



## Elements levels in dogs from “triangle of death” and different areas of Campania region (Italy)



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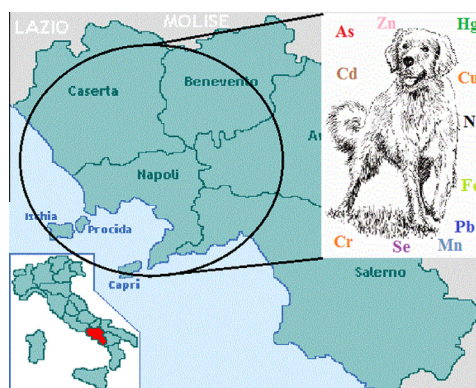
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### HIGHLIGHTS

- Elements in hair and blood of dogs from Campania region.
- Identification of a hot spot of contamination in the less contaminated area.
- Concern about the levels of chromium, nickel and arsenic.
- Possible “background levels” contamination due to volcanic emissions.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 2 September 2013

Received in revised form 5 March 2014

Accepted 8 March 2014

Handling Editor: Susan Jobling

#### Keywords:

Dog  
Blood  
Hair  
Elements  
Campania  
Pollution

### ABSTRACT

In the last twenty years, many concerns have raised in Campania region (Southern Italy) about illegal waste dumping and toxic waste and their possible adverse effects on health. Many human activities are considered to be important sources of environmental pollutants, elements among them. In this study, pet dogs were enrolled as environmental sentinels from three different areas of Campania, with a different degree of pollution, evaluating elements in blood and hair. The obtained data indicated that dogs from less polluted area were exposed to a hot spot of pollution, as only animals from one city (Sessa Aurunca) presented elements concentrations very close to toxic levels. When excluding these animals, the area proved to be the less contaminated. The present report confirm the higher degree of pollution of the most industrialized areas, and a certain concern originates from Cr, Ni and As, which are present as levels well above toxic thresholds.

These data are indicative of a reduced pollution of the areas considered by Cd and Pb, but arise concern for Hg, As, Cr and Ni, which reach concentrations high enough to impact dogs and humans health, in term of acute (in the city of Sessa Aurunca) and chronic toxicity (i.e. reproduction impairment, endocrine disruption, immunosuppression).

Additional studies are necessary to better define not only the precise distribution of hot spots of pollution, but also the real impact of such an exposure on the health of dogs, in term of endocrine balance and/or immune system activity.

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## 1. Introduction

In the last twenty years, many concerns have raised in the Campania region (Southern Italy) about illegal waste dumping and its possible adverse effects on human health. Particularly, in 2004 an area located in the North East of Naples was referred to as the “triangle of death” (a geographic area encompassing three villages: Nola, Marigliano and Acerra where most waste disposal sites are concentrated) since it was supposed that illegal waste dumping and toxic waste could have a possible effect in cancer mortality (Senior and Mazza, 2004). Many studies have been carried out in order to establish possible cancer risk deriving from living in these villages (Comba et al., 2006; Albanese et al., 2008; Barba et al., 2011; Filippelli et al., 2012). Although no cause-effect relationship has been demonstrated, the studies show that in the North East of Naples people have higher cancer mortality rates; additionally, a negative correlation between lead levels and semen quality was observed (Giaccio et al., 2012).

Moreover, in this study, there are many environmental stressors, deriving from intensive agriculture, widespread industrial activities, and a very high population density. Intensive agriculture and industry are considered to be a source of environmental pollution, and an anthropogenic source of elements, which are released into the atmosphere and consequently settle as dust and precipitations.

Due to the close association and common environment shared with humans, dogs are exposed to similar pollutants and have been suggested as sentinels for biohazards from toxic pollutants (Zook, 1978; Berny et al., 1995; Swarup et al., 2000; Santin et al., 2005). Companion animals can be particularly valuable sentinels for human exposures because they share much of their environment (air, water and food) with people, and may be even more exposed than their owners to some contaminants and vectors, such as soil or house dust, especially if they are allowed to free-range in the environment. Therefore, the assessment of the environmental load of elements in dogs may serve as a possible indicator of their load in humans.

It is recognized how hair gives a better estimate of the total body intake of certain elements than blood or urine (Wilhelm et al., 1989). Anyway, few attempts have so far been made to study the content of elements in companion animals and to evaluate any potential correlation between blood and hair levels (O'Brien et al., 1993; Berny et al., 1995; Kozak et al., 2002; Park et al., 2005a, 2005b).

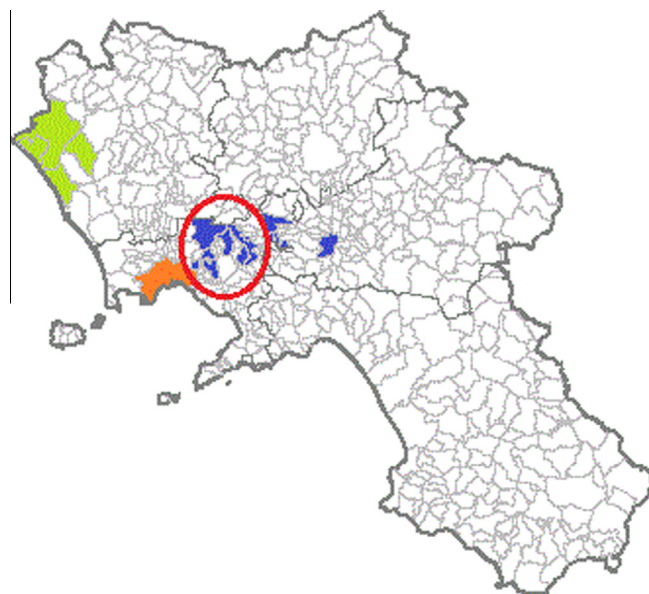
No studies have so far investigated the impact of element pollution in those areas of Campania that are considered contaminated due to illegal waste dumping. Indeed, some studies can be found concerning the impact of total pollution on pathologies incidence, but little detailed investigations, focusing on the effect of specific contaminants on health parameters, were performed (Comba et al., 2006; Albanese et al., 2008; Barba et al., 2011; Filippelli et al., 2012; Giaccio et al., 2012). Thus, it is also difficult to get precise geo-referenced “background” values for the region, when elements are of concern (Cicchella et al., 2005, 2008).

The aim of this study was to assess and compare the levels of elements in dog hair and blood from three different areas of Campania, evaluating the existence, if any, of hot spots of pollution and of exposure levels that can raise concern for animals and humans. It will also provide additional information on the degree of contamination in the districts considered, improving the scarce existing monitoring database for the area.

## 2. Materials and methods

### 2.1. Geographic areas and sampling

Three different geographic areas in the Campania region were selected (Fig. 1): A: the area including villages encompassed in



**Fig. 1.** Sampling area. The circle (red in the web version) identifies the so called “triangle of death”. Dark grey: area A (blue in the web version). Black: area B (orange in the web version). Light grey: area C (green in the web version).

the so-called “triangle of death”; B: the city of Naples and C: the area encompassing rural villages in the province of Caserta. Before conducting the survey, the villages from the above mentioned areas were geo-referenced by GIS database using Arc-GIS 9.2 GIS software (ESRI, Redlands, CA, USA).

During normal health control examination of pet dogs, 30 dogs for each area were recruited at Department of Veterinary medicine and Animal productions. All dogs under investigation had been living in the areas since they were pups. Each animal underwent clinical examination before sampling and only healthy animals were enrolled in this study. Blood samples were opportunistically collected from those used for health parameters evaluation, so no animal was specifically sampled for this study. Blood was stored at  $-20^{\circ}\text{C}$  until elements quantification. During examination, age and gender of each animal were also registered. The age ranged from 2 to 15 years with dogs five to seven years old being the largest category. Gender were equally distributed in all the areas (Table 1).

Hairs were sampled in all the cases from the dorsal area and individual samples weighed around 3 g. Hair samples were prepared following the method by Chyla and Żyrnicki (2000) and Hayashi et al. (1981), previewing a triple washing with water and acetone. Washed samples were then stored in plastic bags and preserved  $-20^{\circ}\text{C}$  until analysis.

### 2.2. Elements analysis

Element analysis was performed using ICP-OES method after microwave digestion of 0.7 g of hair and 0.5 mL of whole blood.

Samples were microwave digested using a Milestone ETHOS ONE oven using 4 mL nitric acid and 1 mL hydrogen peroxide. All reagents were from Merck, Darmstadt (Germany); acids were of Suprapur grade.

Elements (As, Cd, Cr, Cu, Mn, Fe, Ni, Pb, Hg, Se, Zn) were quantified by Inductively Coupled Plasma-Optic Emission Spectrometry technique (ICP-OES) using a Perkin Elmer Optima 2100 DV instrument, coupled with a CETAC U5000AT+ ultrasound nebulizer for mercury. Two blanks were run during each set of analysis to check for chemicals purity, and the accuracy of the method was verified

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