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Monitoring of polycyclic aromatic hydrocarbons in vegetables in 'Land of Fires' area and assessment of benzo(a)anthracene bioavailability *in vitro* and soil cultivation

Ph.D. Dissertation

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Chapter 1

1 INTRODUCTION

1.1 Persistent organic pollutants

Contamination of environment with hazardous and toxic chemicals is one of the major problems facing the industrialized world today. Among the contaminants present in the environment, Persistent Organic Pollutants (POPs) are a group of toxic, bioaccumulative substances including a wide variety of compounds belonging to different chemical classes, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs). They are easily available to environmental transport over long distances once introduced in the environment by human activities (Figure 1.1).

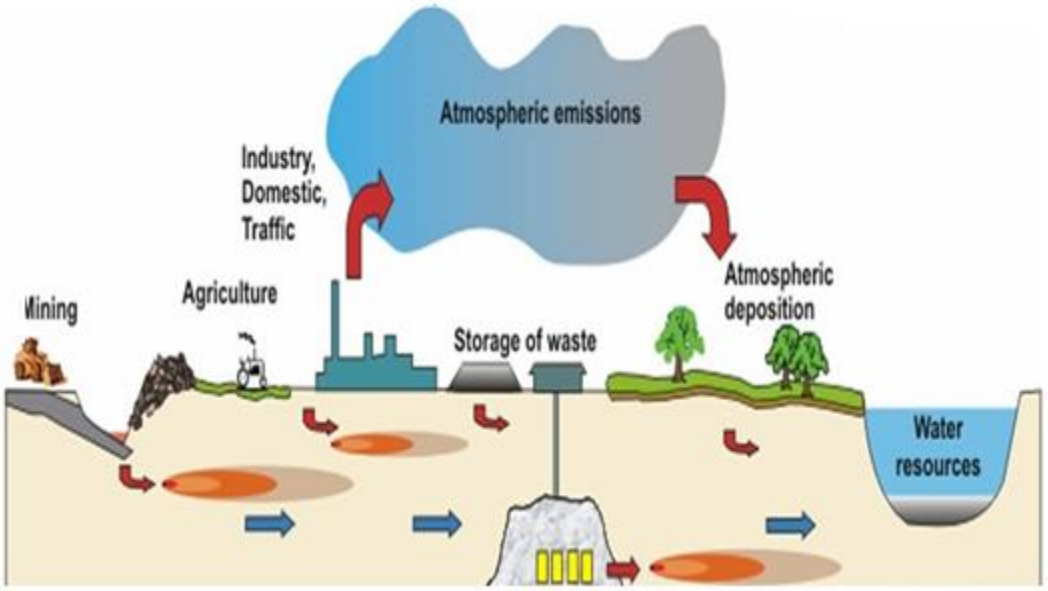

Figure 1.1: *Transport of pollutants (from www.anka.kit.edu/28.php).*

The atmospheric transport has been identified as the main cause of long-range transport and global dispersal of POPs (Teran *et al.*, 2012). The latter can move (indirect transport) from source to pristine and remote areas by a phenomenon called global distillation (Wania and Mackay, 1995). Pollutants released into the environment vaporise when temperature raises and are carried around by winds until temperature cools and condensation occurs. Drop deposition can occur when chemicals are blown to cooler climates or when seasons change. A further effect is the atmospheric transport from low to high latitude and altitude (Wania and Mackay, 1995).

Since global distillation is a relatively slow process depending on successive

evaporation/condensation cycles, it is only effective for semi-volatile chemicals that break down very slowly in the environment, such as PAHs, dioxin and polychlorinated biphenyls (Wania and Mackay, 1995).

Atmospheric deposition of pollutants can occur simply by gravity (dry deposition), through the dissolution of organic vapours into rain and cloud droplets (washout and wet deposition) (Bidlemann, 1988). Vegetables, in particular, plants having a relatively large surface area covered with waxes that facilitates the accumulation of hydrophobic chemicals, capture particulates through leaves then falling and releasing them to the soil (Figure 1.2).

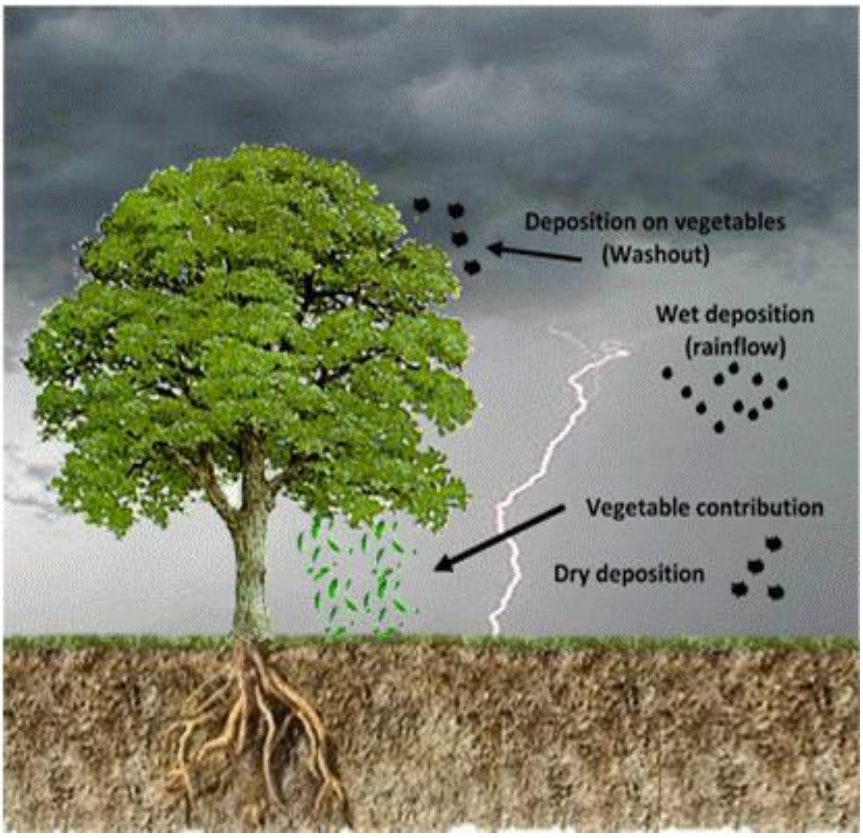

Figure 1.2: *Indirect deposition of POPs by air transport*.

POPs released into the environment tend to be diluted in matrices such as water and air, while they tend to accumulate in the soil for various periods and they are subject to partitioning, degradation, and transport processes depending on their physical-chemical properties (Cetin and Odabasi, 2007).

The extent to which POPs react in the atmosphere can dictate their long-term environment fate. If the atmospheric degradation reactions are slower than the deposition

rates, a greater amount of the emitted POPs could reach food chains. In fact, due to their low solubility in water and high lipophilicity, they tend to cross the phospholipid structure of biological membranes and accumulate in living organisms. Bioaccumulation leads to high concentrations so high exposures to higher levels of the trophic chains.

Therefore, the magnitude of soil pollution depends on several factors: the chemical properties of POPs, their concentration and persistence in the environment, the formation of toxic secondary compounds, the synergistic effect with other chemical compounds, the time of exposure, and the vulnerability of exposed populations (Cetin and Odabasi, 2007).

1.2 Ubiquitous environmental pollutants: polycyclic aromatic hydrocarbons

PAHs are organic compounds containing two or more fused aromatic rings of carbon and hydrogen atoms without any heteroatom or substituent (Figure 1.3).

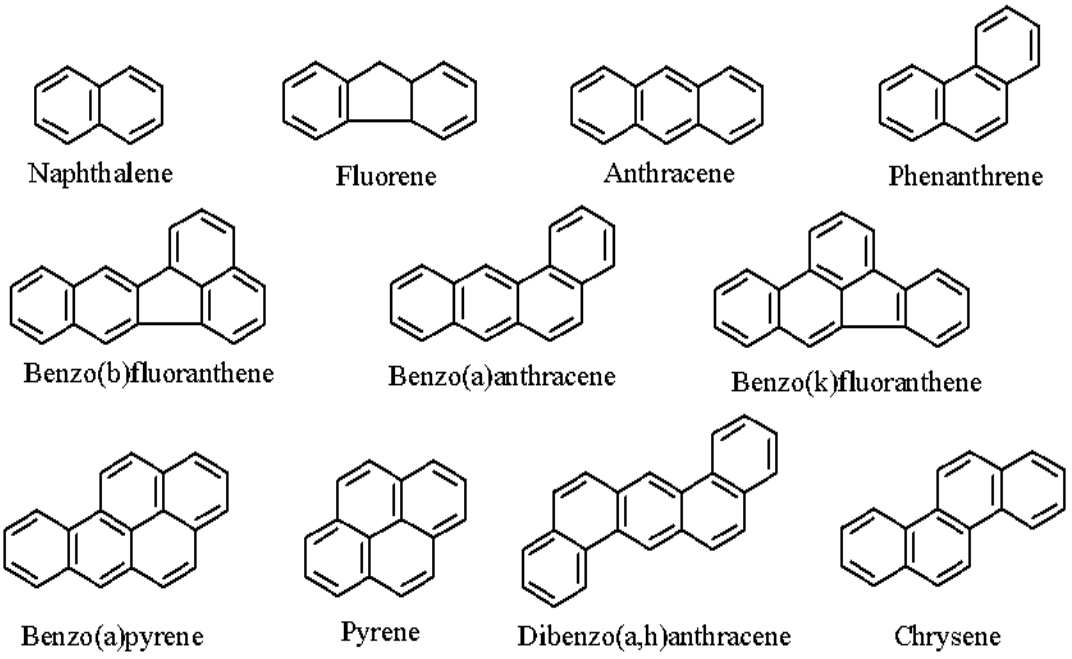

Figure 1.3: *Polycyclic aromatic hydrocarbons.*

PAHs are colourless, white, or pale yellow-green solids generally occurring as complex mixtures. The physical and chemical properties of PAHs are linked to the conjugated systems of π electrons and they vary rather regularly with the number of rings and the molecular weight. They have low vapour pressure, high melting and boiling points, therefore all PAHs are solid at ambient temperature. They are poorly water-soluble: the higher their molecular weight the lower their water solubility.

The transport and fate of PAHs released into the environment are related to their chemical and physical properties, such as vapour pressure, Henry's law constant, water solubility, octanol-water partitioning coefficient (Kow), and organic carbon partitioning coefficient (Koc) (Simon and Sobieraj, 2006). Vapour pressure and Henry's Law constants indicate the chemical tendency to volatize when in their pure form or dissolved in water. The Kow value provides an indication of the potential of the organic compounds to partition from water into lipids and provides a correlation for bioconcentration in aquatic organisms. The Koc value indicates the compound potential to bind organic carbon in soil and sediments. PAHs have relatively low water solubility, vapour pressure and Henry's Law constant and high Kow and Koc values (Simon and Sobieraj, 2006) (Table 1.1).

Table 1.1: *Physical and chemical properties of PAHs.*

Compound	**Molecular Weight (MW)**	**Water Solubility ($\mu g\ L^{-1}$)**	**Vapour Pressure (mm Hg)**	**Henry's Law Constant ($atm - m^3 \cdot mol^{-1}$)**	**Log K_{ow}**	**Log K_{oc}**
Low MW (2-3 rings)						
Anthracene	178.20	76	$1.7 \cdot 10^{-5}$	$1.77 \cdot 10^{-5}$	4.45	4.15
Phenanthrene	178.20	1200	$6.8 \cdot 10^{-4}$	$2.56 \cdot 10^{-5}$	4.45	4.15
Medium MW (4 rings)						
Benzo(a)anthracene	228.29	10	$2.2 \cdot 10^{-8}$	$1.00 \cdot 10^{-6}$	5.61	5.30
Chrysene	228.30	2.8	$6.3 \cdot 10^{-7}$	$1.05 \cdot 10^{-6}$	5.16	5.30
High MW (≥ 5 rings)						
Benzo(a)pyrene	252.30	2.3	$5.6 \cdot 10^{-9}$	$4.90 \cdot 10^{-7}$	6.06	6.74
Benzo(b)fluoranthene	252.30	1.2	$5.0 \cdot 10^{-7}$	$1.22 \cdot 10^{-5}$	6.04	5.74
Benzo(k)fluoranthene	252.30	0.76	$9.6 \cdot 10^{-11}$	$3.87 \cdot 10^{-5}$	6.06	5.74
Dibenzo(a,h)anthracene	278.35	0.5	$1.0 \cdot 10^{-10}$	$7.30 \cdot 10^{-8}$	6.84	6.52

The main source of PAH emissions into the atmosphere is represented by combustion processes and some industrial activities. The PAHs, which naturally occur in fossil fuels, were produced primarily during pyrolysis, a thermochemical decomposition of organic materials at high temperatures (650-900 °C) in the absence of oxygen. Another

mechanism for natural formation of PAHs is petrogenesis (Stou *et al.*, 2001), more specifically diagenesis that is the formation of magma and fossil fuels deriving from burial of organic material under quite high temperature (about 200 °C) and pressure (about 2-3 bar) conditions; therefore, coal, oil and bitumen are rich in PAHs. In addition, PAHs can be formed via biogenesis by aerobic and anaerobic bacteria, fungi and plants (Brooks, 2000; Silliman, 2001).

PAHs are relatively reactive in the atmosphere, incurring degradation caused by chemical and photochemical transformations. However, the persistence time in atmosphere of some PAHs is long enough (4-12 days) to allow the transport over long distances.

Even though their natural origin, anthropogenic PAH emissions are the main sources of environmental contamination, such as residential heating, illegal waste incineration, coal gasification, coal tar pitch and asphalt production, coke and aluminum production, catalytic cracking towers and related activities in petroleum refineries as well as motor vehicle exhaust; also tobacco smoke is a significant source of PAH intake. Furthermore, some of the cooking mode such as barbecued/grilled/broiled and smoke-cured meats, roasted, baked, fried foods (high-temperature processing), and smoked cheeses can contribute to the formation of PAHs (Esposito *et al.*, 2015).

The composition of PAH emissions depends on the combustion source. For example, emissions from wood combustion contain more acenaphthylene than other PAHs (Perwak *et al.*,1982), whereas auto emissions contain more benzo(g,h,i)perylene and pyrene (Rogge *et al.*,1993; Santodonato *et al.*,1981). PAHs in diesel exhaust particulates are represented mainly by three- and four-ring compounds, primarily fluoranthene, phenanthrene, and pyrene (Rogge *et al.*,1993; Westerholm and Li 1994). Diesel exhaust vapor emissions are composed mainly by phenanthrene and anthracene (Westerholm and Li 1994). Acenaphthene, fluorene, and phenanthrene have been found to be predominant in total (particulate- and vapour-phase) diesel emissions (Lowenthal *et al.*,1994). Fluoranthene, benzo(a)fluoranthene, benzo(g,h,i)perylene, indeno(1,2,3-c,d)pyrene, phenanthrene, and chrysene were predominant in emission particulate samples collected from a municipal waste incinerator, whereas benzo(g,h,i)perylene and benzo(a)anthracene were predominant in emission particulate samples collected from a municipal and illegal waste incinerator (Williams *et al.*,1994).

In the environment, PAHs held on dust and other particles in the air but also can

contaminate surface water through discharges from industrial plants and wastewater treatment plants, and therefore be released to soils. The movement of PAHs in the environment is extensively influenced by their concentrations in the atmosphere; even the season can affect their environmental level. In winter, for example, PAH concentrations are higher because of increased domestic heating, reduced thermal and photo-decomposition (Menichini, 1992).

Their movement in the environment depends on how easily they dissolve in water and how easily they evaporate into the air. They are present in air as vapours or stuck to the surfaces of small solid particles. Generally, PAHs do not easily dissolve in water. On the contrary, in soils, PAHs are more adhered to particles, but some PAHs evaporate from surface soils to air or migrate through lower layers contaminating the underground water.

1.3 Toxicity and bioavailability of PAHS

PAHs were the first class of atmospheric POPs to have been identified as suspected carcinogens (IARC, 1983).

Numerous studies indicated that PAHs with two and three rings are extremely toxic, while the higher molecular weight PAHs are considered genotoxic (Agency for Toxic Substances and Disease Registry [ATSDR], 1995). The condensation of the rings decreases their aromaticity and makes easier the metabolite reactions of epoxidation resulting in formation of compounds with greater carcinogenicity. Therefore, not all PAHs show the same toxicity because of differences in structure that affect metabolism; however, among them, the benzo(a)pyrene (BaP) is the most studied.

The metabolites, deriving from respective PAHs, are the real carcinogenic agents. PAHs generally lack characteristics of electrophylicity that enable covalent interaction with DNA nucleophilic centers (nitrogen, oxygen and phosphorus atoms). However, metabolic conversion of PAHs by microsomal oxidative systems (epoxide hydrolase and cytochrome P-450 system) generates intermediates that can form highly reactive species responsible of interaction with the DNA. The addition products result in a distortion of the DNA helix structure, compromising its function. These adducts are very difficult to be fixed by DNA repairing enzymes so they act as starters in chemical carcinogenesis, leading to cancer development (Song *et al.*, 2012).

Several experimental evidences indicate that benzo(a)anthracene (BaA), through oxidation by Cytochrome (CYP-P450) enzymes together with epoxide hydroxylase, leads to a reactive carcinogenic metabolite (3,4- diol-1,5-epoxide-benzo(a)anthracene) that binds DNA forming an adduct with it (BaA-DNA-adduct) then causing cancer (Mi-Kyung Song *et al.*,, 2012) (Figure 1.4).

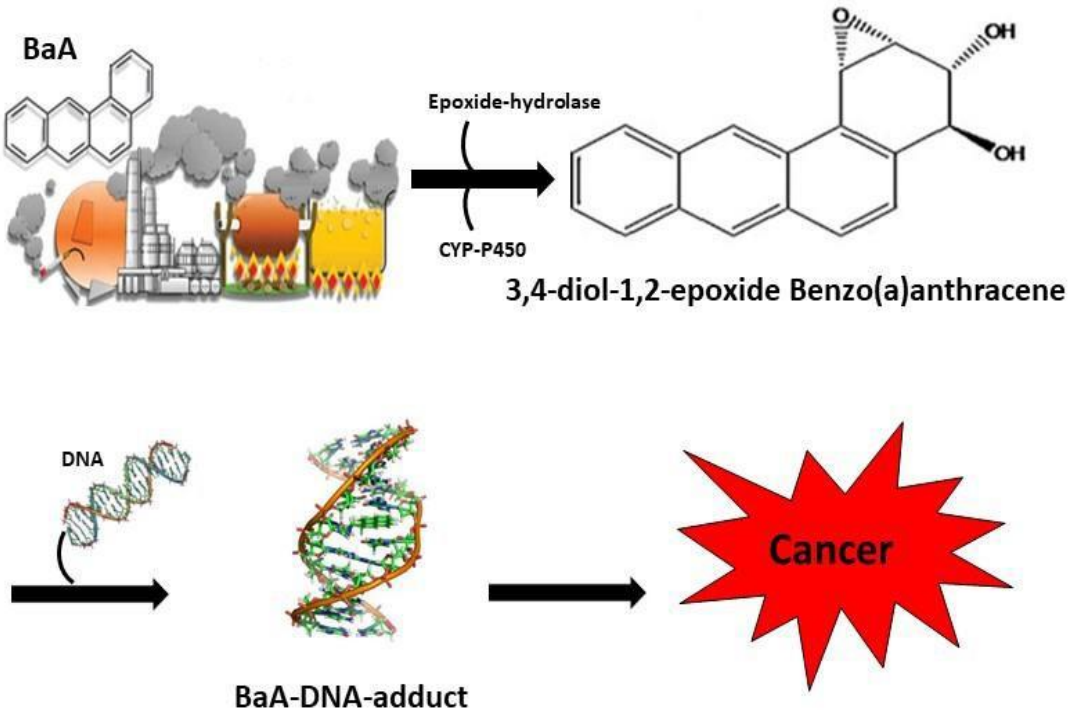

Figure 1.4: *Metabolic activation of BaA.*

Some others PAHs are weak carcinogens that require metabolism to become more potent. Therefore, the International Agency for Research on Cancer (IARC) has identified benzo(a)pyrene (B(a)P) as “carcinogenic to humans” (group 1; IARC 2010). Other PAHs have been recognised as “probably carcinogenic to humans” and included in groups 2A (dibenzo(a,h)anthracene) and 2B (chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene) (Table 1.2) (www.iarc.fr/index.php).

Table 1.2*: IARC Classification of PAHs.*

N° CAS	Compounds	Group	Definition
50-32-8	Benzo(a)pyrene	1	Carcinogenic to humans
53-70-3	Dibenzo(a,h)anthracene	2A	Probably carcinogenic to humans
56-55-3	Benzo(a)anthracene	2B	Possibly carcinogenic to humans
85-01-8	Phenanthrene	3	Not classifiable as to its carcinogenicity to humans
91-20-3	Naphthalene	2B	Possibly carcinogenic to humans
120-12-7	Anthracene	3	Not classifiable as to its carcinogenicity to humans
129-00-0	Pyrene	3	Not classifiable as to its carcinogenicity to humans
189-55-9	Dibenzo(a,i)pyrene	2B	Possibly carcinogenic to humans
189-64-0	Dibenzo(a,h)pyrene	2B	Possibly carcinogenic to humans
191-07-1	Coronene	3	Not classifiable as to its carcinogenicity to humans
191-24-2	Benzo(g,h,i)perylene	3	Not classifiable as to its carcinogenicity to humans
191-26-4	Anthanthrene	3	Not classifiable as to its carcinogenicity to humans
205-99-2	Benzo(b)fluoranthene	2B	Possibly carcinogenic to humans
207-08-9	Benzo(k)fluoranthene	2B	Possibly carcinogenic to humans
218-01-9	Chrysene	2B	Possibly carcinogenic to humans

Food ingestion is an important PAH source of human exposure, therefore a better understanding of PAH accumulation in food, in particular fruit and vegetables, is required.

Plants play an important role in the global cycling of PAHs; however, the uptake, accumulation, and biochemical transformation of PAHs within plants need a deeper knowledge.

Organic pollutants can enter plants from contaminated soils through plant roots, but they can also derive from the atmosphere by deposition on leaves or by uptake through

the stomata. In many cases, once pollutants enter plant tissues, they can migrate from roots to leaves (acropetal), and vice versa (basipetal), depending on chemical processes.

In general, to evaluate the effects of contaminants such as PAHs on the environment, it is appropriate to define their bioavailability and bioaccessibility. Semple *et al.* (2004) have defined the term bioavailability as the fraction of substance that, at a given time, is free to cross the cell membrane of an organism, while bioaccessibility means that is certainly and potentially bioavailable, distinguishing between what is measured in a chemical test and in a biological assay. Therefore, the fate of organic contaminants in soil is a function of bioavailability. In fact, a large part of contaminants is adsorbed mainly by the organic matter of the soil (Nam *et al.*, 1998; Alexander, 2000) and partly by interacting with the fine-grained mineral component (Ball and Roberts, 1991). Nam *et al*. (1998) and Yang *et al.* (2010) found a positive correlation between the fraction of sequestrated PAHs and the organic matter content, reducing their accessibility to biota (Alexander, 2000; Reid *et al.*, 2000).

PAHs outcome in soil is strongly associated with the organic matter fraction. PAH accumulation in soil depends on their physico-chemical properties that affect binding with colloidal fraction, nonpolar compounds (hydrophobic interactions), anions and cations (ionic interactions) within the soil.

Therefore, PAHs might not be expected to be susceptible to plant uptake and subsequent translocation. As consequence, if plant root uptake and translocation are inefficient processes, PAHs in shoot and leaf tissues should derive from atmosphere as a result deposition on particle-bound compounds and the retention of vapour-phase PAH on the waxy leaf cuticle (Kipopoulou *et al.*, 1999). It has been suggested that lipophilic organic pollutants include PAHs partition to the epidermis of the root or to soil particles and are not drawn into the inner root or xylem, because this part of translocation system is water-based (Simonich and Hites, 1995).

Later the knowledge advanced: PAHs enter plants through two main routes: (i) root uptake and subsequent translocation into various plant parts through the transpiration process and (ii) foliage uptake of atmosphere PAHs from the surroundings (Collins *et al.*, 2006; Srogi, 2007).

The first route is usually predominant for low-volatile PAHs and the degree of PAH uptake is influenced by the contaminant properties, plant species and soil in which they are strongly associated with the organic matter fraction. Highly lipophilic or low water-

soluble PAHs generally have high tendency to bioaccumulate from soil and water into plant roots (Gao and Zhu, 2004).

Plant composition and lipid content, in particular, also influence the uptake of PAHs. For example, Simonich and Hites (1994) observed higher concentrations of PAHs in vegetation with higher lipid content. This finding was further supported by previous observations that plant root contamination and the root contamination factor are significantly and positively correlated with root lipid content (Gao and Zhu, 2004), suggesting a primary role of plant lipids for PAH retention.

1.4 European policies on PAH content in food

On the basis of examination of PAHs outcomes in food and the evaluation of carcinogenicity studies in animals, the Scientific Committee on Food (SCF) suggested the use of B(a)P as a marker of the occurrence and the effect of carcinogenic PAHs in food. Therefore, since 2002, B(a)P has been listed in European legislation as a marker of the presence of carcinogenic PAHs in food, thus leading to also establish maximum limits in certain foodstuffs. Subsequently, on the basis of the European Food Safety Authority (EFSA) conclusion, the European Commission determined that B(a)P alone was not a suitable marker of the occurrence of PAHs in food. Based on the currently available data relating to occurrence and toxicity, the EFSA, concluded that a set of four specific substances (4 PAHs: benzo(a)pyrene, benzo(a)anthracene, chrysene, and benzo(b)fluoranthene) are the most suitable indicators of PAHs in food (EFSA, 2008).

New maximum levels for the sum of the above mentioned four PAHs should be introduced, whilst maintaining a separate maximum level for B(a)P to ensure comparability of previous and future data.

To date, the content of PAHs in cereals and vegetables is not that high and the available data indicate low levels of PAHs, so currently available occurrence data do not justify the immediate setting of maximum levels. Nevertheless, EFSA identified cereals and vegetables as important contributors to human exposure due to their high consumption, and then the PAH levels in these two product groups should be further monitored. Based on future data, the need for setting maximum levels should be required (Reg. (EU) No 835/2011).

1.5 Aim

Humans are exposed to PAHs through different pathway, but the major route of exposure is consumption of food. Food can be contaminated from environmental sources expecially anthropogenic sources without exclude natural ones. PAHs can enter the food chain by deposition from air or by deposition and transfer from soil and water. While low molecular weight PAHs (two- and three-rings) occur in the atmosphere in the vapour phase and multi-ringed PAHs (five-rings) are bound to particles, intermediate molecular weight PAHs (four-rings) are partitioned between the vapour and particulate phases, depending on atmospheric temperature (Howsam *et al.*, 2000).

Therefore, PAHs from polluted atmosphere can be transferred to plants by particle-phase deposition on the waxy life cuticle or by uptake in the gas phase through the stomata in according with PAH chemical characteristics. However, PAHs can also enter plant tissues by partitioning from contaminated soil to the roots and translocation into the shoot. However, the extent of PAH uptake by roots is still debated in the literature: because these organic compounds are highly lipophilic and little hydro soluble. PAHs would be adsorbed through the epidermis of roots in contact with soil particles, but not drawn along the inner root (Kipopoulou *et al.*, 1999).

Soil is the natural substrate for the growth and productivity of most plant organisms that inhabit the earth as plants derive nutrients essential to their development from soil; therefore, each bioavailable plant nutrient in the soil is potentially intended to enter the food chain of animals and humans. The current concerns occurring always more often in several regions of the Earth derive from anthropic activities leading to the accumulation of potentially toxic substances in environmental compartments as the wicked practices of illegal spills and waste abandonment may result.

Unfortunately, in the case of illegal practices causing environmental pollution, the problem is complicated by the extremely heterogeneous nature of the potential pollutants and by the lack, in most cases, of scientific research to determine their behavior in the agro-ecosystem and the consequent vulnerability for crops. In recent years, the awareness of undeniable relationship between environment and human health resulted in a growing interest in public opinion.

The region of Campania is the third region by number of inhabitants (about 6 million) and the first by demographic density (432 inhabitants km^{-2}) in Italy. It represents the most industrialized region of Southern Italy and, in particular, Naples was one of the

most industrialized areas until the beginning of the twentieth century, since the last few years witnessed a phase of deindustrialization. Moreover, Campania is the region that mostly contributes to the national agricultural income, thanks also to the high fertility of the territory positively affecting the regional and national economy.

In recent years, the Campania region was involved in some environmental emergencies, some regarding public health, such as the alert for dioxin contamination in buffalo milk and derivatives or the problem of illegal dumping and burning waste. These latter events led to a real environmental emergency called "Land of Fires" that have been faced through synergistic activities of research and monitoring on a specific area of the Campania region. The complexity of the action made necessary to involve the Ministry of Health, the Campania Region, the Agenzia Regionale per la Protezione dell'Ambiente - Regione Campania (ARPAC), the University of Naples "Federico II" and the Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM). In this context, a partnership started between the Department of Agricultural Sciences of Portici of the University of Naples "Federico II" and the Department of Chemistry of the IZSM.

The present thesis aimed to monitor the vegetable contamination in this specific area of Campania region, in which repeated and numerous pollution events occurred. The investigations regarded the contaminant impact on agricultural products grown in this area and in particular the PAH contamination of various fruit and vegetable crops collected in the LoF within a random sampling in farms sited in the risk areas. The Commission Regulation (EC) No 1881/2006 of 19 December 2006 (Commission Regulation (EC) No 1881/2006, 2006) was the first Europe regulation to establish maximum limits for PAHs in food and benzo(a)pyrene, which was recognized as suitable marker for the occurrence and impact of carcinogenic PAHs. In 2011, the 1881/2006 was amended by the Regulation (EU) 835/2011 (Commission Regulation (EU) 835/2011, 2011) and identified benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene as markers for the occurrence of the 16 EU priority PAHs.

The thesis addressed also the uptake and accumulation from/in vegetables and fruits cultivated in the presence of BaA, one of the six congeners more frequently found in the monitoring phase in LoF in several types of vegetables and fruits. The specific objective was to verify if the soil could be the possible source of organic compound contamination for vegetables. The study consisted in the cultivation of two vegetables, turnip and tomato plants, in a spiked agricultural soil and the analysis of plant tissue (root and shoot)

contamination after a 28 days growth in the presence of BaA. The fate of BaA in the pot soil was also studied. As soil is a complex heterogeneous system able to affect strongly the fate, degradation, translocation of BaA in the experiments, the fate of BaA in plants grown in axenic condition required attention to try to nullify the complex interactions between contaminant molecules and soil colloids.

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Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 2006/1881 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs.

Commission Regulation (EU) No 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and

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Chapter 2

2 MONITORING OF PAH CONTAMINATION IN VEGETABLES AND FRUITS: CASE STUDY "LAND OF FIRES"

2.1 Introduction

In ancient times, the Campania Region of Italy, more precisely, the area ranging from Mount Massico, in the North, to Campi Flegrei and Vesuvius area in the South, was known as *Campania Felix*, i.e. fertile Campania thanks to the volcanic nature of the soil as well as to the presence of the Volturno River (Figure 2.1).

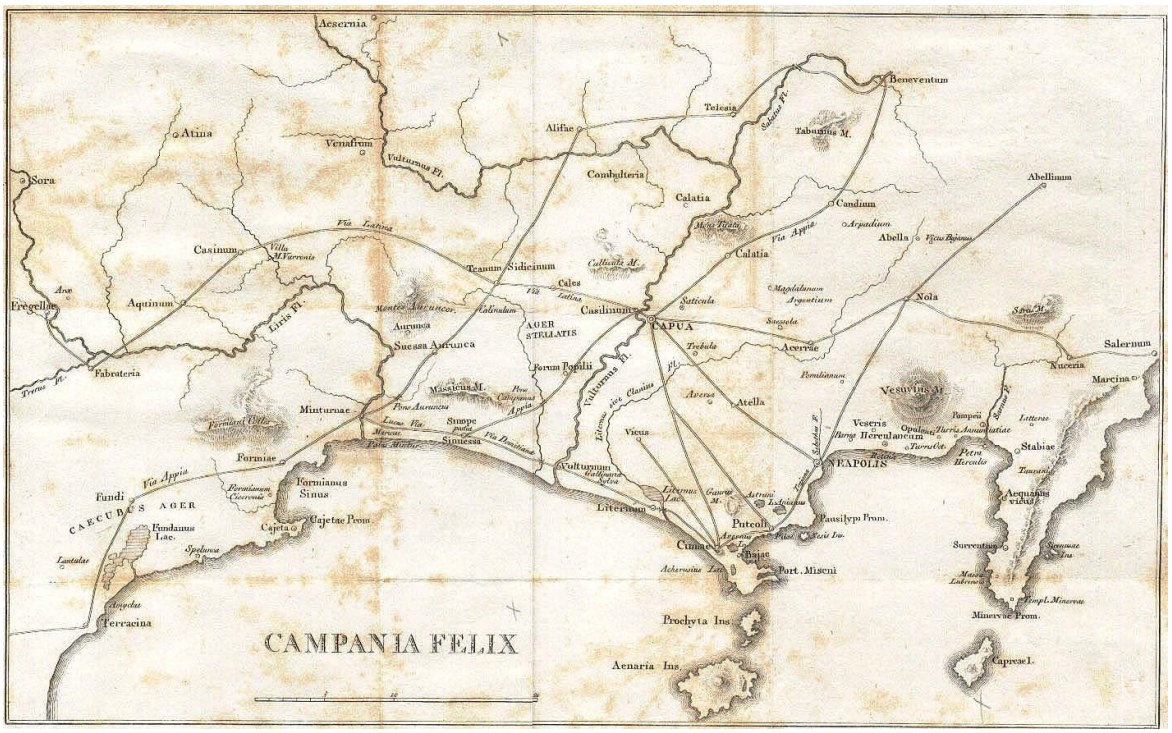

Figure 2.1: *Etching from "Campania felix" of De Laurentis – Naples 1826 (http://www.ulixes.it/italiano/cartografia/cartografia_mappa_5.html).*

Today, unfortunately, the same area is known as "Land of Fires" (Terra dei Fuochi in italian). The definition "Land of Fires" was mentioned first time in 2003 in Ecomafie Report from Legambiente. (Ecomafie Report Legambiente, 2003).

This definition identifies a wide area located between the provinces of Naples and Caserta in the region of Campania. This territory is characterized by the illegal dumping and burning of waste. In many cases, the piled of waste, illegally dumped in the countryside or by the roadside, are set on fire, releasing smoke full of toxic substances and giving rise of the famous "burning".

This phenomenon has become not only an environmental problem, but also a real health emergency.

An extended number of people are undergoing consequences: not only those who are directly exposed to and in contact with the source of risk, but also people who live geographically far from what the community identifies it as a "dangerous" area. (Abdel-Shafy and Mansour, 2016). Environmental and social aspects, such as the community health, the food quality, the impact on the environment, and the resilience of ecosystems, representing only some of the themes taken into account when speaking of "Land of Fire", raise public interest.

On the other hand, media have amplified the phenomenon. Media, exploiting the potential of the events, have made the phenomenon as a succession of events.

This image does not capture all aspects of the phenomenon, often deliberately filtered by the newspapers and television needs. A distorted image of the reality rises up. In fact, the aim of media is often to report only news at broadcasting that create sensation. The result was turning this health emergency in a collective psychosis having as direct consequence a huge economic damage for the farms working in this area. Some major brands excluded for their production products cultivated in those territories. Other companies have checked the quality of products from Campania through chemical analyses at their own expenses. The results of the monitoring performed by Coop (Italy) (Coop, 2014) showed that the content of toxic chemicals such as heavy metals, polychlorinated biphenyls (PCBs), dioxins, polycyclic aromatic hydrocarbons (PAHs) and radionuclides in both vegetable and fruit samples, waters, and soils was within the law levels.

Therefore, on the one hand media amplify the phenomenon “Land of fires” and, on the other hand, no significant pollution in the affected areas has been found, if the presence in the area of major industrial agglomerations should be considered.

In this area, urbanized at more than 40%, there is a very high population, as well as productive and infrastructural-density. In fact, the area involved in the phenomenon “Land of Fires” is less than 15% of the Campania region, where, however, over 4 million people (75% of the entire population of Campania) live. Therefore, it is obvious that the level of environmental quality is somehow affected by this, as it has clearly explained by the results of the “Life Ecoremed” project. (Project Life Ecoremed, 2012).

The problem of illegal landfill waste not only affects the region of Campania, but it

concerns the entire national territory. In the 2013 news related to toxic waste also includes "the unspoiled Trentino", as defined in an article published on inquiries.repubblica.it, where it is stated that in the land of Trentino there are thousands of tons of toxic waste. Ex-cave "huge quantities of substances, residues from industrial processing, coming from half Italy" are stored. Not only waste, but also possible emissions beyond the law limits by local steel mills (La Repubblica, Le inchieste, 2013).

Therefore, it would not be appropriate to consider a unique phenomenon of "Land of Fires", identifying it with a particular area, but rather a spread problem of waste management throughout Italy and far from being solved.

Since the year 2008, in fact, there have been over time a number of sampling plans, both national and regional, to search for environmental contaminants such as dioxins, PAHs, PCBs and heavy metals.

The goal of each plan has been to analyze raw materials in order to identify the batches that may be still contaminated before they were processed into a finished product. Thus it is prevented contaminated materials arrive into the production circuit, trying to preserve firstly the food security as well as the local economy.

Initially an expanded sampling started throughout the region of Campania and then the activities progressively focused on the areas with "high risk" for the environment, such as the “Land of Fires”. To date, the actions related to the crisis were carried out at national and regional level.

The national regulations are:

- The issuing of the Legislative Decree n.136 dated 10 December 2013 “Terra dei Fuochi”, and then turned into Law n.6 dated 6 February 2014.
- Government Directive that specifies the guidelines of technical surveys for mapping the agricultural soils of Campania Region (article 1, paragraph 1 of the Decree-Law n°136 dated 10 December 2013).
- Implementation of the mapping of land and publication of the Official Report program.
- Implementation of the specific investigation program in potentially contaminated agricultural areas (sampling and laboratory analysis).

As stated by the Intergovernmental Directives a Working Group (WG) was established. This WG consists of the following entities:

- ✓ Agenzia per le Erogazioni in Agricoltura (AGEA);

- ✓ Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA);
- ✓ Agenzia Regionale per la Protezione Ambientale - Campania region (ARPAC);
- ✓ Istituto Superiore di Sanità (ISS);
- ✓ Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA);
- ✓ Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM);
- ✓ Istituto Zooprofilattico Sperimentale di Abruzzo e Molise (IZSAM);
- ✓ Region of Campania;
- ✓ University of Naples "Federico II".

The objectives of WG are:

- to identify sites affected by abusive spills and disposal;
- to map and classify soils included in these municipalities into six groups according to aptitude to be suspicious areas (Table 2.1).

Table 2.1: *Classification of soils in LoF plan.*

Class	Typology
1	Presence of only superficial waste
2	Presence of only excavations and soil movements
3	Excavations / movements of soil and coatings
4	Excavations / movements of soil and surface coatings with waste
5	Excavations / movements of soil and surface coatings with waste and fires
6	Anthropic suspicious activity that determines the abandonment of agricultural activity

This WG has the task to provide a scientific reference model aimed at defining the criteria for the land evaluation, in order to ensure the health and quality of the agricultural food crops.

This model is not limited to classify sites depending on absolute concentration of any contaminants, but it takes into account also the transfer of pollutants from soil to plants and therefore in food crops. In fact, the bioavailability of contaminants in soil does not depend on their absolute concentrations, but rather on their chemical and mineralogical forms and on the chemical, physical and microbiological properties of soil (Summary Report, 2013).

At the local level, several focused activities can be mentioned:

- Project LIFE ECOREMED "Sviluppo di protocolli eco-compatibili per la bonifica di suoli agricoli contaminati nell'ex SIN Litorale Domizio Agro-Aversano". The project has as main objective the creation of an operational link between the technological and scientific results produced by the project and land management policies. Thus, it intends to support farmers with regulatory and financial tools, to promote remediation techniques of degraded agricultural soils, and to restore the agronomic fertility, multi-functionality and landscape identity. Moreover, the skills involved in the different phases of validation of remediation protocols converge towards information and technical assistance to local authorities at different levels (municipal and regional) (Project Life Ecoremed, 2012).
- Control system "QR Code Campania", set up by the Campania Region, through the Istituto Zooprofilattico Sperimentale del Mezzogiorno, in collaboration with the Department of Agricultural Sciences and the Department of Veterinary Medicine and Animal Production of the University Federico II of Naples. The QR CODE is a system of certification for healthiness of food products. This project supports and accompanies the Companies of the Campania region on a virtuous path to protect consumers.

  The purposes of the Certification “QR Code Campania secure” are the following:

  - ✓ guarantee of the productions in the sales and processing of food products at national and international level;
  - ✓ transparency and increased consumer confidence;
  - ✓ corporate image commercial opportunities.

  The procedure for the issue of this certification involves a cognitive stage company with which it analyzes the entire production process and the target market for products.

  Then a specific analysis plan is defined. Once food products are healthy it is released the brand "QR Code Campania". Therefore, the consumer buying products from Campania can choose those with the QR Code mark. Framing with the camera of the smartphone to the QR Code on the packaging, the consumer may know all information (such as traceability, tests and analyses latest dates) on the quality of the product (www.qrcodecampania.it).
- Monitoring plan “Land of Fires”, carried out by Campania Region through the

Istituto Zooprofilattico Sperimentale del Mezzogiorno, the Osservatorio Regionale sulla Sicurezza Alimentare (ORSA), the ASL Napoli 2 North, ASL Caserta (Regione Campania Extraordinary monitoring plan on food matrices, 2011). The monitoring plan "Land of Fires" was carried out on animal and vegetable food. The chemical analyses focused not only on substances already normed by European regulations, such as lead and cadmium, but also on analytes not regulated like PAHs in vegetable food.

In the three years 2014-2016 this monitoring plan included the area that the coast Domitio-Flegrea, the Agro Aversano-Atellano, the Agro Nocerano-Nolano, the Vesuhatvian land, and the city of Naples delimit. Initially, the municipalities involved were 57 (Zone A) to which other 66 municipalities joined subsequently (Zone B) selected depending on their environmental criticality. Finally 120 municipalities were included (Figure 2.2). The municipalities have voluntarily signed the so-called "Land of Fire Pact", a document that requires them to adopt measures against to the waste burning phenomenon in streets and public areas.

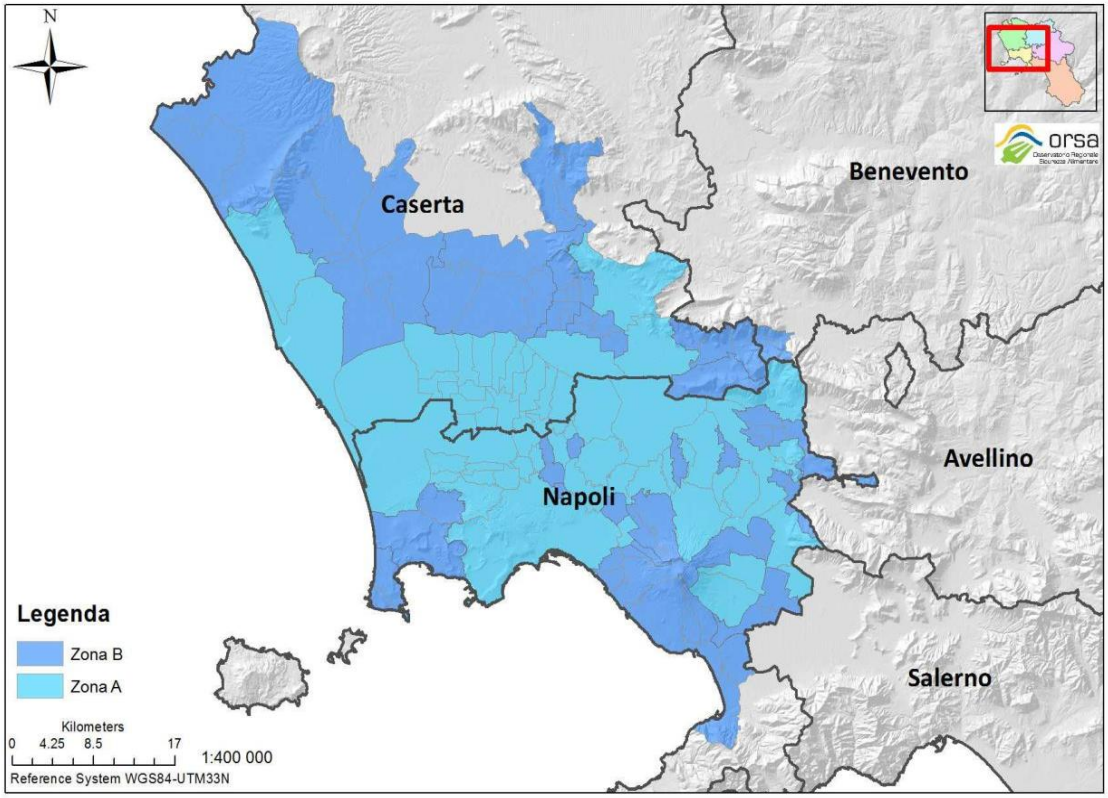

Figure 2.2: *Map of the Municipalities included in the project LoF*

2.2 Aim

The current legislation EC Regulation n° 835/2011 of 19 August 2011 (amending regulation EC n°1881/2006) defines the maximum levels of benzo(a)pyrene (BaP) and the sum of benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, and crysene (BaA, BaP,, BbF and Cry respectively) in some types of foodstuffs (Table 2.2).

Table 2.2: *Maximum levels of BaP and ∑ 4PAHs (BaP, BaA, BbF, and Cry) in some types of foodstuffs*

Foodstuffs	**BaP** **µg kg^{-1}**	**∑ 4PAHs** **µg kg^{-1}**
Oils and fats (excluding cocoa butter and coconut oil) for food	2.0	10.0
Cocoa beans and derived products	5.0*	30.0*
Coconut oil for direct consumption or as an ingredient in human nutrition	2.0	20.0
Muscle meat of smoked fish and smoked fishery products	2.0	12.0
Smoked meat and smoked meat products	2.0	12.0
Shellfish and smoked sprats	5.0	30.0
Smoked shellfish	6.0	35.0
Dietary foods for special medical purposes intended specifically for infants	1.0	1.0
Infant formulae and follow-on formulae, including infant milk and follow-on milk	1.0	1.0
Cereal-based foods and foods for infants and children	1.0	1.0

***** These values are expressed as µg kg^{-1} fat.

Furthermore, the available data on PAHs in cereals and vegetables are scarce and indicate that cereals and vegetables contain rather low levels of PAHs. Hence, the low levels do not urge to define maximum levels. Nevertheless, EFSA identified cereals and vegetables as important contributors to human exposure due to their high consumption. Therefore, PAH levels in these two food groups should be further monitored, so to understand maximum levels should be set.

The purpose of this study was to determine the distribution and levels of PAHs in vegetable and fruit samples collected in the region of Campania, in the area called “Land of Fires”, where the high risk of contamination is due to the illegal disposal of toxic wastes and the subsequent combustion in land for agricultural use.

2.3 Materials and methods

2.3.1 Sampling sites

In the 3-year period 2014-2016 the samples of fruit and vegetables analyzed to find and quantify six PAHs (BaA, Cry, BaP, BbF, BkF, and dBahA) were collected in 90 municipalities (44 municipalities of Naples and 46 of Caserta) included in the area LoF (Figure 2.3 and Table 2.3).

Samples were saved at the laboratory of the Department of Chemistry of Istituto Zooprofilattico Sperimentale del Mezzogiorno of Portici (Naples) where chemical analysis was performed.

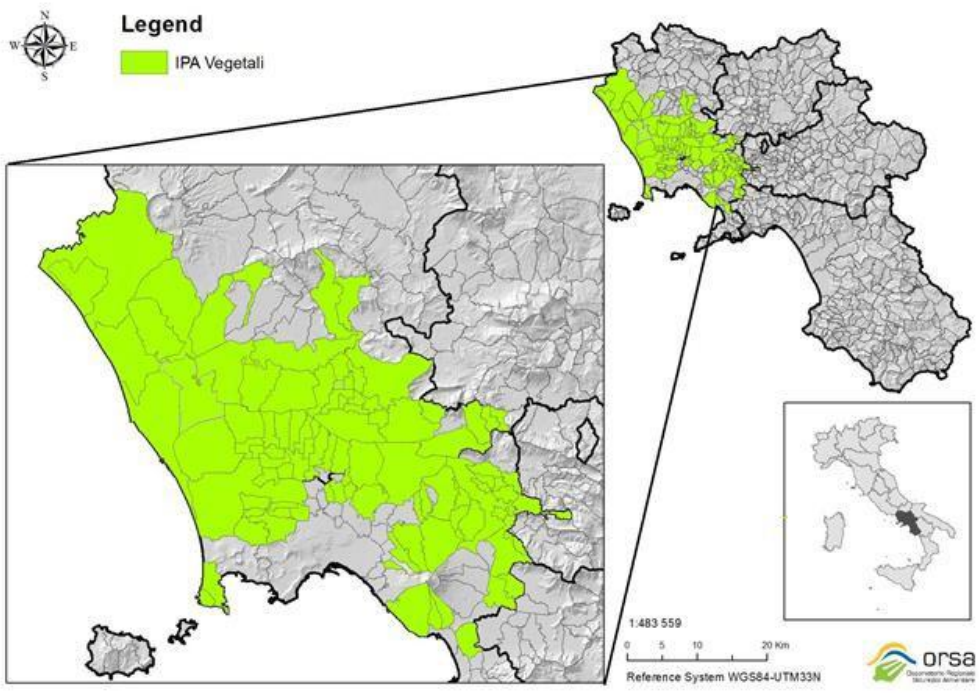

Figure 2.3: *Sampling area*

Table 2.3: *Municipalities included in the monitoring project LoF.*

Province	No.	Municipalities
Naples	44	Acerra, Afragola, Bacoli, Boscotrecase, Brusciano, Caivano, Calvizzano, Carbonara di Nola, Cardito, Casamarciano, Castello di Cisterna, Cicciano, Cimitile, Comiziano, Crispano, Frattamaggiore, Giugliano in Campania, Grumo Nevano, Liveri, Marano di Napoli, Marigliano, Monte di Procida, Mugnano di Napoli, Nola, Palma Campania, Poggiomarino, Pollena Trocchia, Pompei, Portici, Qualiano, Quarto, San Paolo Belsito, San Sebastiano al Vesuvio, San Vitaliano, Sant'Anastasia, Saviano, Scisciano, Somma Vesuviana, Striano, Torre del Greco, Trecase, Tufino, Villaricca and Visciano.
Caserta	46	Arienzo, Aversa, Calvi Risorta, Capodrise, Capua, Carinaro, Carinola, Casal di Principe, Casaluce, Casapesenna, Casapulla, Caserta, Castel di Sasso, Castelvolturno, Cellole, Cervino, Falciano del Massico, Francolise, Frignano, Grazzanise, Villa di Briano, Gricignano d'Aversa, Lusciano, Macerata Campania, Maddaloni, Marcianise, Mondragone, Orta d'Atella, Parete, Pontelatone, Portico di Caserta, Recale, San Cipriano d'Aversa, San Felice a Cancello, San Marcellino, San Marco Evangelista, San Nicola La Strada, San Prisco, San Tammaro, Santa Maria Capua Vetere, Santa Maria La Fossa, Sessa Aurunca, Succivo, Teverola, Trentola-Ducenta, Villa di Briano, Villa Literno.

2.3.2 Sampling methods

Sampling of plant products for human consumption is classified by Reg. (CE) 2005/396. The measurement of PAHs was performed on a "global sample" of fruits and vegetables. The "global sample" is defined as the representative sample of the collection area and consists of one or more "primary samples". The “primary sample” is a sample taken in one geo-referenced place. A pick-up system was applied depending on the type of the sample and the extension of the agricultural plot in order to make the global sample more representative.

The agricultural plot was identified tracing a hypothetical form "X" (X-sampling scheme, Figure 2.4). Depending on the area size, along the lines in the X, from 5 to 15 incremental samples per hectare were collected (e.g., for areas < 1 hectare 5 incremental samples were taken (4 at the vertices of X and 1 in the centre).

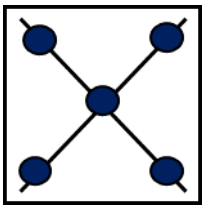

Figure 2.4: *X-sampling scheme*

2.3.3 Sampling in orchards

Depending on the size of the area, fruits deriving from one or more trees correspond to a single sampling point. The amount of fruits necessary to obtain a significant incremental sample depends on the type, shape and size of fruit as well as the characteristics of the place.

In general, a number of units comprised between 5 and 10, coming from one or more trees, had to be reached and, in any case, the amount of the primary sample had to be of the order of 1-2 kg. The fruit must be collected from the outer crown, the top and the bottom as well as inside the canopy itself.

The aggregate sample was made of all the incremental samples; if the aggregate sample was too large, aliquots had to be taken from the previous partition in quarters, collecting two quarter and discarding those diametrically opposed, mixing the remaining amounts up to the attainment of the required amount (about 5 kg of wet substance). The amount of laboratory samples had to be 1-2.5 kg.

2.3.4 Sampling in vineyards

The sampling in vineyards was carried out with the same procedures described for orchards; in this case the primary sample units were 5-10 bunches per group of grapevines, for a total weight of 1-2 kg. Any reduction of the bulk sample, if too large, was realised as indicated for orchards. The amount of laboratory sample consisting of only berries had to be 1 kg.

2.3.5 Sampling in vegetable crops

Depending on the size of the sampled area, vegetable matrices arising from one or more rows of vegetables corresponded to individual sampling point. Similarly to orchards, the amount of matrix necessary to obtain a significant incremental sample depended on different types, shape and size of vegetable and the characteristics of the surrounding places. In particular, the size of the primary sample are the following:

- roots, bulbs and big tubers (potatoes, turnips, onions, etc.): 2 kg or more, having not less than 5 units;
- roots, bulbs and small tubers (carrots, radishes, onions, etc.): 1 kg;
- vegetables with leaf or with large stem (cabbage, broccoli, etc.): 2 kg having not less than 5 complete vegetables;
- vegetables with leaf or medium sized stem (asparagus, lettuce, spinach, etc.): 1kg of complete vegetable;
- vegetables with leaf or with small rod (arugula, etc.): 0,5 kg of complete vegetable;
- vegetables and large fruit (pumpkins, melons, eggplant, etc.): 2 kg having at least 5 fruits;
- vegetables with a medium-sized fruit (peppers, tomatoes, cucumbers, etc.): 1 kg of fruits;
- vegetables and small fruit (peppers, etc.): 250 g of fruits;
- legumes with or without pods (beans, peas, etc.): 1 kg.

The same procedure was adopted in any reduction of a too big global sample. The amount of laboratory samples was 1 kg except for the large bulbs (2 kg), vegetables with leaf or with small rod (0,250 kg), vegetables with fruits of large dimensions (5 kg), vegetables with small size fruits (0,100 kg).

2.3.6 Sampling in greenhouses

The sampling procedures in the greenhouse were similar to the specific procedures above mentioned for sampling in the field.

2.3.7 Sampling of arable crops

To obtain a representative sample of cereals and wheat and/or corn the whole area was divided into 5-10 squares, each having a side of 50 cm (Figure 2.5). These squares were sampled following a W scheme for wheat, barley and wheat in general (Figure 2.5a), and a X scheme for the corn (Figure 2.5b), with 50 cm x 5 m rectangles.

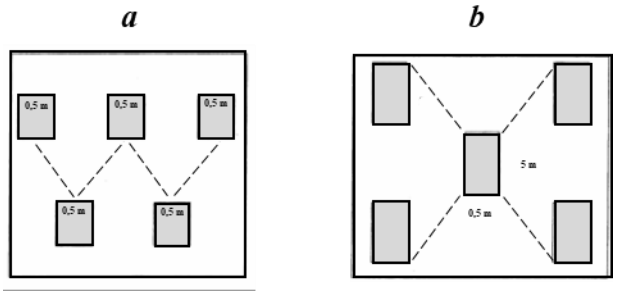

Figure 2.5: *Sampling of arable crops: a) wheat and b) corn.*

The amount of the collected sample had to be at least 20 kg to obtain the bulk sample. The separation of the grain from the whole plant was performed directly in the field or in the laboratory. After that, the quartering and training of the laboratory sample (1 kg) was performed.

Soil residues were removed from all primary samples that were mixed to form the aggregate sample from which the sample laboratory was formed and stored in polyethylene bags at -20 °C before analysis.

2.3.8 Vegetables and fruits

The matrices of plant origin collected within this study were the following:

a. Fresh fruits and nuts

- citrus fruits (oranges, lemons, mandarins, etc.),
- nuts (with or without nuts: walnuts, chestnuts, almonds, hazelnuts, etc.),
- pomacee (apples, pears, loquats etc.),

- drupacee (apricots, prunes, etc.),
- berries and small fruits (grapes, strawberries, etc.),
- fruit varies (loti, etc.).

b. Fresh vegetables

- root and tuber vegetables (potatoes, carrots, beetroots, turnip, radishes, etc.),
- bulb vegetables (garlic, onion, etc.)
- fruiting vegetables (tomatoes, pepperoni, aubergines, zucchini, pumpkin, corn, etc.),
- cabbage,
- leafy vegetables and fresh herbs (broccoli, lettuces, endive, spinach, chicory, etc).

c. Arable crop

- grain cereals (wheat, barley, etc.),
- corn.

2.3.9 Chemicals

The solvents cyclohexane (pesticide residue analysis grade), acetonitrile (HPLC grade), and etanol 98%, hydroxide potassium RPE, and sodium sulphate anidrous RPE were purchased from Carlo Erba (Milano, Italy). Sep-Pak silica cartridges were purchased from Waters (Dublin, Ireland). Purified water was obtained through a Milli-Q water system (Millipore, Billerica, MA, USA).

The standard solution of six PAHs – benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene and dibenzo(a,h)anthracene (Figure 2.6) – was purchased from Dr. Ehrenstorfer (Augsburg, Germany).

The PAH recovery standard mixture was prepared by mixing all six PAHs in acetonitrile (ACN) at 100 $\mu g \cdot L^{-1}$. Stock solutions of each PAH having 100 $\mu g \cdot L^{-1}$ concentration were prepared in ACN and stored in dark at 4 °C. From these, working standard mixed solutions were prepared daily and used to construct the calibration curve by diluting with ACN (Figure. 2.7).

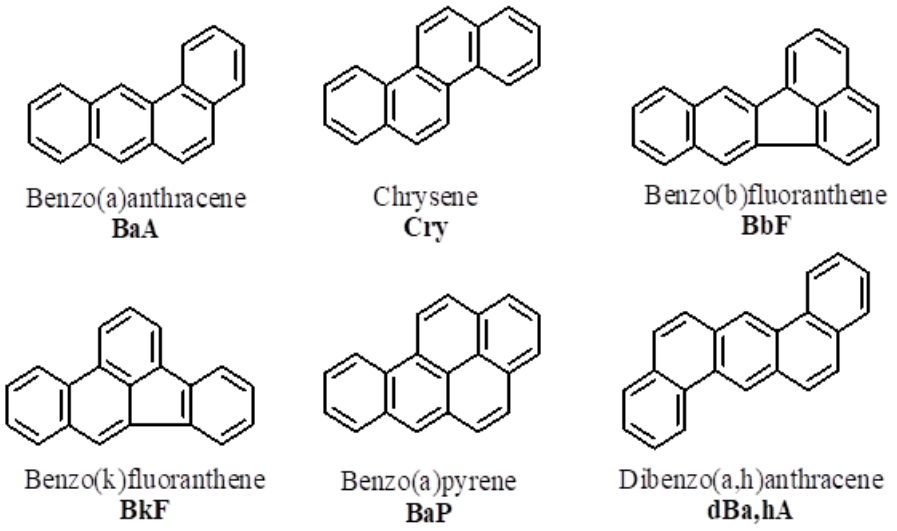

Figure 2.6: *PAHs analysed in fruit and vegetable samples.*

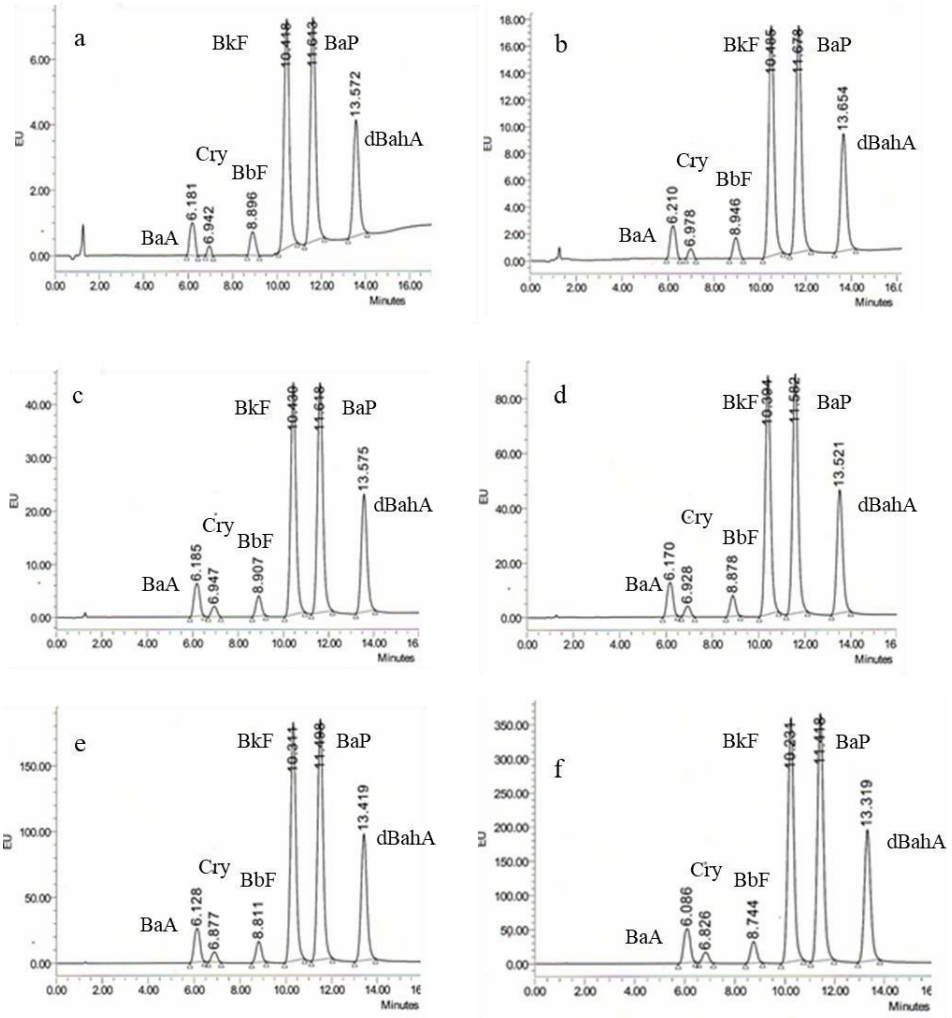

Figure 2.7 *The calibration curve of mix 6 PAH standards at a) 0.4, b) 1.0, c) 2.5, d) 5.0 , e) 10.0, and f) 20.0 µg* L^{-1}.

2.3.10 Sample preparation, extraction and clean-up

After removing skins and the non-edible parts from samples, where possible, they were washed. Then a knife mill Grindomix (Retsch, Haan, Germany) homogenised them. About 2.0 g (fresh weight) of each homogenized sample was placed in a 25 mL Pyrex tube and saponifed with 10 mL of a solution of potassium hydroxide (2 N in ethanol) in a water bath at 80 °C for 2 h. The digest was cooled to room temperature and 10 mL of ultrapure water were added. The digest was extracted with 20 mL of cyclohexane and then centrifuged at 2000 rpm for 5 min at 4 °C. The extraction step with cyclohexane was carried out three times.

The supernatants were assembled (about 60 mL), filtered through a filter paper containing anhydrous sodium sulphate, and reduced to a small volume (about 0.2 mL) by a rotary evaporator at 40 °C (Büchi, Flawil, Switzerland). The evaporated extract were dissolved in 3 mL ACN, transferred onto a Sep-Pak silica cartridges conditioned with 3 mL ACN and eluted with another 3 mL ACN. The solvent of the extract was evaporated in a thermoblock at 40 °C under nitrogen flow; the residue was dissolved in 1 mL ACN and transferred into vials for HPLC-FLD analysis.

Since vegetables and fruits are consumed normally fresh, all results are expressed on fresh weight.

2.3.11 High-performance liquid chromatography (HPLC) analysis

Instrumental analysis were performed by means of HPLC system Alliance E2695 Waters (Waters, Dublin) equipped with a Waters 2475 fluorescence detector (FLD) (Figure 2.8). The HPLC column was an EnvirosepPP 125 mm x 3.20 mm, 5 µm (Phenomenex, Castelmaggiore, Italy). The mobile phase was ACN/water in gradient mode at a flow rate of 0.5 mL min^{-1} with the column oven set at 25 °C. The gradient elution programme, started with an initial mobile phase ACN/water (80:20, v/v), reached linearly 100% ACN in 15 min and, after 5 min, went back to the initial phase (80:20 v/v). The total run time of each analysis was 30 min. The injection volume was 50 µL.

For the FLD-detection of PAHs, excitation and emission wavelengths were set at 294 and 404 nm, respectively. Identification of sample peaks was based on the retention time of standard peaks.

An external standard method was used to determine PAH concentrations in the samples

Figure 2.8: *Waters Alliance HPLC-FLD System (Waters, Dublin)*

2.3.12 Validation method

The performance criteria set out in Commission Regulation EU 836/2011 for the analysis of PAHs were evaluated.

The linearity of the method was verified in the range of 0.0004 – 0.020 mg kg^{-1}, corresponding to concentration values between 0.20 and 10 µg kg^{-1}. Linear least square regression was applied to construct a calibration line showing peak area vs. PAH concentration. The value Rr^2 was higher than 0.99 for all six PAHs, thus demonstrating good linearity.

The limit of quantification (LOQ = 0.2 µg kg^{-1}) was calculated for each PAH using a blank sample fortified with a solution of the six PAHs; the limit of detection (LOD) was 0.1 µg kg^{-1}.

The precision of the analytical method was determined by spiking a blank sample with the 0.1 µl L^{-1} calibration standard solution, carrying through the entire extraction, and clean-up procedure as for the samples. Precision was evaluated by calculating the coefficient of Horrat for each PAH at a concentration of 5.0 µg kg^{-1}, under conditions of

repeatability (Horrat r) and reproducibility (Horrat R). In terms of repeatability, precision was also assessed through the analysis of the blank sample spiked at concentrations of 0.5, 2.0, and 5.0 μg kg^{-1} in at least five replicates. Other five replicates for each concentration level were analysed by a different operator with different lots of reagents and solvent, on different days, to obtain the reproducibility value. Accuracy was calculated as the mean recovery percentage for all individual PAHs compared with the interval of acceptability provided by EU regulation 836/2011: in all cases, the recovery values obtained were in the range 50-120%, as required.

Specificity was assessed by verifying the absence of interfering peaks at approximately ± 2.5% of the retention time (Rt) of the individual PAH in the chromatogram samples of PAH-free dairy products.

For analytical quality assurance measures in each batch of samples, a procedural blank and a spiked sample were included.

2.4 Results and discussion

In the three years 2014-2016, 202 samples of fruit and vegetable were analyzed in the 90 Municipalities (Naples and Caserta) of LoF.

2.4.1 PAH contamination in vegetables and fruits in Naples province

In the province of Naples 117 samples of vegetable matrices were analyzed (56 vegetables and 61 fruits) (Table 2.4). The Figures 2.9, 2.10, and 2.11 show the distributions in the Neapolitan territory of the positive samples for PAHs analyzed. In the Tables 2.5 and 2.6, the PAH content in analyzed vegetable and fruit samples, respectively, are reported.

Table 2.4 *Distribution of sampling in 44 municipalities of province of Naples.*

Municipalities	No samples	No Positive samples	% Positive samples	Positive vegetables	Positive fruits
Acerra	13	4	31	Corn (3)	Lotus (1)
Afragola	1		0		
Bacoli	1	1	100		Orange (1)
Boscotrecase	2	1	50		Orange (1)
Brusciano	1	1	100	Endive (1)	
Caivano	5	4	80	Turnip top (4)	
Calvizzano	1		0		
Carbonara di Nola	1		0		
Cardito	1		0		
Casamarciano	2		0		
Castello di Cisterna	1		0		
Cimitile	2	2	100	Potato (2)	
Comiziano	1	1	100		Halzenut (1)
Crispano	1		0		
Frattamaggiore	2		0		
Giugliano	32	20	63	Aubergine (3) Pumpkin (2) Potato (1)	Peach (6) Prune (8)
Grumo Nevano	2	2	100	Aubergine (1) Endive (1)	
Marano di Napoli	1	0	0		
Marigliano	3	1	33	Tomato (1)	
Monte di Procida	1	1	100		Mandarin (1)
Mugnano di Napoli	1	0	0		
Nola				Pepper (1) Tomato (1) Curgette (1)	Olive (1)
Palma Campania	1	1	100		Walnut (1)
Poggiomarino	1	0	0		
Pollena Trocchia	1	1	100	Apricot (1)	
Pompei	1	1	100	Aubergine (1)	

Table 2.4: *Distribution of sampling in 44 municipalities of province of Naples.*

Municipalities	No samples	No Positive samples	% Positive samples	Positive vegetables	Positive fruits
Portici	6	6	100	Fennel (1) Lettuce (1) Tomato (2)	Apple (1) Walnut (1)
Qualiano	2	0	0		
Quarto	1	1	100		Apple (1)
San Paolo Belsito	1	0	0		
San Sebastiano al Vesuvio	2	2	100	Tomato (2)	
San Vitaliano	2	1	50	Tomato (1)	
Sant'Anastasia	1	1	100		Apricot (1)
Saviano	1	1	100		Halzenut (1)
Scisciano	1	1	100		Halzenut (1)
Somma Vesuviana	1	1	100		Olive (1)
Striano	1	0	0		
Torre del Greco	1	1	100	Tomato (1)	
Trecase	1	1	100		Grape (1)
Tufino	2	1	50		Halzenut (1)
Villaricca	1	0	0		
Visciano	3	1	33		Halzenut (1)

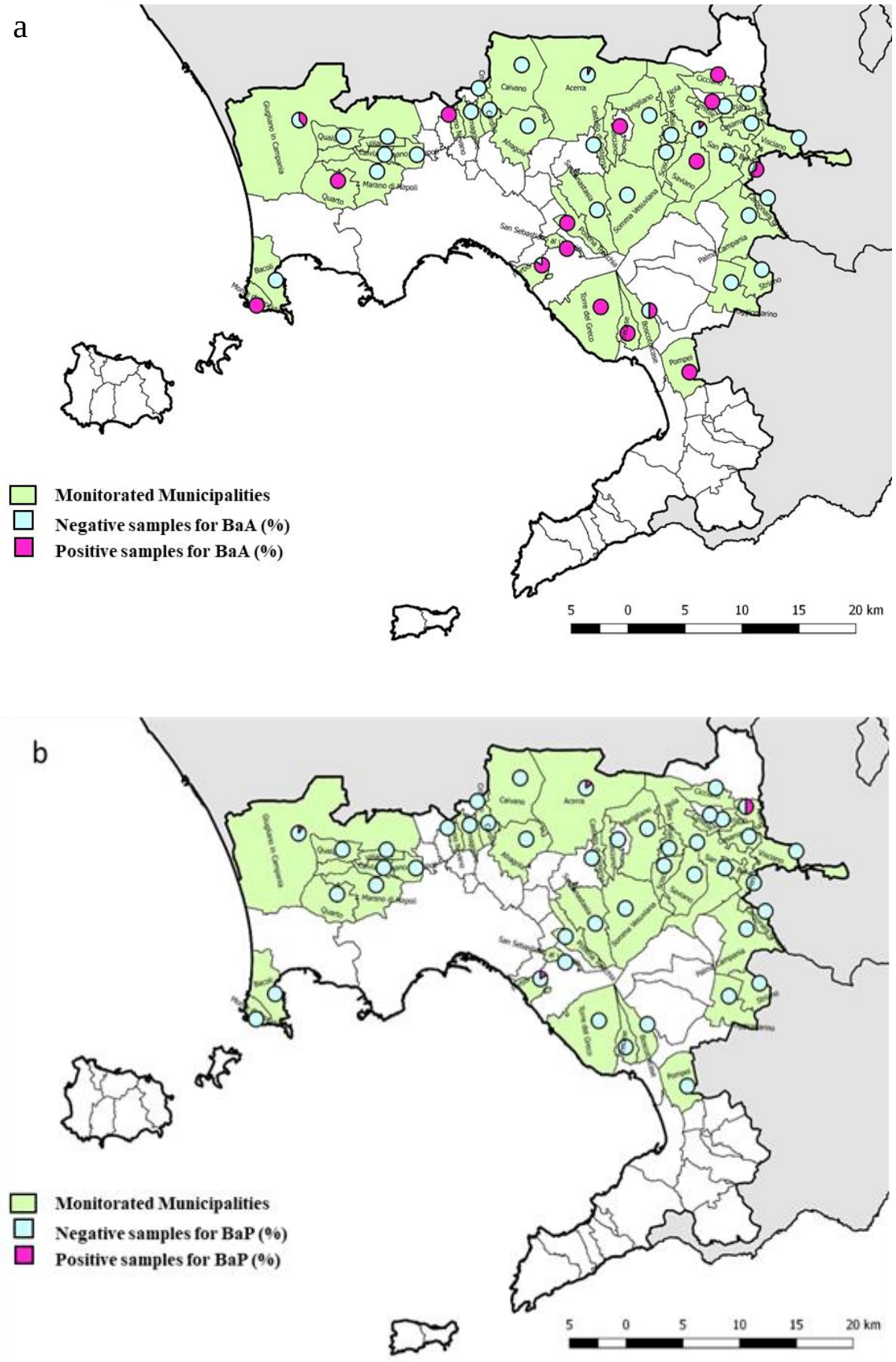

Figure 2.9 *Distribution of positive results (vegetables and fruits) for a) BaA and b) BaP in the Municipalities of Naples*

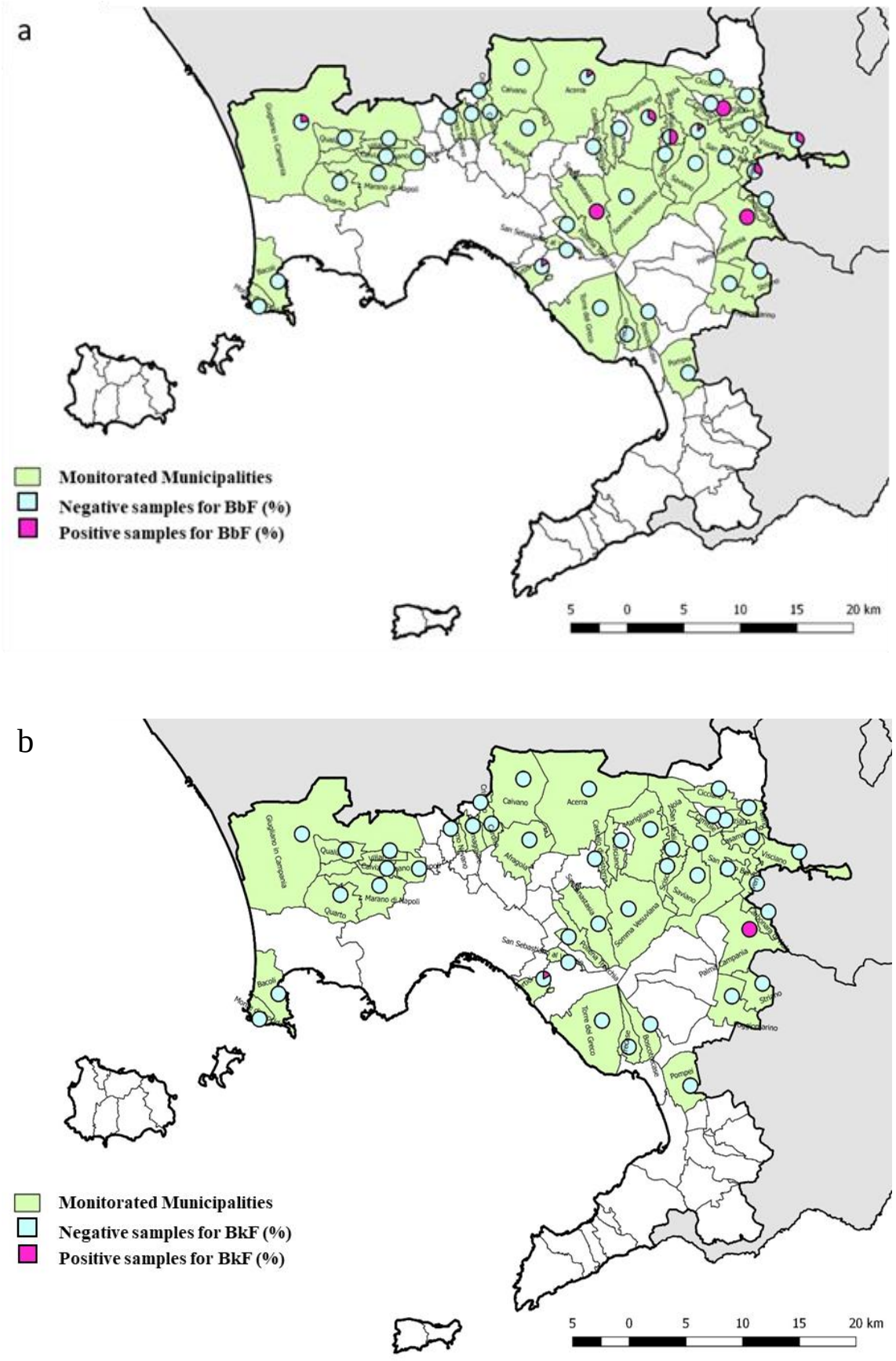

Figure 2.10 *Distribution of positive results (vegetables and fruits) for a) BbF and b) BkF in the Municipalities of Naples.*

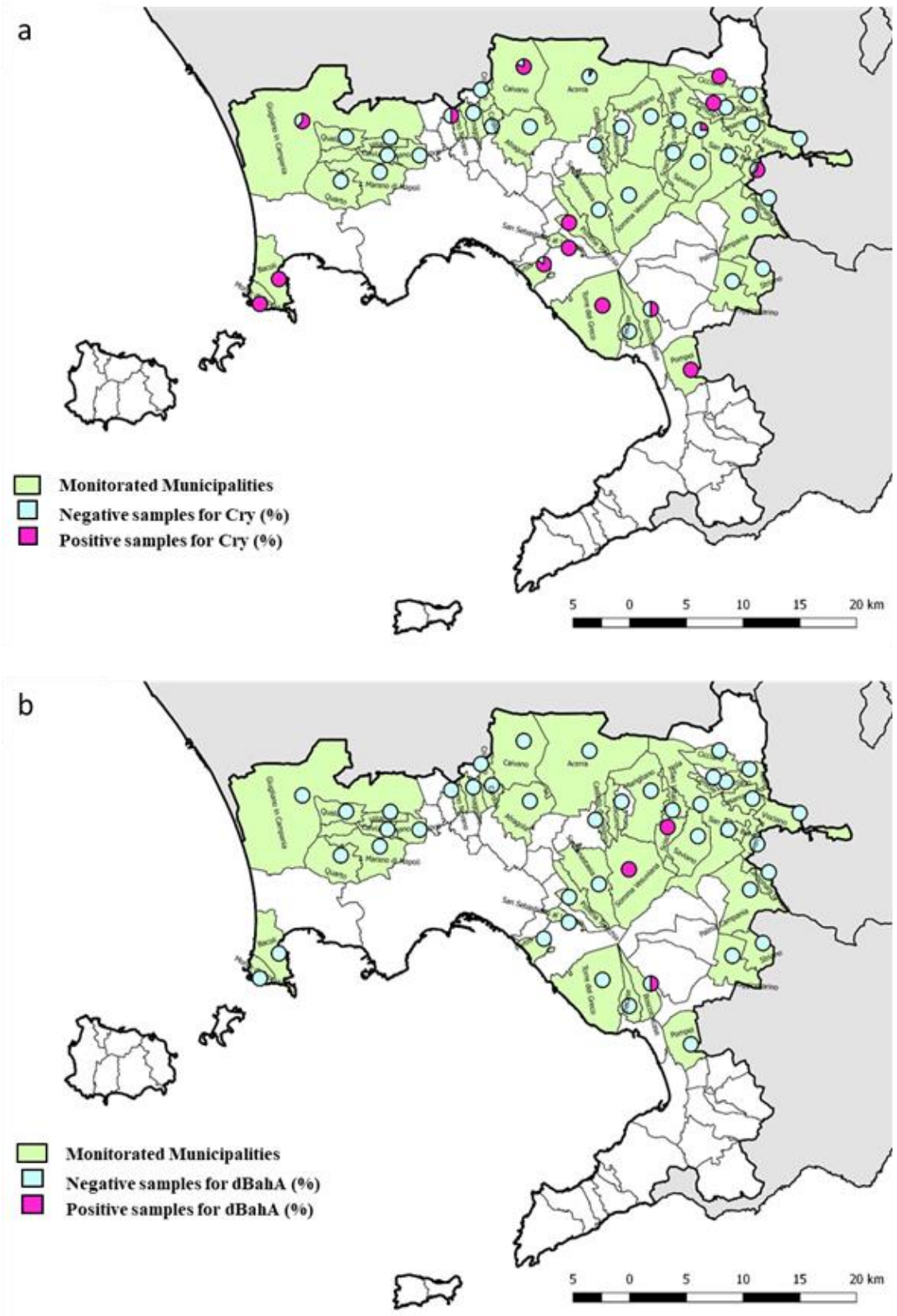

Figure 2.11 *Distribution of positive results (vegetables and fruits) for a) Cry and b) dBahA in the Municipalities of Naples.*

Among 56 samples of analyzed vegetables, only 32 (57%) were positive for PAHs (Table 2.5). Among 61 samples of analysed fruits, only 31 (51%) were positive for PAHs (Table 2.6). The percentage of positive samples within each site number of samples varied markedly as the number of samples is variable and not fixed for each site. In several case the exiguous number of samples determined a very high percentage of positive samples. (Table 2.7). The monitoring activity was not planned in according to a fixed grid with a well distributed number of sampling. Sampling were carried out where criticism occurred.

The positive cases of BaA and Cry contaminated samples were more present whereas the almost absence of BaP and BkF contaminated samples occurred in the map of the Municipality of Naples (Figures 2.9, 2.10 and 2.11). Therefore, BaA and Cry were the most commonly encountered PAHs (38% and 43% of positive samples, respectively) ranging between 0.2 - 1.9 µg $kg^{-1}$and 0.2 - 3.8 µg kg^{-1}, respectively (Table 2.7). BaP was found only in 5 samples (9% of positive samples) ranging between 0.2 - 0.7 µg $kg^{-1}$and this amount is significantly below the maximum permissible limit for the category of childhood foods, where the limits are much more restrictive (Table 2.2). The Σ 4PAHs (BaA, BaP, Cry and dBahA) ranged between 0.2 - 6.9 µg kg^{-1} (Table 2.7).

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Table 2.5: *PAH content in vegetable samples of Municipalities of Naples ∑=BaA+BaP+BbF+Cry)*

Vegetables	No Samples		Municipalitie	6 PAHs (µg kg^{-1}) -LOQ < 0.2 µg kg^{-1}						
	Total	Sites		BaA	BaP	BbF	BkF	Cry	dBahA	∑
Aubergine	5	3	Giugliano	0.2	0.6	< LOQ	< LOQ	0.2	< LOQ	1.0
				0.5	< LOQ	< LOQ	< LOQ	1.0	< LOQ	1.5
				0.6	< LOQ	0.4	< LOQ	1.2	< LOQ	2.2
		1	Grumo Nevano	0.4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.4
		1	Pompei	0.3	< LOQ	< LOQ	< LOQ	0.6	< LOQ	0.9
Cauliflower	1	1	Acerra	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
Curgette	3	1	Cardito	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
		1	Castel di Cisterna	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
		1	Nola	< LOQ	< LOQ	< LOQ	< LOQ	0.3	< LOQ	0.3
Corn	4	1	Giugliano in Campania	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
		3	Acerra	0.3	< LOQ	< LOQ	< LOQ	0.3	< LOQ	0.6
				< LOQ	0.3	0.4	< LOQ	< LOQ	< LOQ	0.7
				< LOQ	0.7	< LOQ	< LOQ	< LOQ	< LOQ	0.7
Endive	2	1	Brusciano	0.4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.4
		1	Grumo Nevano	0.6	< LOQ	< LOQ	< LOQ	1.4	< LOQ	2.0
Fennel	1	1	Portici	0.3	< LOQ	< LOQ	< LOQ	0.6	< LOQ	0.9
Lectuce	1	1	Portici	1.9	0.4	0.8	< LOQ	3.8	< LOQ	6.9
		1	Poggiomarino	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
Onion	1	1	Striano	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
Pea	2	2	Giugliano in Campania	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
Pepper	1	1	Nola	< LOQ	< LOQ	< LOQ	< LOQ	0.3	< LOQ	0.3
Potato	15	7	Acerra	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
		1	Afragola	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
		1	Cicciano	0.3	< LOQ	< LOQ	< LOQ	1,0	< LOQ	1.3
		2	Cimitile	0.3	< LOQ	< LOQ	< LOQ	0.6	< LOQ	0.9
				0.5	< LOQ	< LOQ	< LOQ	1.0	< LOQ	1.5
		1	Crispano	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ
		1	Giugliano in Campania	0.2	< LOQ	0.3	< LOQ	0.4	< LOQ	0.9
		1	Marigliano	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
		1	Portici	0.3	< LOQ	< LOQ	< LOQ	0.8	< LOQ	1.1
Pupkin	2	2	Giugliano in Campania	0.5	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.5
				0.2	0.2	0.4	< LOQ	0.7	< LOQ	1.5

Table 2.5: *PAH content in vegetable samples of Municipalities of Naples ∑=BaA+BaP+BbF+Cry)*

Vegetables	No Samples		Municipalities	6 PAHs (µg kg^{-1}) -LOQ < 0.2 µg kg^{-1}						
	Total	Sities		BaA	BaP	BbF	BkF	Cry	dBahA	∑
Tomato	12	2	Frattamaggiore	< LOQ	<LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
		2	S. Sebastiano al Vesuvio	0.3	< LOQ	< LOQ	< LOQ	0.6	< LOQ	0.9
				0.3	< LOQ	< LOQ	< LOQ	0.7	< LOQ	1.0
		1	S. Vitaliano	< LOQ	< LOQ	0.8	< LOQ	< LOQ	< LOQ	0.8
		1	Torre del Greco	0.4	< LOQ	< LOQ	< LOQ	0.4	< LOQ	0.8
		1	Portici	0.7	< LOQ	< LOQ	< LOQ	2.2	< LOQ	2.9
		1	Castel di Cisterna	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
		2	Marigliano	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
				< LOQ	< LOQ	0.3	< LOQ	< LOQ	< LOQ	0.3
		2	Nola	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
				< LOQ	< LOQ	0.2	< LOQ	< LOQ	< LOQ	0.2
Turnip	5	5	Caivano	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
				< LOQ	< LOQ	< LOQ	< LOQ	0.2	< LOQ	0.2
				< LOQ	< LOQ	< LOQ	< LOQ	0.2	< LOQ	0.2
				< LOQ	< LOQ	< LOQ	< LOQ	0.2	< LOQ	0.2
				< LOQ	< LOQ	< LOQ	< LOQ	0.5	< LOQ	0.5

Table 2.6: *PAH content in fruit samples of Municipalities of Naples (∑= BaA+BaP+BbF+Cry)*

Fruits	No Samples		Municipalities	6 PAHs (µg kg^{-1}) - LOQ < 0.2 µg kg^{-1}						
	Total	Site		BaA	BaP	BbF	BkF	Cry	dBahA	∑
Apple	2	1	Quarto	0.5	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	0.5
		1	Portici	0.5	<LOQ	<LOQ	< LOQ	1.0	0.3	1.5
Apricot	3	1	Liveri	0.3	<LOQ	<LOQ	< LOQ	0.7	< LOQ	1.0
		1	Pollena Trocchia	0.4	<LOQ	<LOQ	< LOQ	0.8	< LOQ	1.2
		1	Sant'Anastasia	<LOQ	<LOQ	0.4	< LOQ	<LOQ	< LOQ	0.4
Grape	2	1	Trecase	0.3	0.2	<LOQ	< LOQ	<LOQ	< LOQ	0.5
		1	Boscotrecase	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Halzenut	16	2	Casamarciano	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Carbonara di Nola	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Comiziano	<LOQ	<LOQ	0.9	< LOQ	<LOQ	< LOQ	0.9
		2	Nola	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Liveri	<LOQ	<LOQ	0.9	< LOQ	<LOQ	< LOQ	0.9
		1	Saviano	0.2	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	0.2
		2	Visciano	<LOQ	<LOQ	0.3	< LOQ	<LOQ	< LOQ	0.3
				<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	S. Paolo Belsito	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	S. Vitaliano	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		2	Tufino	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
				<LOQ	0.5	<LOQ	< LOQ	<LOQ	< LOQ	0.5
		1	Scisciano	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	0.3	<LOQ
Lemon	1	1	Liveri	0.5	<LOQ	<LOQ	< LOQ	0.9	< LOQ	1.4
Loti	1	1	Acerra	<LOQ	<LOQ	0.7	< LOQ	<LOQ	< LOQ	0.7
Mandarin	1	1	Monte di Procida	0.3	<LOQ	<LOQ	< LOQ	0.4	< LOQ	0.7
Olive	2	1	Somma Vesuviana	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	0.4	<LOQ
		1	Nola	1.1	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	1.1
Orange	2	1	Boscotrecase	0.3	<LOQ	<LOQ	< LOQ	0.7	0.2	1.0
		1	Bacoli	<LOQ	<LOQ	<LOQ	< LOQ	0.4	< LOQ	0.4
Peach	10	1	Marano di Napoli	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		1	Qualiano	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		8	Giugliano in Campania	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
				<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
				0.2	<LOQ	<LOQ	< LOQ	0.7	< LOQ	0.9
				0.2	<LOQ	0.3	< LOQ	0.5	< LOQ	1.0
				0.4	<LOQ	<LOQ	< LOQ	0.9	< LOQ	1.3
				0.4	<LOQ	<LOQ	< LOQ	1.6	< LOQ	2.0
				0.2	<LOQ	<LOQ	< LOQ	0.8	< LOQ	1.0
				0.7	<LOQ	<LOQ	< LOQ	0.8	< LOQ	1.5

Table 2.6: *PAH content in fruit samples of Municipalities of Naples (∑= BaA+BaP+BbF+Cry)*

Fruits	No Samples		Municipalities	6 PAHs (µg kg^{-1}) - LOQ < 0.2 µg kg^{-1}						
	Total	Site		BaA	BaP	BbF	BkF	Cry	dBahA	∑
Prune	18	1	Calvizzano	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		7	Giugliano in Campania	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1		<LOQ	<LOQ	0.6	< LOQ	0.5	< LOQ	1.1
		1		<LOQ	<LOQ	0.9	< LOQ	0.9	< LOQ	1.8
		1		0.2	<LOQ	<LOQ	< LOQ	0.4	< LOQ	0.6
		1		<LOQ	<LOQ	<LOQ	< LOQ	0.4	< LOQ	0.4
		1		0.3	<LOQ	<LOQ	< LOQ	0.5	< LOQ	0.8
		1		<LOQ	<LOQ	<LOQ	< LOQ	0.6	< LOQ	0.6
		1		0.2	<LOQ	<LOQ	< LOQ	0.2	< LOQ	0.4
		1		<LOQ	<LOQ	0.6	< LOQ	0.5	< LOQ	1.1
		1	Qualiano	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Villaricca	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Valnut	3	1	Nola	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Portici	<LOQ	<LOQ	<LOQ	< LOQ	0.4	< LOQ	0.4
		1	Palma Campania	<LOQ	<LOQ	1.7	< LOQ	<LOQ	< LOQ	1.7

Table 2.7: *Distribution of positive vegetable samples respect single PAH*

	No Positive samples	% Positive samples	Range µg kg^{-1}
BaA	21	38	0.2 – 1.9
BaP	5	9	0.2 – 0.7
BbF	8	14	0.2 – 0.8
BkF	0	0	0
Cry	24	43	0.2 – 3.8
dBahA	0	0	0
∑ 4PAHs	32	57	0.2 – 6.9

The highest value of BaA, Cry and Σ 4PAHs was found in Portici, which is the second Italian town, after Casavatore (Naples), for population density. It was a sample of lettuce, a leafy vegetable that appears to have suitable properties to collect dust and fine dust due to its conformation, therefore it tends easily to accumulate any contaminants propagated by air.

The samples showing values of the Cry content > 1.0 µg kg^{-1} were:

- 2 samples of aubergines cultivated in Giugliano in Campania (1.0 < Cry < 1.2 µg kg^{-1});
- 1 sample of endive (Cry = 1.4 µg kg^{-1}) cultivated in Grumo Nevano;
- 2 samples of potatoes, one from Cimitile and the other one from Cicciano (Cry = 1.0 µg kg^{-1});
- 1 sample of tomato cultivated in Portici (Cry = 2.2 µg kg^{-1}).

The samples showed values of Σ 4PAHs > 1.0 µg kg^{-1} were:

- 3 samples of aubergines cultivated in Giugliano in Campania (1.0 < Σ 4PAHs < 2.2 µg kg^{-1});
- 2 samples of potatoes, one from Cimitile (Σ 4PAHs = 1.5 µg kg^{-1}) and the other one from Cicciano (Σ 4PAHs = 1.3 µg kg^{-1});
- 1 sample of pumpkin cultivated in Giugliano in Campania (Σ 4PAHs = 1.5 µg kg^{-1});
- 2 samples of tomato, one from S. Sebastiano al Vesuvio (Σ 4PAHs = 1.0 µg kg^{-1}) and the other one from Portici (Σ 4PAHs= 2.9 µg kg^{-1}).

The results highlighted and that there is a strong correlation between BaA and Cry

(0.939) and a slightly lower correlation between BaA and BbF (0.733) and between BbF and Cry (0.755) (Table 2.8). The value of Σ 4PAHs > 1.0 μg kg^{-1} in potatoes could be ascribable to soil contamination (Table 2.5); conversely the contamination of other vegetables could be due to air distribution.

Table 2.8: *Pearson's correlation matrix of PAHs in vegetable samples of Municipalities of Naples*

	BaA	**BaP**	**BbF**	**BkF**	**Cry**	**dBahA**	**∑ PAH**
BaA	1.000						
BaP	0.286	1.000					
BbF	**0.733**	0.306	1.000				
BkF	0.000	0.000	0.000	1.000			
Cry	**0.939**	0.121	**0.755**	0.000	1.000		
dBahA	0.000	0.000	0.000	0.000	0.000	1.000	
∑ PAH	0.972	0.311	0.829	0.000	0.973	0.000	1.000

Within fruits (Table 2.6), PAHs were detected in 35 samples on 61 samples (57% of positive samples). Once again, among these, BaA and Cry were the ones most commonly encountered (31% and 36% of positive samples, respectively) ranging between 0.2-1.1 µg kg^{-1} and 0.2-1.6 µg kg^{-1} respectively (Table 2.9).

Table 2.9: *Distribution of positive fruit samples respect single PAH*

	No Positive samples	**% Positive samples**	**Range µg kg^{-1}**
BaA	19	31	0.2 – 1.1
BaP	2	3	0.2 – 0.5
BbF	11	18	0.3 – 1.7
BkF	0	0	0
Cry	22	36	0.2 – 1.6
dBahA	4	7	0.2 – 0.4
∑ 4PAHs	34	56	0.2 – 2.0

The positive samples of BaA contamination > 1.0 µg kg^{-1} regarded a sample of olive cultivated in Nola (BaA), whereas of Cry contamination > 1.0 µg kg^{-1} regarded a sample of apple cultivated in Portici and a sample of peach cultivated in Giugliano in Campania. Only in two cases BaP contamination was found however having very little concentrations (0.2 µg kg^{-1} in a sample of grapes and 0.5 µg kg^{-1} in a sample of hazelnuts) (Table 2.9).

The samples showed values of Σ 4PAHs > 1.0 µg kg^{-1} were:

- 1 sample of apple cultivated in Portici;
- 2 samples of apricot cultivated in Liveri and in Pollena Trocchia;
- 1 sample of lemon in Liveri;
- 1 sample of olive in Nola;
- 1 sample of orange in Boscotrecase;
- 5 samples of peach cultivated in Giugliano in Campania (1.0 < Σ 4PAHs < 2.0 µg kg^{-1});
- 3 samples of prune cultivated in Giugliano in Campania (1.1 < Σ 4PAHs < 1.8 µg kg^{-1}).

In general, a more relevant contamination level in fruits was observed compared to

vegetables in terms of positive case abundance, but fruits showed a lower contamination levels in terms of contaminant concentration.

Even in the case of fruit, the results highlighted a correlation between BaA and Cry contamination (0.733) (Table 2.10).

Table 2.10: *Pearson's correlation matrix of PAHs in fruit samples of Municipalities of Naples*

	BaA	**BaP**	**BbF**	**BkF**	**Cry**	**dBahA**	**Σ PAH**
BaA	1.000						
BaP	-0.266	1.000					
BbF	-0.289	0.506	1.000				
BkF	0.000	0.000	0.000	1.000			
Cry	**0.733**	0.000	0.120	0.000	1.000		
dBahA	0.140	0.300	0.000	0.000	0.000	1.000	
∑ PAH	0.652	-0.027	0.429	0.000	0.933	-0.191	1.000

2.4.2 PAH contamination in vegetables and fruits in Caserta province

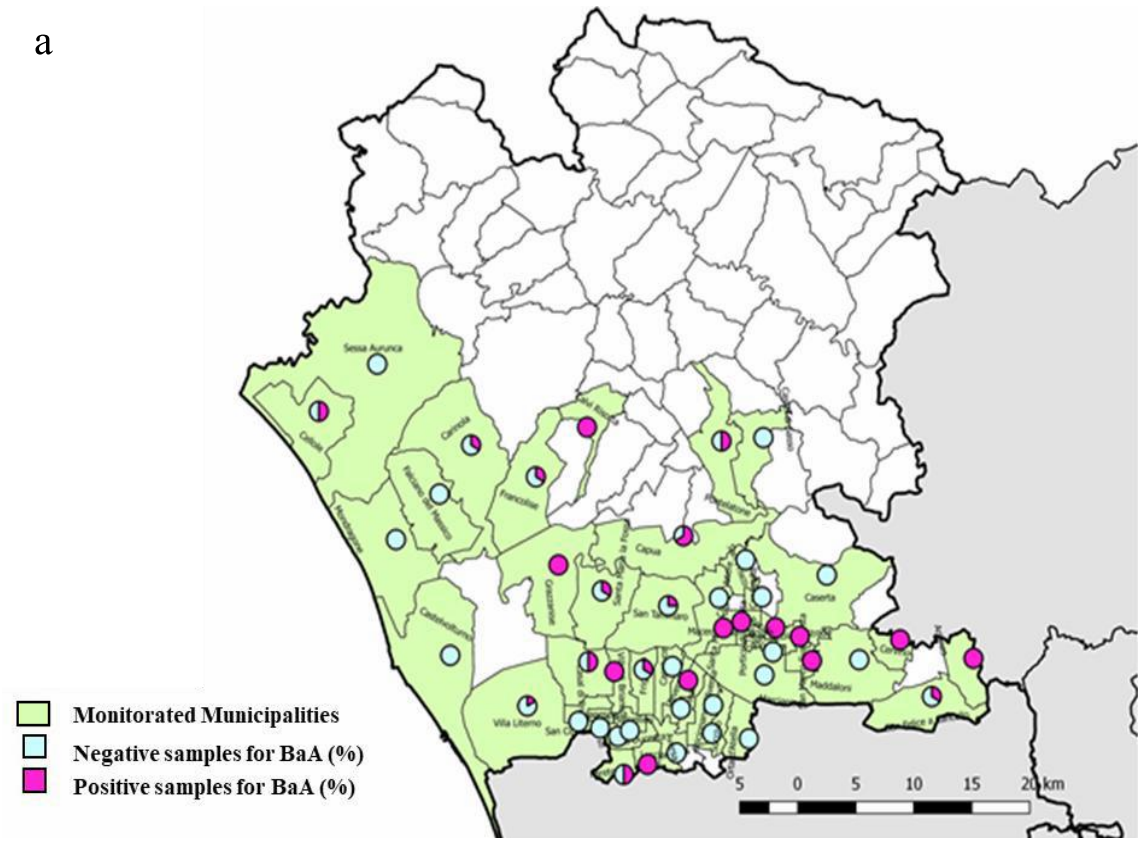
In the 46 Municipalities of the province of Caserta, 86 samples were analysed (62 vegetables and 25 fruits) (Table 2.11). The Figures 2.12, 2.13 and 2.14 indicate the distribution of positive samples in the territory of Caserta. The Tables 2.12 and 2.13 show the results of PAHs analyzed in the vegetable and fruit samples respectively cultivated in territory of Caserta.

Table 2.11 *Distribution of sampling in 46 municipalities of province of Caserta*

Municipalities	No samples	No Positive samples	% Positve samples	Positive vegetables	Positive fruits
Arienzo	3	3	100		Limon (1)
					Loti (1)
					Orange (1)
Aversa	1	0	0		
Calvi Risorta	2	2	100		Olive (2)
Capodrise	1	1	100	Wheat (1)	
Capua	3	3	100	Cauliflower (1)	
				Turnip (1)	
				Curgette (1)	
Carinaro	1	0	0		
Carinola	3	2	67	Aubergine (1)	
				Green (1)	
Casal di Principe	4	3	75	Tomato (3)	
Casaluce	1	0	0		
Casapesenna	1	0	0		
Casapulla	1	0	0		
Caserta	1	1	100		Pear (1)
Castel di Sasso	1	1	100	Fava bean (1)	
Castel Volturno	2	0	0		
Cellole	2	1	50		Strawberry (1)
Cervino	2	2	100	Potato (1)	Mandarin (1)
Falciano del Massico	2	2	100	Pea (1)	Strawberry (1)
Francolise	3	3	100	Lettuce (1)	
				Green beans (1)	
				Tomato (1)	
Frignano	3	2	67	Tomato (2)	
Grazzanise	1	1	100		Apricot (1)
Gricignano	1	0	0		
Lusciano	2	2	100	Artichokes (1)	Peach (1)

Table 2.11 *Distribution of sampling in 46 municipalities of province of Caserta*

Municipalities	No samples	No Positive samples	% Positve samples	Positive vegetables	Positive fruits
Macerata Campana	1	1	100	Tomato (1)	
Maddaloni	2	0	0		
Marcianise	2	0	0		
Mondragone	3	2	67	Chycory (1)	
				Pepper (1)	
Orta di Atella	3	1	33	Lettuce (1)	
Parete	2	1	50	Pepper (1)	
Ponte Latone	2	2	100	Fennel (1)	
				Pea (1)	
Portico di Caserta	1	1	100	Wheat (1)	
Recale	1	1	100	Wheat (1)	
S. Cipriano d'Aversa	5	5	100	Tomato (3)	Limon (1)
					Watermelon (1)
S. felice a Cancello	3	3	100	Lettuce (1)	Apricot (1)
				Potato (1)	
S. Marcellino	1	1	100		Peach (1)
S. Marco Evangelista	2	2	100	Corn (2)	
S. Nicola la Strada	1	1	100	Corn (1)	
S. Prisco	1	1	100	Pepper (1)	
S. Tammaro	4	2	50	Green beans (1)	
				Potato (1)	
S. Maria C.V.	1	0	0		
S. Maria la Fossa	3	1	33		Apricot (1)
Sessa Aurunca	1	1	100		Strawberry (1)
Succivo	2	0	0		
Teverola	1	1	100		Prune (1)
Trentola Ducenta	1	1	100	Aubergine (1)	
Villa Briano	1	1	100	Tomato (1)	
Villa Literno	6	2	33	Tomato (1)	Peach (1)



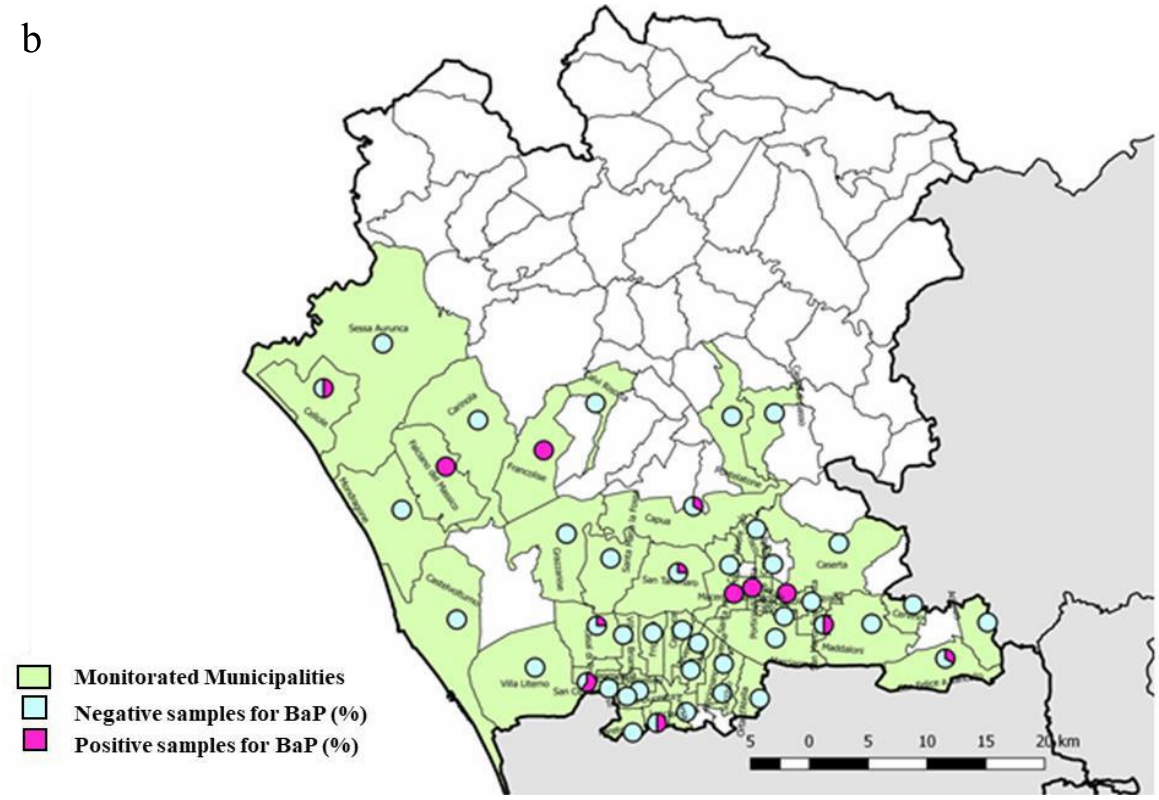


Figure 2.12: *Distribution of positive results (vegetables and fruits) for a) BaA and b) BaP in the Municipalities of Caserta*

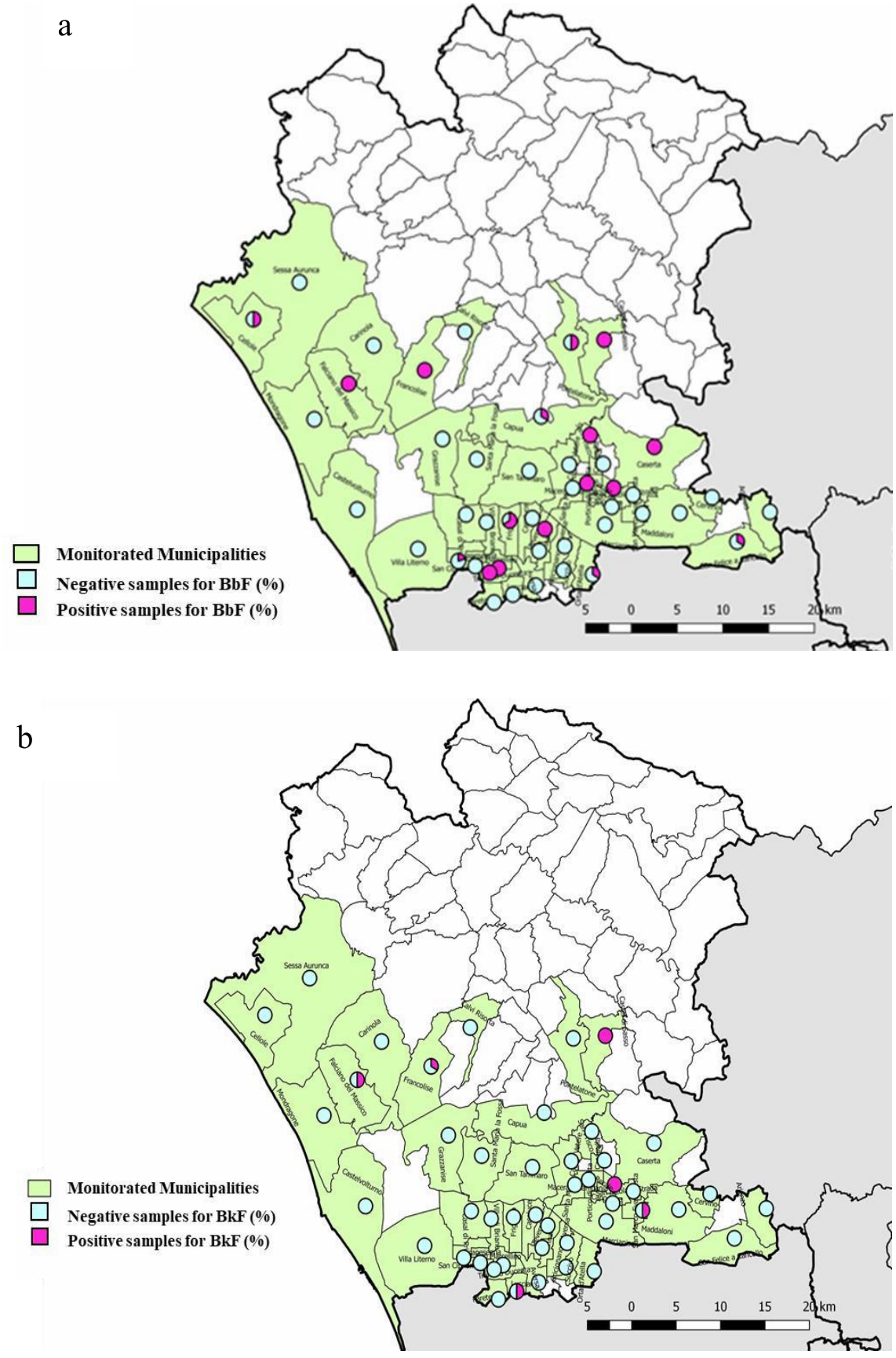


Figure 2.13 *Distribution of positive results (vegetables and fruits) for (a) BbF and (b) BkF in the Municipalities of Caserta*

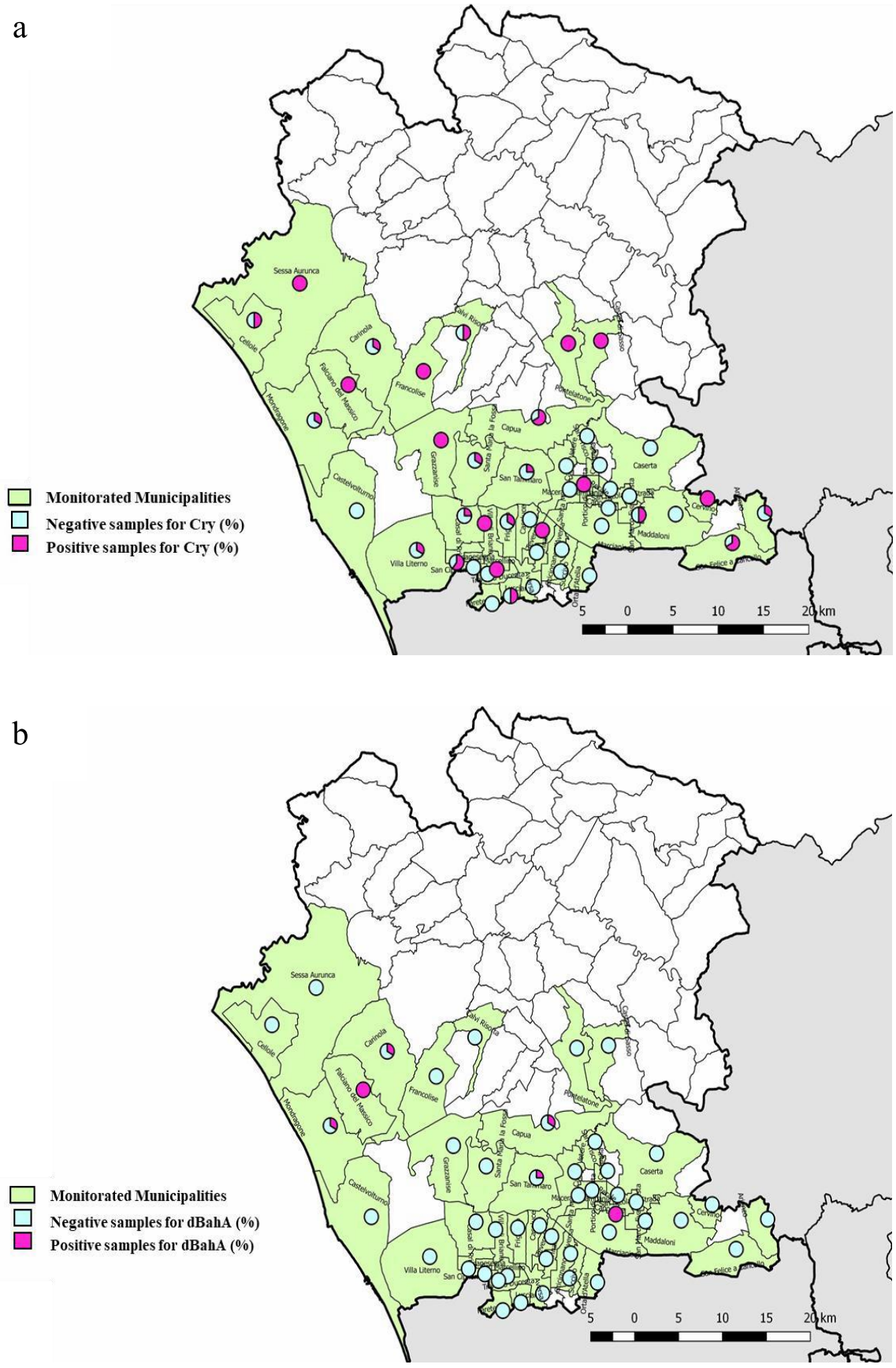


Figure 2.14 *Distribution of positive results (vegetables and fruits) for a) Cry and b) dBahA in the Municipalities of Caserta*

Among 62 samples of vegetables analyzed, only 37 (60% of positive samples) were positive for PAHs (Table 2.12). Among 25 samples of analysed fruits, 19 (76%) were positive for PAHs (Table 2.13).

Table 2.12: *PAH content in vegetable samples of Municipalities of Caserta (∑= BaA+BaP+BbF+Cry)*

Vegetables	N° Samples		Municipalities	6 PAHs (µg kg^{-1}) - LOQ < 0.2 µg kg^{-1}						
	Total	Sities		BaA	BaP	BbF	BkF	Cry	dBahA	∑
Artichokes	1	1	Lusciano	2.4	4.5	<LOQ	4.1	<LOQ	< LOQ	6.9
Aubergine	7	1	Trentola Ducenta	<LOQ	<LOQ	0.5	< LOQ	<LOQ	< LOQ	0.5
		1	Mondragone	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Orta di Atella	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Carinaro	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Carinola	0.9	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	0.9
		1	Maddaloni	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Succivo	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Cauliflower	1	1	Capua	1,0	0.3	<LOQ	< LOQ	0.7	0.3	2.0
Chicory	1	1	Mondragone	<LOQ	<LOQ	<LOQ	< LOQ	1.7	< LOQ	1.7
Corn	4	1	Maddaloni	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		2	S. Marco Evangelista	0.3	1.2	<LOQ	0.3	<LOQ	< LOQ	1.5
				0.4	<LOQ	<LOQ	< LOQ	0.8	< LOQ	1.2
		1	S. Nicola la Strada	0.3	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	0.3
Courgette	4	1	Capua	<LOQ	<LOQ	0.2	< LOQ	<LOQ	< LOQ	0.2
		1	S. Maria C.V.	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	S. Tammaro	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Cellole	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Garlic	2	2	S. Maria la Fossa	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Green beans	2	1	S. Tammaro	<LOQ	0.2	<LOQ	< LOQ	0.5	< LOQ	0.7
		1	Francolise	<LOQ	0.6	1.3	< LOQ	1.1	< LOQ	3.0
Fava bean	2	1	Castel di Sasso	<LOQ	<LOQ	0.8	0.2	0.2	< LOQ	1.0
		1	Carinola	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Fennel	2	1	Pontelatone	0.7	<LOQ	0.5	< LOQ	0.9	< LOQ	2.1
		1	Orta di Atella	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Lettuce	3	1	Orta di Atella	<LOQ	<LOQ	2.9	< LOQ	<LOQ	< LOQ	2.9
		1	Francolise	<LOQ	0.7	2.6	0.3	1.0	< LOQ	4.3
		1	S. Felice a Cancello	<LOQ	0.7	<LOQ	< LOQ	0.9	< LOQ	1.6
Pea	3	1	Falciano del Massico	<LOQ	0.6	1.5	< LOQ	1,0	0.2	3.1
		1	Carinola	<LOQ	<LOQ	<LOQ	0.2	0.2	0.2	0.2
		1	Pontelatone	<LOQ	<LOQ	<LOQ	0.2	<LOQ	< LOQ	<LOQ

Table 2.12: *PAH content in vegetable samples of Municipalities of Caserta (∑= BaA+BaP+BbF+Cry)*

Vegetables	N° Samples		Municipalities	6 PAHs (µg kg^{-1}) - LOQ < 0.2 µg kg^{-1}						
	Total	Sities		BaA	BaP	BbF	BkF	Cry	dBahA	∑
Pepper	5	1	Parete	0.6	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	0.6
		1	Castel Volturno	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	S. Prisco	<LOQ	<LOQ	0.4	< LOQ	<LOQ	< LOQ	0.4
		1	S. Tammaro	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Mondragone	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	0.3	<LOQ
Potato		1	Cervino	0.2	<LOQ	<LOQ	< LOQ	0.4	< LOQ	0.6
		1	S. Tammaro	0.2	<LOQ	<LOQ	< LOQ	<LOQ	0.3	0.2
		1	S. Felice a Cancello	<LOQ	<LOQ	0.2	< LOQ	<LOQ	< LOQ	0.2
Pumpkin	1	1	Castel Volturno	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Tomato	16	1	Macerata Campania	0.3	0.8	<LOQ	< LOQ	<LOQ	< LOQ	1.1
		3	Villa Literno	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
		1	Villa Literno	0.3	<LOQ	<LOQ	< LOQ	0.6	< LOQ	0.9
		1	Villa Literno	<LOQ	1.5	<LOQ	< LOQ	<LOQ	< LOQ	1.5
		2	Casal di Principe	<LOQ	<LOQ	<LOQ	< LOQ	0.5	< LOQ	0.5
		1		<LOQ	0.9	<LOQ	< LOQ	<LOQ	< LOQ	0.9
		1	Villa di Briano	0.2	<LOQ	<LOQ	< LOQ	0.5	< LOQ	0.7
		1	Frignano	0.2	<LOQ	0.2	< LOQ	0.4	< LOQ	0.8
		1		<LOQ	<LOQ	0.6	< LOQ	<LOQ	< LOQ	0.6
		1		<LOQ	<LOQ	<LOQ	< LOQ	0.5	< LOQ	0.5
		1	S. Cipriano d'Aversa	<LOQ	1.5	<LOQ	< LOQ	<LOQ	< LOQ	1.5
		1	S. Cipriano d'Aversa	<LOQ	0.9	<LOQ	< LOQ	<LOQ	< LOQ	0.9
		1	Succivo	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Turnip	2	1	Capua	0.3	<LOQ	<LOQ	< LOQ	0.7	< LOQ	1.0
		1	Villa Literno	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ
Wheat	3	1	Portico di Caserta	0.3	0.3	0.3	< LOQ	0.5	< LOQ	1.4
		1	Recale	0.8	3.4	0.3	0.3	<LOQ	< LOQ	4.5
		1	Capodrise	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	0.3	<LOQ

Table 2.13: *PAH content in fruit samples of Municipalities of Caserta (∑= BaA+BaP+BbF+Cry)*

Fruits	No Samples		Municipalities	6 PAHs (µg kg^{-1}) - LOQ < 0.2 µg kg^{-1}						
	Total	Sities		BaA	BaP	BbF	BkF	Cry	dBahA	∑
Apple	1	1	Casaluce	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ
Apricot	3	1	S. Felice a Cancello	0.3	<LOQ	<LOQ	<LOQ	0.6	< LOQ	0.9
		1	S. Maria la Fossa	0.4	<LOQ	<LOQ	<LOQ	0.7	< LOQ	1.1
		1	Grazzanise	0.5	<LOQ	<LOQ	<LOQ	0.9	< LOQ	1.4
Grape	1	1	Gricignano d'Aversa	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ
Lemon	2	1	Arienzo	0.4	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	0.4
		1	S. Cipriano d'Aversa	<LOQ	0.2	0.8	<LOQ	0.8	< LOQ	1.8
Loti	1	1	Arienzo	0.5	<LOQ	<LOQ	<LOQ	0.9	< LOQ	1.4
Mandarin	1	1	Cervino	0.5	<LOQ	<LOQ	<LOQ	0.6	< LOQ	1.1
Olive	2	1	Calvi Risorta	0.3	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	0.3
		1		1.1	<LOQ	<LOQ	<LOQ	1.5	< LOQ	2.6
Orange	1	1	Arienzo	0.4	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	0.4
Peach	4	1	Aversa	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ
		1	S. Marcellino	<LOQ	<LOQ	0.4	<LOQ	0.3	< LOQ	0.7
		1	Villa Literno	<LOQ	<LOQ	<LOQ	<LOQ	0.7	< LOQ	0.7
		1	Lusciano	0.4	<LOQ	<LOQ	<LOQ	0.7	< LOQ	1.1
Pear	1	1	Caserta	<LOQ	<LOQ	0.3	<LOQ	<LOQ	< LOQ	0.3
Prune	4	1	Frignano	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ
		1	Teverola	0.3	<LOQ	0.2	<LOQ	0.6	< LOQ	1.1
		1	Parete	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ
		1	Casapesenna	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ
Strawberry	3	1	Cellole	0.5	0.6	1.4	<LOQ	1.1	< LOQ	3.6
		1	Falciano del Massico	<LOQ	0.3	0.5	0.2	0.9	0.2	1.7
		1	Sessa Aurunca	<LOQ	<LOQ	<LOQ	<LOQ	0.2	< LOQ	0.2
Watermelon	1	1	S. Cipriano d'Aversa	<LOQ	<LOQ	<LOQ	<LOQ	0.5	<LOQ	0.5

In the Table 2.14 the number of positive samples, the positivity percent and the range of concentration of single PAHs congeners are summarized.

As shown in Table 2.12 BaA was found in higher concentrations in artichokes (2.4 µg kg^{-1}). BaP was found in higher concentrations in artichokes (4.5 µg kg^{-1}), wheat (3.4 µg kg^{-1}), corn (1.2 µg kg^{-1}), and tomato (1.5 µg kg^{-1}). BbF was found in two samples of lettuce (2.6 and 2.9 µg kg^{-1}), in one sample of pea (1.5 µg kg^{-1}) and in one sample of green beans (1.3 µg kg^{-1}). The highest value of BkF was found in artichokes (4.1 µg kg^{-1}). Cry was found in sample of chicory (1.7 µg kg^{-1}), green bean (1.1 µg kg^{-1}), lettuce and pea (1.0 µg kg^{-1}). The Σ 4PAHs ranged between 0.2 µg kg^{-1} and 6.9 µg kg^{-1}. The higher values (> 1.0 µg kg^{-1}) were found in large leafy vegetables such as lettuce and chicory (1.6 µg kg^{-1} < Σ 4PAHs < 4.3 µg kg^{-1}), similarly to Naples province, corn (0.3 µg kg^{-1} <Σ 4PAHs < 1.5 µg kg^{-1}), artichoke (6.9 µg kg^{-1}), cauliflower (2.0 µg kg^{-1}), green beans (3.0 µg kg^{-1}) and finally fennel (2.1 µg kg^{-1}).

Table 2.14: *Distribution of positive vegetable samples respect single PAH*

	No Positive samples	**% Positive samples**	**Range µg kg^{-1}**
BaA	17	27	0.2 – 2.4
BaP	15	24	0.2 – 4.5
BbF	13	21	0.2 – 2.9
BkF	7	11	0.2 - 4.1
Cry	19	30	0.2 – 1.7
dBahA	6	10	0.2 – 0.3
∑ 4PAHs	36	58	0.2 – 6.9

This more diffuse positivity among samples from Caserta indicated a greater diffusion of PAH contamination in the territory of Caserta. In addition, in this area higher concentration level were measured respect to the territory of Naples.

The presence of PAHs in broadleaf vegetable samples (chicory and lettuce) in both provinces suggested that there might be some critical issues whose origin could be related to the environment and/or the morphology and metabolism of these species.

The analysis of fruit samples showed a 76% of positivity to the presence of PAH

(19 samples on 25 analyzed) (Table 2.13).

In particular, in the Table 2.15 the number of positive samples, the positivity percent and the range of concentration of single PAHs congeners in fruit samples cultivated in territory of Caserta are summarized

Among them the 50% have presented positivity for BaA, and 62% for Cry BaP was found in 3 cases: one sample of lemon (0.2 µg kg^{-1}) and two samples of strawberries (0.5 µg kg^{-1} and 0.6 µg kg^{-1}). The Σ 4PAHs was found to be much more widespread with values between 0.2 µg kg^{-1} and 3.6 µg kg^{-1}. Therefore, fruits would appear to be most affected by PAH contamination than vegetables.

Table 2.15: *Distribution of positive fruit samples respect single PAH*

	No Positive samples	**% Positive samples**	**Range µg kg^{-1}**
BaA	12	50	0.3 – 1.1
BaP	3	12	0.2 – 0.6
BbF	6	25	0.2 – 1.4
BkF	1	4	0.2
Cry	15	62	0.2 – 1.5
dBahA	1	4	0.2
∑ 4PAHs	19	79	0.2 – 3.6

Even in the case of vegetables, the results not showed a significant correlation between the six PAH congeners. (Table 2.16).

Table 2.16: *Pearson's correlation matrix of PAHs in vegetable samples of Municipalities of Caserta.*

	BaA	**BaP**	**BbF**	**BkF**	**Cry**	**dBahA**	**∑ PAH**
BaA	1.000						
BaP	0.555	1.000					
BbF	-0.228	0.119	1.000	1.000			
BkF	0.625	0.532	-0.049	1.000	1.000		
Cry	-0.166	0.390	0.508	-0.185	1.000	1.000	
dBahA	0.029	-0.019	-0.023	-0.090	-0.071	1.000	1.000
∑ PAH	0.411	0.855	0.571	0.382	0.654	0.017	1.000

P<0.005

Even in the case of fruit, the results highlighted a correlation between BaP and BbF (0.956), BaP and BkF (0.975) and BaA and Cry contamination (0.709) (Table 2.17).

Table 2.17: *Pearson's correlation matrix of PAHs in fruit samples of Municipalities of Caserta*

	BaA	**BaP**	**BbF**	**BkF**	**Cry**	**dBahA**	**∑ PAH**
BaA	1.000						
BaP	0.042	1.000					
BbF	-0.021	0.956	1.000				
BkF	0.047	0.975	0.897	1.000			
Cry	0.709	0.493	0.486	0.500	1.000		
dBahA	0.000	0.000	0.000	0.000	0.000	1.000	
∑ PAH	0.628	0.737	0.724	0.719	0.000	0.000	1.000

The results of this study have highlighted the widespread diffusion of contamination by PAHs into the environment. Figure 2.15 shows the percent distribution of positive vegetable sample compared to six PAH congeners in Naples and Caserta. Comparing the data found in the provinces of Naples and Caserta (Figure 2.15), the territory of Caserta appears to be more positive. Results showed that BaP turns out to be quite infrequent, and the BaA and Cry were the most abundant between the analysed PAHs (Table 2.15). In any case, the values found in vegetables and fruits are generally quite low, as suggested by other studies and as supported by EFSA. Comparing the results obtained in this study with the limits for the most restrictive food category such as childhood foods (EU Regulation, 2011), it appears that most vegetables have lower BaP and Σ 4PAHs < 1.0 μg kg^{-1}.

Moreover, leaf vegetables showed high values probably due to their morphology: this indicates that gaseous deposition is the principal pathway of PAH accumulation in plants (Kipopoulou *et al.*, 1999). The only sample of artichokes analyzed (coming from the province of Caserta) showed values of BaP and Σ 4PAHs, which were certainly high to be a plant sample. The possible explanation might lie in the fact that it is a multi-year species and thus able to accumulate pollutants over time.

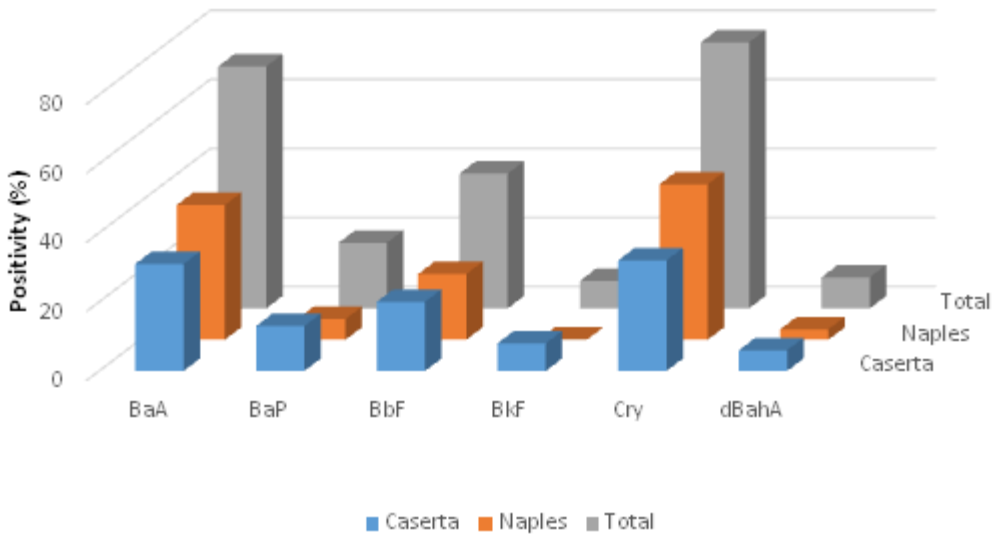


Figure 2.15: *Profiling of number of positive samples for six PAH congeners in the 3-years 2014-2016 in vegetable matrices growing in LoF.*

2.4.3 Dietary exposure assessment

Humans are exposed to PAHs through various pathways. The dietary source represents the major route of exposure to PAHs in non-smokers and in non-occupationally exposed people (EFSA, 2008; Domingo and Nadal, 2015).

In this context, the results of the monitoring activity carried out between 2014 and 2016 in the provinces of Naples and Caserta provided important indications on exposure to PAHs.

In order to evaluate the impact of vegetables on human diet the samples (n=203) collected during the monitoring activities were grouped into 4 classes:

- leafy vegetables (artichokes, chicory, endive, lettuce and turnip);
- fruit vegetables (aubergine, cauliflower, courgette, corn, green beans, fava beans pea, pepper, pumpkin and tomato);
- rhizome vegetables (fennel, garlic, onion and potato);
- fruits (apple, apricot, grape, hazelnut, lemon, loti, mandarin, olive, orange, peach, pear, prune, strawberry, walnut and watermelon).

The analysis of the results showed that BaA appeared more frequent in both rhizome vegetables (45%) and fruits (46%) followed by fruit vegetables (31%) and leafy vegetables (27%). The concentration was larger in leafy vegetables (1.5 μg kg^{-1}) than the other three categories, where it was ≤ 0.5 μg kg^{-1}. Conversely, Cry was more frequent in leafy vegetables (41%) than fruit vegetables (32%), rhizome vegetables (40%) and fruits (32%). The concentrations found in the four types of matrices showed that leafy vegetables are more contaminated (1.5 μg kg^{-1}), followed by fruits (0.7 μg kg^{-1}), fruit vegetables and rhizome vegetables with 0.6 μg kg^{-1}. BaP, not quantified in rhizome vegetables and fruits, was detected more frequently in fruit vegetables (19%) and leafy vegetables (18%) at 0.6 μg kg^{-1} and 1.5 μg kg^{-1} concentration, respectively. Finally, BbF was detected more frequently in fruit vegetables (18%) compared to other matrices (14-15%). The largest concentration (1.8 μg kg^{-1}) was measured among leafy vegetables, followed by fruit vegetables (0.7 μg kg^{-1}), and rhizome vegetables and fruits (0.3 μg kg^{-1}) (Figures 2.16 and 2.17).

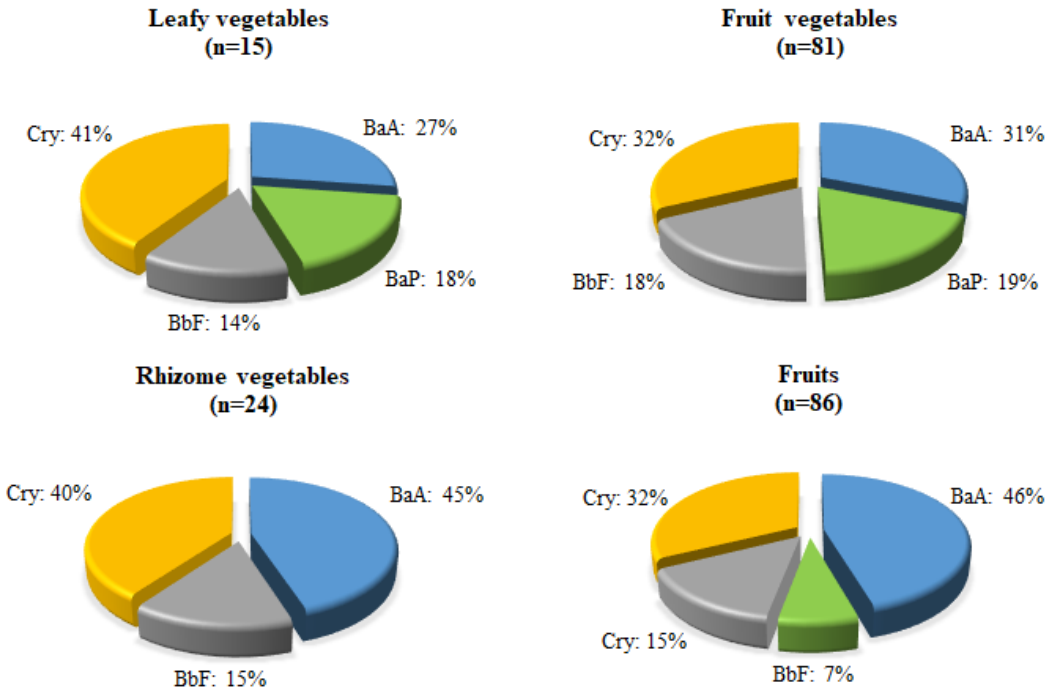


Figure 2.16: *Percentage distribution of 4 PAHs.*

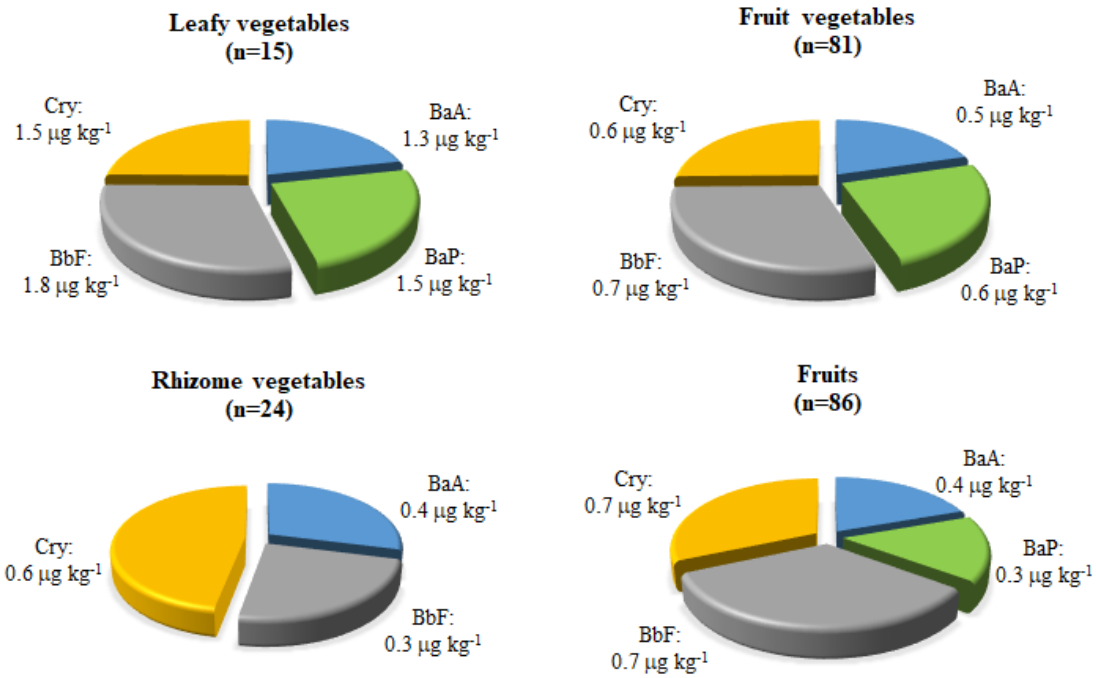


Figure 2.17: *Distribution of 4 PAHs concentration ($\mu g\ kg^{-1}$)*

Data analysed above are referred to all samples collected and analysed during the monitoring activity. When the analysis was limited to the positive samples to PAH contamination with no distinction of plant classes, the distribution of the four congeners, included in ∑4PAHs (BaA+BaP+BbF+Cry), showed that Cry was present in 37% (0.8 μg kg^{-1}) of the positive samples. It was followed by BaA (59% with 0.5 μg kg^{-1}), and then by BbF (30% with 0.7 μg kg^{-1}) and BaP (20% with 0.6 μg kg^{-1}) (Figures 2.18 and 2.19).

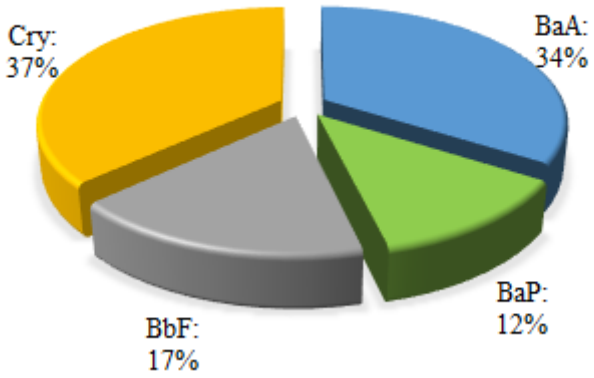


Figure 2.18: *Distribution (%) of 4 PAHs (BaA, BaP, BbF and Cry) in positive samples of collected in LoF.*

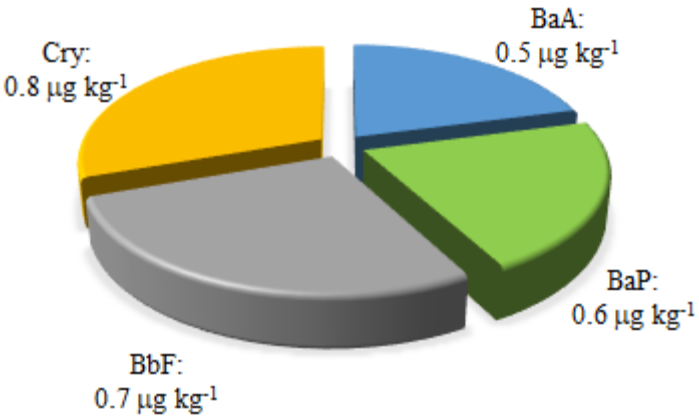


Figure 2.19: *Concentration* (μg kg^{-1}) *of 4 PAHs (BaA, BaP, BbF and Cry) in positive samples of collected in LoF.*

The PAH-positive samples generally showed contaminant concentration below 1 μg kg^{-1}, maximum value admitted for serial based food for infant and children (EC Reg. 835/2011). This Regulation was taken into account because in absence of a specific indication for fruit and vegetable food, it is the more restrictive currently. As reported in Figure xx the PAH contamination of monitored products followed this order: Cry > BbF > BaP > BaA.

The EC Reg. 835/2011 takes into account not only BaP as PAH marker but also ∑4PAHs (BaA+BaP+BbF+Cry) that should not exceed 1 μg kg^{-1} in food for infant and children. This indicator is important to highlight possible additive effect from the four PAHs and not only from BaP that is a not sufficient indicator for carcinogenic risk. By analysing ∑4PAHs of the positive samples (the mean value), the value was lower than 1 μg kg^{-1}.

Guidelines on healthy nutrition suggest that at least five servings (400 g) per day of fruit or vegetables should be taken and each portion (80 g) is defined as the quantity of raw fruit or vegetables that can be contained on the palm of a hand or half a plate of cooked vegetables (Ministry of Health of Italy, 2013).

Campania region shows a consumption (5 portions) of fruit and/or vegetables lower than the national average (Figure 2.20; Epicentro - Istituto Superiore della Sanità).

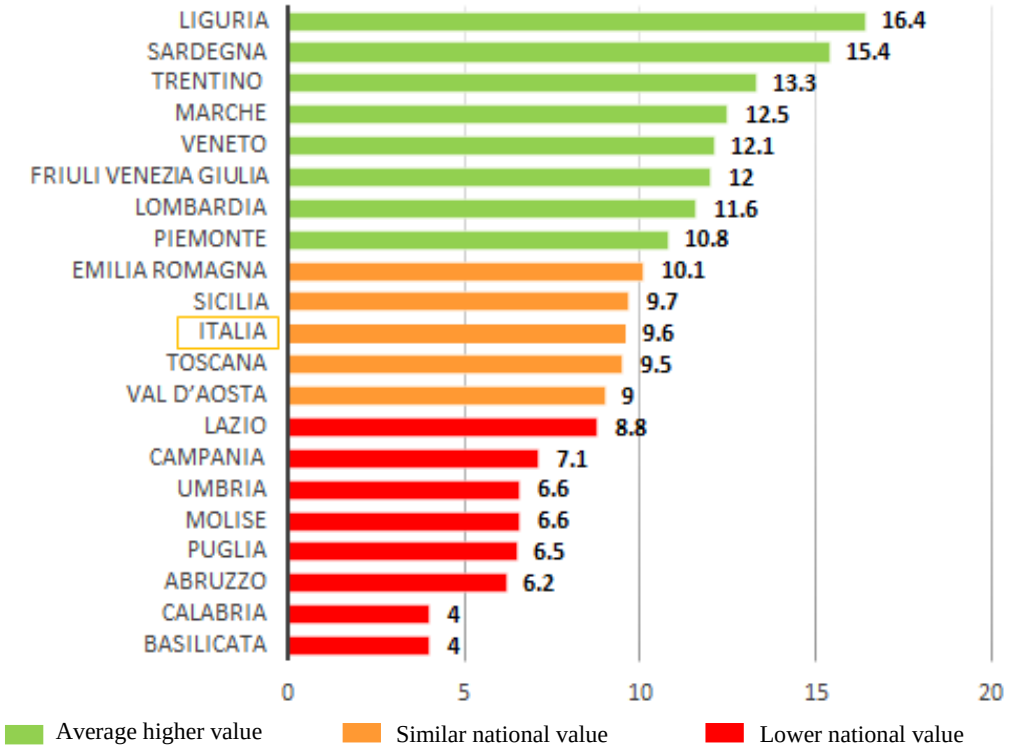


Figure 2.20: *Daily consumption (%) of 5 servings of fruits and/or vegetables in percentage in the 20 regions and in Italy*

In Italy, the average consumption of fruit and vegetables accounts 49% of the population consuming 1-2 servings (80-160 g), 38.6% consuming 3-4 servings (240-320 g), and only 9.6% of the population assumes an amount equal to or greater than that indicated in the

Guidelines of the Istituto Superiore della Sanità. As far as the Campania region is concerned, 57.3% of the population consumes 1-2 servings, followed by 31.6% with 3-4 servings and only 7.1% of Campania follows the guidelines for healthy eating (Table 2.18).

Table 2.18: *Daily consumption of servings of fruits and/or vegetables in percentage in Campania and in Italy*

	Italy (n=146450) % mean value	**Region Campania (n= 6789) % mean value**
0 serving	2.8	4.0
1-2 servings (80-160 g)	49.0	57.3
3-4 servings (240-320 g)	38.6	31.6
5+ servings (≥400 g)	9.6	7.1

Therefore, by combining the overall results obtained in this monitoring activity on LoF (Figure 2.19) with the average consumption in Italy and Campania (Table 2.18), it was possible to calculate the daily dietary exposure level (*E*) of BaA, BaP, BbF and Cry:

$$E = C \times IR$$

where E is daily dietary PAH (μg day^{-1}); C is PAH concentration in vegetables (μg kg^{-1}); IR is ingestion amount of vegetables per day (g day^{-1}) (Wu *et al.*,, 2016) (Table 2.20).

Table 2.20: *Mean estimated daily intake (E) calculated in this study for 4 PAHs (BaA, BaP, BbF and Cry, according to the portions of fruit and/or vegetables daily consumed.*

	Exposure level (μg day^{-1})				
	BaA	**BaP**	**BbF**	**Cry**	**∑4PAHs**
1-2 servings (80-160 g)	0.04-0.08	0.04-0.08	0.06-0.11	0.06-0.13	0.08-0.16
3-4 servings (240-320 g)	0.12-0.16	0.12-0.16	0.17-0.22	0.19-0.26	0.24-0.32
≥5 servings (≥400 g)	≥ 0.2	≥ 0.2	≥0.3	≥0.3	≥0.4

This field monitoring study confirmed the presence of PAH contamination in cereals, vegetables and fruits in LoF, but this contamination resulted not considerable as risk for human health. Therefore, the results are in line with EFSA's definition concerning these vegetable matrices as possible contributors to human PAH exposure due to their high consumption. Nevertheless, PAH levels in these food groups should be further monitored in order to define, in the next future, the limit values to which we can refer as it already occurs currently for other food categories.

The presence of environmental pollutants, e.g. PAHs, in food is generally an issue of concern that requires continuous monitoring activity. In recent years, a number of epidemiological studies have shown that a large percentage of human cancers are attributable, at least in part, to dietary factors, including dietary exposure to PAHs, potentially carcinogenic compounds (Abid *et al.,* 2014).

Fruits, cereals and vegetables can be consumed as such and/or as ingredients (e.g. in milk and meat/fish based baby foods). The exposure via food to contaminants mainly depend on the category and on the amount of foodstuff, frequency of intakes as well as the type of food processing that may influence the xenobiotic concentrations (Santonicola *et al.*, 2017). Phillips (1999) concluded that diet was the major source of human exposure to PAHs, with cereals and vegetables showing the greatest contribution to PAHs exposure.

2.5 Conclusions

The territory covered by monitoring activity turns out to be highly urbanized and industrialized, and thus more exposed to contamination of anthropogenic origin. Moreover, the presence of smoke freed from the burning of waste occurred thrown everywhere in the countryside and in suburban areas due to uncivilized people.

This sad habit often hides the attempt of some industrial companies with the complicity of criminal organizations to get rid of waste that should be disposed in according to costly protocols. The uncontrolled abandonment and burning of theses wastes involve the release into the environment (air, soil and water) of highly toxic substances, such as, PAHs.

Therefore, in this three-year period 2014-2016 the results indicate that PAH contamination in plant matrices is a definitely spread phenomenon. Moreover, the contamination levels result to be quite low when compared with those found in other food matrices (smoked or not meat and fish). In fact, although most of the analyzed samples showed PAH values below the limit of quantification, few cases with exceeding values have sporadically occurred (artichokes, broad-leaved vegetables, wheat, corn, strawberries).

In general, the results obtained in monitoring activity show that the amount of contamination by PAHs was close to those of plant products grown in Saudi Arabia (Ashraf 2012) and was significantly smaller than that found in other studies conducted in China (Zhang and Wang, 2002).

In Italy, the Mediterranean diet provides a great deal of consumption of both raw and cooked vegetable foods. Vegetables and fruits are considered cancer protective and therefore their consumption is strongly recommended. However, if this food is not under a specific regulation it could not be excluded that this food can contribute to the onset of cancer due to the presence of PAHs. A valuation of the dietary intake is therefore fundamental in order to define the real contribution of these matrices to the PAHs assimilation.

However, the current European legislation (Reg. UE 835/2011) has not yet fixed the maximum levels for PAHs in vegetables, so this lack makes not possible to establish the compliance of the samples analysed in this study.

Therefore, it is considered necessary to examined well the results obtained in this phase of the thesis, focusing on some of the critical emerged issues.

Specifically, we wanted to investigate whether the positivity emerged in some matrices, such as tomato and lettuce, may be due to a translocation of the contaminant from the environment to the plant through root absorption or if the morphology of the plant can promote the concentration of these contaminants.

Irrespective of pathways of such accumulation, information on potential exposure of PAHs is of particular interest because the general population is frequently exposed to PAH through food.

At the light of these results on the distribution of PAHs in the vegetable and fruit products, a study that determines the degree of absorption of PAHs, according to different vegetables and fruits assumed, should be performed. These results are based upon the fact that vegetables are in general consumed raw. In fact, vegetables are cooked, which may substantially affect the final content of eaten vegetables.

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Chapter 3

3 BIOAVAILABILITY OF BENZO(A)ANTHRACENE IN VEGETABLES

3.1 Introduction

Industrial and urban activities often release in the environment several types of persistent pollutants including mainly aromatic compounds. Since the industrial revolution, the production and release of aromatic compounds in the environment increased basing on multitude of end products.

The use of fossil fuels for industrial activities generates a massive production of aromatic compounds such as polycyclic aromatic compounds (PAHs). These carbon-based molecules are ubiquitous and are produced in nature during wood fires and volcanic activities. The spatial distribution in the environmental compartments (air, water, and soil) is highly heterogeneous and depends mainly on their physical and chemical properties, source, location and abundance of emission. Low molecular weight PAHs (two or three rings) occur in the atmosphere predominantly in the vapour phase, whereas PAHs with more rings are largely bound to particles (Srogi, 2007). The PAHs with four rings are portioned between the vapour and particulate phase. The multitude of different PAHs molecules increase as they can be found as substituted molecules with polar functions (Chibwe, 2017). Due to their high recalcitrance and toxic effects such as genotoxicity these molecules are a worldwide concern. The high recalcitrance in the environment is due to the low bioavailability, which reduces the biotransformation (Semple, 2004).

The xenobiotics entering in soil are affected by different processes, such as volatilization, up- take by living organisms, degradation by soil microorganism, or immobilization as non-extractable residues (Kästner *et al.,* 2014).

Microorganism and plants thanks to many evolutionary and co-evolutionary processes are able to live in ecosystem containing complex aromatic molecules. Secreting enzymes, active migration, expression of high affinity PAHs uptake system are some examples of organism adaptation in this type of environment. These biological processes reduce the low bioavailability of PAHs (Cristaldi, 2017).

Despite this, PAHs metabolism and accumulation in plants are not well documented, also because the interaction among plants and microorganisms present in the rhizosphere is highly complex. The release of root exudates by plants has been shown to depend on the diversity and the total number of microorganisms (Cristaldi, 2017). The

main studies that have been performed include plants in bioremediation systems. These studies have found out that non-food/feed species such as Populus spp. and Salix spp. are able to grow on contaminated soil by reducing also the PAH content. Other evidence suggests that a strong interaction of fungi and/or bacteria with plants induces a rapid degradation of aromatic complex molecules (Cristaldi, 2017).

The uptake and transport of PAHs by/into plants still remain unknown processes. Phenanthrene absorption and accumulation studies were carried out on the model plant Arabidopsis thaliana and on wheat roots (Alkio *et al.*, 2005). Gao and Zhu (2004) found that the root uptake of PAHs in different plant species was correlated with PAH concentration in soil and plant composition.

The monitoring activity in LoF, reported in Chapter 2 of the present thesis, showed that BaA, followed by Cry, was the most frequently present in vegetables and fruits among the six studied PAHs. Therefore, BaA was chosen as the model molecule in a study focused to better understand mechanisms responsible for this widespread plant contamination. Therefore, the aim of this work was to understand the origin of the widespread contamination of BaA, in particular which kind of absorption process was involved (deposition on leaves, root absorption, etc.).

Turnip (*Brassica rapa*) and tomato (*Solanum lycopersicum*) was chosen as typical crops of Campania region. These plants were grown in the presence of the BaA in a controlled environment (in vitro) and in a more complex system as soil (in vivo). In both the approaches two BaA rates were applied (50 e 100 mg kg^{-1}) by exceeding contamination level reached in other studies (Khan *et al.*, 2008).

In particular the specific objectives of this work were:

- to grow the seedlings of turnip and tomato in BaA contaminated soils at 50 and 100 mg kg^{-1} (*in vivo* experiment);
- to grow seedlings of turnip and tomato in BaA contaminated culture medium (in vitro experiment) at 50 and 100 mg kg^{-1} to simulate the BaA contamination in an axenic and controlled condition (plant tissue) and nullify possible phenomena due to soil, a very complex matrix;
- to study possible translocation of BaA from soil and culture medium to root and shoot;
- to highlight the specific sensitivity of the different vegetable species by phytoxicity test with turnip and tomato seeds.

3.2 Materials and methods

3.2.1 Chemicals

High purity water (18.2 MΩ cm) was obtained through a Milli-Q water system (Millipore, Billerica, MA, USA). The solvents cyclohexane (pesticide residue analysis grade), acetonitrile (ACN, HPLC grade), n-hexane (RPE grade), and acetone (RPE grade), were purchased from Carlo Erba (Milano, Italy). Sep-Pak silica cartridges were purchased from Waters (Dublin, Ireland). Purified water was obtained through a Milli-Q water system (Millipore, Billerica, MA, USA).

The standard of benzo(a)anthracene (BaA) was purchased from Sigma-Aldrich (Saint Loius, Missouri, United States). Its characteristics are listed in Table 3.1.

Table 3.1: *Physical and chemical properties of BaA.*

Compound	Molecular weight g mol^{-1}	Water Solubility µg L^{-1}	Vapour pressure mm Hg	Henry's law constant atm $m^3 \cdot mol^{-1}$	Log K_{ow}	Log K_{oc}
Benzo(a)anthracene	228.29	10	$2.2 \cdot 10^{-8}$	$1.00 \cdot 10^{-6}$	5.61	5.30

3.2.2 Soil

The experiments *in vivo* were performed with soil collected in Sant'Angelo in Formis (Caserta), 2 mm sieved and dried. The soil had pH 7.88, organic matter content of 38.7 g kg^{-1}, total carbon content of 22.5 g kg^{-1}, total nitrogen content of 2.45 g kg^{-1}, and a water holding capacity of 46% (Table 3.1).

Table 3.1 *Physical and chemical soil properties.*

Parameters		Value
pH		7.88
Conductivity	dS m^{-1}	0.171
Coarse sand	g kg^{-1}	223
Fine sand	g kg^{-1}	534
Silt	g kg^{-1}	186
Clay	g kg^{-1}	57
Total limestone	g kg^{-1}	32.8
Active limestone	g kg^{-1}	6.2
Total Organic carbon	g kg^{-1}	22.5
Organic matter	g kg^{-1}	38.7
Total N	g kg^{-1}	2.45
C/N ratio		9.2
P (P_2O_5)	mg kg^{-1}	301
CEC	meq 100 g^{-1}	29.3

3.2.3 Germination test

Germination tests were performed with turnip and tomato seeds by using BaA uncontaminated soil (0 mg kg^{-1} of BaA), BaA contaminated soil (50 mg kg^{-1} of BaA), and BaA contaminated soil (100 mg kg^{-1} of BaA). Seeds of turnip (*Brassica rapa*) and tomato (*Solanum lycopersicum*) were supplied from Agrisementi Lebbioli srl (Caserta, Italy).

Soil (10 g) was added in Petri dishes (10 x 90 mm). A disc of filter paper was placed above the soil to level out the soil surface. In each Petri dish 10 seeds were spread evenly across the surface of the soil whose water field capacity was maintained by adding deio nized water. All four replicates were stored for 5 days at 25 ± 2 °C in the dark, in a climate chamber.

A primary root >1 mm was considered as the end germination point. The relative germination RG = 100 (Gs/Gc), the relative length RL= 100 (Ls/Lc), and the germination index GI = 100 (Gs/Gc) were calculated for each treatment (0, 50 and 100 mg kg^{-1}). Gs and Gc are the numbers of seeds germinated in the contaminated soils (50 or 100 mg kg^{-1}) and control, respectively; Ls and Lc are the length of root in the contaminated soils and control, respectively (Rao et al, 2017; Smith et al., 2006).

3.2.4 *In vivo* cultivation of turnip and tomato plants in BaA contaminated soil

3.2.4.1 Soil spiking

Before spiking, the absence of BaA in soil was verified by analysing the soil sample according to the procedure described in Paragraph 3.2.6.1. The sieved soil (approximately 5.4 kg) was rewet to 30% of water holding capacity (WHC) before spiking procedure. The BaA standard solution at 10000 mg L^{-1} in acetone (stock solution) was prepared in the laboratory and stored in dark at 4 °C.

Sieved soil was split in three aliquots (1.8 kg per each): two aliquots were spiked with BaA standard solution to reach a final concentration of 50 mg kg^{-1} and 100 mg kg^{-1} BaA, respectively. The third aliquot was used as control sample in which suitable amount of acetone was added in order to reproduce the same conditions.

In according to the spiking procedure, 10% of soil aliquot to contaminate was placed in a beaker and a mixture of 7 mL of acetone and 1 or 2 mL of BaA stock solution depending on the final BaA contamination rate, 50 mg kg^{-1} or 100 mg kg^{-1}, respectively, was added. Further small soil amounts (about 10 g) were added until to mix the entire soil amount and to achieve a final concentration of 50 mg kg^{-1} or 100 mg kg^{-1} BaA, respectively.

After that, the soil sample was placed in a sealed glass jar, the plastic seal was protected with an aluminum foil to prevent the absorption of contaminants on the rubber. This sample was kept under stirring at room temperature for one night to allow complete

Figure 3.1: *Soil shaking system*

homogenization of the contaminated sample (Figure 3.1). After that, in order to remove acetone, soils were laid under hood on an aluminum paper at 25 °C.

3.2.4.2 Cultivation of turnip and tomato plants in BaA contaminated soil

The experimental design for each species was planned as follow:

- unplanted pots with unspiked soil (S), 50 mg kg^{-1} spiked soil (S50), and 100 mg kg^{-1} spiked soil (S100);
- planted pots (turnip and tomato) with unspiked soil (SP), spiked soil at 50 mg kg^{-1} (SP50) and spiked soil at 100 mg kg^{-1} (SP100) (Figure 3.2).

Three replicates were performed for each BaA concentrations (0, 50 and 100 mg kg^{-1}).

Figure 3.2: *Transplant of a) turnip and b) tomato seedlings in pots with soil at 0, 50 and 100 mg kg^{-1}*

Turnip and tomato seedling were purchased with approximately 4-5 and 15-20 cm height, respectively. The seedlings were transplanted in plastic pots containing 300 g of uncontaminated or contaminated soil in according to experimental design.

All pots were stored in a greenhouse for 28 days under sunlight (12 h), with day temperature about 25 ± 4 °C and night temperature about 19 ± 3 °C and relative humidity of 65 ± 2%. Soils were irrigated with tap water to maintain the water content at 70% of the WHC. To compensate the difference in terms of light exposure all pots were re-randomized at regular interval.

Turnip and tomato plants were harvested after 28 days and separated into shoots and roots. In order to remove soil particles, shoots and roots were washed with tap water and then with deionized water, and dried. Shoots, roots and soils were frozen at -20 °C until they were analysed.

3.2.5 *In vitro* cultivation of turnip and tomato plants in BaA contaminated culture medium

3.2.5.1 Seeds

Prior to proceeding with the experiment, damaged and small seeds were removed. Seeds were sterilised and washed 2-3 times with tap water. The seeds were left in ethanol solution (70%) for 1 minute. Ethanol solution was discarded and a 0.5% (v/v) sodium hypochlorite solution with 0.1 mL of Tween-20 was added and shaked for 20 min. The sterilization solution was removed under a laminar flow hood and seeds were washed five times with sterilized MilliQ water.

3.2.5.2 Culture medium

Seeds were sow in sterilised plant medium containing Murashige and Skoog salt (purchased from Duchefa Biochemie, Haarlem, Netherlands), vitamins (Table 3.1), sucrose 20 g L^{-1} and plant agar 8 g L^{-1} (MS medium) (Table 3.2).

MS medium was sterilized in autoclave (15 min. at 1.2 atm). In each glass jar 30 mL of sterilized MS medium was added. The experiment was performed in triplicate for each BaA contamination rate (0, 50, and 100 mg kg^{-1}).

Table 3.2: *Composition of MS medium*

Macroelements	mg L^{-1}	Microelements	mg L^{-1}	Vitamins	mg L^{-1}
$CaCl_2$	332.02	$CoCl_2$ 6 H_2O	0.03	Myo-Inositol	100.00
KH_2PO_4	170.00	$CuSO_4$ 5 H_2O	0.03	Nicotin acid	1.00
KNO_3	1900.00	FeNaEDTA	36.70	Piroxine HCl	1.00
$MgSO_4$	180.54	H_3BO_3	6.20	Thiamine HCl	10.00
NH_4NO_3	1650.00	KI	0.83		
		$MnSO_4$ H_2O	16.90		
		Na_2MoO_4 2 H_2O	0.25		
		$ZnSO_4$ 7 H_2O	8.60		

3.2.5.3 BaA contamination of MS culture medium

A suitable volume of the BaA standard solution in acetone (10000 mg L^{-1}) was added in sterilised MS medium in order to obtain 50 and 100 mg kg^{-1} BaA concentration.

3.2.5.4 Turnip and tomato cultivation in BaA contaminated MS culture medium

The sterilized turnip (Figure 3.3a) and tomato (Figure 3.3b) seeds were grown in uncontaminated MS medium in climatic chamber for 10 d at 25/21 °C (day/night, temperature), with 16 h of light (200 µmol^{-2} s^{-1}) and 8 hours of dark. Five seedlings of turnip (Fig. 3.3b) or tomato (Fig. 3.3e) with similar growth in terms of shoot and root length (Figure 3.3d) were transplanted into sterilized glass jar with MS medium with or without BaA (0, 50 and 100 mg kg^{-1})

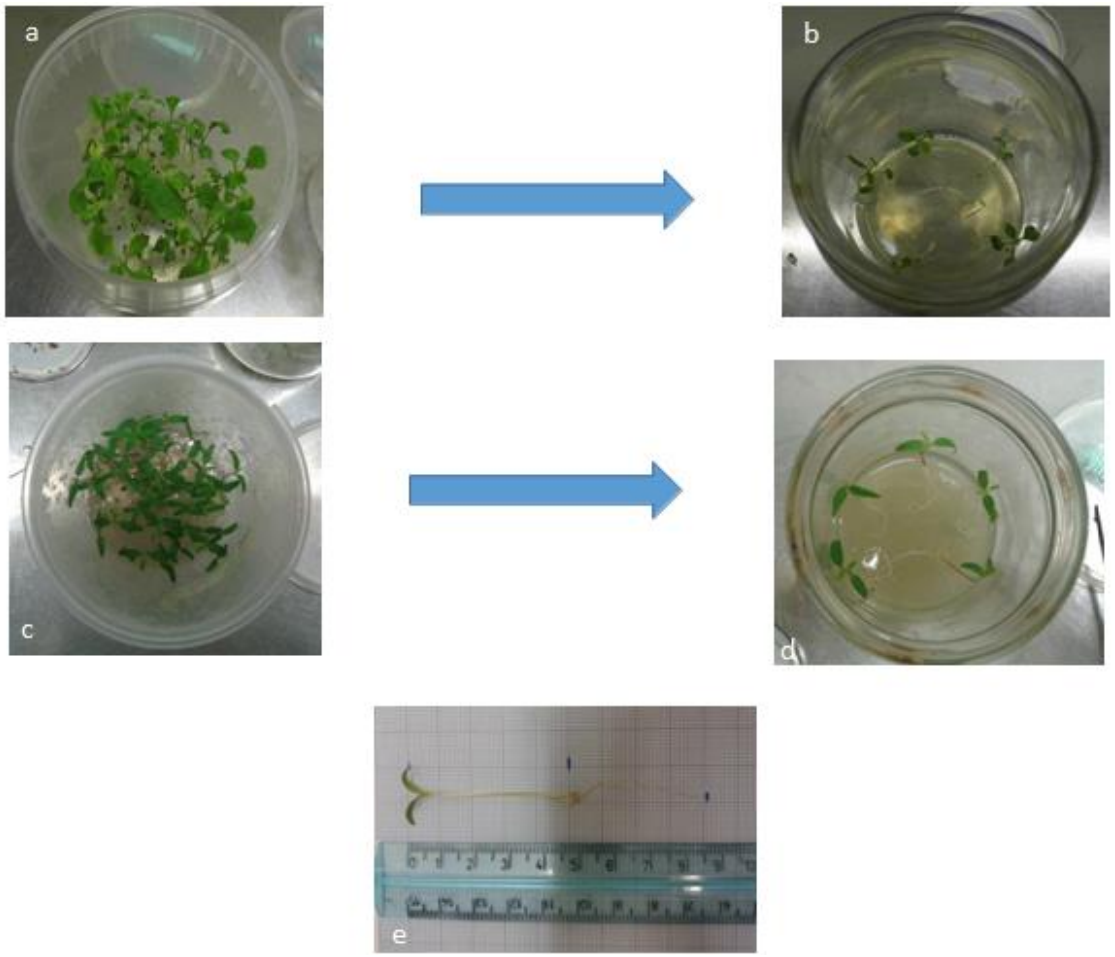

Figure 3.3: *Seedlings in MS medium cultivated for 10 days: a and c) seedlings of turnips and tomatoes grown in medium without BaA, b and d) seedlings of turnips transplanted in BaA contaminated medium. e) Measurement of seedlings to evaluate their suitability to transplant.*

Three replicates for each BaA concentration were prepared. The seedlings were cultivated for 28 days at the same conditions of photoperiod and temperature described above.

At the end of the growth cycle, the plants were removed from the jar. The length and the weight, of the shoots (stem and leaves) and roots were measured as well as the weight of the medium (Figure 3.4). BaA extraction and analysis were performed for each sample (medium, shoot, and root), the procedure was described in Paragraphs 3.2.4.5 and 3.2.4.6. Before the analysis, roots were washed several time with deionized water in order to remove residues of the medium.

Figure. 3.4: *Tomato plants after 4 weeks: a) plants of tomato with medium, b) shoots and roots separated from medium, c) weight of shoot, and d) shoots prepared for the storage at -20 °C.*

The recovery of BaA in the extraction method was evaluated by spiking MS medium, turnip and tomato samples (shoots and roots) with BaA standard solution with a concentration of 5 mg kg^{-1} and 1 mg kg^{-1} for MS medium and plant tissue, respectively. For turnip, the recoveries were of 90% for MS medium and of 60% for shoots and roots; while for tomato the recoveries were of 80, 60 and 60% for MS medium, shoots and roots, respectively**.**

3.2.6 BaA measurement

3.2.6.1 BaA measurement in soil

The extraction procedure of BaA was performed in according to EPA (Environmental Protection Agency - United States, Test Methods: EPA3550c, EPA3630c and EPA8270d).

Soils sampled from unplanted or planted pots were collected and homogenized. Five g of dried soil was transferred in a 50 mL Falcon tube and extracted with 40 mL of cyclohexane-acetone (70:30; v:v) in ultrasonic bath (1 h). The extract was centrifuged at 2000 rpm for 5 min at 4 °C. The supernatant was concentrated (about 1 mL) by a rotary evaporator at 40 °C (Büchi, Flawil, Switzerland). Concentrated extract was purified with Sep-Pak silica cartridges conditioned with 5 + 5 mL of n-hexane and eluted with 5 mL of n-hexane and then with 5 mL of ACN. The eluate was evaporated in a thermoblock at 40 °C under nitrogen flow, dissolved in 1 mL of ACN and transferred into vials for HPLC-FLD analysis.

Recovery of BaA was calculated by spiking soil with BaA standard solution (5 mg kg^{-1}). In the soils cultivated with turnip the recovery was 70%, while in the soil cultivated with tomato was 91%.

3.2.6.2 BaA measurement in culture medium and plant tissues

Since vegetables and fruits are consumed normally fresh, all results are expressed on fresh weight. The shoots and roots were washed and crumbled. Two grams (fresh weight) of each homogenized sample was transferred in a 25 mL Pyrex tube, 10 mL of potassium hydroxide solution (2 N in ethanol) was added. The samples were placed in a water bath at 80 °C for 2 h. The samples were cooled at room temperature and 10 mL of ultrapure water were added. The samples were extracted with 20 mL of cyclohexane and

centrifuged at 2000 rpm for 5 min at 4 °C. The extraction step with cyclohexane was carried out three times.

The supernatants were assembled (60 mL), filtered through a filter paper containing anhydrous sodium sulphate, and reduced to a small volume (about 0.2 mL) by a rotary evaporator at 40 °C (Büchi, Flawil, Switzerland). The extract was dissolved in 3 mL can and purified through Sep-Pak silica cartridges. The cartridges were conditioned with 3 mL ACN and the sample was eluted with another 3 mL ACN. The eluate was evaporated in a thermoblock at 40 °C under nitrogen flow, dissolved in 1 mL ACN and transferred into vials for HPLC-FLD analysis.

3.2.6.3 High-performance liquid chromatography analysis

High-performance liquid chromatography (HPLC) analysis was performed by Alliance E2695 Waters (Waters, Dublin) equipped with a Waters 2475 fluorescence detector (FLD) (Figure 3.9). The column was an EnvirosepPP 125 mm x 3.20 mm, 5 µm (Phenomenex, Castelmaggiore, Italy). ACN/water (80:20; v:v) were used a the mobile phase in isocratic mode with flow rate of 0.5 mL min^{-1}. The column oven was set at 25 °C. The injection volume was 50 µL.

In the FLD-detection of BaA, excitation and emission wavelengths were set at 280 and 385 nm, respectively. Identification of sample peak was based on the retention time of BaA standard peak.

An external standard method was used to determine PAH concentrations in the samples.

3.2.7 Statistical analysis

Statistical analysis was performed by SPSS (23.0 version).

For germination test the data of each species was compared across the soil treatments using one-way analysis of variance. The significant differences between means at $P < 0.05$ were assessed according to Tukey test.

Two-way ANOVA (BaA concentration, part of plants) was used for in vivo and in vitro experiments to test the results from bioavailability of BaA in vegetables (turnip and tomato). The significant differences between means at $P < 0.05$ were assessed according to Duncan test.

3.3 Results and discussion

The study of the fate and effect of BaA in/on vegetables followed a wide approach because it started from phytotoxicity test based on the response of turnip and tomato seeds in the germination stage and then the study continued by following the turnip and tomato seedlings for one month to highlight possible effect on the plant biomass and the differentiated BaA accumulation in plant tissues (shoots and roots) and also between two analysed species. As contaminated soil could affect the availability, translocation and accumulation of BaA molecules in plant tissue, *in vitro* experiments were also performed to be able to observe possible different behaviour of the contaminant in a simpler environment without all well-known phenomena characterizing soil system.

3.3.1 Germination test in presence of BaA contaminated soil

In turnip samples the presence of 50 and 100 mg kg^{-1} BaA reduced not significantly the relative germination (RG) (Table 3.3). Conversely, the relative length (RL) significantly shortened to 63 and 65% at 50 and 100 mg kg^{-1} BaA rate, respectively. The same trend was obviously observed in GI (60 and 68% in Petri dishes containing 50 and 100 mg kg^{-1} BaA contaminated soil, respectively) (Table 3.3 and Figure 3.5).

In tomato samples, the RG value showed no significantly differences between the control sample and the treatments with 50 and 100 mg kg^{-1} BaA (Figure 3.3). In this case, the RL value increased significantly to 143 and 163% with 50 and 100 mg kg^{-1} BaA contaminated soil, respectively. The same trend was observed for the GI (126% and 151% with 50 and 100 mg kg^{-1} BaA contaminated soil, respectively) (Table 3.3 and Figure 3.5).

Table 3.3 *Relative germination percentage (RG), relative length percentage (RL) and germination index percentage (GI) of turnip and tomato seeds. Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}BaA).*

	Turnip			Tomato		
BaA rate mg kg^{-1}	**RG**	**RL**	**GI**	**RG**	**RL**	**GI**
	----------%			----------%		
0	100 b	100 b	100 b	100 ab	100 ab	100 ab
50	97 b	63 a	60 a	89 a	143 cd	126 bc
100	103 b	65 a	68 a	93 ab	163 d	151 cd

These results clearly indicate that BaA did not affect the germination of both the species as the number of germinated seeds significantly remained unchanged in all samples. This was contrasting with data reported by Adam and Duncan (2002), showing a negative effect of PAHs on germination with 22 different species including grasses, legumes, herbs and commercial crops. (Adam, *et al.*, 2002). Conversely Henner *et al.* (1999) and Korade and Fulekar (2009) explained that volatile, water-soluble low molecular-weight hydrocarbons (<3 rings) such as benzene, toluene, xylene (BTX), styrene, indene, naphthalene and other toxic substances strongly inhibited plant germination and growth in contrast to the high molecular weight PAHs (3-5 rings) which did not show any phytotoxicity under the conditions studied.

Two species showed different response in RL: it reduced markedly in turnip seedlings, whereas it raised significantly in tomato seedlings. The differernt fat content of two species could help to understand the different behaviour: the tomato seeds (b cv. Principe Borghese) had 23.44% (dw) fat content (Giuffrè and Capocasale 2016) against 9.36% of turnip seeds (Lopez- Cervantes, *et al.*, 2013), a value markedly larger that can explain the differentiate behavior in the germination stage in the BaA presence.

Seed germination is strictly related to seed metabolism (Rosental *et al*, 2016). Maturing seeds accumulate transcripts and metabolites necessary for seed germination. During germination, glucose at high levels can support abscisic acid signaling, delaying germination and starch degradation in tomato (Rosental *et al*, 2016). Intermediates of the tricarboxylic acid cycle accumulate during seed priming, likely in preparation for the high energy demands of germination. Amino acids are also used as energy production sources during the early stages of germination via various pathways. Cell wall metabolism is essential for the loosening of the endosperm cap in tomato and for the elongation of the radicle leading to germination (Martínez-Andújar *et al*, 2012).

The presence of BaA led seedlings to exibit species dependent stress characteristics. In fact, PAHs could affect quantitatively and qualitatively several biochemical and physiological processes taking part in biomass production (Kummerová *et al.*, 2007).

PAHs as well as their products deriving from their photo-modification can affect structures and functions at cellular and subcellular levels (Kolb and Harms 2000). These lipophilic substances interact mainly with plasma membrane, where phospholipids could be oxidized. The disturbance of this membrane and the inner subcellular membranes and changes in enzyme activities may cause an inhibition of photosynthetic and respiration

processes (Kummerová *et al*., 2007). In particular, intact and photo-modified PAHs accumulate preferentially in the thylakoids of the chloroplasts (Kummerová e*t al.*, 2007).

Huang *et al.* (1997) found fluoranthene could cause a reversible inactivation of PSII on thylakoid membranes. Kummerova *et al.* (2012) showed that fluoranthene inhibited pea and maize germination. It is common that PAHs dramatically inhibit root growth (Alkio *et al*., 2005) but there were also exceptions regarding *Helianthus annuus* (Smreczak and Maliszwska-Kordybach, 2003) and *Lepidium sativum* (Rao *et al.*, 2017).

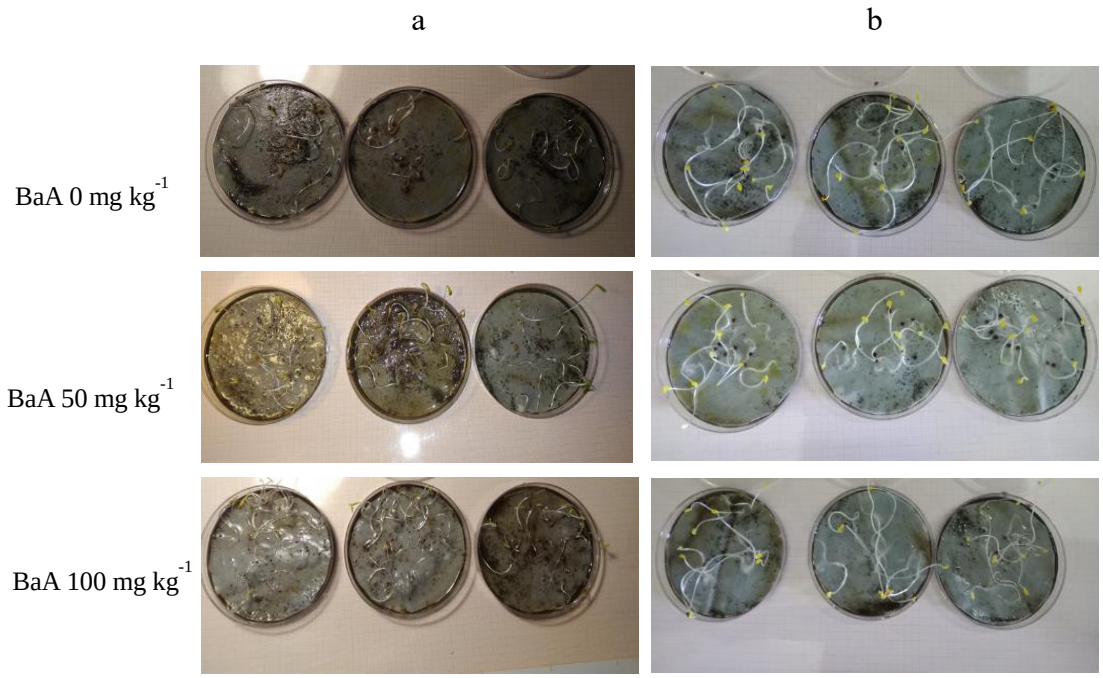

Figure 3.5 *Germination test of a) turnip and b) tomato seeds at 0, 50 and 100 mg kg^{-1}* BaA.

3.3.2 Cultivation of turnip and tomato plants in BaA contaminated soil

The growth of one seedling of turnip and tomato was performed in soil pots at two BaA contamination levels (50 and 100 mg kg^{-1}) besides the control without BaA.

After 28 days of cultivation time under greenhouse, the BaA did not affected the fresh biomass of turnip's root samples regardless of its concentration (Figure 3.6). In fact, the value remained not significantly different from 0.18 ± 0.05 g of control. The same trend was observed in biomass's shoot samples: the biomass weight remained significantely close to the control (1.58 ± 0.49 g) although with larger variability (Figure 3.6).

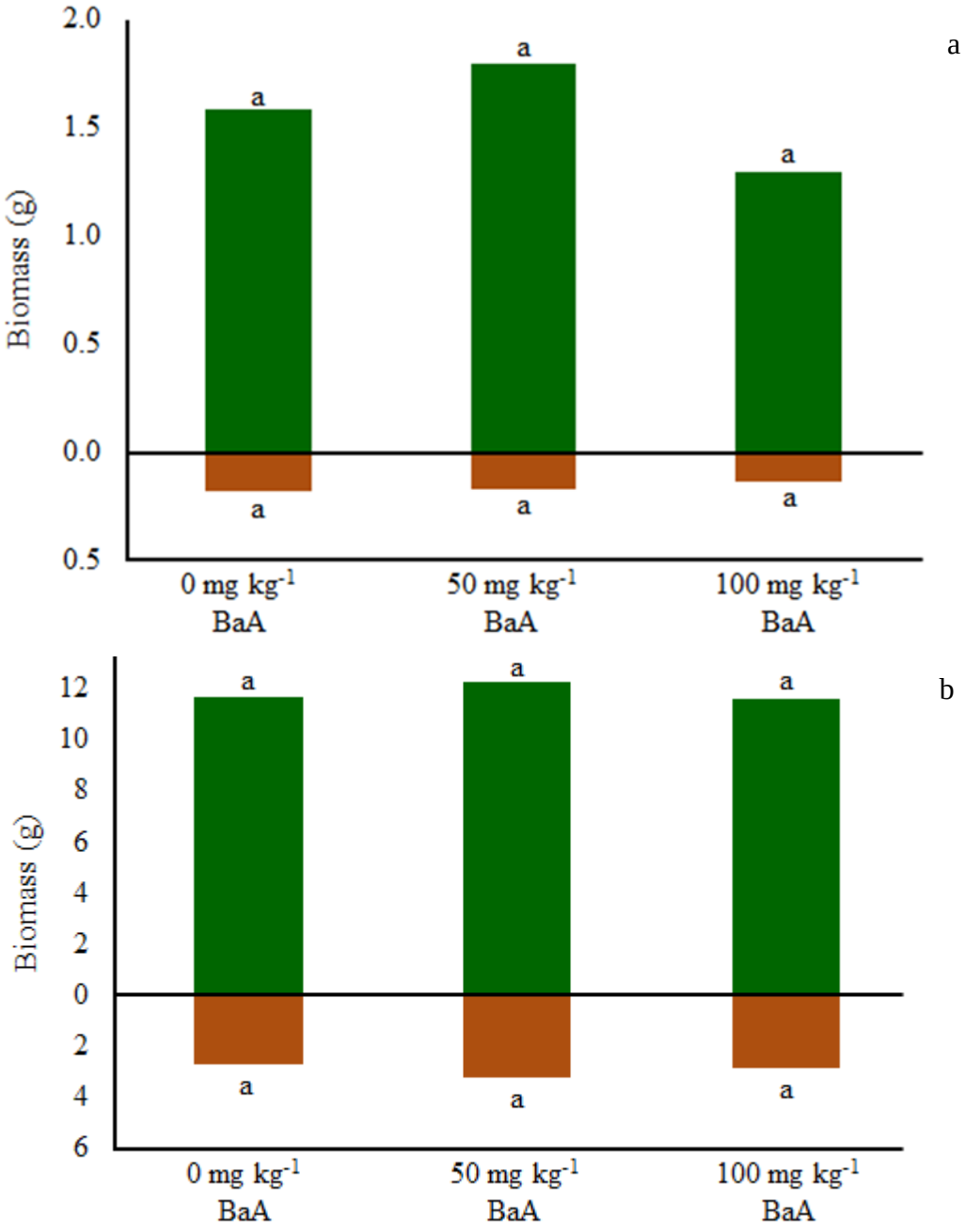

Figure 3.6 *Root (brown) and shoot* (green) *biomass in a) turnip and b) tomato plant cultivated in BaA contaminated soil. Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1} BaA).*

A different trend of biomass dry weight was observed expecially in tomato plants (Table 3.4). In fact, although roots had the same fresh weight regardless of BaA contamination rate of soil, the root biomass as dry weight strongly increased by increasing the soil contamination to indicate the development of root tissue with reduced water content as response to toxicity stress. Turnips behad differently since the values of biomass dry weight did not change at 50 mg kg^{-1} BaA rate but increased significantly at the

highest contamiantion rate. The major affinity of tomato plants to BaA (larger BaA content in the plant tissue) could also affect the growth and the characteristics of hypogeal structures, those in direct contact and interaction with BaA molecules. Conversely, no changes in biomass dry weight of shoot was observed. These results are contrasting weith Gao and Zhu (2004). These authors found no significant biomass disparities between plants grown in the spiked and unspiked soils with PAHs (133 mg kg^{-1} phenanthrene and 172 mg kg^{-1} pyrene). Whereas in heavily spiked soils, plant biomass were significantly lower than in non-spiked soils. In all tested 12 plants, the root systems developped more densely in the presence of contaminants.

After 28 days under greenhouse, the BaA concentration in soil samples in pots was 34.24 and 80.25 mg kg^{-1} in 50 and 100 mg kg^{-1} contaminated soil, respectively. As percentage, the values were 69 and 80%, respectively.

Table 3.4 *Biomass (dry weight)* of *roots and shoots in a) turnip and b) tomato plant (in vivo). Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}BaA).*

	Biomass dw (%)			
	Turnip		Tomato	
BaA rate	**Roots**	**Shoots**	**Roots**	**Shoots**
0 mg kg^{-1}	9.5a	8.5a	29.4a	10.6a
50 mg kg^{-1}	8.2a	7.6a	42.9a	8.4a
100 mg kg^{-1}	13.2b	11.6b	78.4c	11.5a

Table 3.5 *BaA concentration in soil, roots and shoots of turnip cultivated in contaminated soil. Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}BaA).*

	BaA concentration					
	Soil				Roots	Shoots
	without plant		with plant			
BaA rate	**mg kg^{-1}**	**%**	**mg kg^{-1}**	**%**	**mg kg^{-1}**	**mg kg^{-1}**
0 mg kg^{-1}	0.00 a		0.00 a		0.00 a	0.00 a
50 mg kg^{-1}	41.76 c	84	34.24 b	69	0.92 a	0.05 a
100 mg kg^{-1}	94.26 e	94	80.25 d	80	2.17 a	0.15 a

We can observe that the BaA concentration in spiked soils was higher (around 15 percentage units) when no plant was cultivated in the soil pot and no great differences in percentage terms was registered between two contamination rates. These aproximatively ten

percentage units represented 8 and 6 mg kg^{-1}, respectively (Table 3.5), very close values indicating how soil physical, chemical and biological properties can affect the fate of the PAH molecules independently on plant growth. The reduction of the extractable BaA is mainly attributable to intrinsic bioremediation processes including a variety of physical, chemical, and biological processes that act to reduce the mass, toxicity, mobility, volume, or concentration of contaminants (Hansen *et al.*, 2004; Smith *et al.*, 2006; Scelza *et al.*, 2007; 2010; Scelza, 2008; Bisht *et al.*, 2015; Bisht *et al.*,2015). During these processes, the indigenous microbial populations degraded recalcitrant compounds based on their natural metabolic processes. The role of organic matter is very important, as well known the beneficial effect on soil properties and as nutrient source for soil microorganisms.

However, entrapment, sequestration, and ageing, of BaA molecules in soil colloids and aggregated can be most likely favored in the special environment close to root-plant adsorption (Scelza, 2008). Moreover, degradation process could not be excluded as the numerous studies on bioremediation of PAH contaminated soil (Scelza *et al.*, 2007; 2010). All this could, therefore, explain the reduced BaA concentration in pot soil having a cultivated turnip plant (Table 3.5).

Microorganisms are also responsible for the intense activity, synergistic with root activity to favour, for example, aggregate formation, organic matter turnover, energy transfer, etc. (Bisht *et al.*, 2015). Rhizospheric phenomena occurr at root/soil interface where numerous chemical and biochemical processes take place (Pii *et al*., 2006). Specific investigations were addressed to identify soil microorganism diversity in the experimental soil pots, through the DNA extraction and further sequencing analysis, but unfortunately setbacks impeded to obtain information about microbial diversity in due time. These future results could help to highlight if the presence and the rate of BaA could affect the biomass population during the experiment.

To understand better the extent of BaA translocation from soil to turnip tissues the presence of BaA in soil and in the vegetal tissues were also expressed as absolute weight (μg) (Tables 3.6). The BaA concentration in turnip roots, grown in 50 and 100 mg kg^{-1} contaminated soil, was 0.92 and 2.17 mg kg^{-1}, respectively (Table 3.5). Lower values were observed in shoots where the BaA concentration reached 0.15 mg kg^{-1} at the highest BaA rate. The amount translocated from soil to plant resulted very little in root and still lower in shoots, equivalent to at least five orders of magnitude lower than the BaA amount

initially added in the soil (Table 3.6).

Table 3.6 *BaA amount in soil, roots and shoots of turnip cultivated in contaminated soil.*

		Soil				Roots		Shoots	
		without plant		with plant					
BaA rate	Added BaA mg	Extracted BaA mg	%	Extracted BaA mg	%	Biomass g	Extracted BaA µg	Biomass g	Extracted BaA µg
0 mg kg^{-1}	0					0.38		0.55	
50 mg kg^{-1}	30	12.53	42	10.27	34	0.40	0.40	0.61	0.00
100 mg kg^{-1}	60	28.28	47	24.08	40	0.28	0.60	0.39	0.40

In tomato, after 28 days under greenhouse, the BaA concentration in soil samples was 39.67 and 80.57 mg kg^{-1} in 50 and 100 mg kg^{-1} contaminated soil, respectively, equivalent to 79 and 81%, respectively. These values distanced themselves from those measured in soil pots cultivated with turnip (Table 3.5) in according to sample variability. Also in the experiment with tomato natural attenuation of soil contamination occurred at two BaA rates: a reduction of 9 and 16% in 50 and 100 mg kg^{-1} contaminated soil, respectively, respect the initial BaA contamination was registered (Table 3.7).

The tomato cultivation determined a further decrease of the residual BaA soil concentration occurring markedly in the 50 mg kg^{-1} BaA pots (Table 3.7), but less intense than in the turnip experiment. This should not be attributable to the root development as tomato roots showed a greater biomass than turnip root, but rather it could be due to different rhizospheric effect induced by Brassicaceae (Gao and Zhu, 2004).

The BaA translocation in tomato roots and shoots occurred more markedly than in turnip plants and especially in root tissues. In fact, BaA recovered from tomato root tissues cultivated in 50 and 100 mg kg^{-1} contaminated soil was 15.43 and 20.36 mg kg^{-1}, respectively (Table 3.7) against 0.92 and 2.17 mg kg^{-1}, respectively, of tomato. In terms of mass balance, the BaA transfer from soil to root related to 49.22 and 73.04 µg in 50 and 100 mg kg^{-1} contamination level, respectively, against 0.40 and 0.60 µg registered in turnip experiment. The accumulation could be attributable to the grater root biomass produced by tomato plants (Figure 3.4) and likely to the root composition, similarly to tomato seeds having a greater lipid content than turnip seed (Lazos *et al*., 1998; Jopez-Cervantes *et al.*, 2013). Gao and Zhu (2004) found a strict relationship between lipid

content of several plants analysed in their experiment and the phenanthrene and pyrene root concentration resulting the lipids in roots, even at small amounts, were usually the major reservoir for highly water insoluble contaminants.

Table 3.7 *BaA concentration in soil, roots and shoots of tomato cultivated in contaminated soil Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}BaA)..*

	Soil					
	without plant		with plant		Roots	Shoots
BaA rate	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
0 mg kg^{-1}	0.00 a		0.00 a		0.00 a	0.00 a
50 mg kg^{-1}	45.33 e	91	39.67 d	79	15.43 b	0.05 a
100 mg kg^{-1}	84.12 f	84	80.57 f	81	20.36 c	0.07 a

Table 3.8 *BaA amount in soil, roots and shoots of tomato cultivated in contaminated soil.*

		Soil				Roots		Shoots	
		without plant		with plant					
BaA rate	Added BaA mg	Extracted BaA mg	%	Extracted BaA mg	%	Biomass g	Extracted BaA µg	Biomass g	Extracted BaA µg
0 mg kg^{-1}	0					2.73		11.64	
50 mg kg^{-1}	30	14.01	47	11.9	40	3.19	49.22	12.26	0.61
100 mg kg^{-1}	60	27.31	46	25.67	43	2.88	73.04	11.62	0.81

When we compare the BaA concentration with contamination levels revealed in vegetables monitored in LoF (Chapter 2), we observed grater BaA concentration (three orders of magnitude) in the turnips of this experiment as demonstrated by the units in mg kg^{-1}against µg kg^{-1}. The correct interpretation of this comparison needs a careful analysis.. First, the experiments carried out with turnip and tomato defined a serious soil contamiantion level to reproduce an extreme situation, athough in literature these PHA concentration were exeeded. Moreover, the monitoring anctivities of LoF considered just the edulis part of the entire plant. In addition, an important issue, missing in the monitoring activities of LoF, is the lack of information, at least until now, about the real contamination of soil where those vegetables were cultivated. The sampling was carried out during monitoring action, but until now the dataset of soil contamination is not

completed yet. These data could be useful for defining the contamiantion level of sampled soils and the relationship between the PAH contamination rate and the extent of the translocation of contaminant molecules as well as other soil characteristics affecting the behavior and the fate of pollutants in the environment.

3.3.3 Contamination of turnip and tomato plants cultivated in BaA contaminated MS medium

The second cycle of experiments was carried out in axenic condition. Turnip and tomato seedlings were cultivated in MS culture medium to avoid all the possible interferences due to soil (Figure 3.5). These experimental conditions should be able to guarantee a direct interaction BaA-plant with no effect from soil colloids, microorganism and rhizosphere.

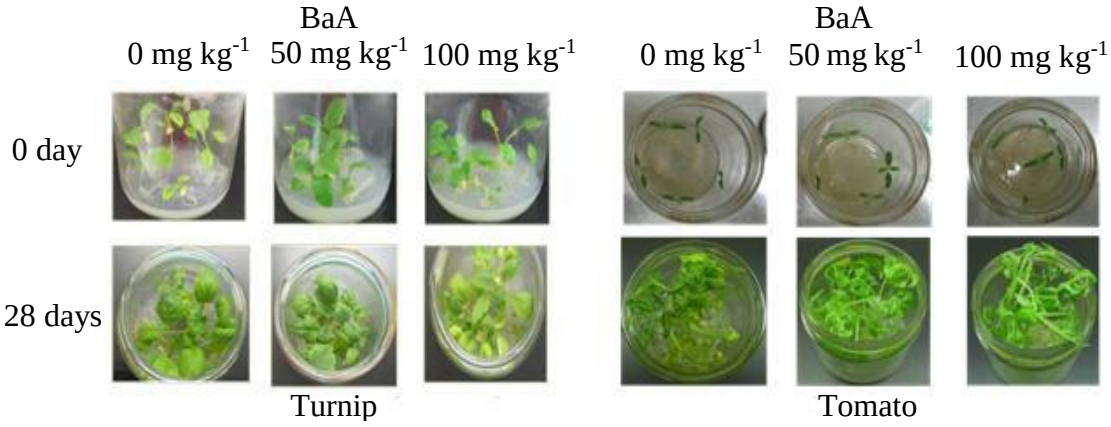

Figure 3.5 *In vitro cultivation of turnip and tomato seedlings in presencce of BaA.*

In these experiment the biomass (gram of biomass per plant) of turnip and tomato roots felt the effect of BaA: turnip root developed significantly less at 100 mg kg^{-1} of BaA (Figure 3.6a), whereas tomato roots developed significantly less at 50 mg kg^{-1} of BaA (Figure 3.6b). Shoot biomass of turnips were consistent with the profile described for roots samples above and, finally, the entire turnip plants cultivated at 100 mg kg^{-1} BaA grew up smaller than the control.

The growth of tomato plants in terms of fresh biomass production followed a different profile. In fact, shoots of plants grown in the presence of BaA reduced significantly biomass regardless its concentration (Figure 3.6b).

When the biomass dry weight of roots and shoots of turnip and tomato plants was taken into account, the data trend changed showing an increase of both turnip root and shoot values at 100 mg kg^{-1} BaA and no difference in biomass dry weight of tomato roots and shoots (Table 3.9).

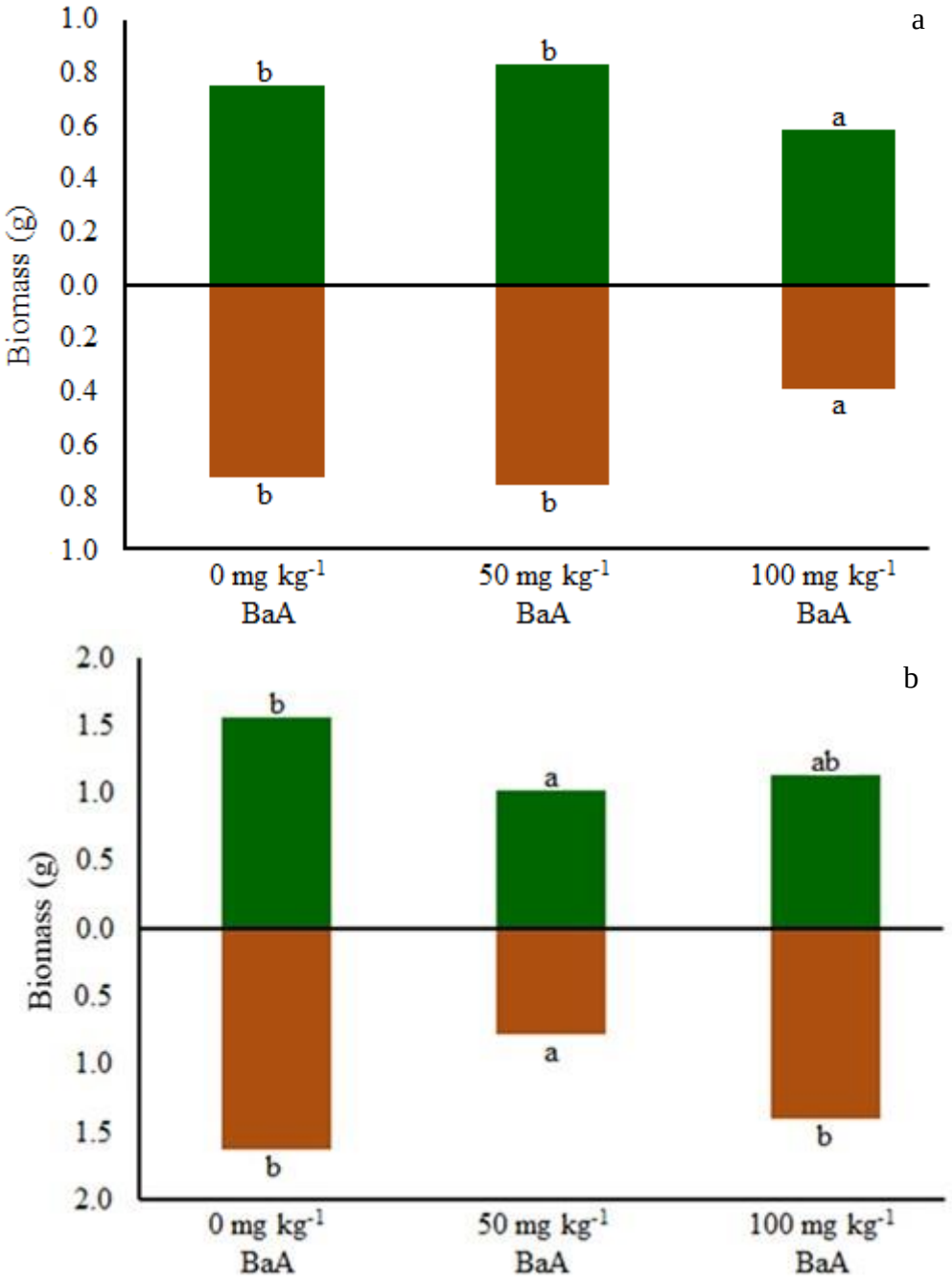

Figure 3.6: *Roots and shoots biomass in a) turnip and b) tomato plant (in vitro). Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}).*

Table 3.9 *Root and shoot* biomass *dry weight of a) turnip and b) tomato plant in vitro. Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}).*

	Biomass dw (%)			
	Turnip		Tomato	
BaA rate	Roots	Shoots	Roots	Shoots
0 mg kg^{-1}	4.76 a	8.39 a	9.55 a	9.42 ab
50 mg kg^{-1}	5.28 a	7.79 a	7.04 a	9.88 b
100 mg kg^{-1}	6.74 b	10.92 b	7.61 a	8.20 a

The availability of hydrocarbons probably increased under i*n vitro* conditions and as consequence contaminant molecules affect more markedly seedling growth (Reynoso-Cuevas *et al.*, 2008). Different response could be attributed to i) the different characteristics between seedling grown in soil and those grown in culture medium; ii) soils characteristics able to affect the biovailability and weathering of PAHs (Reynoso-Cuevas *et al.*, 2008).

In fact, seedlings used in this experiment were from seed germination in culture medium MS and they reached a size smaller than that of seedlings cultivated in soil experiment. The different development of seedlings could also affect the turnip and tomato response to PAH contamination.

On the other hand, since PAHs in culture medium could more negatively affect root development, probably morphology and architecture of roots could result altered with an modified ability to acquire water and nutrients from the medium (Merkl *et al.*, 2005). BaA and the contamination rate affected the root architecture of both turnip and tomato: a strong inhibition of root volume, in the first case, whereas more dense hair roots in the second case (Figure 3.4) occurred. This behaviour was consistent with Reynoso-Cuevas *et al.* (2008). The response changed depending also on the plant species. After all, it is for this reason that plants are classified as tolerant or sensitive: each species has peculiar characteristics making them able to resist or be tolerant to specific stress or in the worst case sensitive. The increase of the biomass dry weight could be index of a defense mechanism of plant able to limit the entrance of contaminant. Besides, the growth of roots is a sensitive indicator of the presence of BaA (Reynoso-Cuevas *et al.* 2008). The short distance between meristematic tissue and contaminant molecules limited the growth and reduced the length. As consequence, the inhibition of the primary root can raise the formation of lateral roots (Figure 3.5).

In order to better understand the mechanism of response to this peculiar stress further studies are still ongoing to analyse in detail the root apparatus as structure and physiology in the presence of increasing BaA contamination level.

BaA concentration in MS culture medium, root and shoot samples under *in vitro* conditions was analysed (Table 3.10). After 28 days under climatic chamber, the BaA concentration in medium samples declined to 26.92 and 45.55 mg kg^{-1} in 50 and 100 mg kg^{-1} corresponding to 54 and 46%, respectively. The control pots without seedling also showed a reduction of BaA concentration, lower than cultured samples, to indicate also in axenic culture a natural contaminant degradation occurred (Table 3.10).

The BaA concentration measured in root and shoot samples of turnip was strongly greater than those detected in soil systems. Roots contained 20.50 mg kg^{-1} and 40.58 mg kg^{-1} in 50 and 100 mg kg^{-1} BaA treatment, respectively, whereas shoots contained 0.52 mg kg^{-1} and 4.96 mg kg^{-1} in 50 and 100mg kg^{-1} treatment, respectively (Table 3.10).

Table 3.10 *BaA concentration in MS medium, roots and shoots of turnip in vitro experiment. Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}BaA).*

	MS medium					
	without plant		with plant		Roots	Shoots
BaA rate	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
0 mg kg^{-1}	0.00 a		0.00 a		0.00 a	0.00 a
50 mg kg^{-1}	40.53 c	81	26.92 bc	54	20.50 b	0.52 a
100 mg kg^{-1}	63.93 d	64	45.55 c	46	40.58 c	4.96 ab

In term of mass balance around the half of BaA initially added to each jar was extracted from MS medium where turnip seedling were cultivated and the value remained greater in the jars without seedlings (81 and 64% at 50 and 100 mg kg^{-1} BaA treatment, respectively (Table 3.11). The translocation of BaA molecules from MS medium to root turnip occurred in terms of 1.22 μg, at 100 mg kg^{-1} BaA rate. This BaA amount was very little if compared to the initially added (3 mg) although it was doubled if compared to the BaA amount measured in turnip roots grown in contaminated soil (Table 3.6). The translocation of BaA to turnip shoots reached under *in vitro* experiment negligible values (Table 3.10).

Table 3.11 *BaA amount in MS medium, roots and shoots of turnip cultivated in vitro.*

		MS medium				Roots		Shoots	
		without plant		with plant					
BaA rate	Added BaA mg	Extracted BaA mg	%	Extracted BaA mg	%	Biomass g	Extracted BaA µg	Biomass g	Extracted BaA µg
0 mg kg^{-1}	0					0.72		0.75	
50 mg kg^{-1}	1.5	1.22	81	0.80	53	0.75	0.62	0.83	0.02
100 mg kg^{-1}	3.0	1.92	64	1.49	48	0.39	1.22	0.58	0.15

The tomato growth experiment showed that the BaA concentration in MS medium samples was 11.83 and 21.18 mg kg^{-1} (24% and 21%) in 50 and 100 mg kg^{-1} treatment, respectively, but not significantly different between each other. The BaA concentration in root samples was 23.81 and 75.53 mg kg^{-1} in 50 and 100 mg kg^{-1} treatments, respectively (Table 3.12). The shoot samples showed a very smaller concentration of BaA: it was not significantly different from the control (Table 3.12).

Table 3.12 *BaA concentration in MS medium, roots and shoots of tomato in vitro experiment. Letters indicate the differences between the treatment (0, 50, and 100 mg kg^{-1}BaA).*

	MS medium				Roots	Shoots
	without plant		with plant			
BaA rate	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
0 mg kg^{-1}	0.00 a		0.00 a		0.00 a	0.00 a
50 mg kg^{-1}	45.80 c	92	11.83 ab	24	23.81 b	0.58 a
100 mg kg^{-1}	86.20 e	86	21.18 ab	21	75.53 d	2.86 ab

The absorption of BaA in root tissues was very low in terms of µg of contaminant translocated from MS medium to plant root: it amounted to 0.005% or 0.007% of the BaA initially added at 50 and 100 mg kg^{-1} treatments (Table 3.13).

Table 3.13 *BaA amount in MS medium, roots and shoots of tomato cultivated in vitro.*

		MS medium				Roots		Shoots	
		without plant		with plant					
	Added BaA	Extracted BaA				Biomass	Extracted BaA	Biomass	Extracted BaA
BaA rate	mg	mg	%	mg	%	g	µg	g	µg
0 mg kg^{-1}	0					1.63	0	1.55	
50 mg kg^{-1}	1.5	1.37	92	0.81	54	0.78	0.71	1.03	0.02
100 mg kg^{-1}	3.0	2.59	86	1.37	46	1.41	2.27	1.14	0.09

Therefore, these experiments showed that two species responded differently to the BaA presence and the response depended on the BaA concentration. Contaminant molecules translocated in turnip roots and just in negligible amount in shoots; simultaneously a reduction of plant biomass occurred, in particular at 100 mg kg^{-1} BaA. Roots in this species modified themselves increasing their dry weight and reducing the volume of MS medium explored.

Tomato seedlings showed a different behavior starting from the unchanged biomass dry weigh, to the higher capacity to absorb BaA in roots. Gao and Zhu (2004) demonstrated the different ability of variious plant species in soil PAH contaminated systems and later Reynoso-Cuevas *et al.* (2008) tested grasses in phytoremediation test under in vitro experiments.

It is evident that the axenic condition where soil is substituted with a medium unable to affect the behaviour and the fate of all system actors could be preferable to study the mechanisms and the potential process driven by plants with the help or better the cooperation of soil microorganisms and soil colloids. In fact, as explained by Reynoso-Cuevas *et al.* (2008) "Plant in vitro cultures are useful systems with practical advantages: i) the plants can grow under laboratory conditions, independent of the weather; ii) are clean, easy for measuring and monitoring systems that often grow more rapidly in comparison to plant-soil conditions; ii) resulting effects are only attributable to plants and not to combined plant-microbial rhizospheric effect; and, iv) are valuable techniques for selecting potential plants for phytoremediation of soil contaminated with hydrocarbons or heavy metals".

3.4 Comparing remarks

Both the approaches followed in this study resulted necessary to investigate complex processes taking place when plants and a PAH contaminant join but at the same time limits of both the approach should not masked. Some observations arose from a comparative analysis.

First, all the experiments led to reach very high BaA contamination levels especially if they are compared with those measured in vegetables sampled in the monitoring activity within LoF plan. In soil and *in vitro* experiments, we detected contaminant concentration three order of magnitude higher. Unfortunately, a complete and correct comparison could not be performed as in the case of the monitoring activity no information about the contamination rate of soil where sampled plants were cultivated. Actually, the source of plant contamination was not always known by means atmospheric pollution could be not excluded. In addition, it is important highlighting that only the edible part of the sampled vegetables were collected in the monitoring activity of LoF whereas in the experiment in soil and under *in vitro* conditions seedlings grew just for 28 days. Therefore, the possible translocation of BaA to shoots of a big turnip plant or to tomato fruits remained just an extrapolation of data obtained in soil and *in vitro* experiments.

On the other hand of this issue, there is the defined and widely demonstrated ability of plant organism to remove various pollutants (organic compounds and heavy metals) from soil, sediment, water and air) (Ouvrard *et al.,* 2014; Cristaldi *et al*., 2017). Many processes were claimed such as rhizoadsorption, rhizodegradation, rhizostabilization without excluding all processes where plants are involved with microorganisms and all together strongly contribute to rhizosphere activity with a very important in the fate of pollutants. Hence, phytoremediation uses the natural mechanisms occurring in plant systems when roots and leaves are in contact with pollutants. In the phytoremediation actions this ability are stressed and facilitate ameliorate and optimize plant conditions.

By comparing the soil and *in vitro* experiments, the important role of soil in the availability of BaA for plant tissues arose. Even only soil was able to contribute to change the fate and the behavior of BaA. The extractable amount was reduced in soil matrix to indicate many possible circumstances. BaA could be entrapped, adsorbed, sequestered in organic colloids, abundantly present in the soil used in the experiment. Once soil colloids bound contaminant molecules, a detoxification process in soil due to their stability and recalcitrance to translocation and breakdown by microbes should be taken into account.

The available fraction was reduced and a direct proportionality missed: by increasing BaA concentration, the BaA translocation in roots and shoots did not increase in the same extent (Tables 3.5 and 3.7). *In vitro* experiments confirmed that the bioavailable fraction of BaA increased and its translocation in plant tissue happened reaching higher contamination levels. Under *in vitro* condition, proportionality between contamination rate of MS medium and Plant tissue contamination could be observed (Tables 3.5 and 3.7).

Even though PAH are organic compound with low solubility in water system and not suitable for the movement within a water-based organism such as plants, in extreme contaminations BaA transfer into plant tissues. Then, the translocation from roots to shoots should be more difficult probably because shoots have not enough the lipidic component like roots. Lipids are able to solubilize PAH and move them within root structure. However, in the present study a serious contamination of turnip and tomato shoots was not observed in soil system or in axenic conditions.

3.5 Conclusions

Therefore, the overall results demonstrated that the contamination of turnip and tomato plants could occur if the BaA contamination levels of matrix where plants live are very high although real data on edible parts are missing. The bioavailability of BaA affected strongly the translocation of contaminant molecules in plant tissues and hence whatever action addressed to limit the BaA mobility should be desirable.

3.6 References

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