has been regulated in several European countries, however, no common European Union (EU) regulation exists on lead ammunition [25]. Although Italy ratified the AEWA agreement in 2006, with Law no. 66 (6 February 2006), the first (partial) concrete provision made by the Italian government regarding hunting equipment was the prohibition of lead shot in wetlands within Special Protection Areas. The Decree of 17/10/2007 by the Minister for the Environment prohibits the ‘use of munitions containing lead shot in wetlands, such as freshwater, brackish and salt water lakes, ponds, marshes, fens, bogs and lagoons, and within a range of 150 meters from their outer banks, as from the hunting season of 2008/9’.

In this context, ecotoxicological studies can be important for the conservation of wetlands. Several methods to detect and assess the impact of lead shot on birds have been suggested, but some of those that may be useful for confirming a case of poisoning with precision are expensive, time-consuming and require trained personnel. Examples of such methods include examination of the gizzard for ingested lead shot and determination of blood concentrations of lead in birds. By contrast, determination of the amount lead shot available to birds (as lead shot density in sediment up to 10 cm depth) cannot accurately assess the magnitude of the problem, but clearly indicates the risk of plumbism in a given area.

In order to evaluate the impact of lead shot from hunting on wetlands, we quantified lead shot density and lead concentrations in sediment in the Padule di Fucecchio (Tuscany), one of the largest inland marshes in Italy. A detailed map of lead contamination was plotted using sediment data. Thematic mapping is a valuable support for environmental remediation and reserve management. Assessment of lead contamination concomitant with the banning of lead shot in the area provides a useful database for long-term monitoring of the effectiveness of the ban.

To evaluate lead accumulation in organisms, the red swamp crayfish, *Procambarus clarkii* (Girard 1852), was chosen as a bioindicator species. This species has often been used in environmental studies to monitor pollution by metals [35–44]. It is a solitary bottom dweller, living in contact with sediment and therefore subject to metal accumulation [41–45]. Lead accumulation in crayfish is dose- and time-dependent, reflecting lead concentrations in contaminated wetlands [35–37,41–45].

2. Materials and methods

2.1. *Study area*

The Padule di Fucecchio (Tuscany) is the largest inland marsh in Italy (2000 ha) and an important stop on bird migratory routes between the Tyrrhenian coast and inland (Figure 1). In the course of a year, more than 190 species of bird can be observed, at least 70 of which nest in the area. Excluding a small portion ($<$250 ha) that is provincial nature reserve for fauna and flora, hunting in Fucecchio marsh is intense.

To quantify lead shot density and lead concentrations, sediments from hunting areas and the nature reserve were sampled. The protected area was established in 1996, however, hunting ceased at different times in the two sub-areas of the nature reserve (La Monaca-Righetti and Le Morette). Sampling points were therefore grouped according to hunting history as follows: (A) hunting conducted for only 2 years; (B) hunting conducted for $>$50 years; (C) protected area, La Monaca-Righetti, where hunting has not been conducted for $\sim$35 years; (D) protected area, Le Morette, where hunting has not been conducted for $\sim$12 years.

2.2. *Sampling and sample preparation*

2.2.1. *Sediments*

Sediments were collected over the 2-year period 2007–2008, avoiding breeding seasons. A portable GPS device was used to obtain the geographic coordinates and to georeference the data. A light dredge of the Ekman-Birge type with a stainless steel sampling box was used to obtain samples of upper undisturbed sediment (approximately the top 5–10 cm).

The samples were placed in metal-free plastic containers. In the laboratory, the macroscopic plant fraction was removed and the sample was homogenised. An aliquot was used to determine

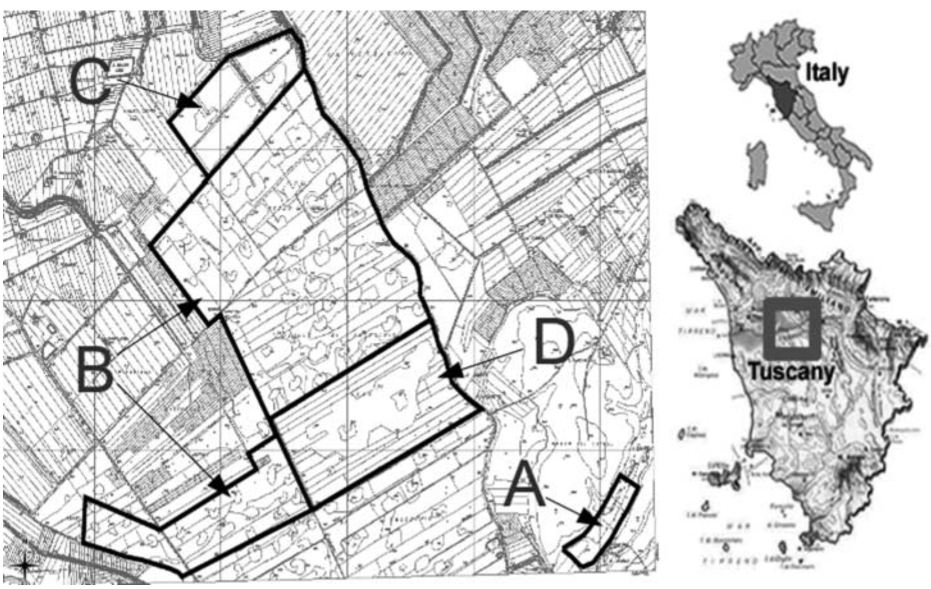

Figure 1. Geographical location of Padule di Fucecchio marsh showing areas with different hunting histories: (A) hunting conducted for only 2 years; (B) hunting conducted for $>$50 years; (C) protected area, La Monaca-Righetti, where hunting has not been conducted for $\sim$35 years; (D) protected area, Le Morette, where hunting has not been conducted for $\sim$12 years.

the frequency of shot in the sediment by sieving (2- and 1-mm mesh) under flowing water. Lead shot were identified and counted among the rock fragments and plant matter retained by the sieves. A second aliquot of homogenised sample was used to determine lead concentrations and was lyophilised to express the results on a dry-weight basis.

2.2.2. *Organisms*

Crayfish were randomly collected throughout the marsh. Field studies on spatial and temporal patterns of movement have shown that *P. clarkii* may travel >3 km per day, particularly in rice fields [46]. Specimens were captured using a hand net and placed in metal-free plastic containers. In the laboratory, biometric parameters were recorded. Length ranged from 5.9 to 11.7 cm and weight from 6.0 to 45.4 g. Specimens were dissected to obtain organs and tissues, specifically hepatopancreas, carapace, muscle and gut contents. Samples were then lyophilised and results expressed on a dry-weight basis.

2.3. *Analytical determinations*

2.3.1. *Mineralisation of samples*

Sediment samples for analytical determinations were obtained by acid digestion using Teflon bombs, including dissolution of heavy metals in hot nitric and hydrochloric acid. Aliquots of ~0.1 g of dried sediment were placed in Teflon containers, to which 0.3 mL hydrogen peroxide was added and left for 20 min. Then 0.9 mL HCl and 0.3 mL HNO_3 were added.

Biological samples (crayfish tissues) were treated with nitric acid and hydrogen peroxide 4:1. For mineralisation, sediment and biological samples were loaded in special steel blocks with pressure seals and held at 160 °C for 12 h. The resulting solutions were transferred to plastic tubes and made up to 10 mL.

2.3.2. *Analytical techniques*

Lead was determined by graphite furnace atomic absorption spectrometry (Perkin–Elmer mod. THGA Analyst 700) with a detection limit of 0.06 ppb. Blanks were run during each set of analyses to check the purity of the chemicals used and any sample contamination. All chemicals were of analytical grade. The accuracy of the results was verified using standard reference materials: estuarine sediments SRM n°1646a from National Institute of Standards and Technology (NIST) Gaithersburg (MD, USA) and dogfish muscle SRM DORM-2 from National Research Council Canada (Ottawa, Ontario, Canada) with recovery >95%. All metal concentrations are mean of three replicates in $mg \cdot kg^{-1}$ dry weight.

2.4. *Data processing*

2.4.1. *Statistical analysis*

All the data were displayed on electronic spreadsheets. Statistica 7 (StatSoft Inc.) and Graph Pad Prism 5 (1992–2004, Software Inc.) were used for statistical analysis that consisted of descriptive statistics and statistical inference. The non-parametric Mann–Whitney *U*-test and Kruskal–Wallis analysis of variance (ANOVA) were used to compare data from areas with different hunting histories. Spearman's rank correlation was used to determine relationships between lead shot density and lead concentrations in sediments.

2.4.2. *Thematic mapping of Pb distribution in sediments*

ArcGis 9.0 (ESRI) (ArcInfo licence 1999-2004 ESRI Inc.) was used for cartography. The types of information were first identified (geometric, topological and informative), then a relational database was built. The data was entered and the algorithm for plotting the map was chosen. Mapping was based on datum and geostatistical analysis.

2.4.2.1. *Datum.* Projected Coordinate System: Transverse Mercator; Projection: Transverse Mercator; Linear Unit: Metre; Geographic Coordinate System: Rome 1940; Datum: D_Rome 1940 Prime Meridian: Greenwich; Angular Unit: Degree.

2.4.2.2. *Geostatistical analysis.* To plot maps of the distribution of lead concentrations we chose a deterministic interpolation method known as kriging. Data from area A was not included because this area was not contiguous with the others.

3. Result and discussion

3.1. *Lead shot density*

Lead shot density in sediment was generally in the range 0–311 shot·m^{-2} (Table 1).

Figure 2 shows the accumulation of lead shot in sediment in areas of Fucecchio marsh with different hunting histories.

The highest mean values were found in area B which has the longest history of hunting (>50 years). Significantly lower values were found in the protected area La Monaca-Righetti (C), where hunting has not been conducted for 35 years (Kruskal–Wallis $H = 13.524$; $p < 0.01$). Intermediate values were found in the area where hunting had only been conducted for 2 years (A) and in the protected area Le Morette (D).

The density of lead shot in wetland sediments has been studied in several countries (Table 1). The highest densities were recorded in southern Europe where up to 398.9 shot·m^{-2} in the upper 30 cm of sediment was reported in the Medina lagoon in southern Spain [22,25].

Although various references are available from all over the world (Table 1), data on lead shot densities in sediment from wetlands is difficult to compare with our study, because of variations in wetland type, soil management, depth of sediment sampled, etc. However, the top density (311 shot·m^{-2}) found in this study is the highest of all, exceeding even the maximum values reported for the Camargue in France [17], Albufera de Valencia [20], the Ebro delta [19,55] and Andalusia in Spain [22]. We did not consider the highest value (398.9 shot·m^{-2}) reported in Medina lagoon [22] because it was calculated as the total density of lead shot down to a depth of 30 cm (with most shot concentrated between 5 and 20 cm), whereas our results were based on sediment sampled down to a depth of 10 cm.

Our maximum lead shot density (311 shot·m^{-2}) was also among the highest reported for Italy [27], despite the fact that data on lead shot densities in Italian wetlands, available for comparison, is only anecdotal, fragmentary and insufficiently out of date (Table 1). The consequences of releasing large quantities of lead shot into wetlands have long been known at an international level, however, in Italy the information published in the scientific literature has mostly regarded cases of poisoning of waterbirds [25–34], and this information has rarely been reported as lead concentrations in sediment or as shot density in sediment.

Regarding areas with different hunting histories, although hunting had only been conducted in area A for 2 years, a relatively large quantity of shot had accumulated with respect to area B with its 50-year history of hunting. In B, shot may have been buried deeply by natural settlement, as well

Table 1. Lead shot density data reported in the world literature.[a]

Reference	Area	Year	Depth (cm)	Shot · ha^{-1}
[8]	Canada	N.A.	N.A.	41,990–125,700
	USA; California	N.A.	N.A.	8608–292,000
[47]	USA; Missouri	1965	0–5	64,515–303,415
		1974	0–5	71,012–92,530
[48]	USA; Colorado	1974	N.A.	9400–43,600
[49]	USA; California	N.A.	N.A.	22,732–859,908
[50]	USA; Maine	1976–1980	0–10	59,541–40,325
[51]	USA Texas	N.A.	N.A.	1,676,300
[52]	USA; California	1997	0–10	342,999
[18]	USA; California	1986–1987	0–10	15,750–2,299,700
[53]	Australia	1988–90	0–20	330,000
[54]	Denmark	N.A.	N.A.	122,000–1,837,000.
[56]	Netherlands	N.A.	0–15 cm	140,000–435,000
[17]	France; Camargue	1987	N.A.	64,000–1,995,000
		1989	N.A.	53,000–852,000
[19]	Spain; Ebro Delta	1993	0–20	<8991–2,661,386
[20]	Spain; eastern, central and southern wetland sites	1993–1997	0–20	<11,988–2,875,984
[21]	Spain; Doñana Park (mobile dune Cerro de los Ansares)	1997	Upper layer	54,000 (mean)
			0–10	125,900 (mean)
			Total 0–20	162,000
[22]	Spain; Andalusia – Doñana, Guadalquivir, and other small closed-basins	2001–2003	0–10	0–1,483,000
[26]	Italy; Saline Margherita di Savoia	1993	N.A.	630,000–1,270,000
	Italy; Saline di Cervia	1995	N.A.	470,000–710,000
	Italy; Valli di Comacchio	1995	N.A.	40,000–430,000
	Italy; Marano lagoon	1994	N.A.	420,000
	Italy; Orbetello lagoon	1994	N.A.	320,000
	Italy; Diaccia Botrona marsh	1994	N.A.	80,000–200,000
	Italy; Po Delta	1995	N.A.	0–200,000
	Italy; Valli Bertuzzi	1995	N.A.	0–190,000
	Italy; Grado lagoon	1994	N.A.	0
This study	Italy; Padule di Fucecchio	2007–2008	0–10	0–3,311,000

Note: N.A. not available.
[a]Further references on European country have been reported in a recent review by Mateo [25].

as by periodic intervention by hunters, including dredging to counteract silting and proliferation of vegetation. Only superficial sediment (depth 5–10 cm) was sampled in this study.

With regard to the protected areas, La Monaca-Righetti (C) was presumably less contaminated and less recently so than Le Morette (D). The difference may be due to the different lengths of time since hunting stopped. Hunting was only banned at Le Morette in 1996, whereas it ceased more than 35 years ago at La Monaca-Righetti. However, in other studies conducted in protected areas, such as the Tablas de Daimiel, almost 30 years after the hunting ban it was found that lead shot density was only slightly lower (994,000 shot·ha^{-1}) than in hunting areas such as Charca Sur de Levante of El Fondo (1,236,000 shot·ha^{-1}) [20]. Moreover, by monitoring lead poisoning from ingestion of lead shot by sentinel mallard at Sacramento National Wildlife Refuge, Rocke et al. [18] demonstrated that exposure to lead and lead poisoning continue to occur, despite conversion to steel shot for hunting waterfowl. They concluded that in some wetlands lead poisoning may remain a significant problem for many years after conversion to non-toxic shot.

By contrast to the findings of the above studies [18,20], we found a lower density of shot at La Monaca-Righetti (C), where hunting has not been practised for more than 35 years. Because lead shot is estimated to persist in sediment for 300 years [12], settling is probably the main way in which lead shot becomes unavailable to birds in Fucecchio marsh. The very soft sediment in our study area depends on factors such as fresh sediment deposition and plant decomposition [17,57] and may allow shot to settle beyond a depth available to ducks. This suggests that in areas where hunting stopped, the density of shot in sediment, at least in the top 5–10 cm, may decrease relatively quickly.

Irrespective of settling rates, lead shot remains available to birds for several years after hunting bans or the introduction of non-toxic shot instead of lead. Thus, for decades after a hunting ban, lead shot in sediment continues to poison waterbirds through ingestion, while chemical dissolution (oxidation) of lead shot continues to make lead available throughout the aquatic ecosystem.

3.2. *Lead concentrations*

Lead concentrations in sediment samples were generally in the range 14–306 mg·kg^{-1} d.w., mean 115.6 mg·kg^{-1} d.w. (Table 2).

In general, lead concentrations were well above natural background levels for the Earth's crust, even considering soil and sediment separately (Table 3).

The vast majority of samples (96.4%) exceeded the concentration of 30 mg·kg^{-1} d.w. established as a quality standard for sediment of marine coastal and transition water bodies by Italian low (Annex 1 Decreto Legislativo n. 152/2006, updated by D.M. 'Ambiente' n. 56/2009), indicating that the whole marsh is heavily contaminated by lead. The results were in line with concentrations reported in sediments of wetlands subject to high metal input, and maximum concentrations were among the highest in the literature for wetlands, even those where hunting is practised, only being lower than maximum concentrations recorded in skeet shooting ranges (Table 2).

Comparison of lead concentrations in sediment from areas with different hunting histories is shown in Figure 3.

Concentrations did not differ among areas except for area A where levels were significantly lower than for area B (Kruskal–Wallis $H = 16.29$; $p < 0.01$). Lead levels can be considered as 'natural' or 'background' in area A, where hunting has only been conducted for 2 years, because mean concentrations were <25 mg·kg^{-1} (Table 3). Because chemical transformation of lead shot is slow [12], 2 years is presumably too short time to detect a rise in concentrations.

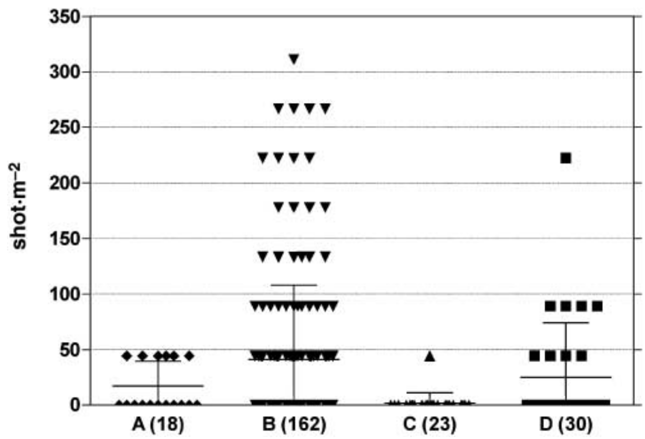

Figure 2. Densities of lead shot per square meter (mean ± SD) in areas with different hunting histories in the Padule di Fucecchio (number of samples in brackets): (A) hunting conducted for only 2 years; (B) hunting conducted for >50 years; (C) protected area, La Monaca-Righetti, where hunting has not been conducted for ∼35 years; (D) protected area, Le Morette, where hunting has not been conducted for ∼12 years.

Table 2. Lead concentrations reported in the literature from different countries.

Reference	Area	Year	Depth (cm)	Pb concentration ($mg \cdot kg^{-1}$ d.w.)
[58]	China; Pearl River Estuary	2005–2006	0–10	13.4–58.9
[59]	Australia; Lake Monger (run-off from nearby freeway)	1987	0–5	192.3 ± 19.3
	Lake Wannamal (hunting area)	1987	0–5	46.3 ± 2.7
	Lake Thomson (no known source of Pb)	1987	0–5	35 ± 4.2
[60]	Australia; Lake Macquarie	N.A.	Superficial	6.7–333.3
[61]	USA; Newark Bay, NJ	N.A.	Superficial	64–2500
[62]	USA; Hackensack area, NJ	N.A.	Superficial	275 ± 138
	Hackensack area	N.A.	Superficial	25–704
[63]	Northwest Mexico Chiricahueto marsh	2002–2003	Superficial	24.1 ± 10.6
	Urias Estuary	N.A.	Superficial	17.4 ± 7.3
[64]	Norway; Pasvik River	N.A.	0–20	29.8 ± 17
			>20	7.3 ± 9.6
[65]	The Netherlands; abandoned clay-target shooting range		Upper layer	360–70,000
[66]	Portugal; Tagus Estuary	2003	0–20	199.3 ± 150.7
[67]	Belgium; Scheldt Estuary			
	Konkelschoor	N.A.	0–20	134 ± 32
	Kijkverdriet	N.A.	0–20	198 ± 25
	Doel	N.A.	0–20	37 ± 7
[26]	Italy; Diaccia Botrona clay-target shooting range	1994	Superficial	123,500
This study	Italy; Padule di Fucecchio			14–306

Note: N.A. not available.

Table 3. Background levels of lead reported in the literature (mean and/or range in $mg \cdot kg^{-1}$ d.w.).

Description	Lead concentrations	Reference
Uncontaminated soil	10–40	[68]
Average concentrations in Earth's crust	14.8	[69]
Average concentrations in Earth's crust	12.5	[70]
Background levels in sediments	21	[71]
Background levels in Earth's crust	35 (2–300)	[72]

When we compared protected areas C and D, lead concentrations in sediment were lower in area C where hunting ceased >35 years ago (La Monaca-Righetti) than in area D where hunting was banned only 12 years ago (Le Morette) (Figure 3), highlighting the fact that chemical dissolution (oxidation) of lead shot in aquatic environments continues to make lead available, spreading it through the aquatic ecosystem for decades after cessation of hunting.

3.3. *Lead shot density–lead concentration relationship*

Lead concentrations and shot density in sediment followed similar patterns, being lower in the area hunted for a short period (A) than in the area with a long hunting history (B), and being lower in the area where hunting ceased >35 years ago (C) than in the area protected only 12 years ago (D) (Figures 2 and 3).

Despite these similarities, no statistically significant correlation was found between shot density and lead concentrations in sediment. This may be due to the long time that lead shot takes to break down. Indeed, although shot density was lower in protected areas than in historical hunting areas

(Figure 2), mean lead concentrations were higher (Figure 3). Area C had a lower mean shot density but lead concentrations in sediment similar to those in area B.

For years after shot falls in wetlands, lead is slowly released into sediment and probably other environmental compartments as well. Total disintegration is estimated to take 30–300 years [12]. Because the Fucecchio wetland has a long history of hunting, the release and spread of lead have occurred over the whole area. Over the years, sediment in hunting areas has been disturbed as explained above, and protected areas have been subject to drainage and naturalistic improvements by park management. This and natural processes (mechanical action of flowing water, input of new sediment, etc.) may mix surface sediment, distributing lead and making its concentrations relatively homogeneous throughout the area, irrespective of hunting history and the shot density detected. Thus, although the ban on hunting has had a positive effect on shot density in a relatively short time, decreasing the risk of ingestion by birds, the same has not happened for lead concentrations in the same period.

Although no direct correlation was found between quantity of shot and lead concentrations in sediment, the widespread nature of contamination and the high density of shot in sediment suggest a link between lead contamination and hunting.

3.4. *Cartography*

The map of lead concentrations (Figure 4) shows highest concentrations in the central area, where the most popular past and present hunting ponds are situated, including most of the protected area Le Morette confirming the spread of lead throughout the wetland.

Concentrations show a slightly decreasing trend towards the north, where the protected area La Monaca-Righetti is situated and towards the east near one of the tributary Canal del Terzo.

Identification and mapping of sites with high levels of contamination can be an important aid for park management when environmental improvement operations (such as creation of basins and water management, remediation of contaminated sites, etc.) are contemplated.

3.5. *Organisms*

Mean lead concentrations in different tissues of crayfish (*P. clarkii*) from Fucecchio marsh is shown in Figure 5.

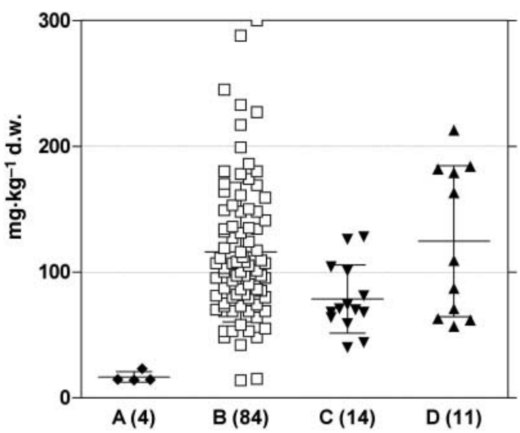

Figure 3. Lead concentrations in mg·kg^{-1} d.w. (mean ± SD) in sediments from areas with different hunting histories in the Padule di Fucecchio (number of samples in brackets): (A) hunting conducted for only 2 years; (B) hunting conducted for >50 years; (C) protected area, La Monaca-Righetti, where hunting has not been conducted for ∼35 years; (D) protected area, Le Morette, where hunting has not been conducted for ∼12 years.

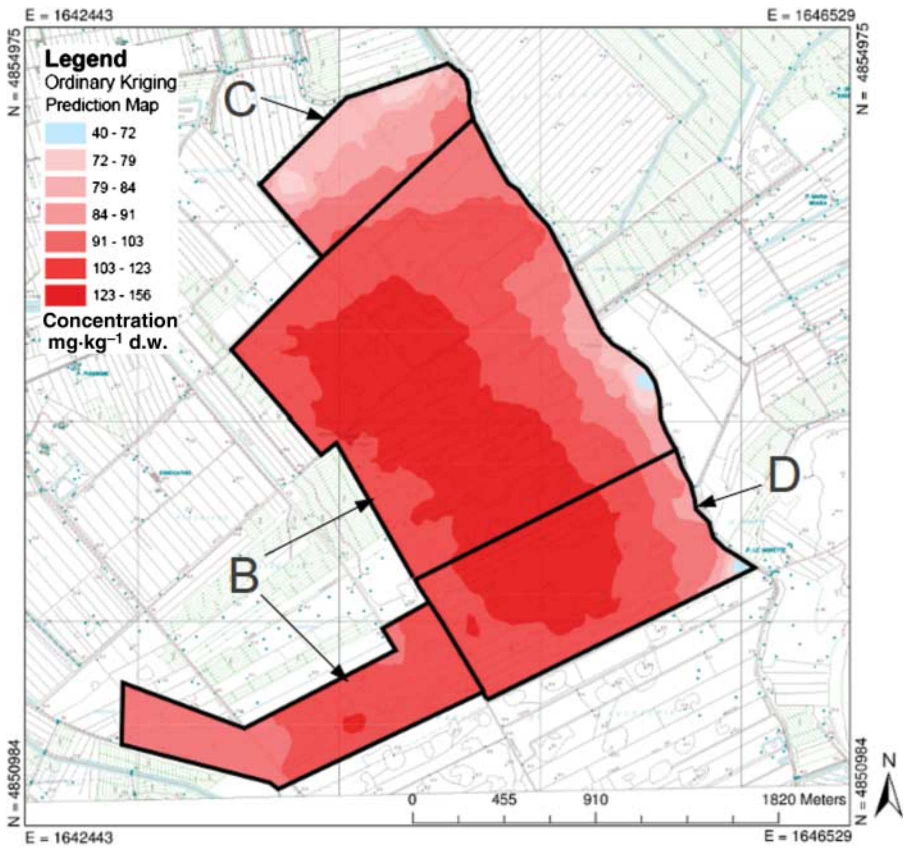

Figure 4. Map of the spatial distribution of lead concentrations in sediment of Padule di Fucecchio marsh. Areas with different hunting histories are also indicated.

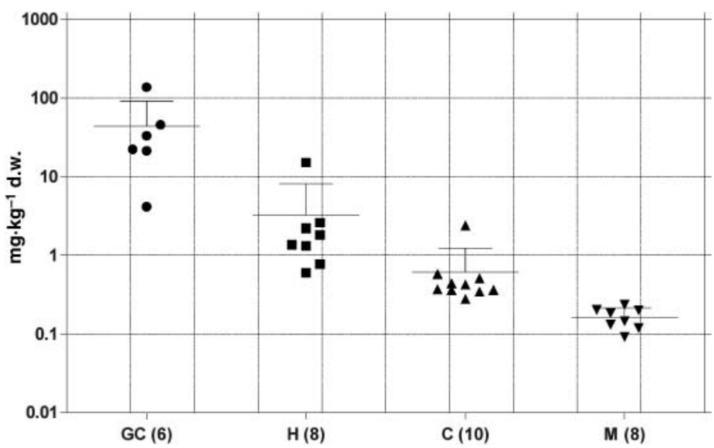

Figure 5. Mean concentrations (mg·kg^{-1} d.w. ± SD) of lead in gut contents (GC), hepatopancreas (H), carapace (C) and muscle (M) of *Procambarus clarkii* (number of samples in parentheses).

Excluding high concentrations in gut contents, which may be largely due to sediment in the digestive tract, the results show a clear accumulation of lead in the hepatopancreas (3.217 ± 4.850 mg·kg^{-1} d.w.), in line with the results of Anderson and colleagues [35]. This organ is involved in various physiological processes including secretion of gastric juices, absorption and accumulation of digested food and detoxification and accumulation of heavy metals [73]. Mean lead concentration for carapace was $0.606 \pm 0.632\,mg{\cdot}kg^{-1}$ d.w., however, high concentrations of metals, especially lead, have been reported to be due largely to absorption rather than bioaccumulation [45]. Abdominal muscle contained the lowest lead concentration of the tissues analysed ($0.164 \pm 0.049\,mg{\cdot}kg^{-1}$ d.w.), as found by other authors [35,36,38,74,75], except in crayfish from the Ebro River where muscle lead concentrations were $1.13\,mg{\cdot}kg^{-1}$ d.w. [41], one order of magnitude higher than in the present study .

On the whole, our results suggest that the population of crayfish in the Padule di Fucecchio is subject to heavy uptake of lead, as deduced from the high levels found in gut contents ($43.84 \pm 47.48\,mg{\cdot}kg^{-1}$ d.w.) and from evident accumulation in the hepatopancreas ($3.217 \pm 4.850\,mg{\cdot}kg^{-1}$ d.w.). Contamination of these organisms, is of little interest for the species itself, but having become a major dietary item of many birds [41,76,77], it can be considered a vector of contamination to higher levels of the food web.

4. Conclusions

Lead concentrations in sediment of the Padule di Fucecchio generally proved to be very high and similar to those found in other areas regarded as contaminated. Although lead concentrations and lead shot density were slightly less in one of the protected area, La Monaca-Righetti, where hunting was banned 35 years ago, contamination was widespread over the whole wetland.

No direct correlation between quantity of shot and lead concentrations in sediment was found, however, the widespread nature of contamination and the high density of shot in sediment suggest a link between lead contamination and hunting.

Lead mapping of the Fucecchio marsh indicated widespread contamination, with higher levels in the central area where hunting has been concentrated, but also in most of the other protected area, Le Morette. Thus, although the ban on hunting has had a positive effect on shot density in a relatively short time, decreasing the risk of ingestion by birds, this has not occurred for lead concentration in the same period. Besides poisoning of birds through direct ingestion of lead shot, attention should also be paid to lead in other forms distributed throughout the sediment and probably other environmental compartments.

Lead concentrations in red swamp crayfish (*P. clarkii*) from Fucecchio marsh, confirmed the utility of this species for biomonitoring studies and demonstrated heavy contamination affecting different levels of the food web.

Although the determination of lead shot available to birds may not accurately assess the magnitude of the problem, it clearly indicates the risk of plumbism to which birds frequenting the area are exposed. Data on shot densities and lead concentrations in Italian wetland sediments have hitherto been insufficient to establish the real entity of problem and formulate further corrective measures. Our data, however, confirm that plumbism is a serious problem in the Mediterranean region and is much higher than in northern Europe and North America.

This study provides evidence of the role ecotoxicology studies can play in the field of wetland conservation. Evaluation of lead contamination status in concomitance with the ban on lead shot provides an extremely useful data base for comparative purposes and long-term monitoring of enforcement of the ban. Identification and mapping of sites with high contamination can also be an important aid for park management, and environmental remediation.

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