Use of pets as indicators of heavy metal exposure across Sydney

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Abstract

Intense urbanization, industrial development and population growth is responsible for reducing environmental quality and in particular increasing the concentrations of contaminants in soils. Exposure to toxic metals and other contaminants in urban soils may be affecting not only the environment, but also the health of humans and other animals. This may especially be the case for those in cities such as Sydney who are living in residential developments that are in close proximity to past and present industrial centres. This research investigates the utility of domestic dog hair as a surrogate method to determine levels of contamination that may present risks to human health. Because domestic dogs live in the same environment as their owners, the presence of metals in dog hair may indicate a potential risk to human health through soil, dust and atmospheric emissions. Positive linear correlations were found between all metal(loid)s analysed (Cr, Cu, Zn, As, Pb and Hg) and domestic dog hair and soil ($p < 0.05$). The strongest correlation between soil and hair concentrations was found for Cr (Pearson correlation coefficient (PCC) 0.355), while there was a weak to negligible relationship for Cu, Zn, As, Hg and Pb (PCCs 0.090, 0.084, 0.137, 0.089 and 0.022, respectively). Linear regression analyses showed that correlations were significantly positive for Cr, Cu, Hg and Pb concentrations in dog hair and residential soil, very weakly positive for Zn concentrations and negative for As concentrations. The result also indicated that physiological, ecological and environmental parameters such as age, hair colour, gender and type of diet affected uptake of metals, although this the effect was less for Zn, which is present in most dry dog foods. While there are no national or international standards or guidelines for metal concentrations in hair, the use of hair as a biological matrix offers significant advantages in terms of ease of collection and ethics approval compared with blood and other body tissues. While the correlation between soil and hair concentrations for Pb, As and Hg was weak, any concentration of these metal(loids) in body tissue represents a health risk, and accordingly further research to develop guidelines for contamination exposure using hair has utility, as does the use of domestic dogs as a sentinel for potential human health risks.

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Statement of Candidate

I certify that the work in this thesis entitled “**Use of pets as indicators of heavy metal exposure across Sydney**” has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by Macquarie University Ethics Review Committee, Human Ethics Approval: reference number: **<5201400297>** on <**4th June 2014**>, Animal Approval: reference number: **< 2014/020 >** on <**21 May 2014**>.

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1. Introduction

The current study investigates the validity of dogs as sentinels for human exposure to heavy metal and metalloid pollution. It seeks to identify the use and utility of domestic dogs as an indicator of contamination risk. Dogs have similar exposure pathways to infants and young children when living in environments with contaminated soil. The study represents the first investigation in Australia of the suitability of the use of domestic dogs' hair as an indicator tissue for metal lead(Pb), mercury (Hg), zinc (Zn), chromium (Cr) and copper (Cu) and metalloid Arsenic (As) exposure and accumulation. The methods used involve sampling soil and collecting hair from dogs in areas that may have higher levels of contamination due to their current and former industrial use. The study also contributes to the broader spatial interpretation of metal contaminants in Sydney.

There has been a large number of studies of heavy metal contamination of soil and the associated correlation to public health (Caussy et al., 2003; Taylor et al., 2010; Wu et al., 2011; Zhao et al., 2012; Xu et al., 2013; Li et al., 2014). Research has often focused on industrial land (Sun et al., 2010; Wu et al., 2011) or areas of known contamination associated with specific industries, such as smelters (Rogival et al., 2007; McLean et al., 2009; Taylor et al., 2010) and chemical manufacturing (Tiller, 1992; Gowd et al., 2010; Yaylali-Abanuz, 2011). Causes of contamination can often be in dispute (Gulson et al., 1981). This may be the case where background concentrations are naturally high (Taylor et al., 2010) or pathways, particularly where dispersal is via wind or water, may be attributed to multiple sources (Birch and McCready, 2009). Further compounding the challenges of research into this area is the difficulty in obtaining health data that provide a link between one or more pollutant sources and their impact on health (Holmes et al., 2009; Yaylali-Abanuz, 2011; Kurt-Karakus, 2012; Zhao et al., 2012; Du et al., 2013; Huang et al., 2014; Liu et al., 2014). For this reason, surrogates such as animals are often used to assess levels of exposure and potential accumulation in tissues that may compromise human health (Berny et al., 1995; Lopez-Alonso et al., 2000; Kozak et al., 2002; Dunnett and Lees, 2003; Lopez-Alonso et al., 2003; Soban´ska, 2005; Scheifler et al., 2006; Rogival et al., 2007; Mclean et al., 2009; Naccari et al., 2009; Filistowicz et al., 2011; Ali et al., 2013; Kuramshina et al., 2014).

Environmental biomonitoring, including the use of domestic pets and wild animals, has been undertaken in a range of studies (Cumbie, 1975; Beyer et al., 1985; Deneman and Douben, 1993; Burger et al., 1994; Kottferova and Korenekova, 1998; Dip et al., 2001; Sobanska, 2005; Rogival et al., 2007; McLean et al., 2009) involving farmland (Petersson Grawé et al., 1997; Lopez-Alonso et al., 2000; Lopez-Alonso et al., 2003; Patra et al., 2006; Patra et al., 2007; Filistowicz et al., 2011) and urban and suburban areas (Ieradi et al., 1996; Komarnicki, 2000; Dunnett and Lees, 2003; Luftl et al., 2003; Scheifler et al., 2006; Beernaert et al., 2007; Lopez-Alonso et al., 2007; Naccari et al., 2009). However, studies involving household pets living in urban areas that may point to a link between exposure pathways and risks to the health of the rising human population are limited (Sakai et al., 1995; Lopez-Alonso et al., 2007; Castro et al., 2013).

In this context, companion animals such as dogs could be beneficial biomarkers of human exposure to potentially heavy metals. Domestic dogs live in the same habitat as their owners, and are accordingly exposed, at least in part, to the same contaminants (Hayashi et al., 1981; O'Brien et al., 1993; Berny et al., 1995; Backer et al., 2001; Kozak et al., 2002; Dunlop et al., 2007; Lopez-Alonso et al., 2007; Schmidt, 2009; Bischoff et al., 2010; Reif, 2011; Serpe et al., 2012; Castro et al., 2013). Household dogs also exhibit similar behaviours to those displayed by young children, such as crawling on the floor and eating or chewing food that may have been exposed to dirt and dust (Berny et al., 1995). Dog can also develop many of the same diseases as humans, such as immunological disorders (Felsburg, 2002; Dunlap et al., 2006; Castro et al., 2013). Several studies have noted a higher incidence of various diseases such as hyperthyroidism, malignant lymphoma, chronic renal failure and cancer in household animals that were attributed to their lifestyle and exposure to chemicals in their diet (Reif et al., 1998; Martin et al., 2000; Hughes et al., 2002).

In this study, pet dogs have been used as a biomarker of human exposure to heavy metals and metalloids. This approach builds on the work of a number of researchers who have examined the accumulation of metals in various parts of the body including the blood (Berny et al., 1994; Berny et al., 1995), kidneys and liver (Lopez-Alonso et al., 2007; Serpe et al., 2012), serum (Park et al., 2005; Venier and Hites, 2011; Ali et al., 2013), bones (Palczewska-Komsa et al., 2014) and hair (Hayashi et al., 1981; Sakai et al., 1995; Kozak et al., 2002; Dunlap et al., 2007; Castro et al., 2013).

Hair is a non-invasive biomarker that has been chosen in this study because it grows at an almost invariant rate and can reflect exposure to metal(loid)s in different periods in the past (Raab et al., 2002; Hasan et al., 2004; Rashed and Soltan, 2005; Patra et al., 2007; Castro et al., 2013). Further, trace levels of contaminants can be distinguished in a single strand (Steely et al., 2007; Castro et al., 2013). In addition, metal(loid) accumulations in hair are generally higher than in body fluids (Teresa et al., 1997; Lui, 2003) and occasionally even higher than in organs (Hariono et al., 1993; Halbrook et al., 1994; Liu, 2003). Furthermore, metal(loid) accumulations in hair have been shown to have a positive and linear correlation with soil contamination (Rashed and Soltan, 2005; D'Have et al., 2006; Beernaert et al., 2007; McLean et al., 2009) and diet (Rashed and Soltan, 2005; Dunlap et al., 2007; Serpe et al., 2012; Castro et al., 2013).

Dog hair offers several advantages over human hair as a biomonitoring tool. Samples are easily obtained, and there are minimal legal issues related to bioethics conventions and principles (Schramm, 1997; D'Have et al., 2006; Castro et al., 2013). In addition, samples do not need refrigeration for storage or transportation, and the preparation and procedure required for elemental analysis of dog hair is considerably easier and less time consuming than those required for organs and body fluids.

In this study, urban suburbs were selected based on their potential for soil contamination reflecting past and present land use (Karim et al., 2013). This includes areas identified by the NSW Environment Protection Authority that are known to be contaminated. The rationale for this approach follows the study of Beavington (1973) where it was shown that the levels of metal concentrations in soil in urban areas in Wollongong were ten times higher than in rural areas (noting that at the time Wollongong was a major iron and steel manufacturing town).

The development pattern of Sydney has seen and continues to see former sites of industrial land use progressively replaced by residential housing as a result of modern urban planning and more recently a focus on consolidation and gentrification (Ashton and Freestone, 2008).

Soils in areas that have experienced longer periods of industrial use may have greater accumulations of metals than those in areas with a shorter industrial history (Chen et al., 2005). This has been seen in urban parks where high levels of heavy metal concentrations have been found, reflecting either the former use of the site for industrial purposes or its close proximity to industry (Chronopoulos et al., 1997; Madrid et al., 2002; Imperato et al., 2003; Chen et al., 2005; Wang et al., 2006; Massas et al., 2010).

1.1. Objectives

The overall aim of this study is to demonstrate the utility of dog hair as a bioindicator of pollution. The specific aims of this research are: first to investigate the usefulness of household dogs' (*Canis lupus familiaris*) hair as a non-invasive indicator of exposure to soil containing lead, mercury, zinc, chromium, arenic and copper; second to determine the extent of spatial differences in metal contamination in soil and dog hair between suburbs known to have higher levels of metal contamination (Surry Hills, Alexandria, Erskineville, Mascot, Botany Bay and St Peters) and suburbs with predominantly residential land use (South Turramurra); and third to detect the impact of a regular dog food regime, including commercial diets and human-like diets, on levels of metal(loid)s in dog hair.

1.2. Hypothesis

Dog hair has the potential to be used as a surrogate method for testing metal(loid) accumulation in and human exposure to contaminated soil.

1.3. Research questions

Can dog hair be used as a non-invasive biomarker in environmental and human health studies related to metal(loid) contamination?

Can dogs be used as a surrogate method to show the level of heavy metal exposure?

Is there any correlation between metal accumulation in dog hair and soil samples across suburbs known to have higher levels of metal contamination as a result of their industrial past?

Is there any correlation between concentrations of metals and As in dog hair and various parameters such as age, hair colour, and gender?

Is there any relationship between diet and the level of metal(loid)s in dog hair?

Is there any relationship between concentrations of metals and As in residential soil, urban parkland soil and dog hair?

1.4. Innovation of this study

This study represents the first investigation of the suitability of domestic dog hair as an indicator for metal and metalloid (Pb, Hg, Zn, Cr, Cu, and As, respectively) exposure and accumulation within the Australian terrestrial environment. The research seeks to provide a surrogate method for establishing the level of exposure to heavy metals of vulnerable sections of the community (e.g., infants) through the use of domestic pets. This approach avoids more complex ethical issues related to obtaining tissue samples from infants. In this study, samples of dog hair were obtained using a new method involving combing, rather than the traditional, more invasive method of cutting or shaving hair (Hayashi et al., 1981; Doi et al., 1986; Burger et al., 1994; Sakai et al., 1995; Rashed and Soltan, 2005; Sobanska, 2005; D'Have et al., 2006; Beernaert et al., 2007; Dunlap et al., 2007; Patra et al., 2007; McLean et al., 2009; Castro et al., 2013).

2. Literature review

2.1. Urban soil contamination

Pollution is the contamination of the environment with materials that interfere with human health, quality of life or the natural functioning of ecosystems. A major form of pollution is soil contamination, which involves materials in the soil, mostly chemicals, that are either out of place or present at concentrations higher than normal (in the naturally occurring background), and may have adverse effects on the environment, humans or other organisms. Anthropogenic activities such as air and soil pollution linked to industry and transport accumulate in soils (Wang et al., 2012). Motorized vehicular traffic is one of the most significant anthropogenic sources of a group of heavy metals including Zn, Cu and Pb that are found in the urban environment (Wei and Yang, 2010). In addition to being affected by industrial and transport-related activities, urban soil is also affected by increased imperviousness and compaction, and other forms of contamination (Biasioli et al., 2006).

Soil is a dynamic natural resource that is essential for human life. Because of its intricate matrix, it is the primary recipient of persistent contaminants such as heavy metals (Luo et al., 2007). All soils contain naturally occurring amounts of various metals, reflecting the composition of the original rock material from which the soil was derived (Scazzola et al., 2003). Nonetheless, metals are one of the most significant contaminants affecting urban soils (Biasioli et al., 2006).

Urban soils play a significant role in maintaining the quality of the environment, and can function as both a source of and a sink for contaminants. Soil contamination from urban development and industrialization affects the health of ecosystems, the environment and humans. As cities expand and change their land-use patterns, for example to accommodate an increasing demand for housing (Gowd et al., 2010; Sun et al., 2010), former industrial land is replaced by residential housing (Lindstorm, 2000; Patz et al., 2004; Pielke, 2005). This change to a more sensitive type of land use is regulated by policies relating to contaminated land and remediation requirements. However, these regulations are relatively recent, leaving many older residential areas exposed to persistent contaminants. The extent to which site clean-up can remediate or remove pollutants remains a public health concern (Jennings, 2013).

Heavy metals are one of many pollutant types that present a concern to the environment and to public health. This is due to their long-term toxicity effects, ability to enter the food chain by ingestion, inhalation or absorption through the skin, and potential for bioaccumulation (Yaylali-Abanuz, 2011). While various metals occur naturally in soils, industrial processes, transport and other anthropocentric activities can contribute to higher concentrations, which can cause significant harm to humans, plants and animals (Gowd et al., 2010).

The magnitude of soil contamination is no less than that of air or water pollution. However, the number of scholarly published articles on air and water pollution in Australia is currently greater than that of articles relating to soil contamination, indicating that it is worthwhile investigating the levels of contaminants in Australian soils due to their ability to affect both human and ecosystem health.

2.2. Australian soil contamination

In Australia, there are a limited number of peer-reviewed published papers on the spatial distribution of urban metal contaminants. These studies have mostly focused on large cities (e.g., Tiller, 1992; Olszowy et al., 1995; Markus and McBratney, 1996; Markus and McBratney, 2001; Snowdon and Birch, 2004; Mival et al., 2006; Birch and McCready, 2009; Ying et al., 2009; Birch et al., 2011) and regional mining centres (Gulson et al., 1994; Martly et al., 2004; Taylor et al., 2010; Laidlaw and Taylor, 2011). It is likely that additional data are available in consultant reports linked to development applications for changes in land use, but this material is difficult to access, and is generally very site specific, reflecting the investigation requirements under the state government's land contamination policy (e.g., Department of Urban Affairs and Planning and Environment Protection Authority, 1998).

Tiller (1992) undertook the first comprehensive study of Australian urban soil contamination and concluded that the most prevalent pollutants were metals and organic materials. His research also showed that more than two-thirds of recorded polluted urban sites in New South Wales, South Australia, Victoria and Queensland were polluted by heavy metals. Of the locations in Sydney, he reported that concentrations of heavy metals such as lead, copper, chromium, arsenic, mercury, cadmium and zinc were significantly higher in industrial locations (Tiller, 1992).

2.3. Sources of high concentrations of heavy metals and metalloids in soil

Metal contaminants accumulate in the soil matrix. This can be particularly problematic where the source of contamination is ongoing, for example emissions by industry or transport. The traditional sources, namely the atmospheric emissions of industry and power generation, can combine with vehicular emissions and poorly regulated disposal of domestic and industrial wastes, such as on-site burial, to amplify the contamination problem (Birch et al., 2011). For example, the levels of some heavy metals, such as lead, are high in Australian soils due to a range of industrial activities (Rouillon et al., 2013). Among various soil contaminants that are persistent in the environment and toxic to humans (Biasioli et al., 2006), lead, mercury, zinc, chromium, arsenic and copper have been identified in urban soils in Sydney (Tiller, 1992; Ying et al., 2009; Birch and McCready, 2009).

2.3.1. Land use

A key feature associated with the urbanization of cities is the continual shift of industrial land to urban use and its subsequent concentration in designated locations. Associated with both land-use these patterns in the displacement and pressure by residential land, e.g., the metropolitan strategies for Sydney (NSW Department of Planning and Infrastructure, 2013). Unfortunately, while industry may move on, its legacy in the form of contaminated soil often remains. This cycle of development and redevelopment tends to expand the contamination footprint left by industrial emissions and waste (Snowdon and Birch, 2004). Soil contamination can also result from direct disposal of materials, such as burial (Irvine and Birch, 1998), or via indirect pathways, such as atmospheric emissions (Snowdon and Birch, 2004).

2.3.2. Roads

"Vehicle emissions play a significant role in the supply and distribution of metals and metalloids to the ambient soil, especially in relation to lead as a result of its use in leaded petrol" (Birch et al., 2011). Traffic volume, density and distance from the roadway are known to be major factors in metal(loid) concentrations in soil (Sunchez-Martin et al., 2000), and Australian soils have accordingly been contaminated by vehicular emissions (Gulson et al., 1981). Following the introduction of unleaded petrol in 1986 (Australian Government, Department of the Environment, 2014), vehicular emissions and consequently the ongoing addition of this contaminant have been reduced (Singh, 2001).

2.3.3. Atmospheric deposition

Soil quality may be affected by the generation of airborne dust and particles in the course of various anthropogenic activities. Atmospheric deposition can facilitate the distribution of metals in urban soils over widespread areas extending a substantial distance from the point of discharge (Snowdon and Birch, 2004;). For example, the levels of cadmium, copper, lead and zinc in the soil at Mount Isa were found to be abnormally high as a result of decades of mining activity (Taylor et al., 2010). Airborne sources of metals and metalloids comprise vehicular emissions, smelters, chemical, electrical and metalliferous industries, scrapyards, mining and extractive industries, paint, pesticide and pharmaceutical manufacturing, coal-fired power stations, manufacture of building materials, urban utilities, food and livestock industries, landfill sites, aircraft, trains and bushfires (Tiller, 1992).

2.4. Concentration and spatial distribution of metals in urban Sydney soils

The rezoning of land from industrial to residential use has resulted in changes in the character and use of many, but not all, suburbs and sites. As a more sensitive type of land use, residential land requires a lower maximum concentration of soil contaminants, and site clean-up is often required for new developments (Department of Urban Affairs and Planning and Environment Protection Authority, 1998;

Environmental Protection Agency (EPA), 2013). Metal content in soils can be extremely high in areas surrounding the Sydney CBD compared with traditional residential areas due to the previous existence of industry, roads and railway lines.

2.5. Comparison of heavy metals in background levels in Sydney soils

Levels of heavy metals in sediment, soil and dust are generally high in industrial areas in Sydney (Tiller, 1992; Markus and McBratney, 1996; Irvine and Birch 1998; Snowdon and Birch, 2004; Birch and McCready, 2009; Ying et al., 2009; Taylor et al., 2010; Laidlaw and Taylor, 2011) (Table 1). Irvine and Birch's (1998) study reported concentrations of copper, lead, chromium and zinc that were 108, 40, 29 and 48 times higher, respectively, than background levels in sediment from Port Jackson. Tiller (1992) also reported high levels of arsenic, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel and zinc. However, these metals also tended to occur at elevated levels across Sydney, suggesting that the natural geology may account for some of the reported levels. Tiller's investigation of the background concentrations of metals in non-industrial locations in Sydney showed heavy levels of elements such as arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel and zinc, in surface soils. He attributed this to higher natural background levels associated with these soils.

Table 1. Heavy metal concentrations in soil in 10 different studies.

Soils	Cr (μg/g)	Cu (μg/g)	Ni (μg/g)	Pb (μg/g)	Zn (μg/g)	Cd (μg/g)
World mean (Bowen, 1966)	not available	20	40	10	50	not available
Lane Cove Valley catchment (sandstone) (Riley and Bank, 1996)	not available	30	Na	10	30	not available
Sydney catchment (shale) (Lester, 1987)	90	39	68	23	120	not available
Iron Cove catchment (soils) (Snowdon, 2001)	not available	30	Na	75	75	not available
Homebush Bay catchment (soils) (Hodge, 2002)	not available	6	5	21	10	not available
Upper Parramatta River catchment (soils) (Olmos, 2004)	20	14	10	48	39	not available
Wollongong (Beavington, 1973)	not available	not available	not available	5	3	not available
Sydney Harbour (Birch and McCready, 2009)	not available	20	10	40	40	not available
Surface soils in the Sydney region (Riley and Banks, 1996)	not available	2–250	not available	2–300	1–900	0.01–2

2.6. Public health risk

The consequences of urbanization and industrialization have been linked to a variety of environmental problems, including the major issue of public health (Due et al., 2013; Huang et al., 2014; Liu et al., 2014). Metals have been widely used for many years in commerce, agriculture, industry and medicine, resulting in their continuous release into terrestrial and aquatic ecosystems, which has led to environmental problems and serious health issues (Due et al., 2013). The spread of heavy metals in terrestrial and aquatic environments can affect animal health through various exposure pathways, such as dust, air, water and direct ingestion (Caussy et al., 2003). Some metals, such as mercury, lead, cadmium and arsenic, have no safe limits, and can damage various organs in the body (e.g., the kidneys, central nervous system and thyroid gland) (Holmes et al., 2009). Others metals, such as iron, copper, cobalt, manganese, zinc and chromium, have maximum exposure limits that are set out in various standards and guidelines (Caussy et al., 2003). Thus, exposure to metals and metal compositions continues to be an important public health issue in many countries (Caussy et al., 2003).

Exposure to metals and metal components depends on the specific route by which they are conveyed, i.e. through air, soil, water, or food. Humans and other animals, including pets, can be exposed to metals by ingestion (drinking or eating), inhalation (breathing) or absorption through the skin. Metals may also be inhaled by animals or they may adhere to an animal's fur and be ingested during grooming. They may also be absorbed through the skin of various species, such as slugs (Ryder and Bowen, 1977), or they may be ingested accidentally through soil remaining on food items.

Worldwide studies of risk assessments in relation to exposure to metal and metalloid concentrations in urban soil and dust has demonstrated that of the various avenues of exposure, oral ingestion is generally the most common acute exposure mode for both children and adults, compared with inhalation and dermal contact (Huang et al., 2014). Living near a current or former industrial site or other pollution source that has discharged metals into the environment increases the risk of exposure. Bioindicators, are known to be one of the most common ways of investigating heavy metal toxicity and assessing the risks to human health.

Biomonitors are used in various fields such as public health and environmental studies. They are especially beneficial for investigating levels of exposure in a given area or population. Biomonitoring can assess the extent to which metals or other contaminants are absorbed by an organism, making it one of the most dependable exposure assessment and appraisement methods available (Castro et al., 2013).

2.7. Importance of bioindicators

Bioindicators are biological processes, species or communities that are used to determine the quality of the environment and how it changes over time. They may include taxa or groups of animals and plants that demonstrate symptoms in response to environmental changes or pressure. The most common causes of such changes are anthropogenic activities and the destruction of the biotic system (Martin and Coughtrey, 1982). In natural ecosystems, various types of bioindicators are used to determine the soil and water quality, and how these affect animal and plant health. In urban environments, soil invertebrates are commonly used to assess urban soil quality due to their sensitivity, representativeness, functional importance in the ecosystem and easy collection and storage (Santorufo, et al., 2012).

Table2. Use of various species as an environmental biomonitors in studies.

Species as a biomonitors	**Authors/ Researchers**
Swedish birds	Berg et al., 1966
Bobcats and raccoons	Cumbie, 1975
Wild and captive birds from Hokkaido	Doi and Fukuyama, 1983
Laboratory animals such as mice	Matsubara and Machida, 1985
Various reptiles and invertebrates	Beyer et al., 1985 and Paoletti et al., 1991
Barn owls (Tyto alba guttatus)	Denneman and Douben, 1993
Mediterranean monk seals (*Monachus monachus*)	Yediler et al., 1993
Guinea pigs	Katz, 1993
Opossums	Burger et al., 1994
Horses	Dunnett and Less, 2003
Wild boars (*Sus scrofa*)	Sobanska, 2005
Sheep, goats and camels	Rashed and Soltan, 2005
Blackbirds (*Turdus merula*)	Scheifler et al., 2006
European hedgehogs (*Erinaceus europaeus*)	D'Have et al., 2006
Cattle	Lopez-Alonso et al., 2000; Lopez-Alonso et al., 2003; Patra et al., 2006
Soil-diet wood mice (*Apodemus sylvaticus*)	Rogival et al., 2007
Wood mice	Beernaert et al., 2007
Marsupials	McLean et al., 2009
Common buzzards (*Buteo buteo*)	Naccari et al., 2009
Red foxes (Vulpes vulpes)	Filistowicz et al., 2011
Household animals	Hayashi et al., 1981; Doi et al., 1986; Berny et al., 1994; Berny et al., 1995; Sakai et al., 1995; Kozak et al., 2002; Park et al., 2005; Dunlap et al., 2007; Lopez-Alonso et al., 2007, Atanaskova et al., 2011; Serpe et al., 2012; Vazquez et al., 2012; Castro et al., 2013; Palczewska-Komsa et al., 2014

2.8. Companion animals as bioindicators

Household pets, including cats and dogs, have been used as biomonitors in a number of studies (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; Castro et al., 2013). The utility of domestic pets is that they exhibit many behavioural characteristics that are similar to those of adults and children. Berny et al. (1995) concluded that cats are more representative of human adults, while dogs exhibit some behaviours that are representative of young children, such as crawling on the floor and consuming food that may have been exposed to dirt and dust. The use of pets to assess human health impacts associated with contaminated soil is related to the fact that exposure pathways may be similar, and animal subjects generally present less ethical issues associated with obtaining tissue samples, particularly where young children and infants are concerned (Needham and Sexton, 2000).

Breny et al.'s (1995) study demonstrated that dogs and cats could be a reliable pathway to assessing lead exposure in humans. Breny reported that juvenile dogs showed clinical symptoms of lead poisoning before young children and infants. This suggests the potential use of domestic dogs as surrogates for lead exposure in children. Breny's study also reported a strong interdependence and correlation between blood lead concentration in younger children (≤6 years old) and school-aged children, and outdoor and indoor domestic animals. This relationship was notable between indoor dogs and younger children, which might be a consequence of the similar characteristics and environment of indoor dogs and young children. Breny suggested that given the same sources of lead, such as soil, dust and paint, domestic pets were at greater risk of having high blood lead concentrations than children.

Lopez-Alonso et al. (2007) also found that domestic animals, especially companion dogs, could be good indicators of human metal exposure because they live in the same environment as their owners, and are exposed, in part, to the same sources. Lopez-Alonso et al. investigated concentration levels of metal(loid)s including arsenic, cadmium, mercury and lead in the liver and kidneys (these being the main organs for metal accumulation) in 57 dogs in Spain. Domestic dogs were categorized according to whether they lived in an urban or a rural environment. The results indicated that the levels of toxic accumulation in pet dogs were generally low. However, the levels of Hg in kidney tissue in dogs that lived in urban areas were three times higher than those in dogs that lived in rural areas. Although mercury levels were higher in dogs from urban areas, there were no differences between dogs in urban and rural areas in terms of the other heavy metals analysed (Pb, As and Cd). The most notable outcome of Lopez-Alonso et al.'s study was that habitat (living in rural or urban regions) had no significant effect on the levels of three heavy metal(loid)s (Pb, As and Cd) in the organs of dogs.

2.9. Hair as a non-invasive indicator

Blood, urine, and liver and kidney tissue samples have traditionally been the sources used for assaying trace levels of heavy metals in the human body. Hair, which can function as an accumulative tissue, has also been used as an excretory indicator of trace levels of contaminants (Matsubara and Machida, 1985; Nowak and Chmieinicka, 2000; Moreda-Pineiro et al., 2007; Mikulewicz et al., 2013; Wolowiec et al., 2013). Some research has identified several advantages of using hair as an indicator of metal contamination, including ease of sampling and storage (IAEA, 1985; Moreda-Pineiro et al., 2007; Schramm, 2008; Esban and Castano, 2009; Wolowiec et al., 2013), and the fact that hair can contain higher concentrations of heavy metals compared with blood and urine samples (Wolowiec et al., 2013). Moreda-Pineiro et al. (2007) and Wolowiec et al. (2013) used hair to assess metabolism status and determine levels of metal exposure in the environment and in the workplace. Hair has also been identified as an appropriate biological material for monitoring and specifying the amount of metal the body contains for various purposes, such as nutritional, toxicological or clinical purposes (Jenkins, 1980). Hair is also used by the International Atomic Energy Agency (IAEA) to monitor trends in metal levels in humans (1985).

As with humans, there are various ways to investigate the levels of metal accumulation in other animals. These may include the blood (which is often used for lead), vital organs such as the kidneys or liver, which accumulate metals, the spine and the hair. Comparisons between human and animal hair show that animal hair can potentially be a better biomonitoring tool for heavy metal assessment (Hayashi et al., 1981; Doi et al., 1986; Sakai et al., 1995; Chyla and Zyrnicki, 2000; Sobanska, 2005; Rashed and Soltan., 2005; Patra et al., 2007; Dunlap et al., 2007; Vazquez et al., 2013) due to its exposure to contaminated soil through food (Smol'ianinov and Ashurbokov, 1974; Ashourbekove, 1989; Rashed and Soltan, 2005). It can also be sampled without permanently damaging the animal, can be correlated with the content of heavy metals in the soil (Rashed and Soltan, 2005; McLean et al., 2009) and can be used as a surrogate method for determining the bioavailability of heavy metals. Animal hair can also reflect long-term accumulation and concentration of heavy metals, and serve as an indicator of exposure (Merian, 1991; Ray et al., 1997; Rashed and Soltan, 2005).

In recent years, dog hair has become one of the most reliable bioindicators of metal concentrations. Since 1981, the use of dog hair in environmental, ecological, hygienic and clinical studies has become increasingly popular. The first study of the levels of heavy metals in the hair of household dogs was completed in 1981 by Hayashi et al. Forty hair samples were collected from pet dogs aged between 6 months and 10 years old (equal numbers of males and females) and analysed using atomic absorption spectrophotometry for zinc, copper, lead, manganese and cadmium. High levels of zinc accumulation were seen in both males and females (205.4 ±39.7 μg/g and 210.2 ± 44.6 μg/g, respectively) and in the 8–10-year-old group (227.0±32.2 μg/g). There was no statistical correlation between white and coloured hair in terms of heavy metal accumulation. Concentrations of lead, copper and zinc were higher in the females (2.06 ± 1.14, 36.1 ± 14.5 and 210.2 ± 44.6 μg/g, respectively) than the males (1.77 ± 1.05, 30.5 ± 10.3 and 205.4 ± 39.7 μg/g, respectively), while concentrations of mangenese were higher in the males.

In 2005, Rashed and Soltan investigated the potential of using animal hair from livestock to determine levels of metal contamination in rural Egypt. This study was conducted to assess the levels of heavy metals such as cobalt, lead, iron, manganese, cobalt and nickel in the hair of goats, sheep and camels, related to locally grown pasture and soil, from four areas in Egypt, Aswan city, Kalabsha, Allaqi and Halaiub. They found that metal accumulation in the hair differed according to the pasture and soil in the various regions. Levels of iron and manganese were markedly higher than those of other metals in sheep, while the accumulation of Pb was significantly high in camels. Levels of cadmium and nickel were elevated in goat hair than the levels of these two metals in other animals. Comparison of the four areas that were studied found that the presence of heavy metals in animal hair might be the consequence of the different levels of accumulation by pasture type from the local soil. Levels of iron and manganese were highest in clover, barley straw and Nile tamarisk. In addition, concentrations of Fe and Mn were markedly high in soil from the various areas studied. The key outcome of the study was the strong correlation between metal concentrations in soil, pasture and hair, suggesting a strong nexus between environmental concentration and absorption by hair.

In 2009, McLean et al. undertook a study using native marsupials and rats to determine the levels of metal contamination related to a nearby lead/zinc smelter in Boolaroo, NSW. Hair from the native marsupial species brown antechinus and two species of eutherian mammals, the black rat and the brown rat, as well as soil samples, were collected from 22 sites at various distances (close (<2 km), medium (2–5 km), far (5–10 km) and very far (>10 km)) from the former smelter. The results showed a direct correlation between concentrations of metals in the soil and distance from the smelter. A positive correlation was reported between hair and metal accumulation in the soil for both cadmium and lead in all experimental animals in this study. The size and weight of the animals had no effect on the accumulation of cadmium or lead in hair. These results revealed that hair of all three species might not be an appropriate biomarker for concentrations of zinc and copper. The concentrations of lead in the hair were similar among the three species, while the concentration of cadmium was markedly higher in the hair of the brown rat. This research did not consider the mobility and potential range of the target animals, i.e., their ability to forage beyond the area that might have been affected by the smelter emissions.

In 2013, Castro et al. investigated the use of household dog hair as a bioindicator of the levels of arsenic in groundwater in Argentina, as well as the degree of absorption. Fifty hair samples (40 samples from As-contaminated areas and 10 samples from As-free zones) were collected. Accumulation of arsenic in the hair of dogs from contaminated areas was elevated compared with that of dogs from the reference sites. The results of Castro et al.'s study indicated that the level of As in groundwater at both types of sites was higher than the levels deemed acceptable by the World Health Organization (WHO) for drinking water, and therefore that all residents might be at risk of exposure to toxic levels of arsenic. Another finding from this study was that the levels of arsenic in female dog hair were almost two times greater than those in male dog hair. According to the guidelines, all dogs sampled in this survey were chronically contaminated by arsenic. Based on the results, this study reported that use of domestic animals as biomonitors is a very reliable method for determining toxic levels of contamination by elements in the environment.

2.9.1. Correlations between hair and other sampling methods

There are various sampling methods that can be used to show the levels of metal accumulation in the body tissues of humans and other animals. For example, levels of potentially heavy metals can be determined through sampling of blood, urine, hair, nails, and critical organs such as the liver and kidneys. Some studies assert that hair samples are a reliable way of demonstrating the levels of metals in the body, and should only be used as an alternative or supplementary approach (Matsubara and Machida, 1985; Kruslin et al., 1999; Nowak and Chmieinicka, 2000; Moreda-Pineiro et al., 2007; Oregon Office of Environmental Public Health, 2011; Mikulewicz et al., 2013; Wolowiec et al., 2013). Other studies have reported a strong relationship between the accumulation of potentially heavy metals in hair and in other parts of the body, e.g., blood (Gyori et al., 2005; Patra et al., 2007; Sanna et al., 2007; Vermeulen et al., 2009), urine (Vahter, 1994), tissues from internal organs (Matsubara and Machida, 1985; Have et al., 2006) and nails (Mehra and Juneja, 2005; Abdulrahman et al., 2012).

In 2007, Patra et al. undertook a study to investigate the correlation between levels of lead and cedmium in the hair and blood of cows in various industrial zones in India. Blood and tail hair were sampled from 287 cows from urban and industrial areas and 30 cows from reference areas. The results showed that levels of lead and cadmium were markedly higher in the hair of cows living in industrial zones close to a lead/zinc smelter (15.09 ± 0.85 and 5.72 ± 2.75 μg/g, respectively). Lead (0.99 ± 0.04 μg/mL) and Cd (0.127 ± 0.017 μg/mL) accumulations in the blood were significantly higher in cows living in areas close to a lead/zinc smelter and steel processing plant. The results also demonstrated a strong positive relationship between levels of Pb in the hair and the blood. No relationship was found between the concentrations of Cd in the hair and the blood.

In 2006, D'Have et al. undertook a study using the European hedgehog (*Erinaceus europaeus*) to determine the levels of endogenous metals and As in the hair and spine in order to investigate the levels of contamination by various metals (silver, aluminium , cadmium, cobalt, chromium, copper, iron, nickel, lead and zinc) and arsenic in terrestrial ecosystems. Forty-four hedgehogs were collected and analysed. All the metals listed above and arsenic were detected in all five tissue types that were tested (hair, spine, liver, kidney and muscle). The results demonstrated that positive correlations existed between metal accumulation in the hair and the liver for several metals (silver, aluminium, cobalt, chromium, copper and lead) and arsenic. The findings also indicated a strong positive relationship between the levels of metal concentrations in the kidney and the hair for aluminium, cobalt, chromium and lead. Metal accumulations in the hair were positively related to the levels of metals in muscle tissue for all metals except cadmium and nickel, as well as for arsenic. The study found that overall there were strong positive correlations between metal and arsenic accumulations in the hair, spine and internal tissues.

2.9.2. Relationship between metal(loid)s in dog diet and hair

Very few studies of the correlation between heavy metal accumulation in dog hair and diet exist. Sakai (1995) found that mercury levels were higher in pets that consumed wet-type commercial pet foods that contain mostly fresh tuna than in those that consumed dry-type pet foods. Comparing the relationship between total hair mercury levels and food preferences, Dunlop (2007) found that mercury concentrations were higher among sled-dog populations that consumed traditional village diets mainly comprising fish than among those that consumed commercial food.

2.9.3. Limitations of hair analysis

The use of hair to report contamination exposure and risk is not universally accepted. Rodushkin and Axelssom (2000) have outlined the primary difficulties associated with hair analysis. Druyan et al. (1998) also reported that the validity of reference ranges depends on the analytical methods used, as well as sampling, sensitivity, accuracy and precision. As reported in Schramm's (2008) investigation, there are various sources of errors that can occur during the various steps of hair analysis. These are summarized in Table 3.

Table 3. Sources of error in hair analysis (Schramm, 2008).

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

3. Materials and methods

3.1. Techniques for the measurement of heavy metal and metalloid levels in hair

Advances in technology have led to greater opportunities for biomonitoring of chemical contamination using techniques involving animals or plants that are both safe and reliable. Several analytical techniques (see Table 4) have been used to assess levels of metal concentration in hair, other tissues (see Table 5) and soil. These include neutron activation analysis (NAA) (Bencze, 1990), various atomic spectrometry methods, including inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Ashraf et al., 1994; Chyla and Zyrnicki, 2000), inductively coupled plasma-mass spectrometry (ICP-MS) (Wolfsperger et al., 1994; McLean et al., 2009), flame atomic absorption spectrometry (FAAS) (Mattera et al., 1981; Eltayeb, 1990; Cheng et al., 1996; Nowak, 1998) and electrothermal atomization atomic absorption spectrometry (EAAAS) (Bermejo-Barrera, 1996; Foo and Tan, 1998). One analytical technique that has recently become widely used for elemental analysis of hair is inductively coupled plasma-optical emission spectrometry (ICP-OES) (Moreda-Pineiro et al., 2007).

Although various techniques have been used in different studies, the determination of heavy metal concentrations in soil and hair is still chiefly performed by spectrometry. However, a few studies in recent years aimed at determining the concentration levels of metal(loid)s in dog hair and soil have used other techniques, such as the flame-free AAS method used by Kozak et al. (2007) and the TXRF and ICP-OES methods used by Castro et al. (2013).

Table 4. Various techniques for measuring the presence of heavy metal(loid)s.

Technique	**Advantages**	**Disadvantages**	**Sample source**
Instrumental neutron activation analysis (INAA)	Multi-element analysis Non-destructive No chemical preparation Small sample sizes (1–200 mg) Very low detection limits for many elements	Sensitivity of the method is dependent upon the sample matrix	Solids, gases or liquids
X-ray fluorescence spectrometry (XRFS)	Portable version available Multi-element analysis Non-destructive Stability and ease of use	High cost Does not work on small spot sizes	Solid
Laser-induced breakdown spectroscopy (LIBS)	Portable version available Multi-element analysis Non-destructive	High cost Large interference effects Poor precision	Solids, gases or liquids
Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)	Multi-element analysis Non-destructive Ultra-highly sensitive chemical analysis (ppb level)	Very high cost	Solids
Flame atomic absorption spectrometry (FAAS)	Low cost Simplicity Rapidity	Single-element analysis Poor atomization efficiency	Liquids
Electrothermal atomic absorption spectrometry (ETAAS)	Moderate cost	Single-element analysis	Liquids
Inductively coupled plasma-atomic emission spectrometry (ICP-AES)	Moderate cost Multi-element analysis Can identify and quantify all elements except argon	Poor precision	Liquids
Inductively coupled plasma-mass spectrometry (ICP-MS)	Multi-element analysis Ability to handle both simple and complex matrices High sensitivity Flexibility	High cost Isobaric interference	Liquids

Table 5. Techniques used in studies to determine the levels of metal(loid)s in various tissues.

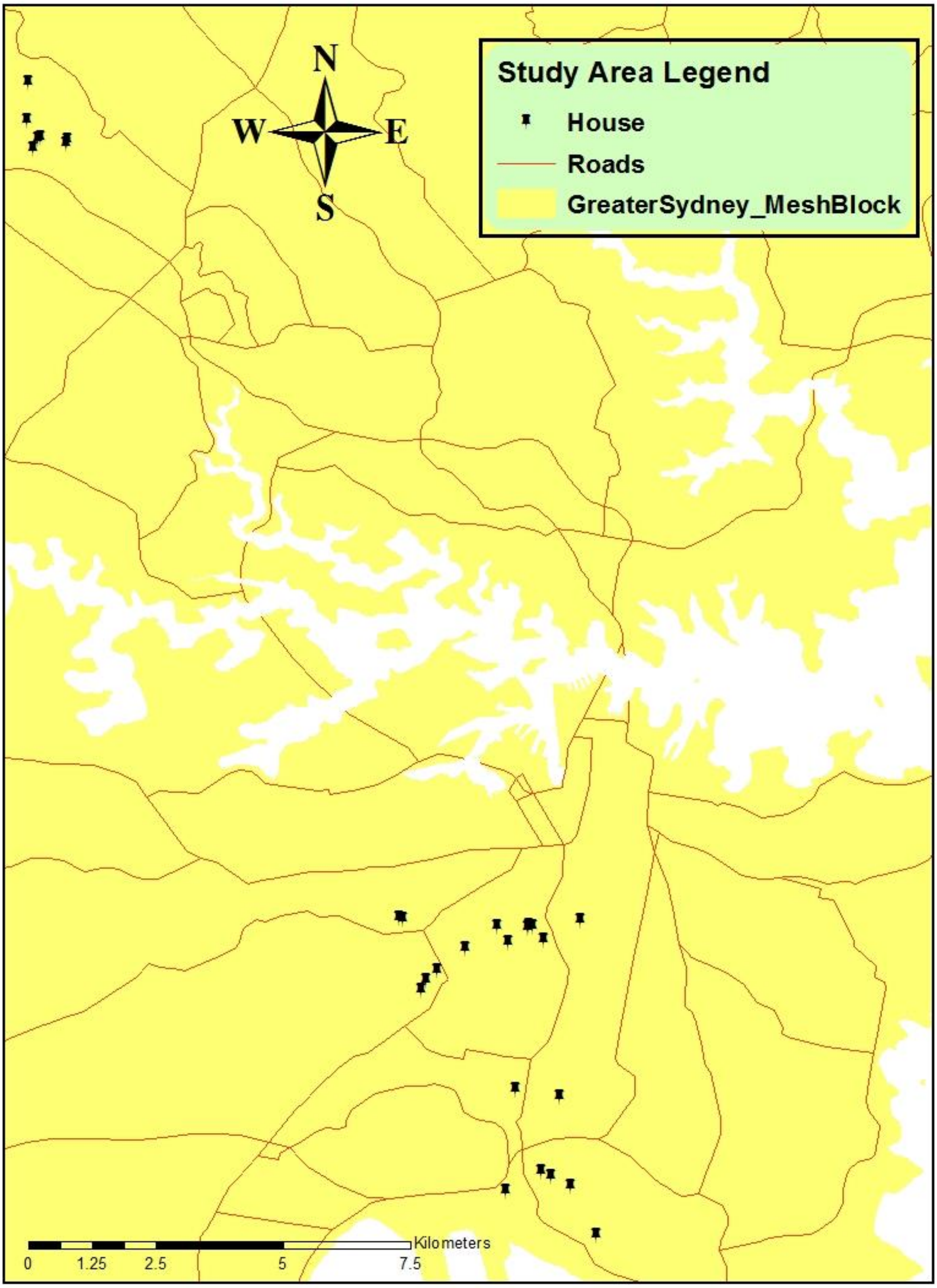
Authors	**Tissues**	**Metal(loid)s**	**Techniques**
Matsubara and Machida, 1985	Hair	Zn & Cd P, Ca, Mg, Fe, Al, Sr, Cu & Mn Ca, Mg, Al, Cu, S, I, Br & Cl	AAS ICP-ES INAA
Yelder et al., 1993	Hair	Zn & Cu Hg	ICP-AES Cold Vapour AAS (CV-AAS)
Berny et al., 1995	Blood	Pb	AAS
Sakai et al., 1995	Hair	Hg	AAS
Kozak et al., 2002	Hair	Cd, As & Pb	Flame-free AAS
Lopez-Alonso et al., 2003	Blood, liver, kidney & muscle	As Cd & Pb Cu & Zn	AAS Graphite furnace AAS (GF-AAS) Flame AAS
Lopez-Alonso et al., 2003	Blood, liver, kidney & muscle	Hg	CV-AAS
Mehra and Juneja, 2004	Hair & nail	Pb, Cd, Cu, Mn, Zn, Fe, Ni, Ca, Mg & Cr	AAS
Park et al., 2005	Serum	Cd, Pb, Hg & Cr	ICP-MS
Sobanska, 2005	Hair, kidney & liver	Hg	CV-AAS
Beernaert et al., 2007	Hair, kidney, liver, lung & muscle	Cd, Cu, Pb & Zn	ICP-MS
Lopez-Alonso et al., 2007	Liver & kidney	As, Cd, Hg & Pb	ICP-MS
Patra et al., 2007	Hair & blood	Pb & Cd	AAS
McLean et al., 2009	Hair	Cd, Cu, Pb & Zn	ICP-MS
Naccari et al., 2009	Feathers, liver, kidney, lung, intestine and muscle	Cd, Pb, Cu & Mn As Zn	GF-AAS Hot Vapour Generation Air-Acetylene Flame
Filistowicz et al., 2011	Hair and skin	C, N, O, S, Cl, Ca, P, Al & Na	ICP-OES
Abdulrahman et al., 2012	Hair and nail	Zn, Fe, Ni, As, Cd, Cr, Mn, Cu & Pb	AAS
Serpe et al., 2012	Liver and kidney	Pb & Cd Hg	GF-AAS CV-AAS
Castro et al., 2013	Hair	As	TXRF & ICP-OES

Nearly all exogenous pollutants can be eliminated from the surface of the hair by a refining and pre-washing process. Therefore, interior pollution levels can be analysed successfully using the residuary hair. On this basis, several studies have already been conducted to investigate the relationship between concentrations in the hair and those in internal tissues (see Table 3), as well as the correlations between concentrations in hair and in soil. As noted in D'Have et al.'s (2007) investigation using the European hedgehog (*E. europaeus*), hair is a better indicator for individual risk assessment than soil alone because hair analysis can provide more direct and specific information on exposure and risk to the animal.

3.2. Sampling sites

The city of Sydney has a long industrial history that has included chemical and metallurgical industries. The study was focused on six the inner city suburbs of Sydney including Alexandria, Botany, Erskineville, Mascot, Newtown, and Waterloo and included 20 residential houses (Figure 1). The inner city suburbs have previously been identified for their potential for soil contamination based on past industrial use and proximity to major transport corridors (Tiller, 1992; Walter et al., 2005). The surveyed area also included 7 urban parks located in Alexandria, Erskineville, Mascot, Botany and Newtown which chosen randomly. A reference suburb, South Turramurra, was located to the north of Sydney that is a residential area with no known former industry and limited agricultural use prior to subdivision in the mid-1900s. Seven dwellings were selected randomly within the residential suburb of South Turramurra (Fig. 1).

Fig 1. Locations of the 27 houses in the inner-west and inner-south areas of the Sydney CBD and South Turramurra. Black pins represent sample locations.



3.3. Sample collection

3.3.1. Hair samples

The Participants in this study were selected randomly among owners that came dog-off-leash parks in Sydney including Mascot Memorial Park and L'Estrange Park in Mascot, Camperdown Memorial Rest Park in Newtown, Alexandria Park in Alexandria, Erskineville Park in Erskineville, Sydney Park in St Peters and Booralee Park in Botany.

Hair samples were collected from dogs (*Canine lupus familiaris*) in winter (June and July) of 2014in accordance with standardized protocolsdue to the time of study is limited to winter . Thirty-six hair samples (28 from surveyed sites and 8 from reference sites) from male and female dogs aged between one and 15 years were collected randomly. This study designed to collect samples from dog hair without considering age, gender, hair colour, also from household soil with different ages. All data for this study was collected randomly and will be used in further investigation by researchers.

A sample of hair was obtained by combing the dog with a plastic disposable plastic brush under the owner's supervision. Each hair sample was placed in a separate metal-free plastic freezer bag prior to analysis. All hair samples were stored at room temperature until analysis. All of the dogs that provided hair samples were assessed by their owners as being healthy and were being fed a normal diet (a combination of dry, wet and fresh food).

A questionnaire was completed by the owner of each dog during the sample collection procedure. The questionnaire provided information on breed, age, gender, hours spent outdoors, use of specific medications, feeding regime, frequency of use of herbicides and pesticides, percentage of garden versus paved surface of the home yard, use of replacement or top-up yard soil in the past 2 years, age of the house and approximate traffic density in their street.

3.3.2. Soil samples

In total 70 samples of surface soil were randomly collected among 27 dwellings, (63 samples from either the rear garden, the front yard or the place in the garden where the dog tended to dig) and urban parkland (one sample from each of the 7 parks surveyed). Surface soil was obtained to a depth of 0–2 cm using a plastic trowel (Standards Australia, 2005). Deionized water and Kimberly-Clark Kimwipes were used to clean the trowel before and after each sample collection. Samples were sealed in plastic zipped bags and stored at room temperature until analysis.

3.3.2. Food samples

Of the 36 dogs sampled, 22 mostly consumed dry food. The remaining 14 dogs were fed fresh meat and vegetables. About 20 grams of dry dog food was collected from each house, sealed in a plastic zipped bag and stored at room temperature until analysis.

3.4. Analytical measurements

3.4.1. X-ray fluorescence (XRF) spectrometry

The technique of X-ray fluorescence (XRF) spectrometry is appropriate for straight analysis for all types of samples. A PANalytical Epsilon 3XL energy dispersive X-ray fluorescence (EDXRF) spectrometer with a Rh anode tube was used for analysis of levels of As, Hg, Zn, Pb, Cr and Cu in the samples of dog food. A plastics calibration was used for quantification, as plastics have matrix compositions (particularly of carbon, hydrogen and oxygen) similar to those found in many foods. Measurement conditions for each element are shown in Table 5. The dog food was placed into a 10 mL polyethylene cup with 3.6 μm polyester X-ray film as the support. The samples were spun at 1 revolution per second, with a total measurement time of 720 s per sample (Table 5). Repeated analysis of ADPOL and ROHS low-density polyethylene certified reference materials (manufactured by PANalytical/DSM Resolve) indicates that accuracy of ±10% is likely. Five sets of measurement conditions for a range of analytes from U to F using EDXRF analysis with a 50 kV, 15 W instrument are listed in Table 6.

Table 6. EDXRF measurement conditions for a range of analytes.

Set No.	Analyte	Line	Voltage (keV)	Current (μA)	Filter	Time (s)
1	As	Kα	50	100	Ag 100 μm	120
	Pb	$L\beta_1$	50	100	Ag 100 μm	120
	Hg	Lα	50	100	Ag 100 μm	120
	Zn	Kα	50	100	Ag 100 μm	120
	Cu	Kα	50	100	Ag 100 μm	120
2	Cr	Kα	20	150	Al 200 μm	120

3.4.2. Hand-held X-ray fluorescence (XRF) spectrometry

Soil aggregates were gently crushed using an agate mortar and pestle prior to sieving in a 2 mm stainless steel sieve to remove debris, stones and large pieces of organic matter. The sieve, pestle and mortar were cleaned in tap water, rinsed with deionised water (American Society of Testing Materials (ASTM) standard) and then dried at room temperature before and after each sample. Soil samples were gently pressed into 10 mL polyethylene cups with 3.6 μm polyester X-ray film (International Atomic Energy Agency, 2005). Samples were analysed using a hand-held XRF spectrometer for a period of 90 seconds. The instrument was recalibrated prior to use each day and after every 27 samples.

3.4.3. Inductivity coupled plasma-mass spectrometry (ICP-MS)

An inductivity coupled plasma-mass spectrometer was used for hair mineral analysis (Yoshinaga, 1996; Kawabata et al., 2000; Yonei et al., 2005). This apparatus is an element analyser that was developed in 1980 and is capable of detecting any elements in concentrations as low as one unit in a billion. The principle is to ionize the atomized sample solution that is placed in the emission source of plasma together with argon gas and then measure the ionized solution with a channeltron. ICP-MS is an industry and research standard method for analysing aqueous solutions of multiple inorganic elements at low levels (one part per billion) in a timely manner. All of the samples of dog hair were analysed using quadrupole ICP-MS. Samples were analysed by the National Measurement Institute (NMI) in Sydney, Australia.

3.4.3.1. Sample preparation procedure

To remove any exterior contamination from the hair, samples were soaked and then washed in 0.5 % Triton X-100 before analysis. This agent is a non-ionic detergent that is highly recommended by Borella et al. (1966) and Chyla and Zyrniki (2000) for removal of trace metals and As from human and dog hair, respectively. After removing excess moisture using filter paper, the hair samples were air-dried at room temperature and then stored in desiccators. Between 0.1 and 0.5 g of dry hair was accurately weighed. Sample digestion was performed by adding between 1 and 2 ml of concentrated nitric acid (HNO_3) through a closed polypropylene tube on top of a boiling water bath (100° C). Digested samples were filtered and analysed using ICP-MS. Indium/iridium was used as the internal standard. Presence of elements was determined using HR-ICP-MS.

3.4.4. Quality assurance procedures

Quality assurance procedures were undertaken for all sampling and analysis techniques. For the soil samples 5 duplicate samples were taken from the material analysed by the hand-held XRF and were analysed by an independent laboratory using standard methods for soil metals using the ICP-MS. The instrument was calibrated at the beginning of the sampling period each day using standard instrument protocols.

Table 7. The data from the handheld XRF and the ICP-MS data and % difference.

Sample description	As (ICP-MS) mg/kg	As (XRF) mg/kg	Cr (ICP-MS) mg/kg	Cr (XRF) mg/kg	Cu (ICP-MS) mg/kg	Cu (XRF) mg/kg	Pb (ICP-MS) mg/kg	Pb (XRF) mg/kg	Hg (ICP-MS) mg/kg	Hg (XRF) mg/kg	Zn (ICP-MS) mg/kg	Zn (XRF) mg/kg
8 Garden Soil	51	65	34	32	180	126	3890	2056	2.55	5.50	782	573
12 In front Soil	1.90	2.30	15.80	22	13	15.20	15	13.20	12.90	20.30	56	47.40
28 In front Soil	5.40	3.60	20	23	45	37	6.60	3.60	1.40	0	470	333
31 Backyard Soil	6.20	5.90	17	48	47	48.20	120	135	0.20	0	210	183
L'Strange Park Soil	8.40	9.80	38.30	47	42	53	140	122	2.20	3.50	95	104
Mean	14.58	17.32	25.02	34.4	65.40	55.88	834.32	465.96	3.85	5.86	322.60	248.08
Standard division	18.33	23.97	9.29	11.25	58.61	37.40	1528.80	796.85	4.59	7.52	271.42	188.71
Experimental F-test	1.71		1.46		2.45		3.68		2.67		2.06	

Comparison between experimental F and critical F with 95 % confidence level (p= 0.05) showed that the experimental F is lower than critical F, so the result of this study had a precision.

Quality assurance for hair samples involved sampling of human-hair-certified reference material (Germany LGC Standard) ERM-DB001 was run once prior to dog hair samples as an external quality control and as a validation of the hair digestion method. The results of the analysis of the hair reference material were 0.29, 32, 210, 0.04, 0.34 and 2 mg/kg for Cr, Cu, Zn, As, Hg and Pb, respectively.

Table 8. The data accuracy from all hair samples by ICP-MS and the hair reference material by ICP-MS.

Metals in hair	Cr	Cu	Zn	As	Hg	Pb
Mean (dog hair)	0.85	14.48	173.61	0.08	0.13	1.91
HRM (Hair Reference Material)	0.29	32	210	0.04	0.34	2
$\lvert \bar{x} - \mu \rvert$	0.56	17.51	36.38	0.045	0.20	0.08

The comparison between mean of each metal(loid) and hair reference material ($\lvert \bar{x} - \mu \rvert$) is low.

For Food sampling, the instrument was calibrated at the beginning of the sampling period each day using standard instrument protocols.

3.5. Metal analysis

3.5.1. Soil samples

The metals lead, mercury, copper, zinc and chromium and the metalloid arsenic were chosen for analysis because these analytes have been identified in previous studies as exhibiting higher concentrations across the Sydney landscape in surficial soil, dust and sediment (Tiller, 1992; Markus and McBratney, 1996; Irvine and Birch 1998; Snowdon and Birch, 2004; Biasioli et al., 2006; Ying et al., 2009; Birch and McCready, 2009; Taylor et al., 2010; Laidlaw and Taylor, 2011).

The assessed metal and metalloid concentrations were compared with the two relevant Australian standards, the National Environmental Protection Council (NEPC, 1999) and the National Environmental Protection Measure (NEPM, 2013). The Canadian, Californian, Norwegian and American soil quality guidelines (CCME, 2013; CEPA, 2005; OEHHA, 2009; NPCA, 2009; EPA, 2013; see Table 9) for residential areas were also examined. These guidelines are widely used because they provide a more conservative standard than the Australian guidelines. The use of these more conservative standards is relevant for metals such as Pb, for which there is no known safe exposure (Lanphear et al., 2005; Jennings, 2013).

Table 9. National and international soil guidelines for residential and parkland areas (mg/kg dry weight).

Element	Australian standard: (NEPC, 1999; NECM, 2013)			Canadian standard: residential/parkland soil (CCME, 2013)	Californian standard: residential soil (CEPA, 2005; OEHHA, 2009)	Norwegian standard: residential soil (NPCA, 2009)	American standard: residential soil (EPA, 2013)
	Residential (with garden/ accessible soil)	Residential (paved yard/with minimal opportunities for soil access)	Recreational (public open space such as parks, playgrounds and playing fields)				
Copper	6000	30000	17000	63	3000	150	310
Zinc	7400	60000	30000	200	23000	300	23000
Mercury	40	120	80	6.6	18	1.5	2.3
Lead	300	1200	600	140	80	90	400
Chromium	100	500	300	0.4	17	75	0.29
Arsenic	100	500	300	12	0.07	12	0.61

3.5.2. Hair samples

The elements lead, mercury, copper, zinc, arsenic and chromium were chosen for analysis because the literature has indicated that these metal(loid)s have been consistently reported to accumulate within mammalian taxa (e.g., Hunter et al., 1989; Talmage and Walton, 1991; Sobanska, 2005; D'Have et al., 2006; Pereira et al., 2006; Dunlap et al., 2007; Patra et al., 2007; McLean et al., 2009; Castro et al., 2013), and were therefore expected to readily accumulate within Australian domestic dogs' hair. Lead and mercury are non-essential and heavy metals, copper and zinc are essential microelements and arsenic and chromium have no known well-defined biochemical function at present. Because of the generally low levels of heavy metals and metalloids in biological materials, especially hair, common analytical methods cannot be used. This is the main reason why ICP-MS was used to detect levels of metal(loid)s in dog hair.

Table 10. Interpretive guideline for hair minerals (Bralley & Lord, 1942).

Name	**Metabolic Association**	**Potential Intervention**
Highly Toxic Heavy Metals		**(High Levels)**
Arsenic	CNS, Hb, GI toxicity	< 0 Avoidance, chelation
Lead	Hb, CNS, toxicity	<0 Avoidance, chelation, Ca
Mercury	Avoidance, chelation, Ca	<0 Avoidance, chelation, Se
Essential Elements		**Repletion Dose Ranges**
Copper	Detox. pathways	3 - 5 mg/d
Chromium	Insulin target cell binding	200 - 400 μg/d
Zinc	Digestive enzymes & many others	15 - 50 mg/d

3.6. Statistical analysis

Statistical calculations in the present study were performed by the Pearson correlation coefficient (PCC) to find the correlation and linear dependence between two variables. Pearson correlation coefficient is a measure of the linear correlation (dependence) between two variables X and Y, giving a value between +1 and −1 inclusive, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation. Linear Regression then applied to detect linear correlations between two variables. All statistical tests were done using IBM Statistics SPSS 19.

In this study, firstly, the correlation of metals and As concentration in the residential soil (both control site and contaminated sites) and hair of dog was calculated by PCC and Linear Regression. Then, to detect correlation between metals and As concentration in dog hair and some parameters such as age, hair colour and gender, PCC was used. Finally, to determine the correlation of metal(loid)s concentration in dog hair and type of dog diet (fresh food such as meat, dry food such as biscuits, or mixed of both), PCC was applied.

4. Results

4.1. Heavy metals and As concentrations in household dog hair

The concentration of metal(loid)s in dog hair is summarised in Table 11 and shown in Figure 2 (Figure 2 excludes Zn and Cu as their concentrations are much higher than the other analytes).

Chromium was present in all hair samples examined at concentrations between 0.1 and 7.8 mg kg $^{-1}$ (mean value = 0.85) dry weight. Highest chromium concentration was observed in Alexandria. Copper was found in all hair samples at concentrations between 7.4 to 62 mg kg $^{-1}$ (mean value = 14.48) dry weight. Highest copper concentration was detected in Botany. Concentration of Zn which was detected in all hair samples was between 110 and 250 mg kg $^{-1}$ (mean value= 173.61) dry weight. Highest zinc concentration in dog hair was observed in Newtown. Arsenic was present in all of hair samples and concentrations were found between 0.03 and 0.30 mg kg $^{-1}$ (mean value= 0.85) dry weight. The highest arsenic concentration was detected in the reference suburb of South Turramurra and Mascot. Mercury was found in all hair samples, and concentrations were within a range from 0.03 to 0.50 mg kg $^{-1}$ (mean value= 0.13) dry weight. The highest mercury concentration was observed in Mascot. Lead was present in all of hair samples and concentrations were found between 0.10 and 16 mg kg $^{-1}$ (mean value= 1.91) dry weight. The highest lead concentration was detected in Mascot (Table 11, Fig 2). Altogether, level of chromium was surprisingly high in Alexandria. Copper concentration was highest in Botany. Newtown contained highest zinc concentration. Mascot had highest concentration of arsenic, mercury and lead. South Turramurra surprisingly represented highest arsenic level.

Metal concentrations (Zn, Cr, Cd, Pb, Hg) across the target sites (located in the inner city suburbs to the south and southwest of Sydney) (n= 36) were all higher than the control sites (South Turramurra). Arsenic was the exception reporting a suburban average equally high in the control suburb of South Turramurra and Mascot. (Table 12, Fig 3).

Table 9. Concentration of heavy metals and As in hair (mg kg^{-1} dry weight) of all dogs from different suburbs (n= 36).

	Dog Hair (mg/kg)					
Suburb	**Chromium**	**Copper**	**Zinc**	**Arsenic**	**Mercury**	**Lead**
Control Site (Turramurra)	0.39	8.10	150	0.03	0.04	0.20
	0.13	18	150	0.04	0.07	0.12
	0.15	10	160	0.03	0.09	0.19
	0.44	7.40	150	0.04	0.03	0.25
	0.17	8.60	160	0.15	0.03	0.31
	0.40	13	180	0.32	0.03	0.84
	0.23	9.60	150	0.10	0.19	0.28
	0.91	12	170	0.23	0.04	0.85
Newtown	0.10	7.90	160	0.03	0.10	0.19
	3.60	15	250	0.04	0.06	4.90
	0.51	10	150	0.10	0.10	0.28
	1.20	17	200	0.20	0.20	0.38
	2.60	20	200	0.10	0.10	1.80
	1.40	14	160	0.03	0.19	1
Botany	0.35	62	150	0.10	0.30	0.15
	0.29	9	170	0.03	0.08	0.22
	0.17	11	220	0.13	0.21	0.40
	0.47	10	160	0.04	0.03	0.53
	0.22	12	180	0.06	0.08	2.70
	0.18	11	190	0.06	0.13	2.40
Alexandria	0.16	11	210	0.11	0.19	0.61
	7.8	15	170	0.05	0.08	1.80
	0.42	11	150	0.14	0.03	3.30
	0.96	15	150	0.05	0.03	2.60
	0.90	12	160	0.06	0.03	5.60
	0.55	15	190	0.06	0.05	4
	0.38	12	190	0.08	0.08	0.94
	0.46	12	190	0.03	0.08	0.59
Erskineville	2.70	19	190	0.06	0.06	1.50
	0.23	15	190	0.03	0.26	0.54
	0.18	15	200	0.03	0.31	1.30
Waterloo	0.23	9.80	150	0.06	0.27	0.78
Mascot	0.38	11	180	0.20	0.52	1.20
	0.19	11	160	0.10	0.31	0.36
	0.85	42	200	0.10	0.14	9.70
	0.30	9.90	110	0.05	0.17	16.0
Mean	0.85	14.48056	173.6111	0.085278	0.130833	1.911389
SD	1.42654	10.02602	26.31162	0.066267	0.110696	3.110613

Fig 2. Concentration (mean value) of metal(loid)s except Zn and Cu in hair (mg kg $^{-1}$ dry weight) of all sampled dogs (n=36).

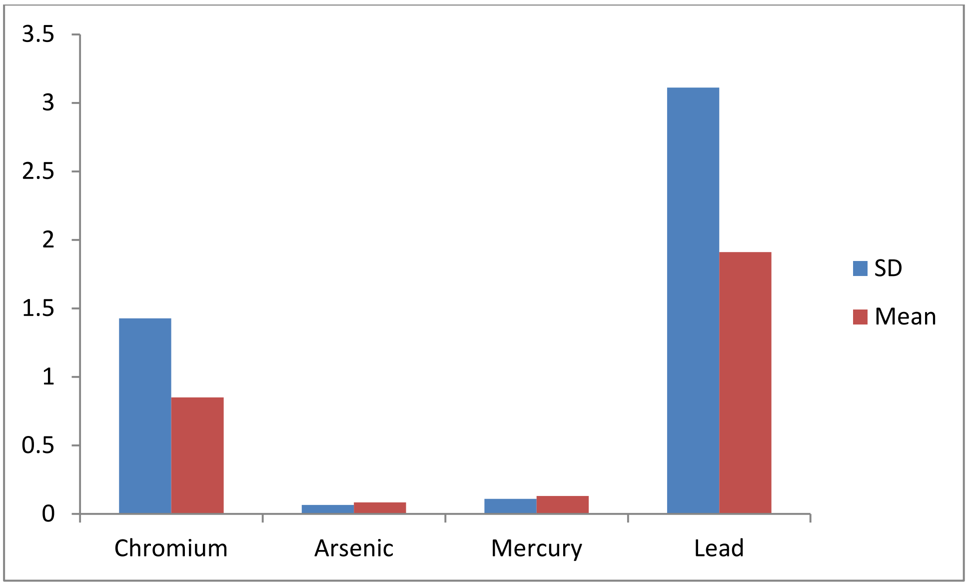
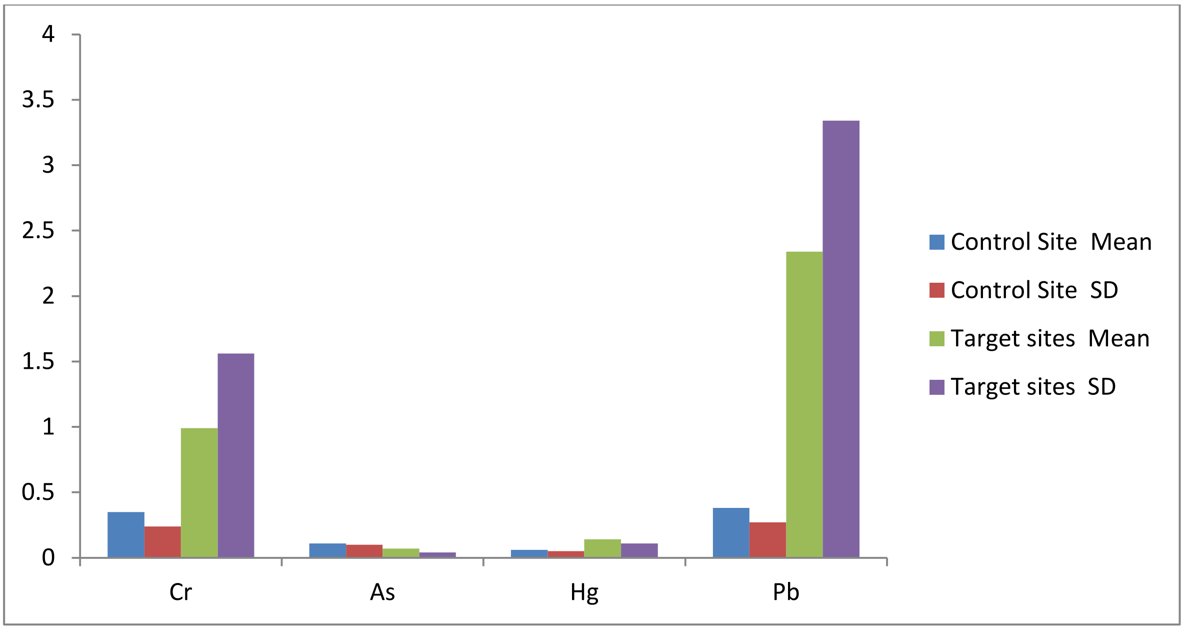


Table 12. Comparison of concentration (mean, Max, Min and Standard Deviation value) of heavy metals and As in hair (mg kg^{-1} dry weight) of all dogs from control site and target sites (n= 36).

		Cr	**Cu**	**Zn**	**As**	**Hg**	**Pb**
Control Site (n=8)	Mean	0.35	10.86	158.75	0.11	0.06	0.38
	Max	0.91	18	180	0.32	0.19	0.85
	Min	0.13	7.4	150	0.03	0.03	0.12
	Standard Deviation	0.25	3.41	11.25	0.10	0.05	0.29
Target sites (n=28)	Mean	0.99	15.52	177.85	0.07	0.14	2.34
	Max	7.8	62	250	0.2	0.52	16.0
	Min	0.1	7.9	110	0.03	0.03	0.15
	Standard Deviation	1.58	11.04	27.93	0.04	0.11	3.40

Fig 3. Comparison of concentration (mean value) of Cr, As, Hg and Pb in hair (mg kg^{-1} dry weight) of all dogs from control site (n=8) and target sites (n= 28).



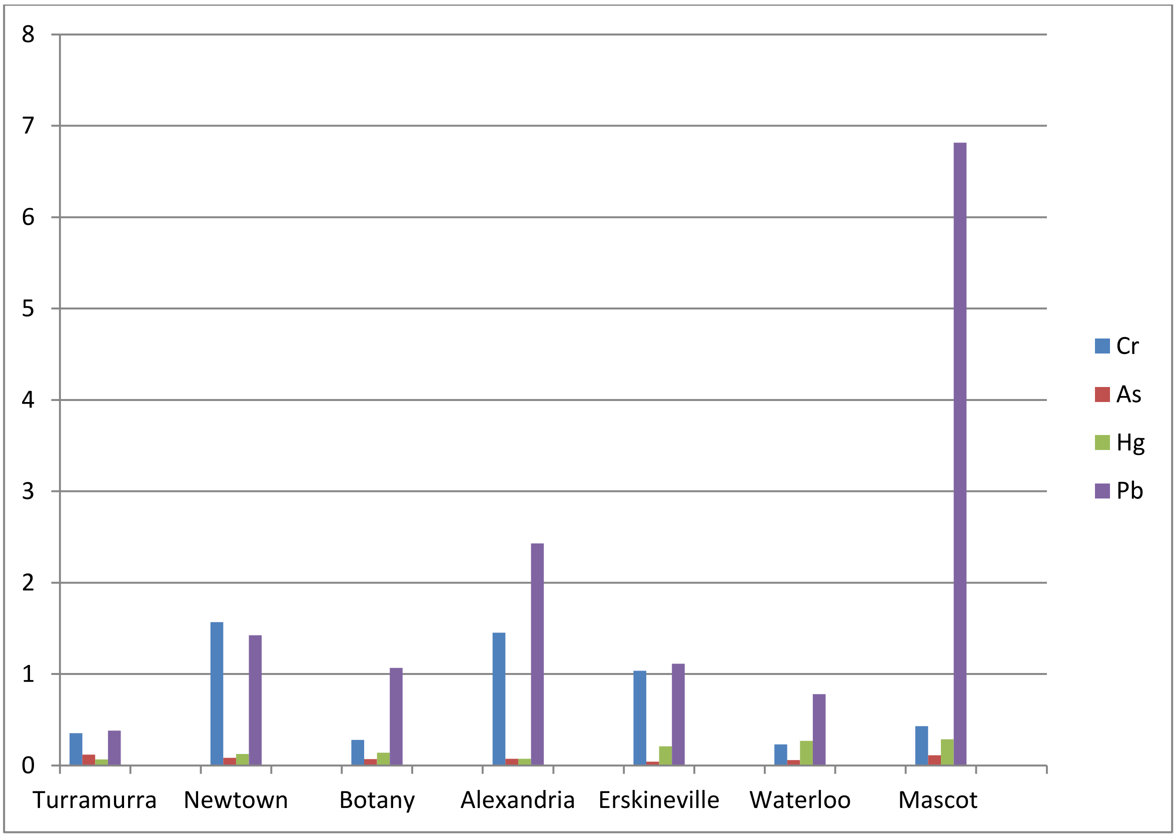
4.2. Comparison of metal(loid)s concentrations in hair according to the area

The results of comparison of metal(loid)s concentrations in hair according to the areas of study are summarised in Table 13. Highest concentrations of chromium in household dog hair were detected in Newtown (mean value= 1.56) followed by Alexandria (mean value= 1.45) and Erskineville (mean value= 1.03), while levels of copper were highest in Botany area (mean value= 19.18) followed by Mascot (mean value= 18.47). Among studied areas, Erskineville had a highest concentration of zinc (mean value= 193.33) followed by Newtown (mean value= 186.66). Highest concentrations of arsenic in dog hair were found in Turramurra and Mascot (mean value= 0.11). Concentrations of mercury was highest in Mascot and Waterloo (mean value= 0.28 and 0.27, respectively. lead was highest in Mascot (mean value= 6.81,) followed by Alexandria (mean value= 2.43) (Table 13 and Fig 4).

Table 13. Mean concentrations of metals and As in dog hair among studied areas (mg /kg d.w.).

Mean value/ Suburb	**Cr**	**Cu**	**Zn**	**As**	**Hg**	**Pb**
Turramurra (n= 8)	0.35	10.83	158.75	0.11	0.06	0.38
Newtown (n= 6)	1.56	13.98	186.66	0.08	0.12	1.42
Botany (n= 6)	0.28	19.18	178.33	0.07	0.138	1.06
Alexandria (n= 8)	1.45	12.87	176.25	0.07	0.07	2.43
Erskineville (n= 3)	1.03	16.33	193.33	0.04	0.21	1.11
Waterloo (n= 1)	0.23	9.80	150	0.06	0.27	0.78
Mascot (n= 4)	0.43	18.47	162.5	0.11	0.28	6.81

Fig 4. Cr, Hg, Pb and As content in dog hair among studied areas (mg /kg d.w.).



4.3. Heavy metals and As concentrations in soil of residential houses

The results of metal(loid)s concentrations in soil of dwellings are summarised in Table 14 and 15. Chromium was present in all examined samples at levels between 165 and 12 mg kg $^{-1}$ (mean value = 37.9) on dry weight in soil. Copper was found in all soil samples, and concentrations were within a range from 285 to 7.4 mg kg $^{-1}$ (mean value = 14.55) on dry weight. Concentration of zinc which detected in all soil samples was between 1014 and 6.8 mg kg $^{-1}$ (mean value= 239.5) on dry weight. Arsenic was present in 90 % of all soil samples and highest concentration was 65 mg kg $^{-1}$ (mean value= 12.8) on dry weight. Mercury was found in 30.5 % of soil samples, and highest concentration was 5.5 mg kg $^{-1}$ (mean value= 1.05) on dry weight. Lead was present in all of residential soil samples and concentrations were found between 2506 and 5.3 mg kg $^{-1}$ (mean value= 267.1) on dry weight.

Table 14. Concentrations of heavy metals and As in residential soil (mg kg $^{-1}$ d.w) from different suburbs (n= 64).

Suburb/ Metals	Cr	Cu	Zn	As	Hg	Pb
Turramurra	23	15.80	33.50	3.40	BD	21
	21	16.80	82.30	3.10	BD	7.60
	31	23.70	48.90	2.20	3.80	16.80
	16	8.50	6.80	BD	BD	10.10
	37	15.50	105	5.10	BD	18.50
	37	37	59.30	4.10	BD	17.60
	20	7.70	13.60	1.50	BD	5.30
	50	10.40	14.80	3.50	BD	12.30
	36	46.10	45.90	6.80	BD	30.50
	33	21.30	38.50	4.10	BD	15
	26	7.40	9.50	3.60	BD	8.10
	17	8.40	13.10	2.20	2.60	14.60
	24	14	38.90	3.90	BD	10
	30	7.40	37	2.30	BD	17
Newtown	32	126	573	65	5.50	2506
	44	115	790	46	BD	1588
	44	107	760	38	BD	1311
	21	48.30	498	10	BD	310
	31	54.10	196	12.80	BD	224
	24	61.90	737	5.70	BD	208
	68	147	597	42	5.40	897

Suburbs	Cr	Cu	Zn	As	Hg	Pb
Newtown	35	79	812	17	BD	402
	55	121	488	27	BD	790
	48	135	431	60	BD	937
	23	48.50	136	8	BD	198
Botany	24	28.90	97.30	7.10	BD	41.20
	25	37.90	94.30	7.80	BD	26.10
	22	15.20	47.40	2.30	3.20	20.30
	28	42.90	65.40	4.30	BD	27.50
	12	258	37.20	2.10	BD	14.80
	53	37.40	66.20	11.20	BD	18.50
	58	51.40	144	7.70	3	100.40
	36	32.30	135	5.20	BD	54.70
	70	63	224	40.80	3.50	161
	15	40.80	116	BD	3.30	292
	42	35.50	223	21.70	BD	178
	45	43.30	521	20	4.30	357
Alexandria	72	57	470	37	BD	774
	60	63	426	15	BD	396
	45	78	463	14	5.10	460
	165	70.90	209	BD	BD	18.70
	103	100	343	15	4.50	460
	45	79	266	9.90	3.60	263
	51	177	625	34	BD	1385
	17	34.40	159	5.70	BD	104.60
	44	88	102	20.80	BD	156
	27	45.20	1014	5.40	3.30	84
	48	48.20	183	5.90	BD	135.50
	64	73	281	13.10	BD	166
	12	11.60	33.30	2.30	BD	19.90
	27	30.40	59.30	3.70	BD	45.80
	24	44	88.70	2.60	3.10	45.80

Suburb/ Metals	Cr	Cu	Zn	As	Hg	Pb
Erskineville	67	72	496	15.0	BD	541
	19	27.40	88.10	BD	BD	51.10
Waterloo	25	151	250	9.50	BD	223
	37	78	169	8.20	BD	67.70
	24	17.30	153	5.70	3.60	118.30
Mascot	19	28.30	159	6.40	3.30	172.70
	41	31.20	110	5.60	BD	94.60
	19	29.60	113	BD	3.20	105.60
	23	37	333	3.60	BD	95.30
	23	40.10	142	BD	BD	94.90
	38	54.10	219	5.10	2.80	138.70

(BD: below of detection level)
The value of BD considered zero (0) for statistical proposes.

Altogether, result of this study indicated that Newtown was notably contaminated site among studied areas, particularly for lead. Chromium significantly was high in Alexandria.

Table 15 .Comparison of concentrations (mean, Max and Min value) of heavy metals and As in residential soil (mg kg $^{-1}$ dry weight) between control site (n= 15) and target sites (n= 49).

		Cr	**Cu**	**Zn**	**As**	**Hg**	**Pb**
Control Site (n= 14)	Mean	28.643	17.143	39.079	3.2714	0.4571	14.6
	Max	50	46.10	105	6.80	3.80	30.50
	Min	16	7.40	6.80	0	0	5.30
	SD	9.46	11.71	28.59	1.63	1.18	6.52
Target sites (n= 49)	Mean	40.696	67.247	300.88	14.392	1.2388	344.48
	Max	165	258	1014	65	5.50	2506
	Min	12	11.60	33.30	0	0	14.80
	SD	26.15	47.23	244.18	15.67	1.863	487.36

4.4. Comparison of metal(loid)s concentrations in soil according to the area (residential and urban parkland)

The results of comparison of metal(loid)s concentrations in soil of residential and parkland, are summarised in Table 16 and 17. In urban parkland, chromium was present in all examined samples and its concentrations were highest in L'Estrange Park (47 mg/ kg) on dry weight soil, whereas level of Copper, Zinc and Lead were found in highest level in Newtown Park (79, 215 and 258 mg/ kg, respectively) on dry weight. Arsenic was present in 71.42 % of all parkland soil samples and highest concentration was 11 mg kg^{-1} on dry weight in Erskineville Park. Mercury was found in 57.14 % of parkland soil samples, and highest concentration was 4 mg kg^{-1} on dry weight in Botany Park (Table 146). Non of determined metal(liod)s are above Australian Standard level.

Table 16. Concentration of heavy metals and As in urban parkland soil (mg kg^{-1} wet weight).

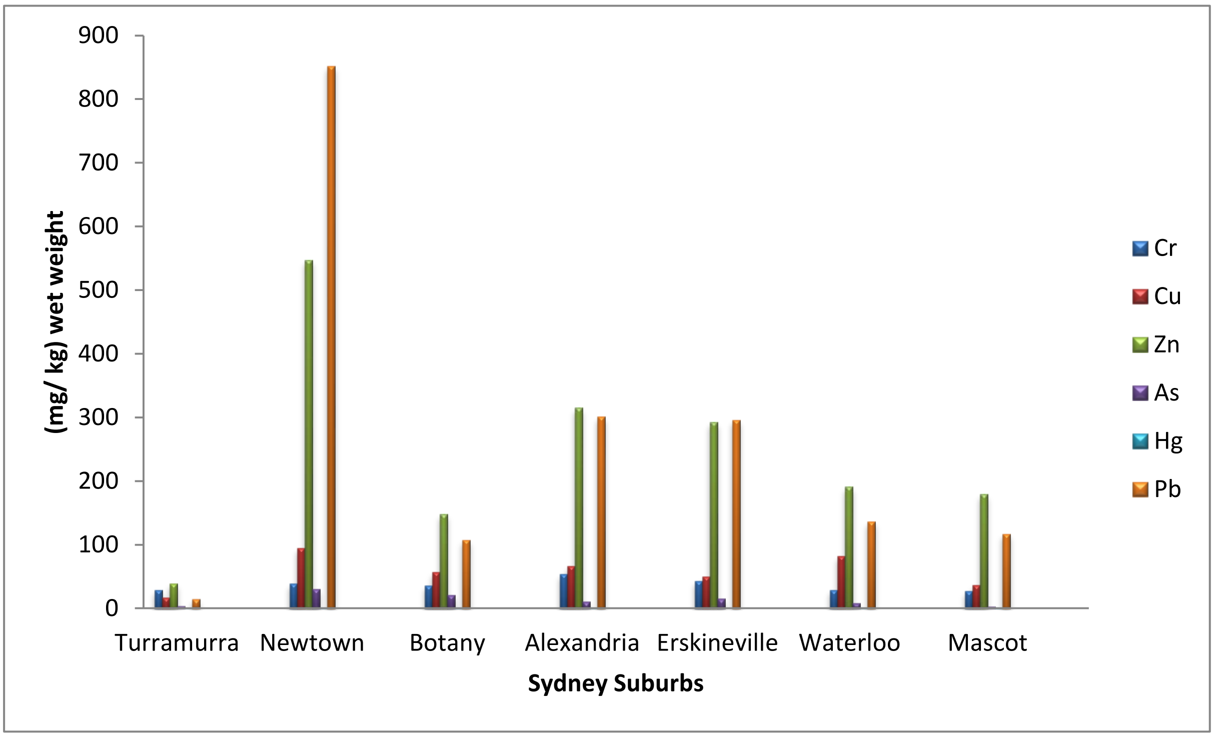
	Park Soil (mg kg $^{-1}$)					
Urban Parkland/ metals	**Cr**	**Cu**	**Zn**	**As**	**Hg**	**Pb**
Botany park	36	32.30	97	5.30	4	51.80
Sydney Park	19	11.30	26.70	0	3.70	24.10
Newtown Park	30	14.30	55	3.50	3.90	61.20
Erskineville Park	34	37.60	158	11	0	161
L'Estrange Park	47	53	104	9.80	3.50	221
Alexandria Park	36	79	215	10	0	258
Mascot Park	38	17.50	109	0	0	33.40

Highest concentrations of chromium in residential soil were detected in Alexandria (mean value= 53.6), while levels of copper, zinc, arsenic and lead were highest in Newtown area (mean value= 94.80, 547.09, 30.13 and 861.90, respectively). Among studied areas, Botany had a highest concentration of mercury (mean value= 1.44) (Table 15 and Fig 4). Newtown surprisingly represented highest concentrations of Pb, which is almost three times more than Australian standard level in residential soil.

Table 17. Mean value of metals and As concentrations in residential and soil among studied areas.

Mean value/ Suburb	Cr	Cu	Zn	As	Hg	Pb
Turramurra (n= 14)	28.90	17	39.10	3.70	0.40	14.30
Newtown (n= 11)	38.60	94.80	547	30.10	0.90	851.90
Botany (n= 12)	35.80	57.20	147.50	20.80	1.40	107.60
Alexandria (n= 15)	53.60	66.60	314.80	10.70	1.30	300.90
Erskineville (n= 2)	43	49.70	292.50	15	0	296
Waterloo (n= 3)	28.60	82.10	190.60	7.80	1.20	136.30
Mascot (n= 6)	27.10	36.70	179.30	2.90	1.55	116.90

Fig 5. Mean concentrations of heavy metals and As in residential soil (mg/ kg d. w.).



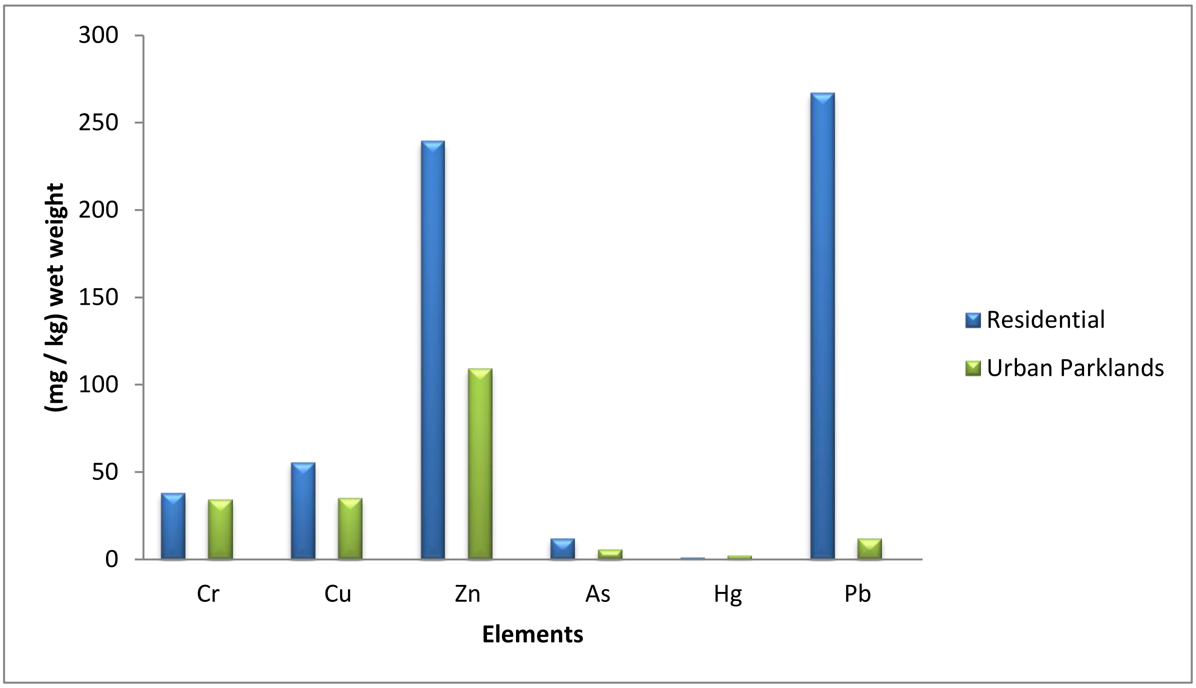
Level of zinc, arsenic, mercury and lead was notably highest in Newtown. Alexandria exhibited highest concentration of chromium and copper in residential soil. Altogether, concentrations of all metals and arsenic were very much higher in the city locations than control site.

Comparison of concentrations of metals and As between residential soil and urban parklands showed average level of mercury was higher in urban parklands, while other metal(loid)s were higher in residential (Table 18 and Fig 6).

Table 18. Comparison of metals and As in soil of urban houses and parklands (mg kg^{-1} d.w.).

Mean value/ Soil	**Cr**	**Cu**	**Zn**	**As**	**Hg**	**Pb**
Residential (n= 63)	37.93	55.47	239.54	11.80	1.04	267.09
Urban Parklands (n= 7)	34.28	35	109.24	5.65	2.15	11.78

Fig. 6. Comparison of metals and As in soil of urban houses and parklands (mg kg^{-1} d.w.).



4.5. Heavy metals and As concentrations in dry dog food

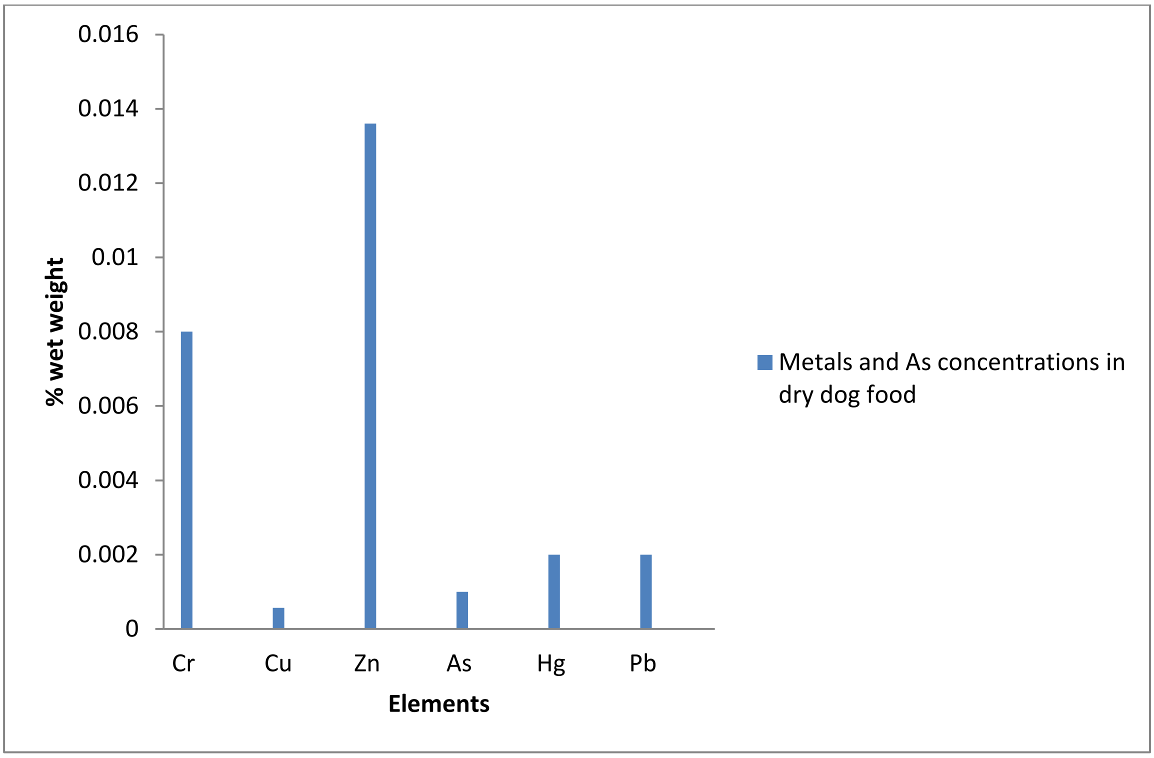
The results of metal(loid)s concentrations in dry dog food are summarised in Table 19. Chromium was present in all examined samples at mean value = 0.008 mg kg^{-1} on wet weight in dry dog food. Copper was found in all samples, and mean concentrations were

0.0005 mg kg $^{-1}$ on dry weight. Mean concentration of zinc, which detected in all samples was 0.0136 mg kg $^{-1}$ on dry weight. Arsenic was present in all samples and mean concentrations were 0.001mg kg $^{-1}$ on dry weight. Mean Mercury concentrations in all hair samples, were 0.002 mg kg $^{-1}$ on dry weight. Lead was present in all of dry food samples and concentrations 0.002 mg kg^{-1} on wet weight (Fig 7).

Table 19. Concentration of metals and As in dog dry food (% d.w.)

	Dog Food (Dry Food)					
Suburb	**Cr (%)**	**Cu (%)**	**Zn (%)**	**As (%)**	**Hg (%)**	**Pb (%)**
Turramurra	0.008	0.0005	0.0192	0.001	0.002	0.002
	0.008	0.0008	0.0119	0.001	0.002	0.002
	0.008	0.0007	0.0142	0.001	0.002	0.002
	0.008	0.0005	0.016	0.001	0.002	0.002
	0.008	0.0005	0.0126	0.001	0.002	0.002
	0.008	0.0005	0.0101	0.001	0.002	0.002
	0.008	0.0008	0.0173	0.001	0.002	0.002
Newtown	0.008	0.0005	0.0086	0.001	0.002	0.002
	0.008	0.0005	0.01	0.001	0.002	0.002
	0.008	0.0005	0.016	0.001	0.002	0.002
Botany	0.008	0.0005	0.0066	0.001	0.002	0.002
	0.008	0.0005	0.0084	0.001	0.002	0.002
Alexandria	0.008	0.0005	0.0099	0.001	0.002	0.002
	0.008	0.0006	0.041	0.001	0.002	0.002
	0.008	0.0006	0.0139	0.001	0.002	0.002
	0.008	0.0005	0.01	0.001	0.002	0.002
	0.008	0.0005	0.0123	0.001	0.002	0.002
	0.008	0.0008	0.0125	0.001	0.002	0.002
Erskineville	0.008	0.0005	0.01	0.001	0.002	0.002
Waterloo	0.008	0.0005	0.0105	0.001	0.002	0.002
Mascot	0.008	0.0005	0.0115	0.001	0.002	0.002
	0.008	0.0008	0.0167	0.001	0.002	0.002
Mean	0.008	0.0005	0.0136	0.001	0.002	0.002

Fig 7. Mean concentration of metals and As in dry dog foods.



4.6. Correlation of metals and As concentrations in hair and soil

In order to find a relationship between level of metals and As in dog hair and residential soil, Pearson correlation coefficient was used. Linear Regression Also was applied to detect each certain metal(loid) correlation in dog hair and residential soil. Each sample of soils was paired with each hair which related to one house. For example, house number one have two soil samples and one hair samples. These samples were paired together. The result is summarised in Table 20 and Fig 8, 9, 10, 11, 12, and 13.

Table 20. Correlation coefficient (r) for metal(loid)s concentrations in dog hair and soil.

	Pearson correlation coefficient (r)	Strength of correlation
Concentrations of Metal(liod)s in dog hair and residential soil	0.263	Weakly positive
Concentration of Cr in dog hair and soil	0.355	Moderately positive
Concentration of Cu in dog hair and soil	0.090	No or negligible relationship
Concentration of Zn in dog hair and soil	0.084	No or negligible relationship
Concentration of As in dog hair and soil	0.137	No or negligible relationship
Concentration of Hg in dog hair and soil	0.089	No or negligible relationship
Concentration of Pb in dog hair and soil	0.022	No or negligible relationship

Results indicated that the calculated correlation coefficient of metal(loid)s concentrations in dog hair and residential soil was 0.263. It was concluded that the total accumulation of metals and arsenic in residential soil and dog hair had a positive but weak relationship. Pearson correlation coefficient of chromium was 0.355 and showed a weak positive correlation. PCC for copper, zinc, arsenic, mercury and lead was 0.090, 0.084, 0.137, 0.089 and 0.022, respectively. The results indicated that negligible positive relationships between copper, zinc, arsenic, mercury and lead in dog hair and residential soil.

In addition, the Linear Regression analyses showed correlation between chromium, copper, mercury and lead concentration in dog hair and in residential soil were significantly positive, while this relationship is negative for As and very weak positive for zinc (Fig 8, 9, 10, 11, 12, 13).

Fig. 8. Correlation between chromium concentration in dog hair and in residential soil.

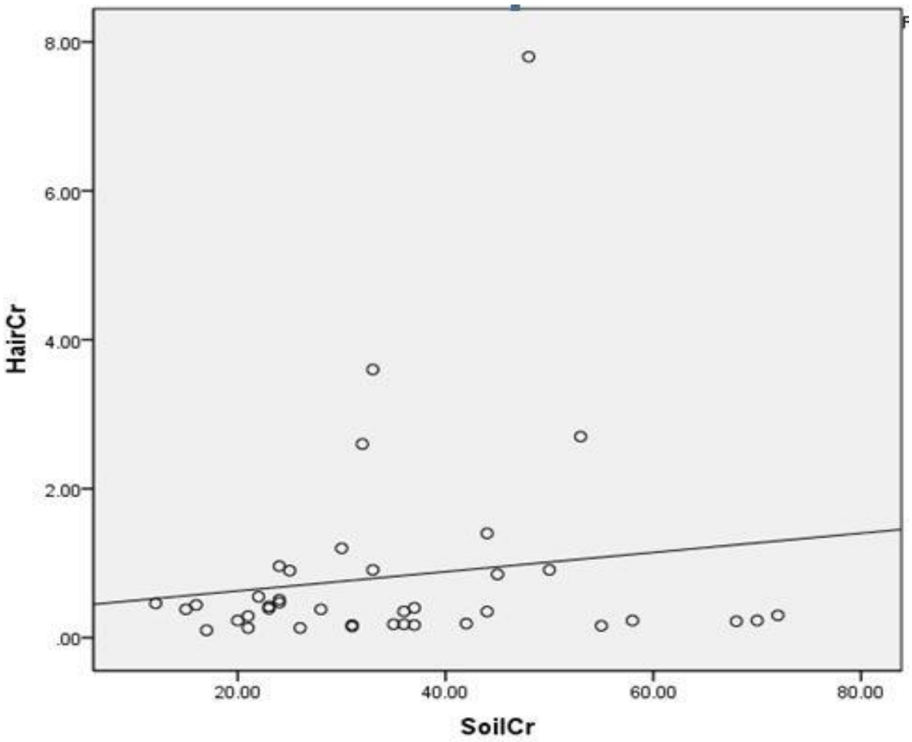


Fig. 9. Correlation between copper concentration in dog hair and in residential soil.

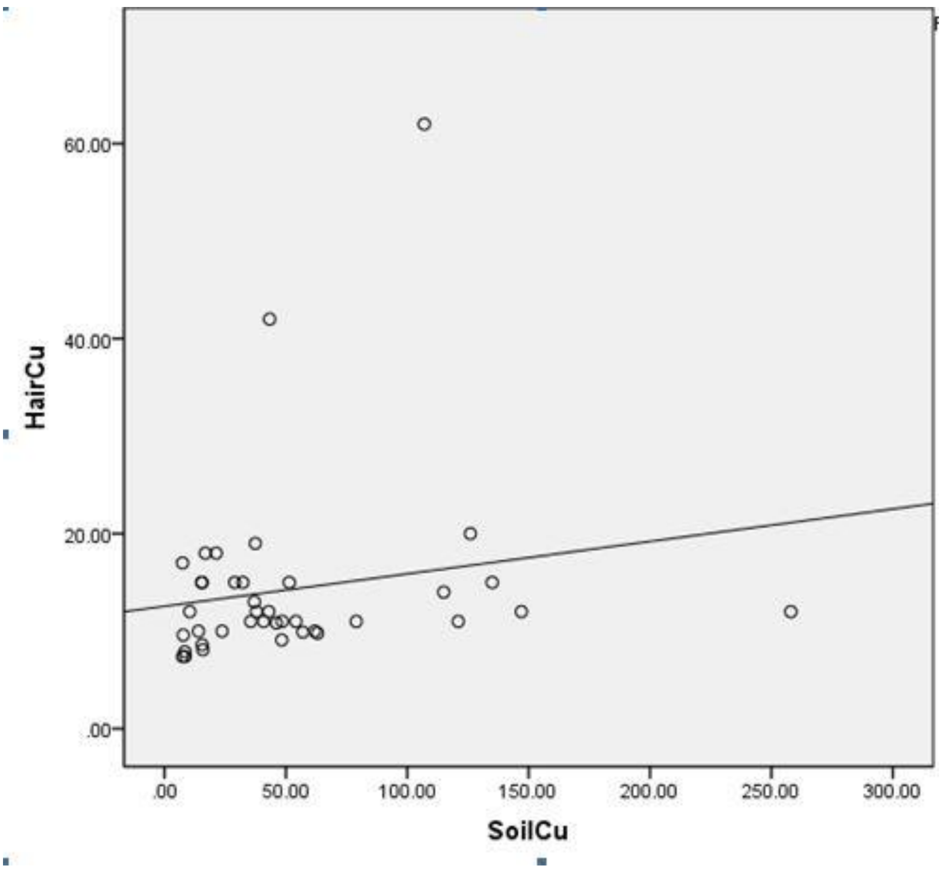


Fig. 10. Correlation between zinc concentration in dog hair and in residential soil.

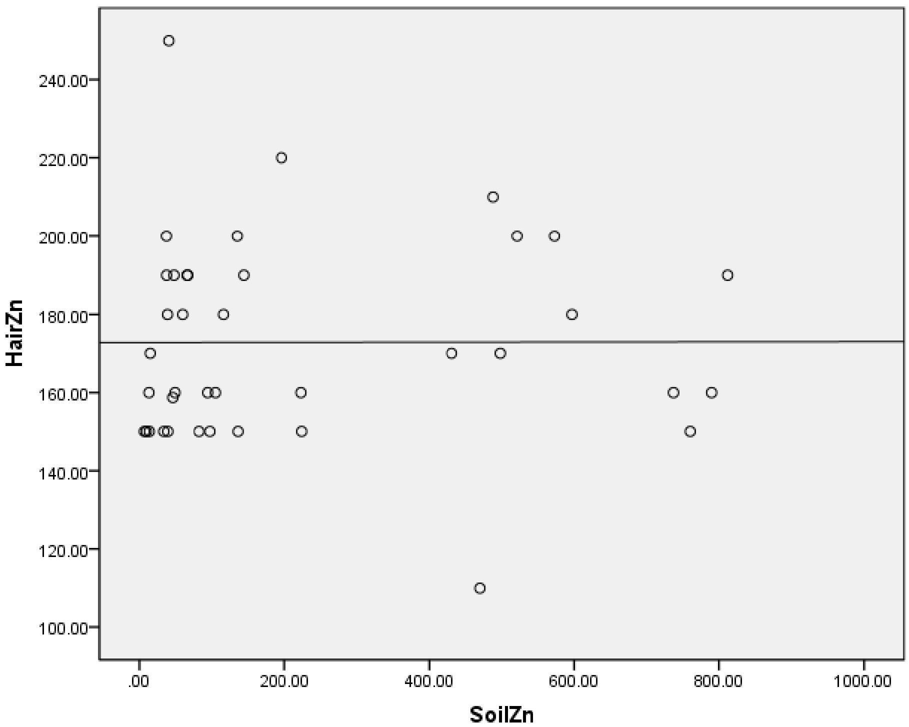


Fig.11. Correlation between arsenic concentration in dog hair and in residential soil.

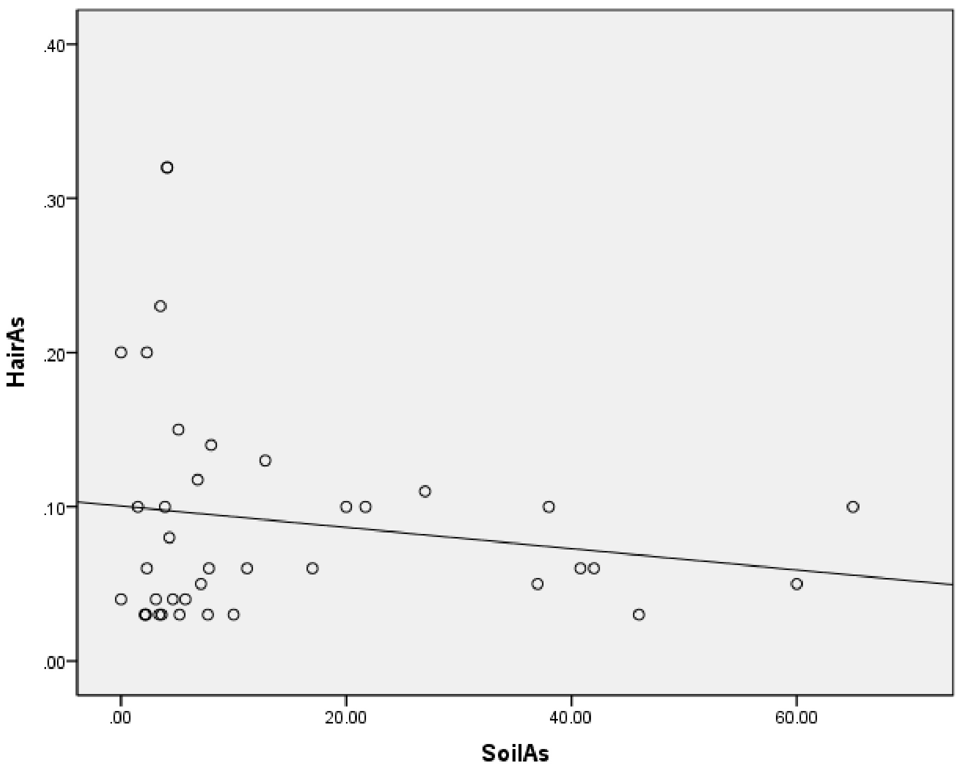


Fig.12. Correlation between mercury concentration in dog hair and in residential soil.

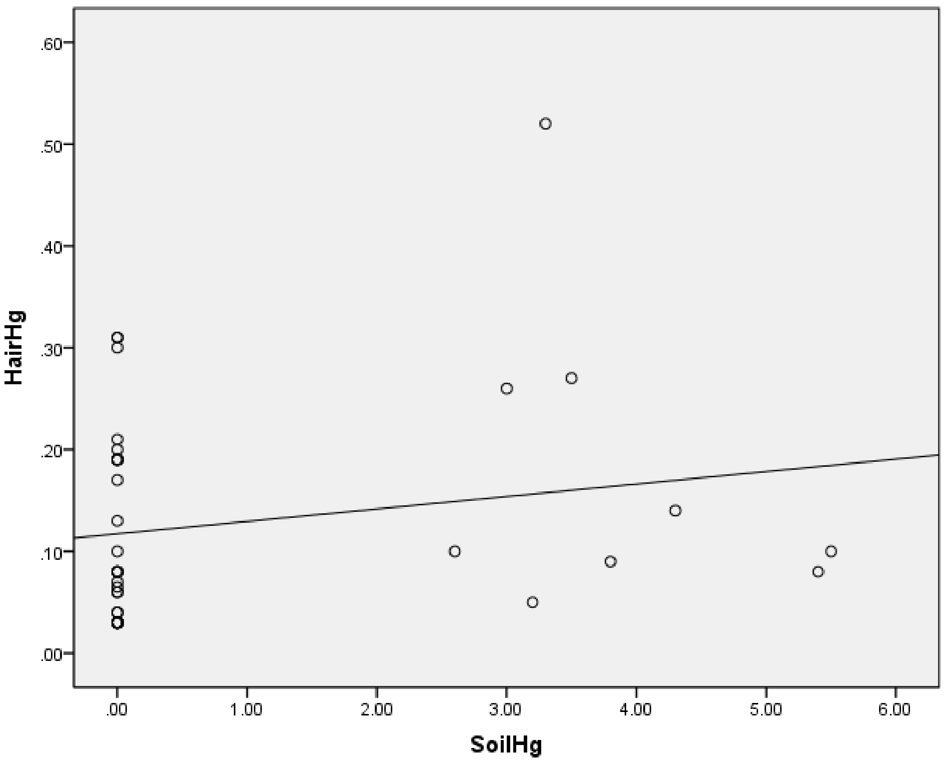
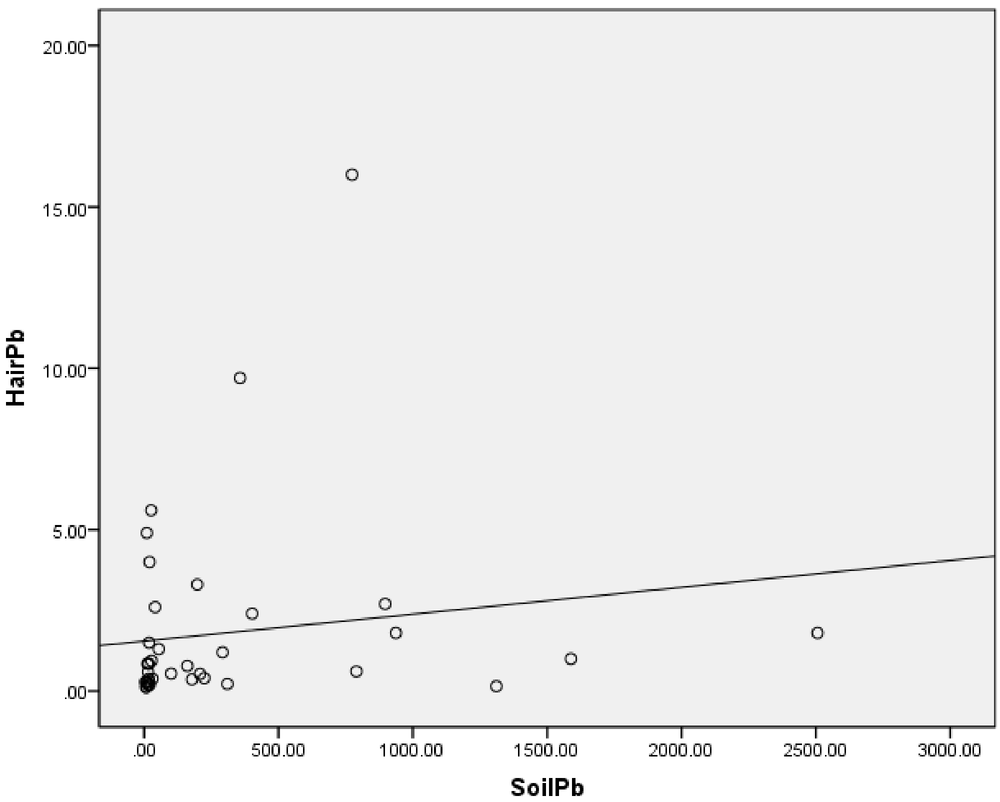


Fig.13. Correlation between lead concentration in dog hair and in residential soil.



4.7. Correlation of metals and As concentrations in hair and various parameters such as age, hair colour and gender

Result of correlation between metals and arsenic concentration in dog hair and age was summarised in Table 21, Fig 14, 15, 16, 17, 18, and 19. In order to find a relationship between level of metals and arenic in dog hair and dog age, Pearson correlation coefficient and Linear Regression were used. Result of correlation between Metals and As concentration in dog hair and hair colour and also gender were also summarised in Table 21 and 22. To detect a correlation between level of metals and As in dog hair and hair colour as well as gender Pearson correlation coefficient was applied (Table 22 and 23).

Table 21. Correlation coefficient for metal(loid)s concentrations in hair and age.

	Total Number (n)	Pearson correlation coefficient (r)	Level of Significance
Concentrations of Metal(liod)s in dog hair and age	36	0.757	0.037

Results indicated that the calculated correlation coefficient between metal(loid)s concentrations in dog hair and dog age was 0.757. It was concluded that the total accumulation of metals and arsenic between dog hair and age had a strong positive relationship. The Linear Regression analyses showed correlation between copper concentration in dog hair and age was significantly positive, while correlation between for arsenic, mercury and lead was weakly positive. There was a negative correlation for zinc and very weak negative for chromium. This meant with age accumulation of copper increased dramatically and for arsenic, mercury and lead increased moderately. Level of zinc was significantly highest in first ages of dog life and with the age, this level was decreased. Same result was observed for chromium. Accumulation of chromium was elevated before adulthood and after that was gradually declined.

Fig. 14. Correlation between Cr concentration in dog hair and age.

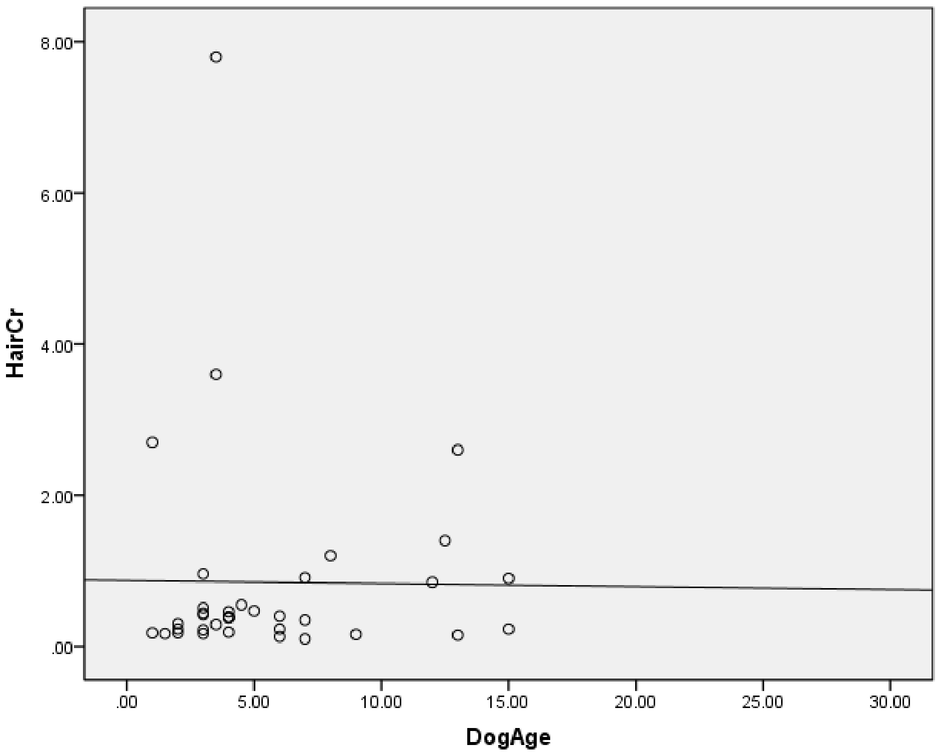


Fig. 15. Correlation between Cu concentration in dog hair and age.

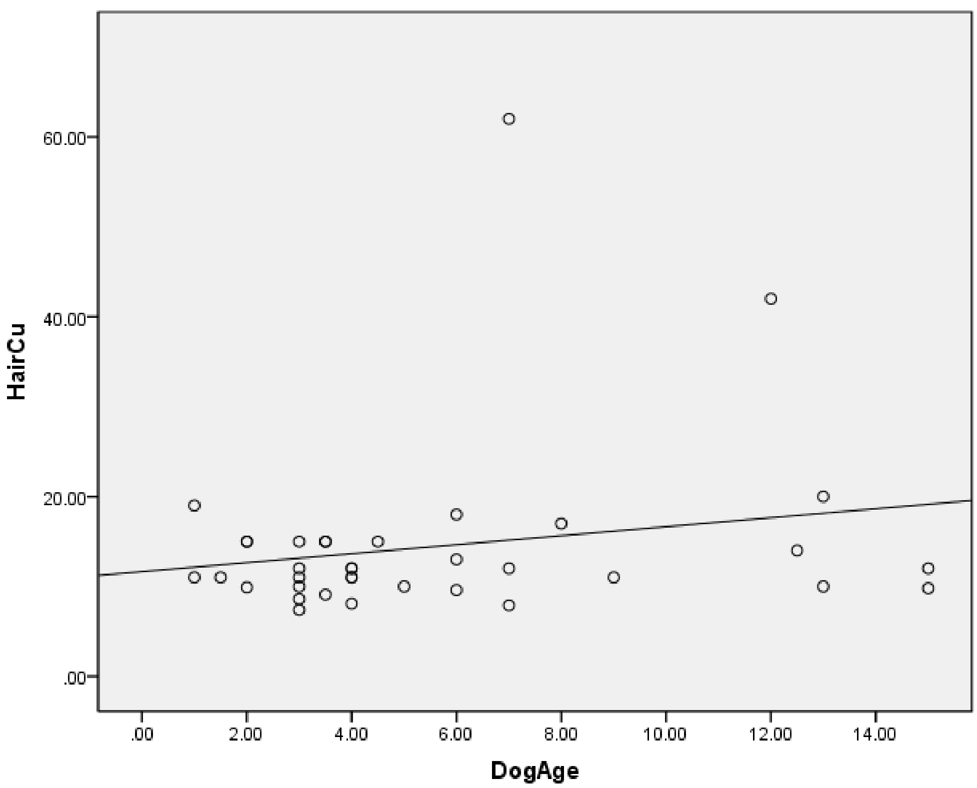


Fig . 16. Correlation between Zn concentration in dog hair and age.

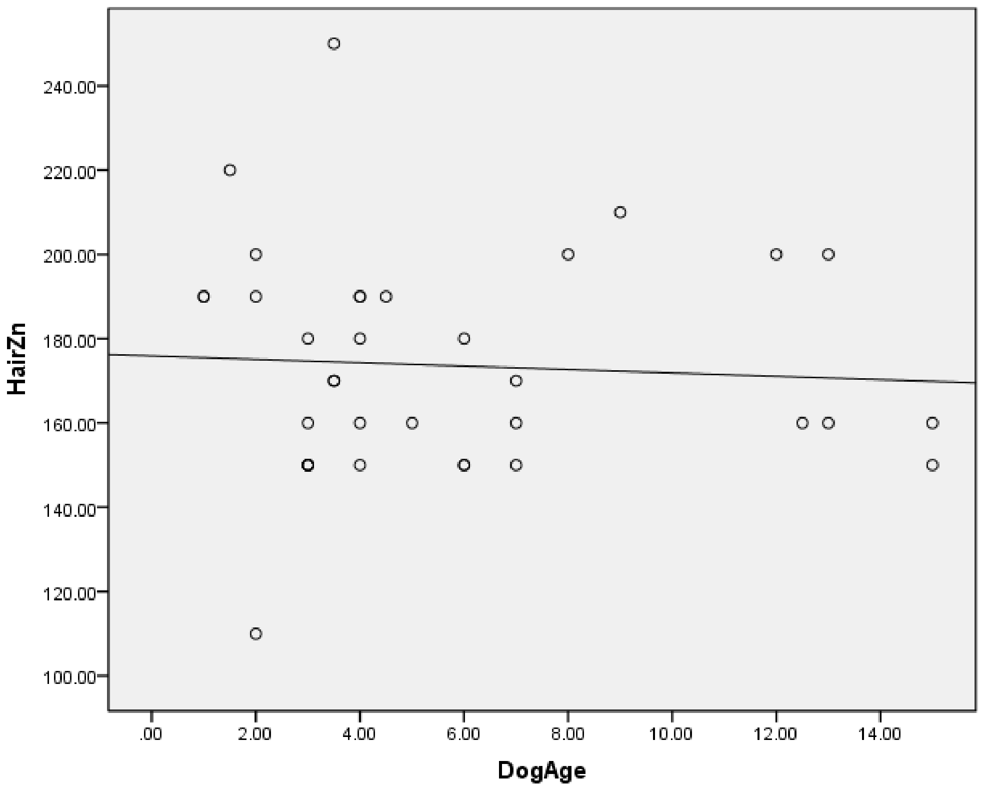


Fig . 17. Correlation between As concentration in dog hair and age.

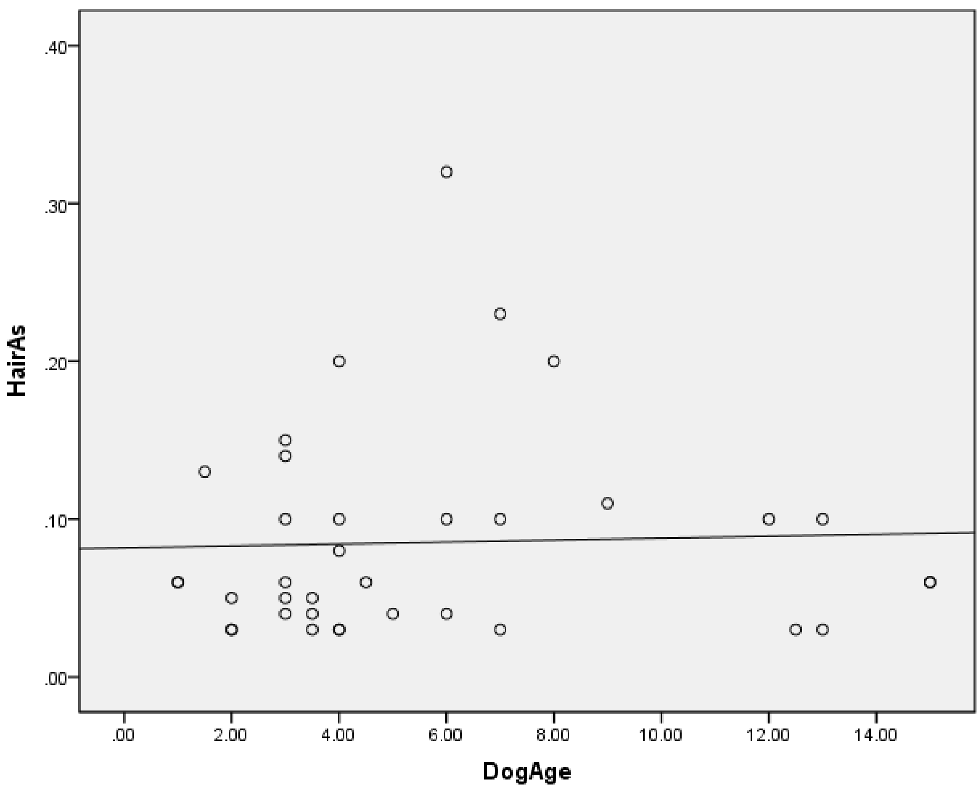


Fig . 18. Correlation between Hg concentration in dog hair and age.

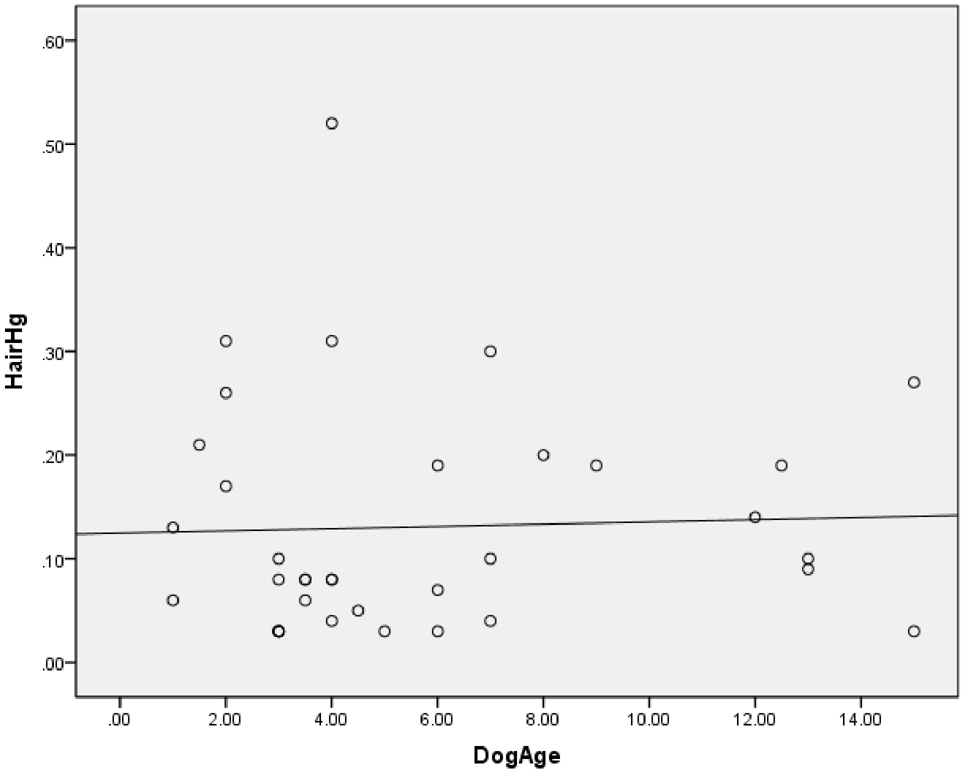


Fig. 19. Correlation between Pb concentration in dog hair and age.

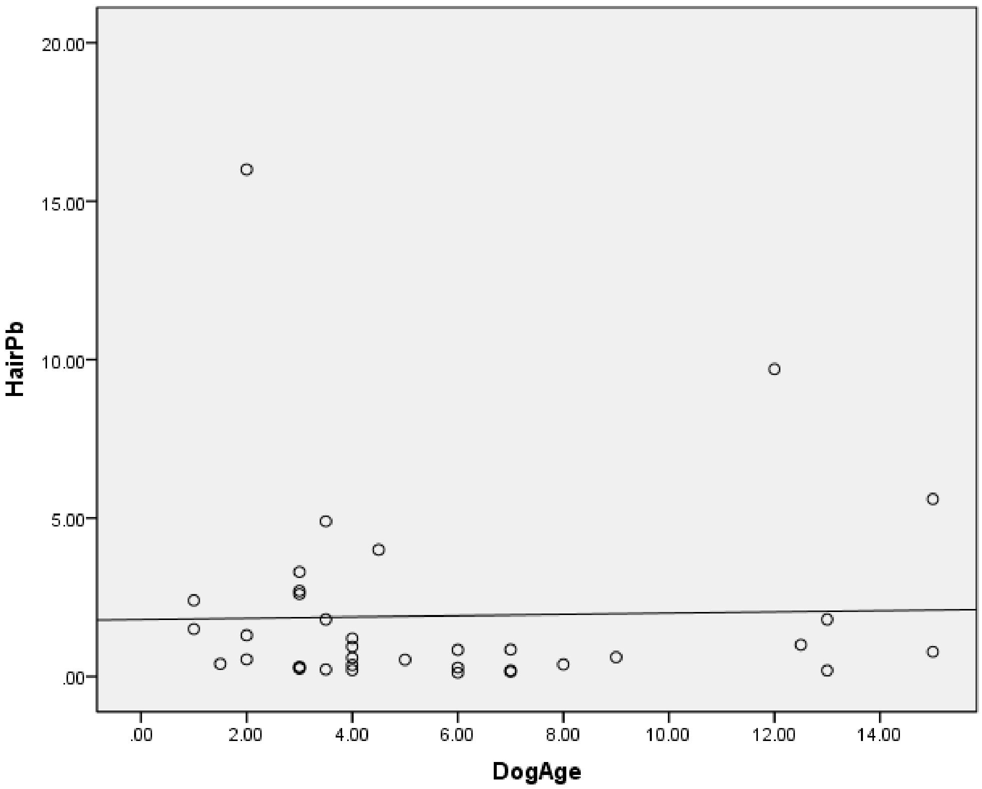


Table 22. Correlation coefficient for metal(loid)s concentrations in hair and hair colour.

	Total Number (n)	Pearson correlation coefficient (r)	Level of Significance
Concentrations of Metal(liod)s in dog hair and hair colour	36	0.632	0.021

Results indicated that the calculated correlation coefficient between metal(loid)s concentrations in dog hair and hair colour was 0.632. It was concluded that there was a positive correlation between the accumulation of metals and As in dog hair and in hair colour.

Table 23. Correlation coefficient for metal(loid)s concentrations in hair and gender.

	Total Number (n)	Pearson correlation coefficient (r)	Level of Significance
Concentrations of Metal(liod)s in dog hair and gender	36	0.802	0.002

Results represented that the calculated correlation coefficient between metal(loid)s concentrations in dog hair and gender was 0.802. It was concluded that there was a very positive correlation between the concentrations of metals and As in dog hair and in dog gender.

4.8. Correlation of metals and As concentrations in hair and type of feed (dry, fresh or mixed of both)

Result of correlation between metals and As concentration in dog hair and in dog food was summarised in Table 24. To detect a correlation between level of metals and As in dog hair and in dog food, Pearson correlation coefficient was applied (Table 24).

Table 24. Correlation coefficient for metal(loid)s concentrations in hair and type of diet.

	Total Number (n)	Pearson correlation coefficient (r)	Level of Significance
Concentrations of Metal(liod)s in dog hair and type of dog diet	36	0.250	0.242

Results indicated that the calculated correlation coefficient for metal(loid)s concentrations(levels) in dog hair and in dog food was 0.250. Thus, there was a weak positive relationship between metal(loid)s concentrations in hair and type of dog feed.

5. Discussion

5.1. Relationship between metal(loid) concentrations in soil and dog hair

Correlations between the concentrations of each metal(loid) in dog hair and residential soil varied. Positive linear correlations were observed for both essential metals (chromium and copper) and non-essential metals (lead and mercury), while the relationship was negative for As and very weakly positive for zinc.

Levels of heavy metals in sediment, soil and dust are generally high in industrial areas in Sydney (Tiller, 1992; Markus and McBratney, 1996; Irvine and Birch 1998; Snowdon and Birch, 2004; Birch and McCready, 2009; Ying et al., 2009; Taylor et al., 2010; Laidlaw and Taylor, 2011). Tiller (1992) also reported high levels of arsenic, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel and zinc. However, these metals also tended to occur at elevated levels across Sydney, suggesting that the natural geology may account for some of the reported levels. Tiller's investigation of the background concentrations of metals in non-industrial locations in Sydney showed heavy levels of elements such as arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel and zinc.in surface soils. He attributed this to higher natural background levels associated with these soils.

Irvine and Birch's (1998) study reported concentrations of copper, lead, chromium and zinc that were 108, 40, 29 and 48 times higher, respectively, than background levels in sediment from Port Jackson.The current study revealed that the soil of target sites was broadly contaminated by metals and arsenic. Concentrations of chromium, copper, zinc, arsenic, mercury and lead at the target sites were 40.6, 67.2, 300.8, 14.3, 1.2 and 344.4 mg/kg dry weight, respectively (mean values). Average copper, zinc and lead concentrations were about six times higher for copper and zinc and ten times higher for lead compared with background levels. Comparisons of control site samples with background levels indicated that the average concentrations of chromium, copper, zinc, arsenic, mercury and lead were close to the background levels. The mean concentration of chromium was lower than the background level in all suburbs studied except Alexandria, where it was the same as the background level. In contrast to chromium, the mean concentrations of copper, zinc and lead were higher than the background levels at all target sites. For example, the mean concentrations of copper, zinc and lead in Newtown were 10, 11 and 25 times higher, respectively, than the background levels. Where high concentrations of chromium, copper, lead, mercury and zinc were reported in residential areas there was a positive correlation between soil and dog hair.

In general, zinc showed the highest concentration levels in dog hair, followed by copper, lead, chromium, mercury and arsenic, while concentration levels in soil were also highest for zinc, followed by lead, copper, chromium, arsenic and mercury. However, concentration levels of all metals and arsenic varied between suburbs. zinc and copper levels were high at the control sites, probably due to high background levels of these metals. Tiller's (1992) study of background levels of heavy metals in non-industrial locations in Sydney showed that these elements such as arsenic, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel and zinc were present in surface soils in Sydney. Riley and Banks (1996) reported that the concentration of zinc in surface soil in the Sydney region was 900 μg/g (mean value). In 2009, Ying et al. found that the concentration of zinc at Sydney's Olympic Park was 124 μg/g. Dry dog food contains high levels of zinc and copper, which are important for dogs' reproductive health, skin and coat health, and ligament strength (Dogs NSW, 2014).

Analysis of various metals and arsenic by Pearson Correlation Co-efficient indicated a weakly positive correlation between all metal(loid)s (chromium, copper, zinc, arsenic, lead and mercury) in dog hair and in residential soil. However, each metal(loid) demonstrated a different relationship. The results showed moderately positive relationship found for chromium and weak positive correlation for copper, and for rest of metal(loid)s no or negligible relationship were detected. Positive linear correlations between accumulation in dog hair and environmental concentrations (in soil) for both the essential metals (chromium and copper) and non-essential metals (lead and mercury) were observed and analysed by Linear Regression, and they were strangely positive, while there was a negative relationship for arsenic and a very weakly positive relationship for zinc. Therefore, based on Linear regression dog hair is good indicator for some analyses metal(liod)s not all.

Published data on the relationship between concentrations of metals in animal or human hair and in soil are sparse, but the findings of this study are in agreement with those of earlier studies. Concentrations of non-essential metals (lead and mercury) in the hair of household dogs in the present study predominantly reflect those found in small mammals in earlier studies. Significant linear correlations have been detected for chromium and lead between soil and the hair of the European hedgehog at a site in close proximity to a non-ferrous smelter in Belgium (D'Have et al., 2005). Rashed and Soltan (2005) found a positive relationship between levels of lead in the hair of sheep, goats and camels and levels in soil in four areas in Egypt. Their results indicated that accumulation of heavy metals in hair reflected the presence of those metals in the surrounding forage and soil. McLean (2009) reported a significantly positive linear correlation between concentrations of the non-essential metal lead in soil and in the hair of Australian marsupials (brown antechinus, black rat and brown rat). Similarly, Xue et al. (2014) found significant positive correlations between concentrations of metals such as chromium, copper, manganese, nickel, lead and zinc in soil, road dust and rice, and in human hair in a rural area in China.

5.2. Factors influencing metal(loid) concentrations in dog hair

5.2.1. Age of the animal

Strong positive correlations were observed between concentrations of all determined metals and arsenic in dog hair and the age of the dog. In particular, positive linear relationships were found between accumulation in the hair and the age of the dog for copper, arsenic, mercury and lead. Other studies have reported similar findings. A significant correlation was found between mercury accumulation in the hair of racoons and age (Cumbie, 1975), while similar results were found regarding Hg accumulation in the hair of cats in Japan (Sakai et al., 1995). Serpe et al. (2012) found that lead accumulation in dogs' kidneys increased with age, and cadmium accumulation increased with age in the hair and other organs of wood mice (Beernaert et al., 2007), in the feathers of buzzards (Naccari et al., 2009) and in the liver and kidneys of dogs (Lopez-Alonso et al., 2009).

Regarding the essential elements zinc and chromium, a negative correlation was observed between accumulation in dog hair and the age of the dog. Again, other studies have reported similar findings. For example, arsenic concentrations were highest in the feathers, liver, muscle and lung of juvenile buzzards (Naccari et al., 2009), while Sobaska (2005) found the highest mercury concentrations in young wild boar. However, some studies did not find significant correlations between age and metal accumulation. For example, age did not influence the levels of lead in racehorses in Japan (Asano et al., 2002).

5.2.2. Hair colour

The results of this study indicated that there was a positive correlation between the accumulation of metals and arsenic in dog hair and hair colour. However, further research is required to determine which particular hair colour shows the strongest correlation. Previous studies have found a relationship between hair colour and metal concentrations in hair. For example, Sobanska (2005) reported that in wild boars, red hair contained higher concentrations of Hg than black hair. Similarly, the results of some epidemiological studies on human hair showed that metal content differed according to hair colour (Dutcher & Rothman, 1951; Bode et al., 2008).

5.2.3. Gender

The current study found a very positive correlation between concentrations of metals and arsenic in dog hair and dog gender. However, more research is required to determine which gender experiences greater metal and arsenic accumulations and gender differences in relation to specific metals. The influence of gender on metal(loid) accumulation in hair is described in the literature. For example, Hayashi (1981) reported that accumulation of lead and zinc is significantly higher in females dogs than in males, while concentrations of chromium, copper, arsenic and mercury were slightly higher in males than in females. Sobanska (2005) reported that mercury levels were higher in female wild boars than in males. A similar result was observed by Castro et al. (2013) regarding arsenic concentrations in dog hair.

5.2.4. Type of diet

There was a weakly positive relationship between metal(loid) concentrations in dog hair and the type of food that the dogs ate. Some previous studies have found a relationship between dietary status and metal concentrations in hair. Sakai et al. (1995) observed that concentrations of mercury were higher in the hair of cats that were fed fresh tuna. Rashed and Soltan (2005) reported a positive correlation between accumulations of iron, manganese, cobalt, nickel, cadmium and lead in goat, sheep and camel hair and the type of fodder that they were fed. Dunlap et al. (2007) found that mercury levels were higher in the hair of sled dogs that were fed fish than in the hair of those that consumed commercial dog food. Serpe's study (2012) showed that fish-based wet pet foods were potentially the main source of high concentrations of mercury in the liver and kidneys. A relationship to diet type can be supposed regarding exposure to all metals and arsenic. Indeed, dogs living in urban environments and consuming commercial dog food showed higher concentrations of copper, arsenic, mercury and lead than dogs fed exclusively with homemade food or with a mixture of commercial and homemade food. The results of the present study are supported by those of Lopez-Alonso et al. (2007). Several other studies have shown that exposure of animals to metals is related to various factors such as environmental contamination and type of diet (Goyer, 2000; Duran et al., 2010; Kerin and Lin, 2010; Markert et al., 2011).

5.3. Use of dogs as a surrogate method to determine the level of human exposure to heavy metals

Based on previous studies throughout the word dogs can exhibit concentrations of various metals and As in their bodies (Berny et al., 1994; Berny et al., 1995; Park et al., 2005; Dunlap et al., 2007; Lopez-Alonso et al., 2007, Atanaskova et al., 2011; Serpe et al., 2012; Vazquez et al., 2012; Castro et al., 2013; Palczewska-Komsa et al., 2014), and that several factors, such as diet, play a role in metal(loid) accumulation. However, studies using dogs as a surrogate method to detect levels of metal(loid) accumulation in humans are scarce, although the number has increased dramatically in recent years (Hayashi et al., 1981; Doi et al., 1986; O'Brien et al., 1993; Berny et al., 1995; Sakai et al., 1995; Swarup et al., 2000; Backer et al., 2001; Kozak et al., 2002; Dunlop et al., 2007;

Lopez-Alonso et al., 2007; Schmidt, 2009; Bischoff et al., 2010; Atanaskova et al., 2011; Reif, 2011; Serpe et al., 2012; Castro et al., 2013).

The findings of this study are similar to those reported by previous studies. Because dogs are companion animals that live in the same environment as their owners and exhibit many behaviours that are similar to those of young children, they appeal as one of the best bioindicators to use instead of humans, particularly infants and young children (Berny et al., 1995). One of the most important reasons for using dogs as a surrogate method to determine the level of exposure of humans to heavy metal(loid)s is that dogs can also develop many of the same diseases as humans, such as immunological syndromes (Felsburg, 2002; Dunlap et al., 2006; Castro et al., 2013). Several studies have reported a higher prevalence of various diseases such as hyperthyroidism, malignant lymphoma, chronic renal failure and cancer in household animals that was attributed to their lifestyle and exposure to chemicals in their diet (Reif et al., 1998; Martin et al., 2000; Hughes et al., 2002).

5.4. Use of dog hair as a non-invasive biomarker in environmental and human health studies related to metal(liod) contamination

The mean concentrations of heavy metals observed in dog hair in this study were generally low (chromium: 0.85, arsenic: 0.08, mercury: 0.13, lead: 1.91 mg/kg dry weight), except for those of zinc and copper (173.61 and 14.48 mg/kg dry weight, respectively), probably because of the importance of zinc and copper for growth. zinc and copper are microminerals that are essential for the body's metabolic processes, although only limited amounts of these minerals are required. To date, very little data on the bioavailability of microelements in dogs that are fed dry food are available. Kastenmayer et al. (2002) reported that of an intake of 3.36 mg of copper, only 0.8 mg was absorbed, and of an intake of 37.0 mg of zinc, only 4.3 mg was absorbed. They concluded that absorption was relatively low in both cases, from commercial dry dog food by adult dogs. The present study found high amounts of zinc and copper in dog hair.

Data concerning levels of Cr, Cu, Zn, As, Hg and Pb in dog hair are also scarce (Zn, Cu, Pb, Mn, Cd: Hayashi et al., 1981; Fe, Hg, Mn, Zn: Doi et al., 1986; Hg: Sakai et al., 1995; Ag, Al, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn: D'Have et al., 2006; Hg: Dunlap et al., 2007; As: Castro et al., 2013), thus evaluation of the results of the current study is difficult. Notwithstanding, the findings of the present study are similar to those of previous studies investigating metal concentrations in other animals. Previous studies have examined the usefulness of various spices hair as a surrogate method to trace metal(loid)s. For example, Rashed and Soltan (2005) focused on hair of sheep, goats and camels; Patra et al. (2007) studied on cows hair as a as a possible biomarker of environmental exposure to various metals and metalloids; Filistowicz et al. (2011) researched on the use of hair of silver and red foxes as a bioindicator.

The use of hair as an indicator of metal contamination offers several advantages. Hair presents relatively few problems in terms of sampling and storage (IAEA, 1985; Moreda-Pineiro et al., 2007; Schramm, 2008; Esban and Castano, 2009; Wolowiec et al., 2013), and can accumulate higher levels of heavy metals compared with blood and urine samples (Wolowiec et al., 2013). Moreda-Pineiro et al. (2007) and Wolowiec et al. (2013) used hair to assess metabolism status and determine levels of exposure to heavy metals in both the environment and the workplace. Hair has also been identified as an appropriate biological material for monitoring levels of various metals in the body for various purposes, such as nutritional, toxicological, or clinical purposes (Jenkins, 1980).

6. Conclusions

The present study has demonstrated that metal accumulation can be found in the hair of pet dogs, thus dog hair might be a suitable biological surrogate that can be used to assess levels of heavy metals and arsenic in humans. However, further investigations to find the relations between different ages, hair color and gender is nessary for next level. The results of this study revealed several important findings. First, for arsenic and a number of metals, accumulation in dog hair was associated with various environmental parameters that play a significant role in determining concentration levels. Dog hair can be regarded as a sensitive bioindicator of contamination by the metal(loid)s analysed in this study, as these essential and non-essential elements are commonly found in dog hair. Concentration levels of the microelements chromium, copper and zinc were highest in the hair of dogs that lived in Newtown, Botany or Erskineville, while concentration levels of the non-essential elements arsenic, mercury and lead were surprisingly high in South Turramurra and were also found in the industrial suburbs of Botany and Mascot.

Second, the regression correlations between dog hair and residential soil demonstrated that the hair of domestic dogs is a useful although not statistically significantly strong indicator of chromium, copper, mercury and lead concentration levels in soil. The result of the present study indicated that soil concentration levels and that in hair samples support the association of lower level accumulation. These correlations are independent of the dogs' normal diet, suggesting that environmental exposure pathways via soil are present. Thus, this study has shown that dog hair may be a suitable biological indicator of toxic levels of the microelements Cr and Cu and the non-essential elements Pb and Hg in humans in residential areas. It seems that dog hair may be able to play a significant role as a useful, non-destructive indicator tissue in future studies.

The results also indicated that domestic dogs may accumulate potentially heavy metal(liod) concentrations in their hair, and thus may be at risk of contamination by the various metals analysed and arsenic. As reported by Backer et al. (2001), Ghisleni et al. (2004) and Schmidt (2009), and confirmed by this study, the use of pet dogs as a sentinel model to evaluate exposure of humans to potentially heavy metals should be considered. This study showed that household dogs are a valuable indicator species for risk assessment regarding concentration levels of various metals and arsenic in the urban environment.

There is a lack of information in the literature regarding the usefulness of household dogs as bioindicators of exposure of humans to potentially heavy metals, and no research has been undertaken to determine whether there is any correlation between concentration levels of heavy metals in dog hair and other organs and human exposure. Further investigations are also necessary to elucidate the causes of differences in the distribution of metal(loid)s in various internal organs. Moreover, the lack of safe limits and standard guidelines for metal(loid)s accumulation in hair is clearly evident, and should be the subject of future research and health and environmental policy deliberations.

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Annexes and Appendices

Annexes A: Dog hair sample

Dog hair Sample (1)

Dog hair Sample (2)

Annexes B: Residential soil samples

Annexes C: Soil sample preparation for hand held XRF


DATE
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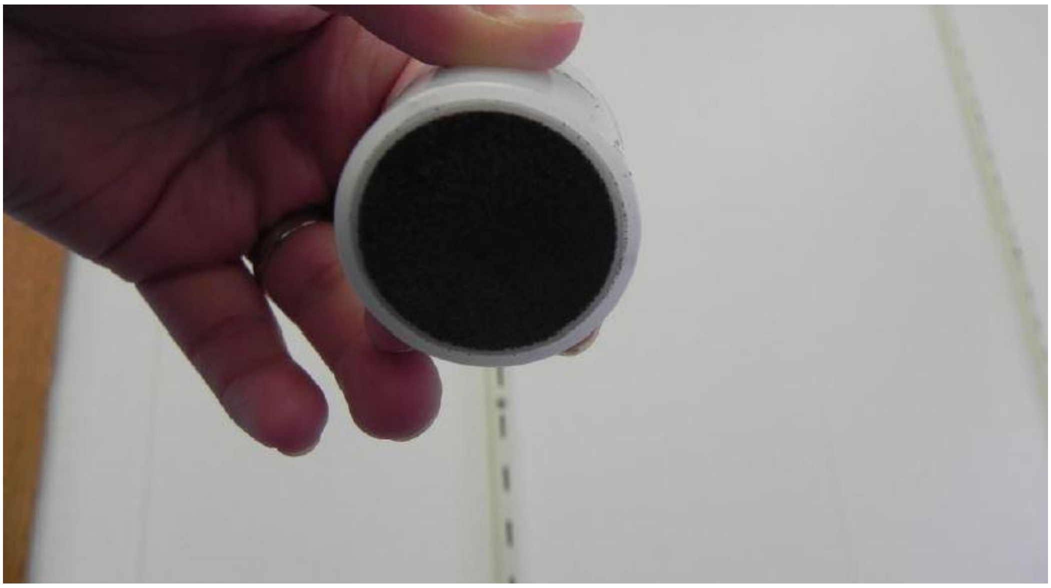
Infront yard



Annexes D: Hand held XRF

Annexes E: Questionary Form

Spatial assessment of metal pollution across Sydney

Questionnaire Number:

Your Dog:

- Breed of dog
- Age of dog
- Gender (F/ M)
- Health of dog
- What do you feed your dog?
- Time spent outdoors / indoors (%)
- Any medication (list if possible)

Your garden

- Use and frequency of pesticides or herbicides (list if possible)
- % garden versus paved surface of yard where dog recreates (front yard or back yard)
- Have you replaced or topped up your soil in the past 2 years (Y/N)

Your house

- Is it on a busy street?
- Do you know if there was any industry nearby to your house and when it finished
- Age of house

Your details

- Name
- address
- Email
- Would you like a copy of the results sent to you be email?

Thank you for your consideration.

Annexes F: Human Ethics Approval

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2014/020 **Date of Expiry:** 1 January 2015

Full Approval Duration: *21 May 2014 to 1 January 2015* ***(7 months and 11 days)***

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

Principal Investigator:
Dr Peter Davies
Macquarie University, NSW 2109
0457 070 702
peter.davies@mq.edu.au

Student:
Mrs Shaghayegh Jafari 0410 401 266

In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above
or **Animal Welfare Officer** - 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Use of household pets as indicators of heavy metals exposure across Sydney

Purpose: 7 - Research: Environmental Study

Aims: To determine the spatial difference of heavy metal contaminants between current and former industrial areas to residential suburbs as measured by soil and how these contaminants are accumulated in hair samples of dogs.

Surgical Procedures category: 1 - Observation Involving Minor Interference

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Age/Sex/Weight	Total	Supplier/Source
32 Canis lupus familiaris	Any	Any	60	N/A
		TOTAL	60	

Location of research:

Location	*Full street address*
Sites located across Sydney, NSW	Sir Joseph Banks Park, Waratah Rd, Botany; James Hilder Reserve, 121/131 Campbell St, Surry Hills, Perry Park, Maddox St, Alexandria; Beaconsfield Park, 54 Queen Street, Beaconsfield; Southern Cross Drive Reserve, Southern Cross Drive, Rosebery; Baker Park, Dudley St, Coogee; Green Ban Park, Erskineville Road, Erskineville; Solander Park, Park Street, Erskineville; Vernon Street Park, Vernon Street, South Turramurra; Bicentennial Park, West Pymble

Amendments approved by the AEC since initial approval: N/A
Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Professor Mark Connor (Chair, Animal Ethics Committee) Approval Date: 15 May 2014

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

Annexes G: Animal Ethics Approval

Dear Dr Davies,

RE: Ethics project entitled: "Use of household pets as indicators of heavy metals exposure across Sydney"

Ref number: 5201400297

The Faculty of Science Human Research Ethics Sub-Committee has reviewed your application and granted final approval, effective 4th June 2014. You may now commence your research.

This research meets the requirements of the National Statement on Ethical Conduct in Human Research (2007). The National Statement is available at the following web site:

http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/e72.pdf.

The following personnel are authorised to conduct this research:

Dr Peter Davies
Mrs Shaghayegh Jafari

NB. STUDENTS: IT IS YOUR RESPONSIBILITY TO KEEP A COPY OF THIS APPROVAL EMAIL TO SUBMIT WITH YOUR THESIS.

Please note the following standard requirements of approval:

1. The approval of this project is conditional upon your continuing compliance with the National Statement on Ethical Conduct in Human Research (2007).

2. Approval will be for a period of five (5) years subject to the provision of annual reports.

Progress Report 1 Due: 4th June 2015
Progress Report 2 Due: 4th June 2016
Progress Report 3 Due: 4th June 2017
Progress Report 4 Due: 4th June 2018
Final Report Due: 4th June 2019

NB. If you complete the work earlier than you had planned you must submit a Final Report as soon as the work is completed. If the project has been discontinued or not commenced for any reason, you are also required to submit a Final Report for the project.

Progress reports and Final Reports are available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

3. If the project has run for more than five (5) years you cannot renew approval for the project. You will need to complete and submit a Final Report and submit a new application for the project. (The five year limit on renewal of approvals allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing, for example, new child protection and privacy laws).

4. All amendments to the project must be reviewed and approved by the Committee before implementation. Please complete and submit a Request for Amendment Form available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

5. Please notify the Committee immediately in the event of any adverse effects on participants or of any unforeseen events that affect the continued ethical acceptability of the project.

6. At all times you are responsible for the ethical conduct of your research in accordance with the guidelines established by the University. This information is available at the following websites:
http://www.mq.edu.au/policy/

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/policy

If you will be applying for or have applied for internal or external funding for the above project it is your responsibility to provide the Macquarie University's Research Grants Management Assistant with a copy of this email as soon as possible. Internal and External funding agencies will not be informed that you have final approval for your project and funds will not be released until the Research Grants Management Assistant has received a copy of this email.

If you need to provide a hard copy letter of Final Approval to an external organisation as evidence that you have Final Approval, please do not hesitate to contact the Ethics Secretariat at the address below.

Please retain a copy of this email as this is your official notification of final ethics approval.

Yours sincerely,
Annabelle McIver, Acting Chair
Faculty of Science Human Research Ethics Sub-Committee
Macquarie University
NSW 2109