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RESEARCH ARTICLE

Domestic cats as environmental lead sentinels in low-income populations: a One Health pilot study sampling the fur of animals presented to a high-volume spay/neuter clinic

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Abstract

Non-human animals serve as sentinels for numerous issues affecting humans, including exposure to toxic heavy metals like lead. Lead plays a role in perpetuating cycles of poverty in low-income communities due to the inequitable distributions of indoor health risks from lower-quality housing and outdoor health risks from industry and polluters, compounded by inequitable distributions of heath care and education. In this pilot study, we explore the potential for studying lead in low-income populations by partnering with nonprofit veterinary outreach programs. We investigate the lead concentration in fur samples of 85 domestic cats (*Felis catus*) presented to a high-volume spay/neuter clinic and report a mean of 0.723 μg of lead per gram of fur. This study reveals new information about lead exposure in cats in the USA, including that females had greater lead exposure than males, lead exposure increased with increasing amount of access to the outdoors, and lead exposure increased in cats with decreased body condition. We propose that pet, feral, and free-roaming cats presented to high-volume spay/neuter clinics could serve as a source of data about lead exposure in disadvantaged communities where these clinics already operate. Such a non-invasive surveillance system using inert, unobtrusively obtained samples could be deployed to detect highly exposed cats, prompting to follow up contact to a cat's caretakers to recommend seeking lead testing for themselves, their families, and their neighbors.

Keywords Environmental justice · Cats · Lead · One Health · Biomonitoring · Fur

Introduction

Non-human animals are considered sentinels for numerous issues affecting humans (Schmidt 2009). There is an increasing push to expand the understanding of connections among animals, humans, and the environment through a One Health "shared risk" paradigm (Rabinowitz et al. 2008). While these approaches often focus on collaborations in tracking infectious diseases (Dórea et al. 2011), another area for which animal sentinels can be used is environmental monitoring for heavy metal exposure, including lead.

Lead plays a role in perpetuating cycles of poverty and underachievement in low-income communities of North America and elsewhere. In addition to being a toxic heavy metal of concern for negative effects in numerous body systems and species (Gómez-Ramírez et al. 2011), lead exposure is a recognized environmental justice issue due to its roles in causing low birth weight, reducing the IQ and cognitive function of children and adolescents, impairing learning ability and motor skills, and altering social behaviors, even at low levels (Kalisinska et al. 2016; Sanders et al. 2012; Sathyanarayana et al. 2006; Schillaci et al. 2011). Minorities make up approximately three-fourths of children with elevated blood lead levels (Reyes 2018), generally defined as being greater than 5 μg/dl (Mannino et al. 2005).

Lead exposure in the USA is associated with living in older housing structures with lead-based paint or areas with contaminated drinking water (Sanders et al. 2012). Lead is part of larger systemic problems of inequitable distributions of indoor health risks from lower-quality housing (Adamkiewicz et al.

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2011) and outdoor health risks from industry and polluters (Reyes 2018), compounded by inequitable distributions of heath care and education (Neuwirth 2018). Lead has been discussed as an additional burden for immigrant families in low-income neighborhoods who may be unaware of lead risks in the USA, struggle to read English language warnings and educational materials, and unable to access healthcare (Neuwirth 2018).

Ranging across wildland and urban areas and sharing human food and water resources to varying degrees, many wildlife species have been studied as potential lead sentinels. Blood lead levels, blood-based biomarkers, and tissue samples have been analyzed across diverse taxa such as owls (Gómez-Ramírez et al. 2011), mink (Ljungvall et al. 2017), and land snails (Regoli et al. 2006). In North America, raccoons (Rainwater et al. 2017) and pigeons (Cai and Calisi 2016) have been proposed as sentinels for infectious disease and heavy metal exposure because of their roles as widely distributed generalists living near humans as commensals and synanthropes. In Asia, rhesus macaque monkeys fill the same role (Engel et al. 2010).

Fur and hair samples are used in heavy metal research as a non-invasive biomarker of chronic exposure to heavy metals (Moreno-Santini et al. 2012). Sulfhydryl groups on keratin proteins of hair strands bind metal cations from the blood supplying hair follicles, creating stable, inert samples that can be acquired non-lethally and do not have special handling and storage requirements (Długaszek and Kopczyński 2014; Hernout et al. 2016b; Asano et al. 2005; Tête et al. 2014; Engel et al. 2010). Fur has been widely subjected to lead concentration testing in wildlife and domestic animals (see Table 1 for a summary).

Domestic cats occupy a number of behavioral and ecological niches, from indoor-only house pets to feral cats that are unsocialized to humans. Indoor cats' lead exposures may include lead-based paint, contaminated drinking water, cigarette smoke, and food. Free-roaming and feral cats' exposures may include contaminated soil and water, paint dust, pollution, and lead in anthropogenic food waste. This positions cats as a unique potential sentinel. Sampling the breadth of cats allows for the combined benefits of sampling both urban wildlife species and domestic pet species, rather than using one species as an indoor sentinel and another species with a different physiology as an outdoor sentinel.

Cats have been studied as sentinels for numerous exposures of concern to humans in environmental toxicology, including methyl mercury (Takeuchi et al. 1977; Aronson 2005), flame retardants (Mensching et al. 2012; Poutasse et al. 2019), perfluoroalkyl substances (Bost et al. 2016), and chlorinated pollutants (Ruiz-Suárez et al. 2015).

Lead concentration has been studied in domestic cats using tissues in Italy (Esposito et al. 2019), the USA (Gilmartin et al. 1985), and Germany (Paßlack et al. 2014); blood samples in the USA (Berny et al. 1994; Berny et al. 1995), and the Czech Republic (Smetková et al. 2002); and using fur in Poland (Rzymski et al. 2015; Skibniewski et al. 2014). There have also been two clinical case studies of lead poisoning or elevated lead levels affecting both cats and humans (Bischoff et al. 2010; Doumouchtsis et al. 2006).

One study of cats and dogs in an Illinois town with a disused lead smelter found that human blood lead levels were lower than those of pets living in the same environment. While human blood lead levels did not correlate to lead concentrations in soil or dust, those of their pets did. These authors concluded that pets are at greater risk of lead exposure than humans, making them valuable sentinels and recommending pets be tested for lead during routine veterinary visits (Berny et al. 1994). This recommendation, however, assumes that people at risk of lead poisoning are visiting a veterinarian at regular intervals.

Expanding access to affordable veterinary care is an issue of rising prominence in the animal welfare world. A study of high-volume spay/neuter clinic clients in the USA revealed, among respondents who provided financial information, that 25% fell below the federal poverty level, defined as $11,880 for a single person and $28,440 for a family of five (White et al. 2018). Many clinics engage in means testing to allow their services to only be accessed by those below a certain income threshold or who have qualified for government benefits. Pets for Life, a social justice approach to providing accessible animal care services, operates in 39 US locations where an average of 33% of residents live below the federal poverty level. Approximately 73% of Pets for Life clients for whom ethnicity data were available were nonwhite (Decker Sparks et al. 2017). Such patrons likely overlap with the population at risk from lead poisoning.

There are other benefits to partnering with high-volume spay/neuter clinics for data collection. Already maximized for efficient operations, many samples from a broader area can be obtained at a central location. Since clinics treat both indoor and outdoor cats, the list of potential sources of the exposure could be narrowed based on the cat's lifestyle and habitat to enable more targeted remediation and education efforts in cases of elevated lead concentrations.

The purpose of this study was to determine (1) whether fur samples collected from cats presented to a high-volume spay/neuter clinic correlated with census tract level human lead risk metrics published by the Washington State Department of Health, (2) which factors about these cats and their environments influenced lead concentrations in their fur, and (3) whether this sampling approach could be incorporated unobtrusively into a high-volume clinic setting. Such a system could serve as a novel means of engaging in non-invasive biomonitoring in partnership with veterinary outreach efforts already being undertaken in many disadvantaged communities around the country.

Table 1 A comparison of the findings from the present study (first listing) and all other known studies of lead concentration in hair and fur of domestic and wild animals. This body of literature does not use a single uniform preparation method to attempt to remove external contamination from hair and fur samples. All studies, unless noted as having analyzing unwashed samples, used some type of washing process. See each individual study for details

Animal	Environment and country	n	Mean (μg/g)	Median (μg/g)	Dispersion	Range (μg/g)
Domestic and feral cats						
Pet, free-roaming, and feral cats *Felis catus*	Mix of urban, suburban, and rural in the USA	85	0.723	0.23	1.717 SD	0.031–14.00
Free-ranging, in/out, and indoor cats *Felis catus* (Rzymski et al. 2015)	Sampled from a veterinary clinic in Poland	44	69.8, 44.5, 95.3*	•	35.5, 19.3, 13.0* SE	•
Pet and feral cats *Felis catus* (Skibniewski et al. 2014)	Urban areas in Poland	10 and 10	1.0 and 2.89	0.90 and 2.20	0.17 and 1.92 SD	0.90–1.30 and 0.90–7.50
Order Carnivora, Family Felidae						
Ocelot *Felis pardalis* (Mora et al. 2000)	Wildlife refuge and surrounding areas in the USA	32	0.56–26.8	•	•	Up to 150
Leopard cat *Felis bengalensis* (Dey et al. 1999)	Unwashed and washed samples from reserves in India	•	110 and 87	•	20 and 6 SD	•
Civet cat *Vivera zebitha* (Dey et al. 1999)	Unwashed and washed samples from reserves in India	•	19.5 and 14.3	•	2 and 2 SD	•
Leopard *Panthera pardus* (Dey et al. 1999)	Unwashed and washed samples from reserves in India	•	Not detected	•	•	•
Order Carnivora, Family Canidae						
Hoary foxes *Lycalopex vetulus* (Curi et al. 2012)	Parks and farms near developed region in Brazil	2	1.5	•	0.99	•
Farmed and wild foxes *Vulpes vulpes* (Filistowicz et al. 2011)	Fur farm and agricultural-woodland areas of Poland	12 and 8	0.642 and 0.633	•	0.034 and 0.078 SD	•
Maned wolves *Chrysocyon brachyurus* (Curi et al. 2012)	Parks and farms near developed region in Brazil	10	2.34	•	0.76	•
Crab-eating foxes *Cerdocyon thous* (Curi et al. 2012)	Parks and farms near developed region in Brazil	14	2.45	•	1.22	•
Order Carnivora, Clade Pinnipedia						
Baikal seal *Pusa sibirica* (Ikemoto et al. 2004)	Lake Baikal in Russia	20	13.4	•	15.3 SD	2.57–58.0
Caspian seal *Pusa caspica* (Ikemoto et al. 2004)	Caspian Sea in Russia	16	3.53	•	2.14 SD	0.047–6.72
Northern fur seal *Callorhinus ursinus* (Ikemoto et al. 2004)	Sanriku in Japan	20	7.68	•	5.60 SD	2.38–26.1
Ringed seal *Phoca htsptda satmensts* (Hyvärinen and Sipilä 1984)	Lake Saimaa in Finland	32	3.6 - 8.5	•	0.58–4.01 SE	•
Mediterranean monk seal *Monachus monachus* (Yediler et al. 1993)	Caves in the Ionian Sea in Greece	•	0.78467	•	0.3106 SD	0.365–1.520
Ringed seals *Phoca hispida ladogensis* (Medvedev et al. 1997)	Polluted lakes in populated areas in Russia	23	6.34	•	9.09 SD	0.34–40.0
Ringed seals *Phoca hispida hispida* (Medvedev et al. 1997)	Polluted lakes in populated areas in Russia	15	1.58	•	1.33 SD	0.36–5.14
Bearded seals *Erignathus barbatus* (Medvedev et al. 1997)	Polluted lakes in populated areas in Russia	3	1.42	•	0.80 SD	0.56–2.11
Pup and adult harbor seal *Phoca vitulina* (Wenzel et al. 1993)	Coast of Germany	47	0.5***	0.6***	0.4 (unspecified)	•
Order Lagomorpha						
	Unpolluted forest and meadows in Poland	11	4.5	3.3	2.9 SD	1.7–9.7

Table 1 (continued)

Animal	Environment and country	n	Mean (µg/g)	Median (µg/g)	Dispersion	Range (µg/g)
Hare *Lepus europaeus* (Długaszek and Kopczyński 2014)						
Order Rodentia						
Wood mice *Apodemus sylvaticus* (Marcheselli et al. 2010)	Urban, suburban, agricultural, and a nature reserve in Italy	26	0.45******	•	•	•
Wood mice *Apodemus sylvaticus* (Tête et al. 2014)	Gradient from polluted smelter area in France	321	•	•	•	UDL - 86.7
Wood mice *Apodemus sylvaticus* (Beernaert et al. 2007)	Gradient from a smelter in Belgium	98	0.36 - 3.22	•	0.04–0.30 SE	•
Black Rat *Rattus rattus* (McLean et al. 2009)	Gradient from decommissioned smelter in Australia	40	•	•	•	1.49–10.6
Black Rat *Rattus rattus* (Pereira et al. 2006)	Abandoned mining area in Portugal	4	•	•	•	0.031–61.290
Brown (Norway) Rat *Rattus norvegicus* (McLean et al. 2009)	Gradient from decommissioned smelter in Australia	16	•	•	•	2.16–20.6
Algerian mice *Mus spretus Lataste* (Pereira et al. 2006)	Abandoned mining area in Portugal	2	•	•	•	0.460–0.946
Flying squirrel *Petaurista magnificus* (Dey et al. 1999)	Unwashed and washed samples from reserves in India	•	45 and 42	•	5 and 5 SD	•
Order Artiodactyla						
Domestic cattle (Gabryszuk et al. 2010)	Organic dairy farms in Poland	33	0.03267	•	0.01615 SD	•
Domestic cattle (Pourjafar et al. 2006)	Dairy farms along gradient from oil industry in Iran	120	1.96–10.40	•	•	•
Domestic cattle (Patra et al. 2007)	Industrial urban and unpolluted areas in India	317	1.82–15.09	•	0.10 to 4.02 SE	0.21–37.24
Sheep (Rashed and Soltan 2005)	Farms with different pollution levels in Egypt	80	0.01–8.9	•	•	•
Sheep (Ward and Savage 1994)	Near and far from a large motorway in England	62 and 10	4.6–22.0 and 1.7–7.1**	•	•	4.0–29.5** (near roadway)
Goats (Rashed and Soltan 2005)	Farms with different pollution levels in Egypt	80	0.35–12	•	•	•
Domestic camels (Rashed and Soltan 2005)	Farms with different pollution levels in Egypt	80	0.90–13	•	•	•
Alpacas (Ward and Savage 1994)	Near and far from a large motorway in England	6 and 4	24.9 and 12.7**	•	•	17.5–47.5** and 8.6–19.5**
Roe deer *Capreolus capreolus* (Maňkovská 1980)	Polluted and nonpolluted areas in Poland	•	1.4–118	•	0.2–9.5 SD	•
Roe deer *Capreolus capreolus* (Długaszek and Kopczyński 2014)	Unpolluted forest and meadows in Poland	13	2.8	3.1	1 SD	1.3–4.6
Reindeer *Rangifer tarandus platyrhynchus* (Pacyna et al. 2018)	Free-roaming on Svalbard Island in Norway	11 and 16	5.14 and 3.20	1.68 and 1.96	2.19 and 0.82 SE	•
Reindeer *Rangifer tarandus platyrhynchus* (Pacyna-Kuchta et al. 2020)	Free-roaming on Svalbard Island in Norway	5 and 3	0.471 and 0.796	0.354 and 0.743	0.327 and 0.444 SD	0.12–1.26
Reindeer *Rangifer tarandus fennica* (Medvedev 1999)	Forested hunting area in Russia	45	5.4	•	0.82 SE	0.10–23.7
Alaskan moose *Alces alces gigas* (Franzmann et al. 1975)	Natural areas and wildlife research facility in the USA	317	5.1–25.5	•	1.8–7.4 SD	•
	Unpolluted forest and meadows in Poland	11	1.1	1.1	0.5 SD	0.6–2.3

Table 1 (continued)

Animal	Environment and country	n	Mean (μg/g)	Median (μg/g)	Dispersion	Range (μg/g)
Wild boar *Sus scrofa* (Długaszek and Kopczyński 2014)						
Order Perissodactyla						
Horses (Iwase and Soska, 2019)	Two horse herds in Poland	30	1.814	•	•	•
Horses (Asano et al. 2002)	Racing horses in Japan	24	0.93	•	0.78 (unspecified)	•
Horses (Asano et al. 2005)	Riding horses in Japan	47	1.43	•	1.3 SD	•
Horses (Ward and Savage 1994)	Near and far from a large motorway in England	10 and 8	20.1 and 10.3**	•	•	12.5–33.4** and 7.3–11.2**
Order Chiroptera		n	Mean	Median	Dispersion	Range
Black flying-fox *Pteropus alecto* (Pulscher et al. 2020)	Wildlife rehabilitation patients in Australia	9	•	1.61	•	0.72–3.75
Gray-headed flying-foxes *Pteropus poliocephalus* (Pulscher et al. 2020)	Newly captive and longer-term captive wildlife rehabilitation patients in Australia	11 and 14	•	0.34 and 0.23	•	0.09–1.35 and 0.06–2.73
Various insectivorous bat species (Mina et al. 2019)	Carcasses found at wind farms in Portugal	51	•	2.50	•	1.18–57.56
Tadarida teniotis and *Miniopterus schreibersii* bats (Andreani et al. 2019)	Unwashed samples from urban and open areas in Italy	3 pools of 10 bats	36.9****	•	18.4 SD	•
Egyptian fruit bat *Rousettus aegyptiacus* (Sheta and Beheary 2019)	Males and females the Nile Delta in Egypt	50	34 and 46*****	•	0.4 and 0.5 (unspecified)	•
Pipistrellus bats (Hernout et al. 2016a)	Across landscape in England and Wales	192	•	28.8	•	0.045–20399
Myotis and *Pipistrellus* bats (Flache et al. 2015)	Urban, agricultural, and forested areas of Germany	41	•	•	•	0.0159–519
Pteropus fruit bats (Hariono et al. 1993)	Urban and non-urban areas in Australia	37	5.82 and 0.85	•	•	0.00–40.26 and 0.85–2.51
Various bat species (Hickey et al. 2001)	Across landscape of two provinces in Canada	110	•	•	•	ND - 11.3
Order Primates						
Macaca fascicularis monkeys (Schillaci et al. 2011)	Nature reserves and city parks in Singapore	27	2.51	2.58	1.86 SD	0.21–6.45
Macaca mulatta monkeys (Engel et al. 2010)	Temple in dense urban area in Nepal	33	4.5	•	•	1.34–10.2
Infraclass Marsupialia						
Opossum *Didelphis virginiana* (Burger et al., 1994)	Males and females in parks and agricultural Costa Rica	12 and 12	0.319 and 0.524	•	0.046 and 0.066 SE	•
Brown Antechinus *Antechinus stuartii* (McLean et al. 2009)	Gradient from decommissioned smelter in Australia	53	•	•	•	1.78–5.54
Order Eulipotyphla						
Hedgehogs *Erinaceus europaeus* (D'Havé et al., 2006)	Roadkill and wildlife rehabilitation patients in Belgium and the Netherlands	43	2.5	•	0.4	0.2–15.4
Hedgehogs *Erinaceus europaeus* (D'Havé et al., 2005)	Along a gradient from a factory in Belgium	83	11.70–0.28	•	1.98–0.06 SE	•
Hedgehogs *Erinaceus europaeus* (Vermeulen et al. 2009)	Reference site and polluted factory site in Belgium	12 and 14	•	0.4 and 7.6	•	0.1–1.1 and 3.6–14.1

Table 1 (continued)

Animal	Environment and country	n	Mean (µg/g)	Median (µg/g)	Dispersion	Range (µg/g)
Hedgehogs *Erinaceus europaeus* (Rautio et al. 2010)	Medium-sized town in Finland	65	0.98	•	0.19 SE	<LD - 7.10

Studies where the mean is listed as a range reported means by subgroups but no grand mean

*Means and SE provided in a personal communication from lead author

**Concentrations based on analyzing unwashed samples

***Concentrations based on wet weight

****Concentrations based on a sample that contained unwashed skin and fur analyzed together

*****Study did not specify whether samples were washed or unwashed

******Lead concentration in hair was presented only in a graph form and appears to be roughly 0.45µg/g

Methods

We obtained data and samples in June and July 2018 at the Feral Cat Spay/Neuter Project, a high-volume feline clinic that serves feral cat caretakers, low-income individuals, and small rescue groups in Washington, USA. During the surgery check-in process, we invited clients to participate in a short electronic survey on a tablet (University of Washington Institutional Review Board STUDY00005055). This process was not randomized or blinded. Our sample size was determined by the number of clinic clients who elected to be part of the study on dates in which a researcher was present in the clinic. We included any cats over the age of approximately 6 months (kittens were excluded to avoid possible confounding with lead sourced from mother's body in the womb or via lactation) and excluded cats about which a previous location and lifestyle were not known. After agreeing to an informed consent, cat caretakers provided information on the cat's lifestyle (100% indoors, mostly indoors with rare outdoor access, 50/50 indoor/outdoor mix, or 100% outdoors) and indicated on a Google Map approximately where the cat lives (which was initially stored as a latitude and longitude before being translated to census tract level lead risk metric rank). We also obtained data from each cat's medical record as to its sex, body condition (overweight, normal, underweight), and whether a female was lactating, in heat, pregnant, and/or postpartum. We did not collect names of cat caretakers, their contact information, or other data about humans in this pilot study.

Fur was obtained by saving material shaved from anesthetized patients as part of routine preparation for ovariohysterectomy (spay) or orchiectomy (neuter) surgery (see Fig. 1). Unlike blood draws, which can require multiple attempts in dehydrated patients, the collection of fur does not interrupt operations in a high-volume clinic or pose any additional risk to patients (the University of Washington's Office of Animal Welfare determined that this study did not require an IACUC protocol for use of non-human animals in research because collecting fur that would otherwise be discarded was considered an incidental sample). Electric clippers were not disinfected or cleaned of hair fragments between healthy patients as was the custom of this and similar clinics, so each cat's sample likely contains a minuscule amount of hair from other patients. Female cat fur samples were from the abdomen, and male cat fur samples were from the scrotum, clearings on either side of the scrotum, and a patch on the abdomen shaved for a tattoo that indicates surgical sterilization.

Our cat data and fur samples were originally collected for carbon and nitrogen stable isotope analysis by mass spectrometry, and as such, we used sample preparation methods drawn from that field which use a 2:1 mixture of chloroform and methanol (Newsome et al. 2015; Savory et al. 2014). In fur sample preparation for both stable isotope analysis and lead

Fig. 1 Example of a fur sample from an anesthetized female cat being shaved in preparation for her ovariohysterectomy (spay) in a high-volume spay/neuter clinic. This fur is normally discarded but could serve as a source of One Health data

analysis, there are no established norms for washing procedures and different authors report use of various methods. We sought to remove surface contaminants and potential exogenous lead from our samples using a 2:1 mixture of chloroform and methanol. We soaked fur samples for approximately 30 min, rinsed with the same solution, rinsed with deionized water, and a final rinse with methanol before transferring samples to plastic containers for shipment to a lab for chemical analysis.

The University of Idaho Analytical Sciences Laboratory analyzed our samples using a 30% nitric acid digestion followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Quality control included initial and continuing calibration verification standards, a standard reference material, method blank, and sample duplicates. A quantification limit of 0.01 ug/g was verified with a low-level calibration standard (for complete details on laboratory methods, please see this article's supplementary files). Laboratory staff were blinded to any characteristics of each sampled cat apart from its sample number and the date the sample was collected.

Little is known about lead contamination in the state of Washington. There is no systematic surveillance of lead found in the environment, such as through soil sampling, or widespread testing of human blood lead levels. Two metrics for relative human lead risks are publicly available from the Washington Tracking Network (Washington State Department of Health 2019). The Lead Risk from Housing metric is determined by an area's percentage of housing units built before 1980. The Lead Exposure Risk metric averages housing-based lead risk and the amount of an area's population living in poverty (defined as 125% or less of the US federal poverty level). Both metrics assign a relative ranking of 1–10 (with 1 being lowest and 10 highest risk) using a decile percentile scale. These rankings are calculated at the census tract level. Each cat's location was tied to a Federal Information Processing Standard Publication (FIPS) code, and we recorded the appropriate risk metric scores for each cat.

US census tracts are delineated by human population size, and tracts occupied by cats in this study varied considerably in geographic size. After converting each tract's area (as listed on censusreporter.org) from square miles to square kilometers, we collapsed them into 5 groups. Group 1 tracts ranged from 1.04 to 2.85 km^2 (n=21), group 2 were 3.11–6.73 km^2 (n=15), group 3 were 14.25–26.42 km^2 (n=22), group 4 were 46.88–67.08 km^2 (n=19), and group 5 were 214.19–333.59 km^2 (n=8). These groups serve as a proxy for human density.

We analyzed data to report arithmetic means, medians, and standard deviations of lead concentrations in cat fur, performed Shapiro-Wilk tests of normality, Kruskal-Wallis analyses of variance, correlation tests using the Pearson method, Pearson's chi-squared tests of independence, two sample F tests of variance, Student's t-tests of differences of means, and one-way analyses of variance. We used R version 3.6.1 (R Core Team 2019) in RStudio version 1.2.1335 with packages ggplot2 (Wickham 2016), maps (Becker et al. 2018), plotrix (Lemon 2006), reshape2 (Wickham, 2007, b), tidyverse (Wickham 2017), and gridextra (Auguie 2017).

Results

We collected and analyzed fur samples from 85 cats (see Table 2) presented from locations

around Western Washington, USA (see Fig. 2). Of these, 84 cats were intact (unsterilized), and one was discovered to be already altered upon shaving. Lead concentrations in fur samples ranged from 0.031 to 14.00 µg of lead per gram of fur based on analyzing 0.25 g of fur on a dry weight basis, with an overall mean of 0.723 µg/g, median of 0.230 µg/g, and a standard deviation of 1.717. Lead data were right skewed and not normally distributed according to a Shapiro-Wilk test of normality (W = 0.370, p-value < 2.2e-16), even with a $\log_{10}$ transformation (W = 0.927, p-value = 0.00014).

We found weak and statistically nonsignificant correlations (using an alpha of 0.05) when testing for associations between cat fur lead concentrations and the two census tract level lead risk metrics, using either raw or $\log_{10}$ transformed lead data (see Fig. 3).

We found variations in lead concentrations when examining cats by subsets based on factors associated with the cats and their environments (see Fig. 4). Because the lead data were not normally distributed, we used Kruskal-Wallis tests to compare lead concentrations in subsets of cats.

By sex, males (n=28) had a mean lead concentration of 0.280 µg/g (SD 0.541), and females (n=57) had a mean lead

Table 2 A summary of the population characteristics of cats included in analysis

Sample population characteristics	n=85
Sex	
Female	57
Male	28
Lifestyle and habitat	
Indoor only	10
Mostly indoor	6
Indoor/outdoor	18
Outdoor only	51
Body condition	
Obese	4
Normal	36
Underweight	45
Status of females (not mutually exclusive)	
Lactating	28
Pregnant	14
In heat	21
Postpartum	25

concentration of 0.939 μg/g (SD 2.034). This difference was statistically significant using a Kruskal-Wallis test (chi-squared = 8.602, df = 1, p-value=0.0034).

By lifestyle/habitat as reported by caretakers, indoor cats (n=10) had a mean lead concentration of 0.113 μg/g (SD 0.0807), mostly indoor cats (n=6) had a mean lead concentration of 0.176 μg/g (SD 0.0857), cats living half indoors and half outdoors (n=18) had a mean lead concentration of 0.462 μg/g (SD 0.447), and outdoor-only cats (n=51) had a mean lead concentration of 0.997 μg/g (SD 2.162). This difference was statistically significant using a Kruskal-Wallis test (chi-squared = 17.616, df = 3, p-value =0.00053).

Body condition category was determined from cat's medical record as assessed by a veterinary medical professional during a routine pre-operative examination. By body condition, obese-only cats (n=4) had a mean lead concentration of 0.0875 μg/g (SD 0.0330), normal cats (n=36) had a mean lead concentration of 0.674 μg/g (SD 2.315), and underweight cats (n=45) had a mean lead concentration of 0.817 μg/g (SD 1.148). This difference was statistically significant using a Kruskal-Wallis test (chi-squared = 17.228, df = 2, p-value =0.00018).

By census tracts grouped by size (with 1 being the smallest and most densely populated and 5 being the largest and least densely populated), cats in group 1 (n=21) had a mean lead concentration of 0.410 μg/g (SD 0.533), cats in group 2 (n=15) had a mean lead concentration of 0.741 μg/g (SD 1.223), cats in group 3 (n=23) had a mean lead concentration of 0.643 μg/g (SD 0.9123), cats in group 4 (n=19) had a mean lead concentration of 0.267 μg/g (SD 0.170), and cats in group 5 (n=8) at a mean lead concentration of 2.800 μg/g (SD 4.817). This difference was not statistically significant using a Kruskal-Wallis test (chi-squared = 5.795, df = 4, p-value = 0.22).

Female reproductive status was determined from cat's medical record as assessed by a veterinary medical professional and were not mutually exclusive. Lactating females (n=28) had a mean lead concentration of 0.993 μg/g (SD 1.322), pregnant females (n=14) had a mean lead concentration of 0.482 μg/g (SD 0.519), postpartum females (n=25) had a mean lead concentration of 0.923 μg/g (SD 1.144), and females in heat (n=21) had a mean lead concentration of 1.193 μg/g (SD 3.017).

This study had one outlier cat with 14.000 μg/g of lead in her fur. The cause of this is comparatively high lead concentration is unknown. She was reported by her caretaker/trapper to be living 100% outdoors, had a normal body condition, was in heat at the time of sampling, and was provided with an ear tip removal by the clinic to indicate her as a sterilized feral cat. Removing this outlier from analysis did not result in

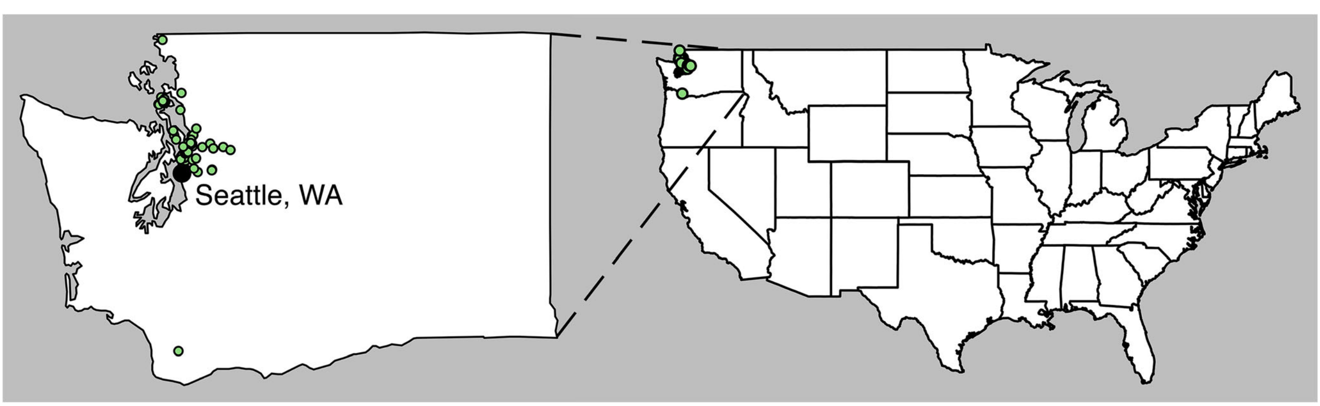

Fig. 2 Map of the state of Washington, USA, where each of 85 sampled cats is represented by a small, green-filled circle, and Seattle is represented by a larger black circle

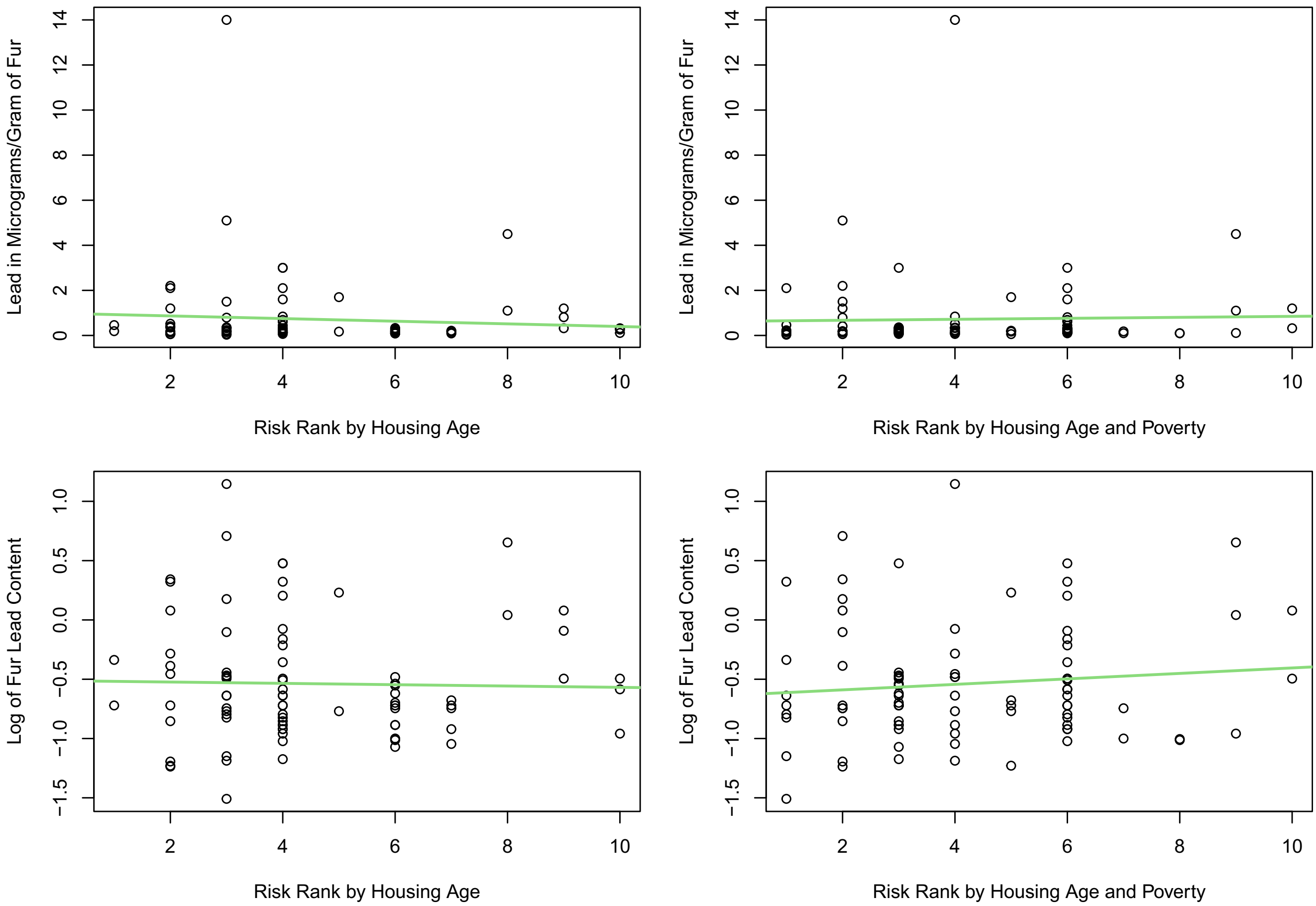

Fig. 3 Lead concentration from 85 cat fur samples was not strongly or significantly correlated with either of two metrics for human lead risk rankings from the Washington State Department of Health: risk based on housing age, and risk based on a combination of housing age and poverty

statistically significant correlations or change overall trends in lead concentration among cat subsets, although it reduced the standard deviations for subsets in which she was included. Removal of the outlier changed the overall mean lead concentration in fur of sampled cats from 0.723 (SD 1.717) to 0.564 μg/g (SD 0.914).

Discussion

This study sought to determine whether fur samples from a high-volume feline spay/neuter clinic could be used for environmental lead surveillance using easily obtained and non-invasive samples. This study reveals new information about

Fig. 4 We found statistically significant differences in lead concentration in cat fur by sex (p-value=0.0034), lifestyle/habitat (p-value=0.00053), and body condition (p-value=00018). The horizontal bar in the middle of each plot shows the mean value, and the shaded area above and below represents the middle half of data points each subset

lead exposure in domestic cats in the USA, including that female cats had greater lead exposure than males, lead exposure increases with increasing amount of access to the outdoors, and lead exposure increases in cats with decreased body condition.

Some commonalities emerged among the most and least exposed cats. The top decile (n=8) had fur lead concentration ranging from 2.100 to 14.000 μg/g. All eight of these cats were outdoor only, seven were underweight, six were lactating, and four were postpartum. The bottom decile (n=8) had fur lead concentration ranging from 0.031 to 0.085 μg/g. Four were outdoor only and four were indoor only, two were underweight, one was lactating, and one was postpartum.

Female cats in this study, which may have experienced pregnancies prior to sterilization, had 3.35 times the lead concentration as males. In humans, pregnancy has been noted for its ability to cause body's lead stores to mobilize into the blood (World Health Organization 2019). Pregnant non-human mammals have been noted to absorb nearly 50% of ingested lead compared to 5–15% in non-pregnant adults (Kalisinska et al. 2016). Pregnant and lactating cats experience high metabolic demands to support their developing fetuses and nursing kittens, which could lead to water- and food-borne lead contamination being amplified in such cats.

There is not a universal pattern in lead concentration by sex in non-human animals. Female opossums in Costa Rica (Burger et al., 1994), female wood mice in Belgium (Beernaert et al. 2007), and female fruit bats in Egypt (Sheta and Beheary 2019) had higher fur lead concentrations than the respective males. No statistically significant differences between the lead concentration of fur from males and females were found in studies of long-tailed macaques in Singapore (Schillaci et al. 2011), brown antechinus in Australia (McLean et al. 2009), red and silver foxes in Poland (Filistowicz et al. 2011), horses in Japan (Asano et al. 2002), rhesus macaque monkeys in Nepal (Engel et al. 2010), reindeer in Russia (Medvedev 1999), hedgehogs in Finland (Rautio et al. 2010), bats in Italy (Andreani et al. 2019), wood mice in Italy (Marcheselli et al. 2010), or in cats in Poland (Rzymski et al. 2015). No statistically significant differences in lead concentrations by sex in cats were found using tissue samples in Italy (Esposito et al. 2019) or Germany (Paßlack et al. 2014) or in blood samples in the USA (Berny et al. 1994) or the Czech Republic (Smetková et al. 2002).

The effect of sex on lead concentration is also variable in research using human hair. Some studies have found that males have higher lead concentration than females (Chlopicka et al. 1995; Shah et al. 2011; Sanna et al. 2003), while others have found that females have higher lead than males (Moreno-Santini et al. 2012; González-Muñoz et al. 2008). Still others found no sex difference (Menezes-Filho et al. 2012), and one found no difference among younger children, but in adolescents, females had higher lead than males (Peña-Fernández et al. 2014). These findings in humans may be due to the amount of exposure, with males potentially having more contact with lead from dust and soil than females (Shah et al. 2011). Further, males are more likely than females to smoke tobacco (Higgins et al. 2015), and blood lead levels are known to be higher in smokers than nonsmokers (Mannino et al. 2005). Unlike animal fur, human hair samples may have been treated with hair dye containing lead acetate, which could affect results.

Among cats in the present study, a positive trend was found between lead concentration in fur and the amount of time spent outdoors. A study of cats as sentinels of perfluoroalkyls, toxicants of concern in indoor environments, mirrored our findings: cats' degree of habitation in the indoors was positively related to their total PFAS in serum (Bost et al. 2016). Two Polish studies similar to the present study reached different conclusions about the effect of cat lifestyle/habitat on lead exposure. One group sampled 10 pet and 10 feral cats and found that feral cats had higher lead concentration in their fur (2.89 μg/g) versus pet cats (1.0 μg/g) (Skibniewski et al. 2014). However, another study of 44 cats examined three cat lifestyle/habitat groups, finding that lead concentration in fur increased in order from "household outgoing" cats to free-ranging cats to "household not outgoing" cats (Rzymski et al. 2015).

The present study was unique in finding differences in lead concentrations by body condition. In another study of domestic cats, there was no association between animal weight and lead concentration in fur or tissues (Rzymski et al. 2015). No association was found between body mass and lead concentration in the fur of long-tailed macaques (Schillaci et al. 2011). In brown antechinus, black rats, and brown rats, neither snout-to-vent length nor weight had an effect on concentration of lead in hair (McLean et al. 2009). Among Egyptian fruit bats, body weight was not correlated with heavy metal content in fur (Sheta and Beheary 2019). In wood mice, no relationship was found between lead concentration in hair or tissue samples and animal weight or length (Marcheselli et al. 2010). A study of northern mockingbirds found no statistically significant relationship between lead concentration in feather and blood samples and body condition (McClelland et al. 2019). In humans, malnourished children lacking in calcium and other beneficial ions absorb more lead ions (World Health Organization 2019), which could be similar for underweight cats with increased lead concentrations in the present study.

Outdoor cats living near each other seem to experience similar exposure to lead. We analyzed sixteen samples from free-roaming cats from a single neighborhood. The standard deviation of the mean of this group's lead concentration was half the standard deviation of the entire study (0.846, compared to 1.717).

Our attempt to correlate cat fur lead concentration with lead risk metrics suffered from a mismatch between the size of census tracts (one as large as 333.59 km^2) compared to the space utilization of cats. While census tracts are smallest scale for which the Washington State Department of Health ranks lead risk, census tracts cover a heterogenous landscape of exposures, and a tract-level score obscures risk localized at the scale of cat's (or a human's) living environment. The study's high-lead outlier cat demonstrates this heterogeneity, as the lead risk rankings for her tract were 3 and 4 out of 10.

Other researchers have had mixed success in finding small and moderate correlations between lead concentration in a sentinel species and traditional means of measuring lead in an area. Even with finer scale environmental lead data, a study in England and Wales did not find a statistically significant correlation between lead concentration in bat fur and soil sampled from 25 km^2 grid cells (Hernout et al. 2016b). In Australia, researchers sampled fur from small mammal species across a gradient from a decommissioned smelter and compared these results to soil concentrations. A statistically significant positive relationship was found in brown antechinus (R^2 = 0.06), brown rat (R^2 = 0.49), and black rat (R^2 = 0.19) (McLean et al. 2009). Pigeons have been studied as lead sentinels in New York City (Cai and Calisi 2016) by analyzing blood from birds submitted to a wildlife center and children with elevated blood lead levels. Here, researchers found a moderate correlation (r=0.647, p-value=0.042) between lead levels in pigeons by neighborhood and rates of elevated lead in children by neighborhood.

Although we were unable to correlate lead concentration in cat fur to census tract-level lead risk metrics, we propose that pet and free-roaming cats presented to high-volume spay/neuter clinics could serve as a valuable source of data about lead exposure in disadvantaged communities at risk of lead poisoning. Such a surveillance system could be deployed without causing harm to non-human animals or infringing on the efficient operations of such clinics, and samples can be collected and stored without special equipment or training. Most cats in the present study had low concentrations of lead in their fur. High-lead outlier cats would stand out as red flags to prompt follow-up contact with residents of a household (with pet cats) or people living in a neighborhood (with free-roaming and feral cats) to alert them that they may be at risk of increased lead exposure and should discuss lead testing with a medical professional. Additionally, if an outlier cat has caretakers, recommendations could be made to present the cat to a veterinarian for chelation therapy or other medical treatment as necessary, as well as to investigate and terminate the source(s) of lead exposure. While this was a somewhat small and low-power study due to the sample size and variation in lead concentrations, it constitutes the largest and most detailed study of lead in domestic cat fur as of this writing.

As social justice-centered animal welfare and veterinary outreach projects gain popularity and funding in the USA (Hawes et al. 2020), we should seek to piggyback intersectional research onto the infrastructure of these programs.

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Author contribution SA conceived and designed the study, collected samples, paid for lead testing of samples, analyzed the data, and drafted the manuscript. TJK supervised the study and contributed to the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information (see S1_aelurocatleaddata.csv).

Declarations

Ethics approval and consent to participate Incidental sample collection did not require an IACUC protocol application for animal use by the University of Washington. Data about cats provided by their caretakers was covered by the University of Washington Institutional Review Board as STUDY00005055.

Consent for publication Not applicable. No data about individual persons were collected for this study.

Competing interests The authors declare no competing interests.

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