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RESEARCH ARTICLE

Heavy metals in organs of stray dogs and cats from the city of Naples and its surroundings (Southern Italy)

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Abstract
The aim of the present study was to assess the concentrations of lead (Pb) and cadmium (Cd) in the liver and kidney from stray dogs (*Canis lupus familiaris*) and cats (*Felis catus*) found dead between 2014 and 2017 in the city of Naples and its surrounding areas. These organs from 290 dogs and 88 cats were collected after ordinary necropsy of stray animals. Heavy metal concentrations were determined by using atomic absorption spectrophotometry (AAS). Concentrations of Pb (up to 5.93 mg/kg) and Cd (ranging from 0.005 to 6.13 mg/kg) were detected in both livers and kidneys analyzed. Differences in concentration were found based on age class, gender, and kind of tissue for both elements, with a trend similar to those already reported in the literature for comparable studies from different countries. Cadmium levels in the kidney were significantly higher ($p < 0.05$) in females than those in males for both species. As regards to Pb, the highest concentrations were detected in the liver (3.45 mg/kg in dog and 5.93 in cat, respectively) followed by the renal tissue, with no significant difference depending on the animal gender. This study can be considered the first one in Italy regarding stray dogs and cats as bio-indicators of environmental contamination due to lead and cadmium, suggesting that pets could be sentinel animals to evaluate human exposure to these heavy metals.

Keywords Stray · Dog · Cat · Pb · Cd · Italy

Introduction

One of the employed systems assessing the exposure levels to environmental contaminants involves the use of so-called sentinel animals; therefore, various animal species have been considered in environmental biomonitoring studies in different habitat (Beyer et al. 2017; De Roma et al. 2017; Naccari et al. 2013; Neo and Tan 2017; Stahl 1997; Van der Schalie et al. 1999). The interest is growing in both wild and stray or domestic animals because they are continuously exposed to toxic compounds (Bilandžić et al. 2009; Naccari et al. 2013) that could bioaccumulate in several organs and tissues.

In particular, dogs and cats meet the criteria that define a sentinel species: in fact, they have adequate body dimensions to provide useful tissues for the analysis of contaminants. Then, they have comparable sensitivity towards specific pollutants to humans and are likely to have similar metabolic and clinical responses to toxicants (Neo and Tan 2017). Moreover, dogs and cats survive a relatively long time compared to other domestic and wild animals, and thus are likely to have long-term exposure to toxicants in food, water, and the environment, allowing for their bioaccumulation (Backer et al. 2001; Buck 1979; Costagliola et al. 2017; O'Brien et al. 1993; Schmidt 2009; Skibniewski et al. 2013; Swarup et al. 2000).

Within environmental contaminants, heavy metals are naturally occurring elements with multiple industrial, domestic, agricultural, medical, and technological applications that have led to widespread contamination of the environment and raised concerns about adverse effects on public and environmental health (Tchounwou et al. 2012).

The Regional Center for Veterinary Urban Hygiene (CRIUV) performs environmental biomonitoring through the

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necropsies of synanthropic animals in the Campania region (Southern Italy). Dogs and cats were used because they are domestic animals or, as in this case, stray animals that share the same habitat with humans and are unavoidably exposed to the same environmental pollutants (Backer et al. 2001; Balagangathara Thilagar et al. 2006; Bischoff et al. 2010; López-Alonso 2011; Reif 2011; Schmidt 2009). Moreover, their diet is linked to the waste of human nutrition, and they spend more time outdoors than humans, hence increasing their exposure risk (Rabinowitz et al. 2010) making them good surveillance tools.

On these bases, synanthropic animals could be good indicators of human exposure to metals; therefore, in the present study, we investigated the content of lead (Pb) and cadmium (Cd) in the liver and kidney of stray dogs and cats.

Our attention was mainly focused on Pb and Cd, as they are ubiquitous pollutants in the environment with a higher tendency towards bioaccumulation. These heavy metals cause environmental hazards and are reported to be exceptionally toxic (Maleki and Zarasvand 2008). Notably, for Pb, developmental neurotoxicity in young children and cardiovascular and nephrotoxic effects in adults have been demonstrated. For Cd, severe risks to human health were reported because of its adverse effects on kidney function and bones (Godt et al. 2006). Several observations suggest that Cd is a widespread pollutant with clear carcinogenic potential in both humans and rodents (IARC 1993, 2012) and may cause cancer in the lung, kidney, and other organs.

In this study, we report the Pb and Cd levels in the liver and kidney of stray dogs and cats from the city of Naples and its surroundings, as well as their relationship with different animal variables such as age and sex. The results of this study could provide new reference data for future investigations on these animals in their natural habitats, giving some useful indication on the environmental biomonitoring.

Moreover, in this study, we use animals already dead and not intentionally exposed; therefore, all the ethical concerns about the deliberate exposure of animals to danger, by placing them at sites of suspected contamination, have been overcome.

Material and methods

Sampling

The liver and kidney analyzed in this study were taken from stray dogs and cats found dead along the streets, usually few hours after the death, both in urban and suburban environments of the metropolitan area of Naples, a densely populated territory (with about 3117 million inhabitants) of Campania region (Fig. 1).

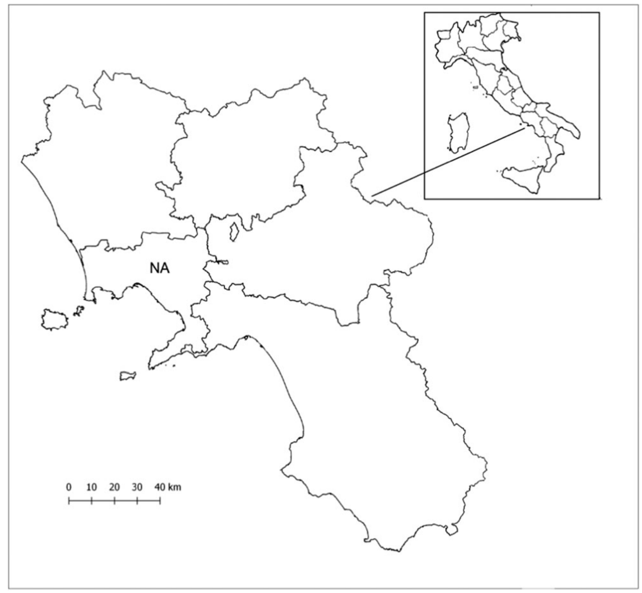

Fig. 1 Metropolitan area of Naples (NA), in Southern Italy

The animal carcasses were collected from the local veterinary authorities and subjected to necropsy in authorized environments. The organs to be examined were immediately frozen at − 20 °C and sent to the laboratory of the Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM) as soon as possible preserving the temperature. In the case of carcasses examined at IZSM, the liver and kidney were removed during necropsy and sent to the Department of Chemistry. Samples were stored at − 20 °C until chemical analysis.

Between 2014 and 2017, organs from 290 dogs (*Canis lupus familiaris*) and 88 cats (*Felis catus*) were collected; in all cases, animals were stray with no known owner. Usually, stray dogs and cats are identified by subcutaneous microchip when they are puppies, registered in a Unified Data Bank (UDB) and then re-routed according to municipal ordinances. If previously identified, all information related to a deceased animal is available on the UDB, and if not, are obtained from the clinical investigation when possible. Sex and age fields are not mandatory in the veterinarian authority reports and unfortunately, information about age and gender were not provided for all animals of the present study: within dogs, 100 were male (34%), 108 female (37%), with 82 unrecorded (28%); within cats, 38 were male (43%), 24 female (27%), with 25 undeclared (29%).

All the samples collected were analyzed for the determination of Cd and Pb, but results of undeclared specimens have not been considered in the present study. Therefore, 208 specimens (corresponding to 416 samples of liver and kidney) of stray dogs and on 62 specimens of stray cats, of different sex and aging between 3 months and 15 years, were taken into consideration in order to evaluate any correlation between the concentrations and sex or age of the animals.

Reagents and chemicals

All reagents and solvents were of analytical grade (Carlo Erba Reagents S.r.l., Milano, Italy). High-purity deionized water (resistivity, 18.2 MΩ cm) was produced in-house using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Perkin Elmer purchased monobasic ammonium phosphate and magnesium nitrate (1% Mg). Standard solutions of lead and cadmium were prepared by dilution of elemental standard solutions at 1000 mg L^{-1} (Merck, Germany) with Milli-Q water. Glassware, before use, was washed with a solution of nitric acid (10% *w*/*v*) then rinsed with Milli-Q water.

Sample preparation

Samples were thawed and homogenized, then 0.50 ± 0.01 g was weighed into a Teflon reaction vessel, added with 5.0 mL of 70% nitric acid, 2.5 mL of 30% hydrogen peroxide, and 2.5 mL of Milli-Q water. After tight closure, the vessels were placed into a microwave Milestone Ethos-One apparatus (FKV S.r.l., Torre Boldone, Italy) with the following thermal program: 5 min at 800 W, from $T = 25$ to $T = 120$ °C; 2 min to 1000 W, $T = 120$ °C; 7 min at 900 W, $T = 120$ °C with $T = 190$ °C; and 10 min at 700 W, $T = 190$ °C. After acid digestion, the vessels were cooled at room temperature, and the samples were quantitatively recovered by filtration in 50.00 mL class A volumetric flasks, then brought to 50 mL with Milli-Q water. Solutions of $(NH_4)H_2PO_4$ and $Mg(NO_3)_2$ were used as matrix modifiers during the determination of Pb and Cd.

Heavy metal analysis

A model PinAAcle 900 T atomic absorption spectrophotometer, with a transverse heated graphite furnace (GF-AAS) with Zeeman effect for electrothermal atomization and equipped with an autosampler, was used in this study (Perkin Elmer, Italy). The system was coupled to an interfaced computer running WinLab software (PerkinElmer). Hollow cathode lamps (PerkinElmer) were used as line sources for both elements. The absorption wavelengths were 247.6 nm for Pb and 228.80 nm for Cd. Argon with an internal flow of 250 mL/min was used as the inert gas.

Each sample solution was analyzed in duplicate, and the mean results were reported as milligram per kilogram fresh weight of both organs. The limit of detection (LOD) for Pb and Cd was 0.0067 and 0.0015 mg/kg respectively. The limits of quantification (LOQ) for Pb and Cd were 0.020 and 0.005 mg/kg respectively.

Statistical data analysis

All data regarding sex, age, and concentrations of Cd and Pb in the liver and kidney of stray animal specimens were statistically evaluated performing the nonparametric comparison test with the Minitab Statistical Software, by Minitab. Inc. Company. Significant differences were calculated to test the significance of variation among the samples. A p value less than 0.05 was considered to be statistically significant ($p < 0.05$), and when the measured value was below the limit of quantification (LOQ) of the test method, LOQ/2 value was used. A nonparametric study was also performed through the box-and-whisker plots that display variation in samples of a statistical population.

Results and discussion

The levels of Pb and Cd in the analyzed organs are reported in Table 1. The data show that heavy metals occur both in the liver and kidney of dogs and cats.

Within dogs, the highest Pb levels were detected in the liver (median, 0.130 mg/kg), while the higher Cd values were found out in the kidney (median, 0.212 mg/kg). Similarly in cats, the highest Cd concentrations were found out in the kidney (median, 0.144 mg/kg), while for the Pb, the two organs, kidney and liver, showed close median values at 0.102 and 0.107 mg/kg respectively.

Figures 2 and 3 show the distribution (median, first and third quartile, minimum and maximum value, and the exceeding values) of Pd and Cd concentrations in the kidney and liver for both cats and dogs, particularly distinguishing between the animal gender.

Table 1 Mean, median, and minimum and maximum concentrations (mg/kg) of lead and cadmium in kidney and liver samples of 88 stray cats and 290 dogs from Naples

	Cats		Dogs	
	Pb	Cd	Pb	Cd
Liver				
Mean	0.268	0.101	0.256	0.098
Median	0.107	0.054	0.130	0.063
Min	0.010	0.003	0.010	0.003
Max	3.450	1.590	5.930	2.200
Kidney				
Media	0.189	0.355	0.147	0.302
Median	0.102	0.144	0.081	0.212
Min	0.010	0.003	0.010	0.003
Max	1.700	6.130	2.260	3.510

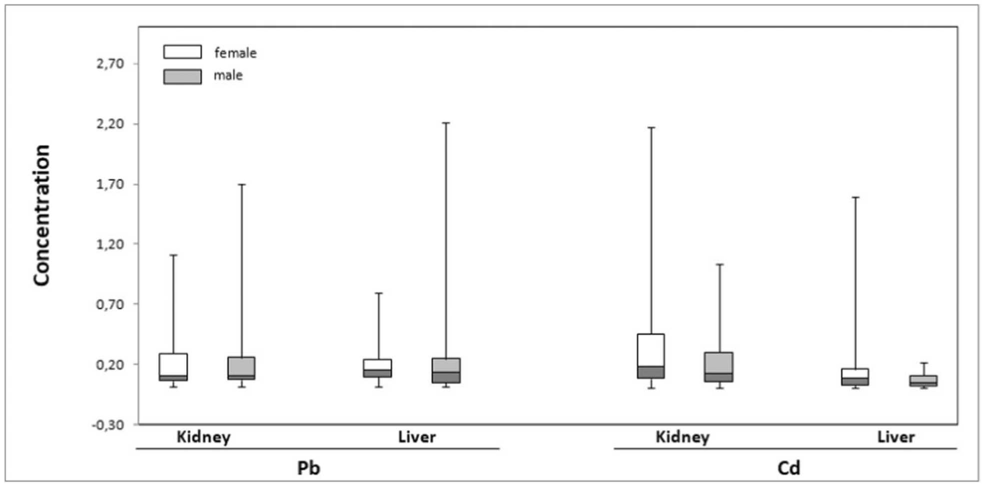

Fig. 2 Box-whisker plots showing the effects of sex on liver and kidney Pb and Cd concentrations, expressed in mg/kg, in the 62 (24 female and 38 male) cats considered in this study. For a better graphic resolution, the following outlier values have been omitted: 6.1 mg/kg of Cd in a kidney sample of female, 3.45 and 3.34 mg/kg of Pb in liver samples of female and male respectively

To the best of our knowledge, only a few data are available about Pb and Cd levels in the liver and kidney of stray dogs and cats living in urban or rural environments. Our results confirmed the presence of Cd in all tissues tested probably because all animals come into contact with contaminated food and water, or Cd in the air. Cadmium is known to accumulate quickly and once absorbed, it is rapidly cleared from the blood and concentrated in various tissues, especially in the main target tissues of the liver and kidney, where it binds to metallothionein (Satarug et al. 2003).

As shown in Figs. 2 and 3, both for cats and dogs, the highest concentrations of Cd were detected in the kidney, ranging between LOQ (0.005 mg/kg) and 3.51 mg/kg in dogs and 6.1 mg/kg in cats (reported as outliers), followed by the hepatic tissue. Cadmium renal levels are much higher than hepatic levels probably due to the high affinity of this element for the kidney, where it is directly deposited from the bloodstream and indirectly from the liver (Chiari et al. 2015).

In this context, a clear and evident gender difference between males and females has been verified (Figs. 2 and 3), which could suggest a sexual dimorphism for the storage of elements in organs. Moreover, all stray animals are sterilized after capture, either they are re-routed or inserted into kennels. Therefore, no evidence of contaminant elimination due to the pregnancy and lactation for the most female was observed.

In particular, a significant difference ($p < 0.05$) was found in the kidney, with a greater presence of this metal in female animals compared to males, similarly to what reported by López-Alonso et al. (2007a, b) in their study on dogs as indicators of metal exposure in rural and urban habitats in Spain.

As regards to Pb, absorbed both through pulmonary exposure or food contamination like Cd (Khazdair et al. 2012), the highest concentration was detected in the liver, ranging between LOQ (0.020 mg/kg) and 3.45 mg/kg for dogs and 5.93 mg/kg for cats respectively, followed by the renal tissue. The concentrations of this element in the liver and kidney of the stray dogs and cats did only slightly differ depending on the sex of the animals. In general, this element is more abundant in males, whereas it is not a statistically significant difference.

Taking into consideration the comparison between the medians of the four age groups, as shown in Tables 2 and 3, for cats and dogs respectively, a different trend for the two metals can be

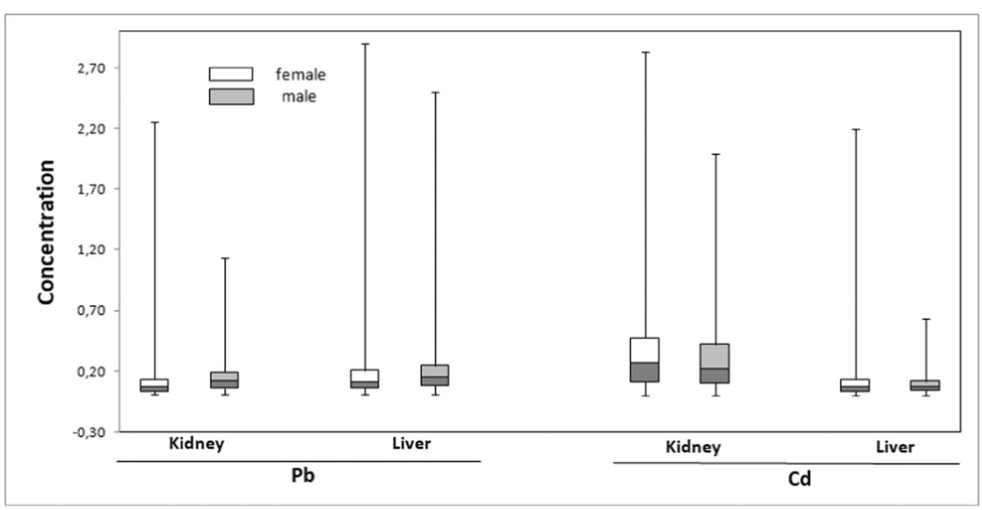

Fig. 3 Box-whisker plots showing the effects of sex on liver and kidney Pb and Cd concentrations, expressed in mg/kg, in the 208 dogs (108 female and 100 male) considered in this study. For a better graphic resolution, the following outlier values have been omitted: 3.5 mg/kg of Cd in a kidney sample of female, 4.50 and 4.1 mg/kg of Pb in liver samples of female and male respectively

Table 2 Median metal concentration (mg/kg) in liver and kidney of selected age groups of stray cats

Age groups	Pb kidney	Pb liver	Cd kidney	Cd liver
≤ 1 year ($n = 3$)	0.348	0.188	0.026	0.032
2–5 years ($n = 34$)	0.106	0.104	0.198	0.059
6–10 years ($n = 11$)	0.195	0.186	0.092	0.036
> 10 years ($n = 14$)	0.064	0.091	0.194	0.085

observed. In the present study, there is a tendency to accumulate Cd with increasing age, as reported by López-Alonso et al. (2007a, b), in the kidney, especially for dogs. Cadmium in the liver gave a similar but less evident trend.

Instead, as far as Pb is concerned, no tendency to bioaccumulate in dog's liver and kidney for different age groups was observed, as variable values were detected. These data are in agreement with the results of two studies in which the authors could not detect an age dependency for the Pb storage (López-Alonso et al. 2007a, b). The Pb measurement in the liver and kidney of cats provided similar concentrations between the two organs as reported by Paßlack et al. (2014) but unlike the latter, no age dependency for the storage of lead has been demonstrated in our study.

Compared to the values reported in literature, the levels of Pb in the organs of cats from Naples and its surroundings are slightly higher than those (0.087 mg/kg) found out in cats in Berlin (Paßlack et al. 2014), while for Cd, the values in the liver are lower (0.054 vs 0.145 mg/kg) and those in the kidney, very close (0.144 vs 0.157 mg/kg in renal cortex).

As regards to the Pb determination in organs of dogs, our results show slightly lower values than those reported for dogs in Germany (Paßlack et al. 2015) both in the liver (0.130 mg/kg vs 0.159 mg/kg) and in the kidney (0.081 mg/kg vs 0.095 mg/kg), but higher than those reported for dogs from rural and urban areas of Spain (0.060 mg/kg in the liver and 0.023 mg/kg in the kidney) (López-Alonso et al. 2007a, b).

Similar results were obtained for cadmium: the dogs from Naples showed lower Cd levels than dogs from Berlin (Paßlack et al. 2015) but higher than dogs from Galicia (López-Alonso et al. 2007a, b).

The average values of heavy metals measured in dogs of this study are lower than those considered high in canine species, both for liver (Cd, 1.0–7.0 mg/kg; Pb, 3.6–5.0 mg/kg) and for kidney (Cd, 4.0–17.0; Pb, 5.0–10.0 mg/kg), as reported in the literature (Puls 1994). These Pb and Cd concentrations found out in samples of the liver and kidney cannot cause acute toxicity, but could be indicative of chronic exposure to the two heavy metals (Serpe et al. 2012).

Table 3 Median metal concentration (mg/kg) in liver and kidney of selected age groups of stray dogs

Age groups	Pb kidney	Pb liver	Cd kidney	Cd liver
≤ 1 years ($n = 23$)	0.109	0.092	0.114	0.056
2–5 years ($n = 34$)	0.096	0.168	0.201	0.054
6–10 years ($n = 90$)	0.104	0.130	0.207	0.058
> 10 years ($n = 61$)	0.078	0.110	0.385	0.096

In general, literature data about toxic metal levels in dogs are scarce; therefore, it is quite difficult to compare the obtained results and evaluate the extent of contamination (Swarup et al. 2000). However, the analysis of our study allows for some observations and considerations.

Compared to the study carried out on dogs living in an area located in Spain (López-Alonso et al. 2007a, b), the Cd values determined in this work were of the same order of magnitude, both in the kidney and in the liver, while considerable differences were observed for the levels of Pb in the liver, about fivefold higher in the Naples area (0.057 vs 0.256 mg/kg), and in the kidney with an average value about tenfold higher than the Spanish data (0.023 vs 0.147 mg/kg).

With regard to the results of heavy metals' determination in cats, to the best of our knowledge, our study reports for the first time the Pb and Cd concentrations in organs of stray cats. The results of this study show that the highest values were observed for Cd in the kidney and Pb in the liver. No significant differences for the gender were observed for both heavy metals, and no apparent trend to accumulate Cd in the kidney was observed as for dogs.

The dogs and cats studied were all stray animals and this suggests two routes of exposure, both environment and food. In particular, for Pb, the correlation between its concentration in the liver and the diet was verified as well as the fact that dogs fed with commercial feeds have higher Pb levels in the liver compared to those fed with homemade food (López-Alonso et al. 2007a, b). Since stray dogs usually feed on waste that is likely scraps from food prepared at home, metal exposure in dogs could be considered similar to that in humans.

In addition to the nutritional aspects, the contribution of contaminants from water and air should not be underestimated considering the habitat of these animals. Both of these compartments are related to geochemical levels and anthropogenic emissions in the environment where animals live, so if their contribution to total exposure is significant, this can lead to essential differences in bioaccumulation.

Analyzing the data obtained in this study, the Cd and Pb presence in the organs of some animals suggests considering also the environmental compartments as a source of contamination. Dust and soil ingested by animals during their grooming activity may involve the addition of a further source of contamination, especially for Cd, usually present in soils with a high degree of pollution.

Although this study has reached its aim to be a first preliminary screening for Cd and Pb in stray animals from Naples area, there were some unavoidable limitations. The research as a preliminary screening analyzed only Cd and Pb on the whole organs (liver and kidney) as required by veterinarian authority for this monitoring plan in such matrices. Therefore, a large panel of elements could be tested in the future evolution of the study.

In conclusion, exposure to these environmental contaminants similar to that observed for dogs and cats can be assumed for man and, even if the determined levels are not such as to cause acute or severe toxic effects, they may over time cause bioaccumulation, and hence, chronic toxic effects cannot be excluded. Therefore, human biomonitoring studies are useful and necessary in order to know the body levels of trace elements, especially those metals recognized as toxic to the body.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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