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Abstract	The idea that animals may be used as sentinels of environmental hazards pending over humans and the associated public health implications is not a new one. Nowadays pets are being used as bioindicators for the effects of environmental contaminants in human populations. This is of paramount importance due to the large increase in the worldwide distribution of synthetic chemicals, particularly in the built environment. Companion animals share the habitat with humans being simultaneously exposed to and suffering the same disease spectrum as their masters. Moreover, their shorter latency periods (due to briefer lifespans) enable them to act as early warning systems, allowing timely public health interventions. The rise on ethical constraints on the use of animals and, consequently, on the sampling they can be subjected to has led to the preferential use of noninvasive matrices, and in this case we are looking into hair. This chapter focuses in three non-essential metals: mercury, lead, and cadmium, due to their ubiquitous presence in the built environment and their ability of affecting the mammal nervous system. There is a fairly short amount of studies reporting the concentrations of these metals in pets' hair, particularly for cats. These studies are characterized, and the metal concentrations corresponding to different parameters (e.g., age, sex, diet, rearing) are described in order to provide the reader with a general vision on the use of this noninvasive matrix on the studies conducted since the last two decades of the twentieth century.
Keywords (separated by " - ")	Surrogacy - Early warning - Latency - Cadmium - Lead - Mercury

Chapter 5
Pets as Sentinels of Human Exposure to Neurotoxic Metals

M. Ramiro Pastorinho and Ana C. A. Sousa

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5.1 Introduction

The idea that animals may be used as sentinels of environmental hazards is pending over humans, and the associated public health implications are not new. The archetypal concept of the *canary in the coal mine* re-claims a new life in the twenty-first century. Miners used canaries in the early decades of the twentieth century to detect high levels of carbon monoxide and other toxicants in mine shafts (Pollock 2016). Nowadays, pets are being used as assessment and prediction tools (bioindicators) for the effects of environmental contaminants in human populations. In an age where increases in synthetic chemicals production and diversification are outpacing "classical" agents of global change (e.g., atmospheric CO_2 concentrations, nutrient pollution, habitat destruction, biodiversity loss) (Bernhardt et al. 2017), the need for sensitive indicators of the presence, and consequently, of the potential (and actual) effects of these chemicals is paramount. The rise on the awareness of the sentient capabilities of other species outside man compelled regulator bodies to impose ethical constraints on the use of animals and consequently on the sampling they can be subjected to. In this context, noninvasive matrices have become preferential targets for the evaluation of tissue contamination on animals (Sousa et al. 2013).

Companion animals share the habitat and are consequently exposed to similar agents as their human counterparts. A particular case is that of children that can be subjected to the same exposure sources (e.g., house dust). The spectrum of disease suffered by pets is similar to that of humans, enabling them as indicators of environmental hazards. Moreover, since they possess shorter latency periods (as they have shorter average lifespans), they can provide an early warning system, enabling timely public health interventions (Wallis et al. 2018). Also, the use of pet sentinels as models for epidemiologic studies of human diseases and environmental exposures has been long proven to present advantages over classical laboratory animal models (Bukowski and Wartenberg 1997). Moreover, the depth of the interface between humans, animals, and the environment is being made more apparent due to the global change our planet is undergoing.

However, these impressive capabilities were not always duly regarded. The notorious episode of the outbreak of neurologic manifestations in the population of Minamata Bay in the 1950s caused by the consumption of fish contaminated by methylmercury was preceded by neurobehavioral disorders in the cat population of the same area. The ataxic, "dancing," cats were a grossly disregarded warning sign, from which resulted a large loss of life (Reif 2011; Tsuchiya 1992).

The currently recognized connection between metals, neurodegeneration, and pets as early warning systems and predictors of human health risk will be the focus of this chapter. The use of a noninvasive matrix – hair – will be highlighted and concentrations found in studies conducted since the last two decades of the twentieth century reported.

Neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease) represent a major threat to human health, with nearly 50 million people across the world suffering from dementia, with this number set to reach

150 million by 2050 (WHO 2019). The increasing proportion of elderly citizens is partially to blame. Conveniently, this reality finds a parallel in pets since their lifespan is also increasing (Wallis et al. 2018). The One Health concept (van Helden et al. 2013) by proposing coordinated efforts between human epidemiology, veterinary epidemiology, and environmental toxicology presents an optimal framework for approaching the current epidemic of non-communicable diseases (of which neurodegenerative disorders are an integral part) and their association with environmental contaminants.

5.1.1 The Importance of Using Bioindicators

The term "bioindicators" has been receiving wider acceptance in recent years, despite its definition being somewhat variable. Here, we define bioindicators as species or communities that are used to identify the influence of an environmental chemical, environmental changes, or pressure, by demonstrating a departure from a normal status. The most common origins of these disrupting elements are anthropogenic activities and the destruction of the biotic system (Martin and Coughtrey 1982). In ecosystems at large, multitudes of bioindicators are used to determine air, soil, and water quality and how they reflect in animal and plant health. This abundance of bioindicators does not have correspondence into heavily humanized areas (e.g., industrial, rural, urban). Far fewer bioindicators used to monitor these ecosystems can be found in the literature. Despite encompassing taxa across different levels of organization, such as lichens (Cicek et al. 2007), plants (Minganti and Drava 2018), soil invertebrates (Santorufo et al. 2012), and bats (Russo and Ancillotto 2015), it becomes evident that they are only remotely related to humans themselves (both in terms of phylogeny and daily habits) so as to become nearly unrepresentative. To circumvent this situation, companion animals can provide a very important contribution to monitor the most common human habitats.

5.1.2 Companion Animals as Bioindicators/Sentinels of Metal Exposure

Humans and animals share the same ecosystem and, in the case of companion animals, the same home environment, and in a large number of times, they share the same food items or entire diets. Pets may therefore serve as sentinels and/or early warning systems for human health hazards, since, as a norm, they are more sensitive to the offending agents, come in closer contact with the hazard (cats groom frequently, dogs crawl and eat food out of the floor), or have shorter latency periods for symptoms and/or disease. Examples include lymphoma in domestic dogs exposed to phenoxy herbicides (Hayes et al. 1991), lung cancer from passive smoking exposure in dogs (Reif et al. 1992), and the mirroring of the human obesity epidemic by pet cats and dogs (German 2006). But probably the better-known example of the forecasting ability of pets regarding the human health was the Minamata incident. A factory (owned by the Chisso Corporation) located in Minamata Bay (Japan) started production of acetaldehyde in 1932. The chemical reaction used to produce the acetaldehyde used mercury sulfate as a catalyst, generating methylmercury (a powerful neurotoxic) as a side product that was discarded into the bay until 1968, contaminating the ecosystem, including the fish consumed by humans. The first patient, reported on April 21, 1956, was a five-year-old girl presenting walking and speaking difficulties and convulsions. Many more would follow with a death rate of 37% by the end of the same year. However, from around 1950 onward, far before the appearance of similar effects in humans, cats had been seen suffering convulsions, "go mad," and die, leading the locals to call the mysterious disease the "cat dancing disease." This exhibition of neurotoxic effects, brought upon by the consumption of very same contaminated fish captured from the bay, occurred years before the first human reported case. If properly contextualized and identified, this could have saved the life of 900 individuals and prevented the effects of poisoning in 2300 others who were left with lifelong sequels (Tsuchiya 1992; Aronson 2005) (Fig. 5.1). AU3

Despite their potential, household pets, mostly cats and dogs, have been used as biomonitors in a limited number of studies, particularly in the context of the built environment (e.g., Hayashi et al. 1981; Doi et al. 1986; Berny et al. 1995; Sakai et al. 1995; Dunlap et al. 2007; Atanaskova et al. 2011; Rodriguez Castro et al.

Fig. 5.1 Indoor cats share the same environment as their human counterparts and thus are exposed to the same indoor contaminants. In the picture, Maria Pia, the cat, sleeps in the bed of her guardians. (Picture by A.C. Sousa)

2013; Sousa et al. 2013; Bischoff et al. 2010; Lanocha et al. 2012; López-Alonso et al. 2007; Park et al. 2005a, b; Tomza-Marciniak et al. 2012; Zaccaroni et al. 2014). Besides superimposition of exposure pathways, the use of pets to assess human health impacts has the added advantage of possessing fewer ethical issues associated with obtaining samples, particularly when compared to the case of young children and infants (Needham and Sexton 2000) (Fig. 5.2).

As early as the 1990s, Berny et al. (1995) demonstrated that dogs and cats represented reliable surrogates to assess lead exposure in humans. These authors reported that juvenile dogs recorded lead poisoning clinical symptoms ahead of young children and infants. This suggests the potential use of domestic dogs as surrogates for lead exposure in children. This study also described a strong correlation between blood lead concentrations (BLC) in indoor pets and younger children and that the presence of one pet with a high BLC in a house increased the likelihood of finding one person in the same house with a BLC > 10 μg/dl was significantly increased. Surprisingly enough, the study suggested that despite living in an area of heavy lead soil contamination, due to the vicinity of a closed lead smelter, the subjects investigated (pets, or their owners) did not show associations with high blood lead concentrations. This led the authors to focus in indoor sources and concluding that, given the same lead sources (e.g., dust and paint), domestic pets would register higher blood lead concentrations than children.

Subsequent studies came to confirm these seminal discoveries, with domestic animals being considered as good indicators of human metal exposure since they live in the same environment as their owners, being exposed, at least in part, to the same sources. Yet, despite such similarities between humans and their pets, some

Fig. 5.2 Barney, the dog, in the living room. Dogs by sharing the same environment as their human counterparts are exposed to the same indoor contaminants. (Picture by R. Teles)

factors may differ. This preoccupation regarding confounding factors led several researchers to investigate specific pet traits on the bioaccumulation of metals. The effect of habitat, food, and sex (rural vs urban areas; commercial, homemade, mixed feeds; male vs female) in metal bioaccumulation (including arsenic, cadmium, mercury, and lead) was investigated by López-Alonso et al. (2007) in the liver and kidneys (main organs for metal accumulation) of pet dogs. The study showed that habitat had no significant effect on the levels of three of the studied metal(loid)s (Pb, As, and Cd) and that overall levels were low. However, marked differences were found when comparing kidney tissue Hg concentration, with urban dogs showing on average three times the concentration of rural dogs, this being attributed to the higher Hg urban environment concentrations due to atmospheric deposition. Commercial diets caused significantly higher liver lead levels (but not the other metals) as opposed to dogs fed homemade or mixed feeds. Finally, females had statistically significant higher kidney levels than males. Such results reinforce the need to take into account potential confounding factors when using pets as biosentinels.

5.2 Metals, the Nervous System, and Neurodegeneration

The nervous system and adjacent structures constitute a highly complex communication network enabling organisms to maintain homeostasis. It consists of sensory components detecting stimulus, pathways that conduct and process the collected information, and effector components that produce a reaction. In its essence, the mechanism is similar in all species. However, morphology and complexity have changed according to levels of organization of each species (Finsterer et al. 2014).

Neurodegeneration represents the malfunction or overall failure of one or all of the components in the nervous system. Presently, neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease) represent a major threat to human health, with as many as 50 million people across the world suffering from dementia, an umbrella term for a series of neurodegenerative conditions that cause memory loss, with the figure set to triple by 2050 (WHO 2019). This increase is, in part, connected with the increasing longevity of humans, which will lead, by 2050, to a proportion of people above 60 years of 22% of the entire world population (WHO 2013). Simultaneously, demographic studies demonstrate that life expectancy of pet dogs and cats is also increasing, with a census conducted in the US indicating that an increase of 15% in the number of cats over 10 years and 6% for dogs over 6 years occurred in the last two decades (AVMA 2012).

Neurodegenerative processes have been observed in human, canine, and feline brains, including the progressive accumulation of β-amyloid (Aβ) as well as Tau aggregates, two signature hallmarks of neurodegeneration, and dementia progression, namely, Alzheimer's disease (AD) (Head et al. 2005; Ambrosini et al. 2019).

A strong correlation has been shown by epidemiological and clinical studies between aberrant metal exposure and a number of neurological diseases, including AD, amyotrophic lateral sclerosis, autism spectrum disorders, Guillain–Barré disease, Gulf War syndrome, Huntington's disease, multiple sclerosis, Parkinson's disease (PD), and Wilson's disease (Chen et al. 2016).

5.2.1 Metals with Neurodegenerative Potential

Metals are naturally present in the environment being released from natural (volcanic activity, erosion of ore-bearing rocks) and anthropogenic sources (burning fossil fuels; mining and processing of metal ores; mechanical, chemical, and automotive industries; transport; and agriculture). Since the industrial revolution that occurred in the XII century, increasing amounts of metals started to be introduced into the natural cycles, registering a sharp increase after World War II (Nriagu 1988). In the present, the anthropogenic mobilization of metals (as compared to natural mobilization) has increased the magnitude of natural metal cycles, sometimes merely by a fraction, but in other cases by factors of over 100. This means that for these metals the forcing of their cycles is not natural but driven by man (UNEP 2013).

From a living organism's perspective, metals are divided into two groups: essential (being part of structural proteins, enzymes, hormones) and non-essential (with no biological function) (Ferrari 2012). All metals entering an organism, despite being essential or non-essential, can exert toxicity after passing specific thresholds, causing disorders at molecular, cellular, tissue, and organ levels, which can lead to illness and death. As can be easily anticipated, this is particularly true for non-essential metals which can have near-zero thresholds. There is a long list of metals with neurodegenerative potential that include essential and non-essential metals and metalloids (e.g., Cu, Fe, Mn, Zn, Al, As, Cd, Pb, Hg, Tl).

This chapter is focusing on three non-essential metals: mercury (Hg), lead (Pb), and cadmium (Cd), due to the frequency of their study and the long-established fact that they possess the ability of affecting mammals (Keil et al. 2011). The major exposure routes for warm-blooded vertebrates to these metals are via food and inhalation. However, the latter is relevant solely at areas with high levels of air pollution. A third, minor, route is dermal contact, being significant only in very specific circumstances (Tchounwou et al. 2012). Once in contact with the gastrointestinal lining, the absorption of the three metals is residual if they are presented in their inorganic forms (below 3% to a maximum of 20%). However, the most common form of organic mercury (methylmercury – MeHg) can be assimilated on upward of 90%. These variations depend not only on the speciation state of the metal, which influences its bioavailability, but also on individual characteristics and physiological parameters of the exposed organism, such as fasting status, presence of competing elements, sex, and age (Keil et al. 2011).

Of these metals, only Hg is object to biomagnification (the presence of increased amounts of a contaminant in the organisms belonging to the highest levels of a trophic chain), being a good example the high concentrations attained by predatory fish, the main source of exposure of piscivorous mammals (Wolfe and Norman 1998; Clarkson and Magos 2006). The other two metals, despite not being object of biomagnification, are (together with mercury) bioaccumulated in the tissues of vertebrates, being the brain the most vulnerable to Hg and Pb, since they can penetrate the brain–blood barrier (as well as the placental barrier, causing teratogenic effects in the developing fetus) (Clarkson and Magos 2006; Caserta et al. 2013).

The effects of Hg in the brain include visual, cognitive, and neurobehavioral deficits, linked to structural degeneration of the organ, whereas Pb causes its weight reduction; lack of coordination; impaired motor skills, visual discrimination, and learning; convulsions; abnormal social behavior; and increased tendency for aggression (Tchounwou et al. 2012). Despite being mostly recognized as a carcinogenic, both in humans and animals, cadmium can also cause olfactory dysfunction, slowing of vasomotor functioning, learning disabilities, and behavioral disturbances due to its effects upon the nervous system (Minami et al. 2001).

The existence of solid evidence of the negative effects of mercury, lead, and cadmium on the human brain (leading to neurologic dysfunction), in parallel with the growing amounts of metals in circulation in the environment creating added opportunities for exposure, compel us to multiply the amount of studies reporting the levels of these metals in humans' brain tissue establishing correlations with the prevalence and incidence of neurodegenerative diseases. The obstacle here is quite evident: the difficulty in obtaining brain tissue samples, particularly in the numbers necessary to generate robust epidemiological studies. Based on the information provided so far, one could argue that if pets share the same habitat (being subjected to the same type of metal exposure), suffer the same type of damage to their nervous system, and can act as early warning systems, then pets' brains should be harvested in order to achieve that objective. Putting aside the doubtful willingness of owners to relinquish part of their pet's central nervous system, ethical constrains apply to that endeavor. As such, these obstacles need to be circumvented by using easily obtainable (i.e., noninvasive) but representative surrogate pet samples.

5.3 Hair as a Noninvasive Indicator

Blood, urine, liver, and kidney samples have normally been used for assessing levels of metals in the human body. In a much smaller measure, hair has also been used for this purpose (Matsubara and Machida 1985; Nowak and Chmielnicka 2000; Mikulewicz et al. 2013; Pozebon et al. 2017). Usually cited advantages of using hair as an indicator of metal contamination are the ease of sampling and storage (Wołowiec et al. 2013) and the fact that hair, being a concentrator tissue, can contain higher concentrations of metals when compared with blood and urine (Mikulewicz et al. 2013; Wołowiec et al. 2013).

Similarly to humans, levels of metal accumulation in animals can be determined by analyzing samples that include blood, vital organs (e.g., kidneys or liver), bones, and hair. Animal hair can potentially be a better biomonitoring tool for metal assessment (Hayashi et al. 1981; Doi et al. 1986; Sakai et al. 1995; Dwivedi et al. 2001; Dunlap et al. 2007; Vázquez et al. 2013) due to the more complex patterns of exposure to contaminated items. No permanent damage is caused to the animal during and after sampling, and it can be used as a surrogate method for determining the bioavailability of metals, can reflect long-term accumulation and concentration, and can serve as an indicator of exposure (Merian 1991; Rashed and Soltan 2005). As a consequence of growing usage in environmental, ecological, hygienic, and clinical studies since the 1980s, dog hair has become one of the most reliable bioindicators of metal concentrations while, on a reverse trend, cat's hair being sparsely used.

5.3.1 Limitations of Hair Analysis

The use of hair to report contamination exposure and risk still has some detractors, not being universally accepted. Early criticism emerged from the validity of reference ranges depending on the analytical methods used, as well as sampling, sensitivity, accuracy, and precision (Rodushkin and Axelsson 2000; Druyan et al. 1998). Additionally, various sources of error could occur during the various steps of hair analysis (Schramm 1997). However, many of these criticisms have been voided by the evolution of techniques and equipment, the establishment of standard operating procedures, and the production of certified reference materials (Yoshinaga et al. 1997). Still, there is room for error during collection, processing, and analysis of the samples. Table 5.1 summarizes potential sources of error.

Table 5.1 Sources of error in hair analysis

Step	Sources of error
Sampling and storage	No unambiguous identification of the individual Insufficient sample amount and order of hair tuft Inadequate labelling, causing mix-ups with other samples Danger of contamination and degradation
Decontamination	Choice of wrong solvent or solvent sequence No analysis of the wash solution
Extraction	Inappropriate choice of extraction or digestion method Incorrect time and temperature of extraction Decomposition of the compounds High levels of impurities
Analysis	Insufficient specificity, sensitivity, and accuracy Loss of substance in clean-up False-positive or false-negative results

Adapted from Schramm (2008)

5.4 Levels of Metals with Neurodegenerative Potential in Pets' Hair

There are a fairly limited number of studies using cat and dog hair as a matrix of metals bioaccumulation and logically an even lower reporting the levels of metals with neurodegenerative potential. Among these there are great variation on the type of information provided, the locations of the studies, the number of animals involved (cats, min 15- max 44; Dogs, min 8- max 204), the presence/absence of discrimination in terms of age or sex being present, and the number of metals analyzed (rarely all three metals – Cd, Pb, and Hg – are reported on the same study).

Other aspect making this description very hard is the unevenness in the way data is presented. Frequently, values in tables are solely presented as an average without standard deviation or standard error, or results are presented plainly on graphs from which values have to be extrapolated. This last fact excluded several studies from this chapter, as it was virtually impossible to read the data with a minimum of desirable accuracy.

Without the intention of being exhaustive (due to the circumstances described above, and because this chapter is not intended as a systematic review), in the following subchapters, we will present studies that determined the concentrations of Cd, Pb, and Hg in this matrix, by pet. The original intention was to solely describe the levels of the metals in hair of healthy dogs (used as reference or specifically selected to act as control) so as to provide the reader with a set of background levels for metals with neurodegenerative potential. However, the sum of all cited constraints together with this intention dramatically reduced the amount of information. This led to the inclusion, when available, of levels in the hair of cats and dogs with specific illnesses in order to complement the information on healthy animals. Hair mercury level is often not correlated with blood mercury concentration or symptoms of mercury toxicity, and reports of hair contamination by exogenous mercury are not uncommon (Nuttall 2006).

The establishment of baseline levels on the hair of healthy animals is a difficult endeavor, due to the lack of information. Additionally, the baseline itself will vary with food (commercial, wet or dry, homemade), breed, sex, age, rearing (outdoors, indoors, a mix of both), and environmental variables (e.g., temperature, which will influence metabolic rates). Normal reported levels (NRL) determined by the Committee on Minerals and Toxic Substances in Diets and Water for Animals from the US Board on Agriculture and Natural Resources will be provided when available. As additional information, ballpark non-peer-reviewed estimates can be mentioned and can indicate population averages at 0.041 ppm for cadmium, 1.3 ppm for lead, and 0.27 ppm for mercury. These values are reproduced here strictly for reference.

5.4.1 Cats

The number of studies using cat's fur in order to describe the contents in terms of neurodegenerative metals is very restricted. After going through the available literature, five studies can be reported and are summarized in Table 5.2.

These are studies that in some cases additionally reported levels of other metals and/or other species. However, since those are not the object of this chapter, they will not be described here.

Badea et al. (2016) aimed to determine the levels of Cd and Pb in the coat of 15 cats (six clinically healthy and nine suffering from renal failure) in Romania. Rzymski et al. (2015) investigated the contents of Cd and Pb in hair of 18 free-ranging and 36 household (14 outgoing and 22 not outgoing) cats from Poland. Concentration values also had to be extrapolated from graphs in this study. Skibniewski et al. (2013) determined the lead content in 10 domestic and 10 urban feral (stray) cats from the Warsaw region. All animals were mature and disease-free cats. Doi et al. (1986) collected fur from domestic cats in Tokyo, Norway, and the Philippines, determining the concentrations of mercury. The results were presented solely as graphs, so we had to visually extract data here presented directly from those reproduced in the paper. The number of animals tested was not indicated. Hair mercury concentrations were also measured by Sakai et al. (1995) in 41 cats from the Kanagawa, Saitama, and Tokyo prefectures (Japan).

The concentrations obtained in each paper are presented by metal in the following sections. The concentration values are reported as averages (due to the inconsistent reporting) in parts per million (ppm), due to the wide variation of units used by the different authors.

5.4.1.1 Cadmium

Two papers reported cadmium values in cats' coats: Badea et al. (2016) and Rzymski et al. (2015). Clinically healthy cats (in particular males which present levels over eight times higher than females) in the study performed by Badea et al. (2016) registered higher cadmium levels when compared with those suffering from renal failure, which were comparatively the same regardless of age. The authors argue that this is because cats suffering renal failure have a reabsorption inability, which will

Table 5.2 List of studies reporting levels of metals with neurodegenerative potential in cats' hair

Ref.	1st author	Year	Profile	Location	Metal	*N*
1	Badea	2016	Pet cats	Romania	Cd, Pb	15
2	Doi	1986	Pet cats	Japan, Philippines, Norway	Hg	nd
3	Rzymski	2015	Free and pet cats	Poland	Cd, Pb	44
4	Sakai	1995	Pet cats	Japan	Hg	41
5	Skibniewski	2013	Pet cats	Poland	Pb	20

impoverish the hair matrix in terms of metals. The results obtained by Rzymski et al. (2015) show a clear influence from the inhabited environment, with free-ranging animals registering lower cadmium levels as compared to animals totally or partially living indoors. This data illustrates the already mentioned difficulty in establishing a baseline for the values of metals in hair. Even more "traditional" matrices like blood or urine values for small animals are poorly defined, with an indication that blood levels ≥100 μg/dL reflect acute exposure, whereas the presence of cadmium in urine indicates chronic exposure (Osweiler et al. 2011) (Fig. 5.3).

5.4.1.2 Lead

Lead toxicity is not well defined in cats with toxicity set at 1000 ppm in diet or 3 mg/kg (Osweiler et al. 2011). Three papers reported lead values in cats' coats: Badea et al. (2016), Rzymski et al. (2015), and Skibniewski et al. (2013). The results obtained for cadmium by Badea et al. (2016), in which males exhibited much higher levels than females, are not replicated for lead, despite clinically healthy males registering slightly higher levels of lead among all sampled animals (Badea et al. 2016). In the study by Rzymski et al. (2015), house cats, once again, registered the highest levels of metal (lead in this case), but, and contrarily to what was noticed for cadmium, the lowest levels were registered in outgoing pet cats. In the study by Skibniewski et al. (2013), feral females registered the highest concentrations of lead for the Warsaw area, with household cats registering, on average, a concentration three times lower (Fig. 5.4).

5.4.1.3 Mercury

Cats are recognized as highly susceptible to mercury. Blood concentrations of >6.0 ppm and urine >1.5 ppm illustrate acute to subacute exposure. Hair concentrations >45 ppm are proof of chronic exposure (Osweiler et al. 2011).

Two papers reported mercury values in cats' coats: Doi et al. (1986) and Sakai et al. (1995). Japanese cats inhabiting Tokyo in the 1980s have, according to Doi et al. (1986), the highest mercury fur contents, followed by Norwegian and Filipino cats. Sakai et al. (1995) resampled Tokyo cats nearly a decade later finding concentrations a full order of magnitude lower and no differences between males and females (Fig. 5.5).

5.4.2 Dogs

The number of studies analyzing dog's fur for neurodegenerative metals is larger than that for cats, but still very limited. The same constraints applied, and after going through the available literature, 12 studies are here reported and summarized in Table 5.3.

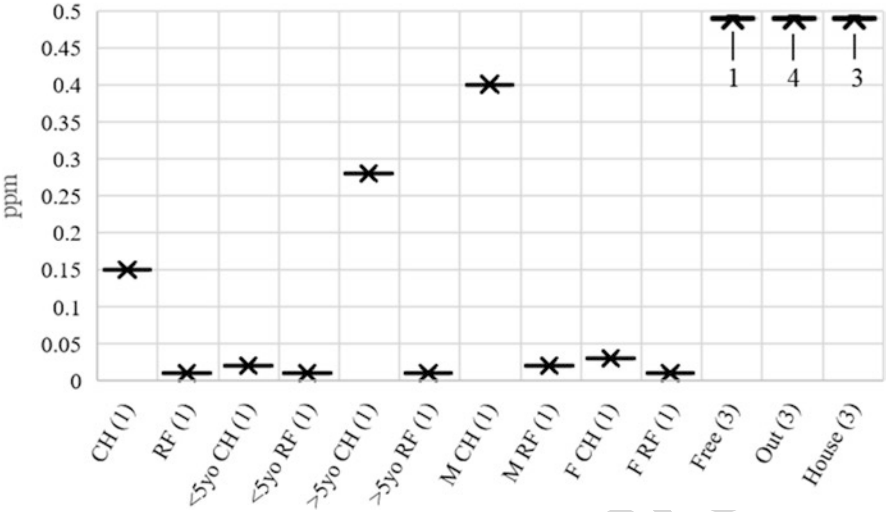

Fig. 5.3 Average cadmium concentrations in cats' hair as reported by (1) Badea et al. (2016) and (3) Rzymski et al. (2015). CH clinically healthy, RF renal failure, <5yo below 5 years of age, >5yo above 5 years of age, M male, F female, Free free-ranging, Out household outgoing, House household not outgoing. (Values from reference 3 were visually extracted from graphs reproduced in the paper)

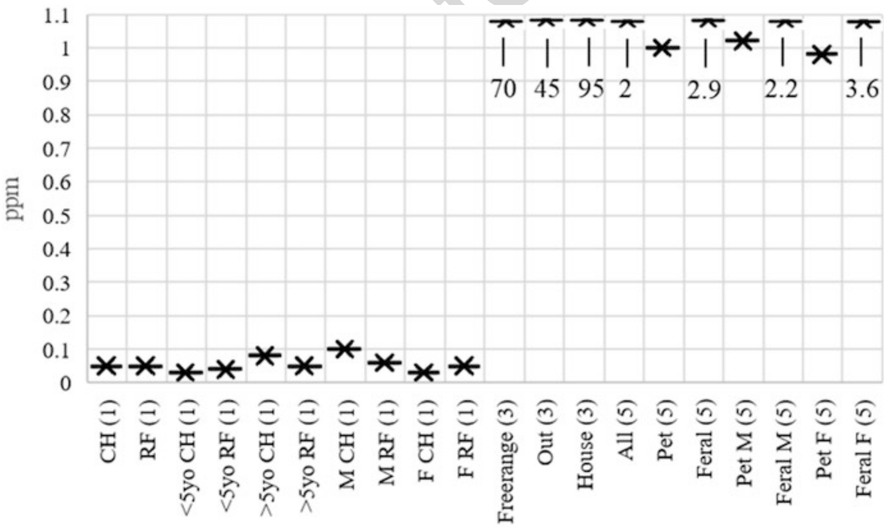

Fig. 5.4 Average lead concentrations in cats' hair as reported by (1) Badea et al. (2016), (3) Rzymski et al. (2015), and (5) Skibniewski et al. (2013). CH clinically healthy, R renal failure, <5yo below 5 years of age, >5yo above 5 years of age, M male, F female, Free-ranging stray cats, Out household out-going, House household not outgoing, All household + stray, Pet household, Feral stray. (Values from reference 3 were visually extracted from graphs reproduced in the paper)

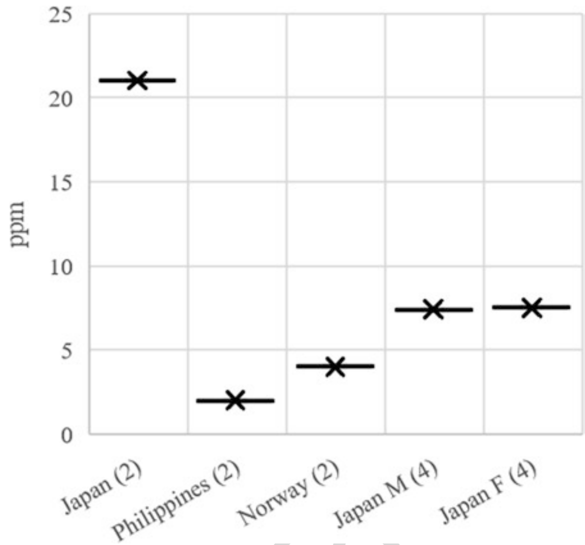

Fig. 5.5 Average mercury concentrations in cats' hair as reported by (2) Doi et al. (1986) (cats from Japan, the Philippines, and Norway) and (4) Sakai et al. (1995) (cats from Japan). M male, F female. (Values from reference 2 were visually extracted from graphs reproduced in the paper)

Table 5.3 List of studies reporting levels of metals with neurodegenerative potential in dogs' hair

Ref.	1st author	Year	Profile	Location	Metal	*N*
1	Atanaskova	2011	Urban dogs	Rep. Macedonia	Cd, Pb	35
2	Badea	2018	Pet dogs (female)	Romania	Cd, Pb	30
3	Doi	1986	Stray dogs	Japan, Norway, Philippines	Hg	nd
4	Dunlap	2007	Sled dogs	USA (Alaska)	Hg	97
5	Hansen	1995	Sled dogs	Greenland	Hg	10
6	Kozak	2002	Pet dogs	Slovakia	Cd, Pb	98
7	Kral	2015	Pet dogs	Czech Republic	Hg	131
8	Lieske	2011	Sled dogs	USA (Alaska)	Hg	8
9	Nikolovski	2011	Pet dog	Rep. Macedonia	Cd, Pb	95
10	Park	2005	Pet dog	Korea	Cd, Pb, Hg	204
11	Sakai	1995	Pet dogs	Japan	Hg	75
12	Sousa	2013	Pet dogs	Portugal	Hg	27
13	Zaccaroni	2014	Pet dogs	Italy	Cd, Pb, Hg	90

Atanaskova et al. (2011) analyzed the content of Cd and Pb in 35 companion dogs' hair from three cities in the Republic of Macedonia. Badea et al. (2018) conducted his study solely in female dogs from Romania. All animals were older than 5 years old, and 15 suffered from mammary neoplasms, whereas 15 were used as a control group. Both groups included animals living indoor and outdoor. The results were presented in the form of graphs, so the results here presented were visually extracted. Kozak et al. (2002) evaluated the content of cadmium and lead mostly in indoor companion dogs' hair from Bratislava (32 individuals) and Kosice (66 individuals). Dogs varied their age between 1–11 and 1–13 years old (respectively), with 4–6 years old constituting the largest category. Zaccaroni et al. (2014) aimed at assessing the levels of Cd, Pb, and Hg in dog hair from three different areas of Campania (Italy) with different profiles of contamination. Thirty healthy dogs from each area (where they had been living since pups) had their hair sampled during normal health control examinations, the ages ranging from 2 to 15 years, with the dogs on the category 5–7 years being the most numerous. In order to quantify the contents of Cd, Pb, and Hg in dog hair from domestic districts and to assess effects of sex and living area, Park et al. (2005a) collected 204 samples from apparently healthy dogs with no history of occupational exposure from different localities of Korea. Nikolovski and Atanaskova (2011) aimed to compare cadmium and lead exposure in different areas of the Republic of Macedonia, using dog's hair while taking into consideration the influence of age. For this purpose, 38 samples of dog hair were collected in low population cities (<20,000) and 57 in higher populated cities (<60,000). The age of the dogs varied between 1 and 10 years, with the largest group being that including dogs between 1 and 2 years old.

Generally, mercury levels in dogs' hair are very rarely reported. That is why the results reported by Doi et al. (1986), despite being determined in the hair of stray dogs (collected in Asahikawa city, Japan), were included in this report. These results were available only as graphs, so we had to visually extract data here presented. Dunlap et al. (2007) reported the contents of mercury in hair from 97 sled dogs fed commercial food and traditional village diets. Thirty-six individuals were fed commercial food (16 from New York and 20 from Salcha, Alaska), whereas 12 from Russian Mission, 12 from Galena, 12 from Rampart, 12 from Fort Yukon, and 12 from Salcha (all in Alaska) were fed a traditional diet. Mercury in sled dogs was also the object of study for Hansen and Danscher (1995). They reported results of hair analysis from 10 individuals (with ages between 6 weeks and >10 years) from the Thule District in Greenland. The work of Kral et al. (2015) was focused on the assessment of mercury contamination of dogs through the analysis of hair. A total of 131 animals were analyzed with 42 being fed granulated feed containing fish and 89 fed fish-free granulated food. Once again, results were presented as graphs so that data here presented had to be visually extracted. The major objective of the study conducted by Lieske et al. (2011) was to characterize changes in total Hg concentrations in hair of sled dogs over time due to long-term piscivory. For that purpose, four dogs were fed a fish diet and four a fish-free diet for twelve weeks. In order to evaluate the effects of environmental contamination, Sakai et al. (1995) analyzed the mercury concentrations in hair of 34 clinically healthy dogs (16 males and 18 females) living in the Kanagawa, Saitama, and Tokyo prefectures. More recently, Sousa et al. (2013) quantified the levels of mercury in the hair of 26 pet dogs from the northern area of Portugal, and the authors concluded that the mercury concentrations were independent of gender, age, and diet types.

The concentrations determined and reported in each paper are presented by metal in the following sections. Concentrations are reported as averages in parts per million (ppm), due to the wide variation of units used and the inconsistent statistical reporting.

5.4.2.1 Cadmium

Six papers reported cadmium values in dogs' hair: Atanaskova et al. (2011), Badea et al. (2016), Kozak et al. (2002), Nikolovski and Atanaskova (2011), Park et al. (2005a), and Zaccaroni et al. (2014).

Values reported for cadmium were generally low for all the studies. As with cats, dogs with renal problems registered lower levels than control animals, but only those living outdoors (Badea et al. 2016). A group of studies centered in central Europe (Macedonia, Romania, Slovakia) present consistent results (Atanaskova et al. 2011; Badea et al. 2018; Kozak et al. 2002). The other study performed on Macedonia dogs (Nikolovski and Atanaskova 2011) presents higher concentrations, which are similar to those registered in Korea (Park et al. 2005a) where the highest levels were registered for the hair of dogs living outdoor in a sandy substrate. Italy, with the exception of animals living near a dumping site, registered the lowest concentrations (Zaccaroni et al. 2014).

Dogs tolerate 10 ppm in diet; chronic toxicity occurs at 50 mg/kg (Neiger and Osweiler 1992). Normal reported levels (NRL) are comprised between 0.1 and 0.9 μg/g (Klasing et al. 2005) (Fig. 5.6).

5.4.2.2 Lead

Six papers reported lead values in dogs' hair: Atanaskova et al. (2011), Badea et al. (2016), Kozak et al. (2002), Nikolovski and Atanaskova (2011), Park et al. (2005a), and Zaccaroni et al. (2014) (Fig. 5.7).

Overall levels of lead were lower when compared to those reported for cats. The group of studies centered in central Europe (Macedonia, Romania, Slovakia) again present consistent results, but this time the second study performed on Macedonia and that in Korea in agreement. However, the value for indoor control dogs in Romania patented higher than average levels (Atanaskova et al. 2011; Badea et al. 2018; Kozak et al. 2002; Nikolovski and Atanaskova 2011; Park et al. 2005a). In the last study, a little nuance is discernible when compared to cadmium results: The animals registering the highest levels are those living indoors. Italy (again with the exception of animals living near a dumping site) once more registered the lowest concentrations (Zaccaroni et al. 2014).

For lead, the acute toxic dose for dogs is approximately 190–1000 mg/kg (dependent on lead form), whereas the chronic cumulative toxic dose is 1.8–2.6 mg/kg/day (Osweiler et al. 2011). NRLs are comprised between 0 and −88 μg/g (Klasing et al. 2005).

5.4.2.3 Mercury

Nine papers reported mercury values in dogs' hair: Doi et al. (1986), Dunlap et al. (2007), Hansen and Danscher (1995), Kral et al. (2015), Lieske et al. (2011), Park et al. (2005a), Sakai et al. (1995), Sousa et al. (2013), and Zaccaroni et al. (2014).

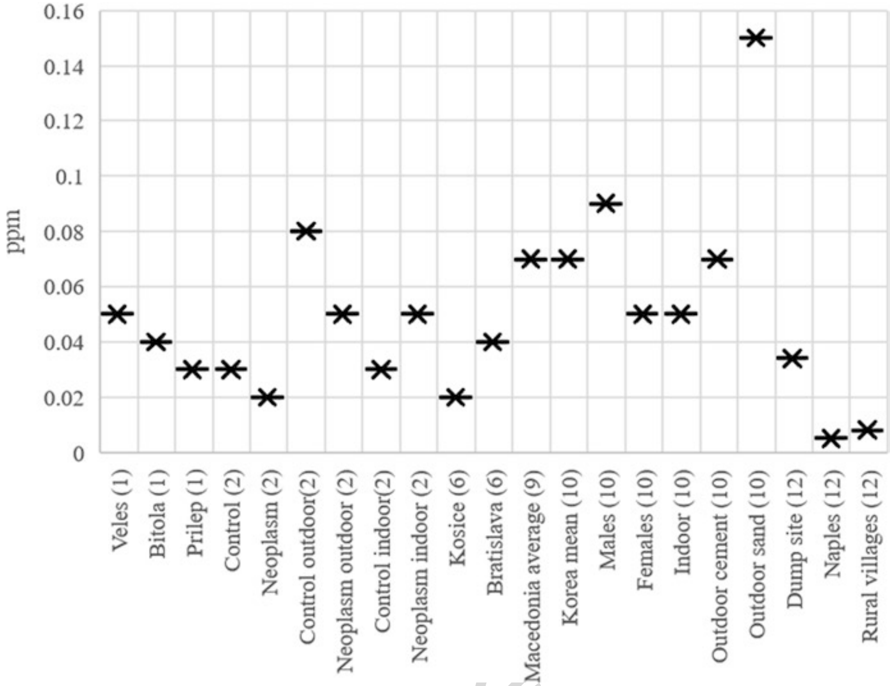

Fig. 5.6 Average cadmium concentrations in dogs' hair as reported by (1) Atanaskova et al. (2011), (2) Badea et al. (2016), (6) Kozak et al. (2002), (9) Nikolovski and Atanaskova (2011), (10) Park et al. (2005a), and (12) Zaccaroni et al. (2014). Veles, Bitola, Prilep: Rep. of Macedonia cities; Neoplasm: female dogs suffering from mammary neoplasm; Outdoor: females raised outdoor; Indoor: females raised indoor; Kosice, Bratislava: Slovakian cities; Outdoor cement: dogs raised outdoor on a cement floor; Sand: dogs raised outdoor on a sand floor; Dump site ("the death triangle"); Naples and rural villages: locations in the Campania Region of Italy. (Values from reference 2 were visually extracted from graphs reproduced in the paper)

There is a wide variation in both inter- and intra-studies regarding mercury in dogs' hair. Observations on sled dogs returned very high fur concentrations (Dunlap et al. 2007), particularly when compared to dogs used as control (by being fed with commercial fodder), and if they were between 1 and 5 years old (Hansen and Danscher 1995). However, sled dogs undergoing a fish diet did not accumulate as much mercury as would be expected (Lieske et al. 2011) being on average, even lower than the study performed in Korean dogs (Park et al. 2005a). In this study and similarly to lead, the animals registering the highest levels are those living indoors.

In Portugal, levels of mercury in dog's hair found by Sousa et al. (2013) varied widely (0.024–0.826 ppm). However, the average levels were overall low, and no significant differences were obtained between average levels for females and males, being the only study to report such results (all the other had clear differences between males and females). Similarly, no differences between the types of diet (commercial or homemade food) could be found. Also, no differences between dogs reared outdoors and indoors (or a mixture of both) were found. The authors suggested that such results could be due to the small sample size. Contrary to the previ-

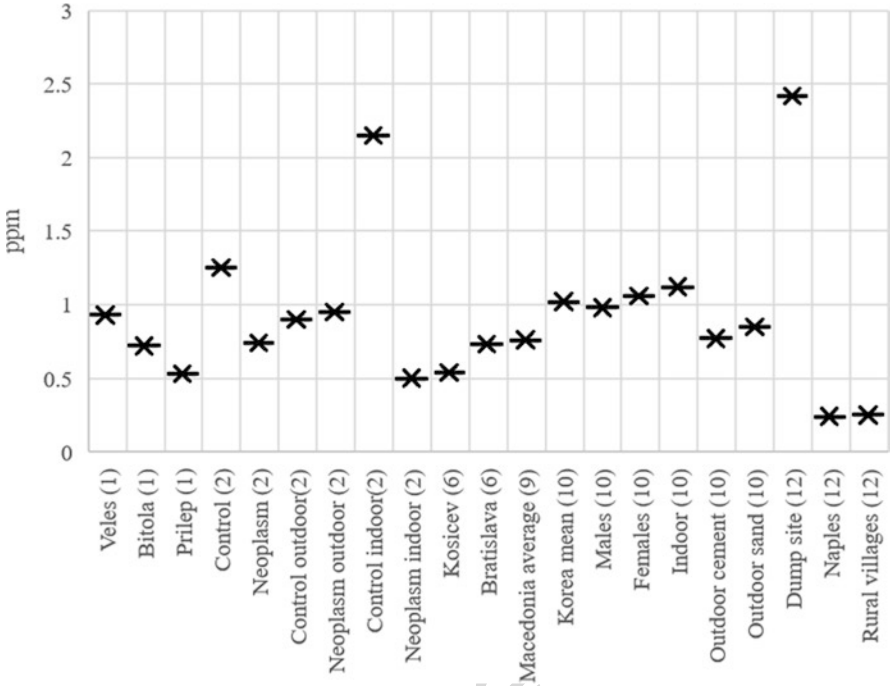

Fig. 5.7 Average lead concentrations in dogs' hair as reported by (1) Atanaskova et al. (2011), (2) Badea et al. (2016), (6) Kozak et al. (2002), (9) Nikolovski and Atanaskova (2011), (10) Park et al. (2005a), and (12) Zaccaroni et al. (2014). Veles, Bitola, Prilep: Rep. of Macedonia cities; Neoplasm: female dogs suffering from mammary neoplasm; Outdoor: females raised outdoor; Indoor: females raised indoor; Kosice, Bratislava: Slovakian cities; Outdoor cement: dogs raised outdoor on a cement floor; Sand: dogs raised outdoor on a sand floor; Dump site ("the death triangle"); Naples and rural villages: locations in the Campania Region of Italy. (Values from reference 2 were visually extracted from graphs reproduced in the paper)

ous metals, the study performed by Zaccaroni et al. (2014) demonstrated that dogs sampled in Naples are the most contaminated among all studies (closely accompanied by those living near the dumping site), whereas the third location used in the study was on level with the one performed in Korea (Fig. 5.8).

5.5 Considerations on the Use of Pets as Sentinels for Neurotoxic Metals

All kinds of animals have been put under consideration for becoming sentinels of human exposure to toxic substances (Reif 2011). The majority of these studies have considered synthetic chemicals, the common conclusion being that, according to each specific contaminant, some species are more useful than others, based on their

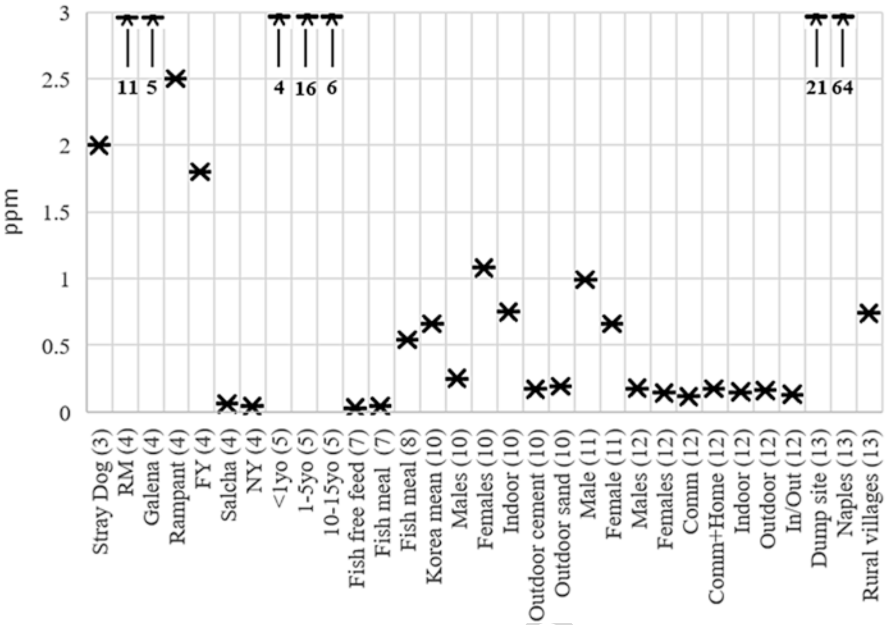

Fig. 5.8 Average mercury concentrations in dogs' hair as reported by (3) Doi et al. (1986), (4) Dunlap et al. (2007), (5) Hansen and Danscher (1995), (7) Kral et al. (2015), (8) Lieske et al. (2011), (10) Park et al. (2005a), (11) Sakai et al. (1995), (12) Sousa et al. (2013), and (13) Zaccaroni et al. (2014). RM (Russian Mission), Galena, Rampant, FY (Fort Yukon), Salcha, NY (New York): Sites of sampling; <1yo: below 1 year of age; 1–5yo: between 1 and 5 years old; 10–15yo: between 10 and 15 years old; Indoor: dogs raised indoors; Outdoor cement: dogs raised outdoor on a cement floor; Sand: dogs raised outdoor on a sand floor; Dump site ("the death triangle"); Naples, and rural villages: locations in the Campania Region of Italy. (Values from reference 2, 3, and 7 were visually extracted from graphs reproduced in the paper)

comparative metabolic capabilities toward man (e.g., D'Havé et al. (2005), Ruiz-Suárez et al. (2016), González-Gómez et al. (2018)).

Metals are probably the oldest known toxins, and the evolution of living entities occurred in the omnipresence of metals. Maybe because of this evidence, they have not been object to the same level of scientific interest. As far as we can determine, only Patrashkov et al. (2003) simultaneously analyzed a non-disclosed number of human, cat, and dog samples for metals with neurodegenerative potential (Cd and Pb) in hair in farms. Results were quite similar for Pb in the three species (humans, 1.93 ± 0.28; cat, 2.42 ± 0.51; and dog, 1.08 ± 0.41), but not for cadmium in dogs, which showed average concentrations lower than the other two (0.06 ± 0.06 mg/kg, versus 0.48 ± 0.22 mg/kg for cat and 0.41 ± 0.07 mg/kg for humans). Despite this, it is evident (at least for this study) that humans exposed to the same exposure environment as pets will end up with very approximate concentrations of metals with neurodegenerative potential in their hair. But this was a single study. Variations between species metal content in hair exist mostly due to differences in metal metabolism. Atop of this, intraspecific differences also occur and can derive from a set of factors such as age, sex, rearing, and type of food consumed, physiological condition, and habitat. In general, the overlapping of all factors contributes to variation in the concentrations within and between species. Further investigations will be necessary to establish robust baselines describing the distribution of these metals among species living in the same environment. There is an imperative need to verify if the strong, positive relationships between metal blood level concentrations in animals and their owners (especially pre-school children) are sustained for hair levels. If they are, the costly and stressful processes of population testing can be immensely simplified. The surrogate testing of cats and dogs instead of their owners can immediately indicate the need for further testing. If none of the animals tested in the household has hair levels that are above identified thresholds for each metal, it will be highly unlikely that the human inhabitants will, as pets integrate them in hair at higher levels than their owners. In either case, the observation by veterinarians of the emergence of neurological symptoms will always pay off, since pets, due to shorter latency periods (as they have shorter average lifespans), can act as early warning systems for human neurodegenerative processes. Fortunately, this trait is more important for chronic rather than acute toxic exposures, which is one of the etiological bases of the dementia disease spectrum.

5.6 Conclusions

The number of studies conducted in pet cats and dogs for the identification and quantification of metals with neurodegenerative potential is very scarce, particularly for cats. The information presented by these studies report (sometimes wide) discrepancies between metals and the influence of sex, age, diet and rearing of the animal. The geographic location of sampling (within and between countries) also clearly influences the concentrations of these metals in pets' hair (but always with significant correlations between this matrix and environmental metal concentrations). Since the number of individuals sampled for each study is generally low (mostly in the tens digit), the results should be carefully interpreted. However, the strong correlation shown in epidemiological and clinical studies between aberrant metal exposure and a number of neurological diseases and, simultaneously, the knowledge that canine and feline brains are subjected to the same neurodegenerative processes as those observed in humans, in a quicker time frame (due to their shorter latency periods), proves that pets should growingly be used as sentinels of exposure to neurodegenerative metals in humans. The archetypal concept of the *canary in the coal mine* re-claims a new life in the twenty-first century.

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Author Queries

Chapter No.: 5 428175_1_En_5_Chapter

Queries	Details Required	Author's Response
AU1	Martin and Coughtrey (1982) is cited in text, but not given in the reference list. Please provide.	
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