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Non-Invasive Matrices Use in Pollution Evaluation at Nanoscale Levels – A Way Forward in Ecotoxicological Studies

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1. Introduction

„For the first time in the history of the world, every human being is now subjected to contact with dangerous chemicals, from the moment of conception until death" – R. Carson, Silent Spring.

This is a quote that summarizes the reality at this moment regarding our safety in our surrounding environment, wherever we are. Unfortunately, pollutants imminence is more increased in developing countries, given that they register higher level of pollutants in all environmental compartments owing to their poor pollution control and pollutants monitoring.

In most cases, environment contamination is a result of humanity's lifestyle resulted from industrial-, agricultural activities and extended urbanization. For most of us it is more and more difficult to imagine our lifestyle from every day without using and profit of products stocked by chemical industry such as pharmaceuticals, petrochemicals, agrochemicals and many other consumer chemicals (Bhandari et al, 2009). Unfortunately together with the rise of chemical manufacture and its use has also come increasing public awareness and concern regarding presence of these chemicals in our surrounding environment. Concernments are mainly caused by the physicochemical properties of these chemical compounds and their possible negative consequence linked to human health and biota.

Owing to awareness's that was attributed by scientific communities and mass media to environmental pollution and living things exposure to such chemicals, has made a clutter regarding the terms like contamination and pollution, terms that tend to be use as synonyms (Hansen, 1993). On our days has been made an agreement between scientific experts, decision-makers and inspectors from different authorities at worldwide level, in that the term contamination should be used where a chemical is present in a given sample with no concrete evidence of harm while the term pollution could be used in cases where was

demonstrated that the presence of the chemical compounds caused harm for humans or to other living things (Neuzil et al, 1996).

Doubtlessness any chemical compounds can become a pollutant in any kind of environmental media (water, soil, air, etc.) causing negative effects if it is at a high enough concentration. Despite the fact that any chemical compounds can be a pollutant, certain chemicals have been identified in regulations or by international agreements as being priority chemicals for control (Harrison, 2001). These chemicals have been selected based on their: frequency found in surrounding environment at global scale; persistence in different media; toxic effects at low concentration (damage of selected organs, mutagenic and teratogenic effects) and carcinogenesis; and not in the last case based on their increased bioaccumulation capacity (Pierce et al, 1998).

Living organisms including humans are exposed to chemical contaminants, called also as environmental toxicants, via environmental media. Exposure might be occurred by breathing, ingestion by drinking liquids or eating nourishments that contain chemical contaminants, or through skin contact – see figure 1.

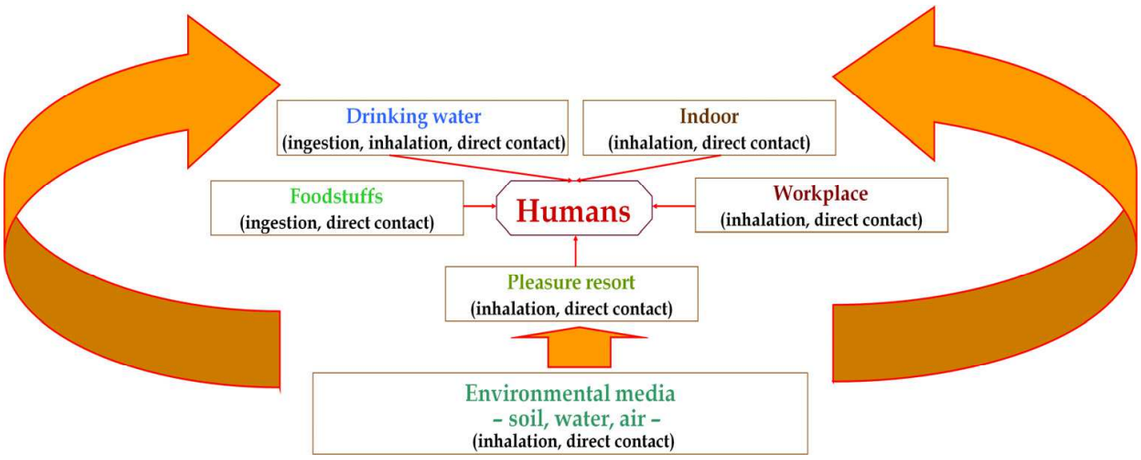


Fig. 1. Humans exposure pathways to organic chemical compounds contaminants.

When living organisms are exposed to environmental contaminants, a cascade of chemical, biological and biochemical events take place in them. These effects intensity depends strongly on exposure conditions – thus when we talk about exposure we must to take into account the dose of chemical contaminants (how much by the chemical substances are ingested, inhaled or imbibed through skin contact), the time period of exposure and the way through that living beings get contact with them. Also is necessary to look at the other chemical contaminants hereat the people are exposed (mixture effects) as well their age, gender, diet, lifestyle, family trails and state of health. Even in our day is difficult to give concrete answers to questions regarding organic chemical contaminants movement and distribution in the living environment – within individual living beings, communities and ecosystems – where multiple factors and events come in to play resulting usually in unwanted events and effects (Walker, 2009). In the last decades many studies underlined that contaminants conspecific to organic chemical classes result in different types of harmful effects on humans or other existences (Byres, 2006).

As regards living beings, concernment regarding our surrounding environment contamination is amplified by issues as movement of toxic contaminants from contaminated media in food products whatever we refers to vegetables or animals grown for consumption purposes. Therefore, humans are not exposed to chemical contaminants just through environmental media or workplaces (particular cases) but also along food chains (Walker, 2009).

Considering the lack of knowledge regarding the additive harm effects of such pollutants on biota (even if we talk about human subjects or animal subjects), becomes more necessary to pay a special attention to identifying, both qualitatively as well quantitatively, the presence of organic chemical contaminants in living beings. Therefore, is important to develop new, accurate and fine analytical methods for analysis of chemical contaminants at very low levels (micro- and nano- orders) from complex biological and environmental matrixes.

Unfortunately even at the present moment, when we want to evaluate the presence of such chemicals in living organisms toward formation of their ecotoxicological profiles, almost all analysis methods use invasive sampling protocols which in a large number of cases, especially in studies on animals, result in causing harm or death of the studied subjects. Introducing of analytical methods which use non-invasive sampled matrixes as saliva, hair, nail, milk, etc., will blot out problems regarding: causing harm (to human or animal subjects) or loss of studied subjects as animals; available number of analysis (referring to the number of human and animal subjects monitoring); and sample collection process.

Development of analytical methods for non-invasively collected biological samples will help in future to provide a better image regarding the contamination with complex chemicals of biota as well will help to get a better understand about their eco-toxicological hall-mark on any living organisms (whether we refer to human or animal subjects).

2. Current obstacles in biota monitoring

Starting from the early of XX century, scientific communities at worldwide scale started to put questions regarding the possibility of humans' exposure to unwanted chemical contaminants as well on the facts that could resulted after exposure events to such chemical contaminants. Thus in 1973, was initiated through World Health Organization the Environmental Health Criteria Programme which presented at that time these emerging objectives:

- to identify new or potential pollutants;
- to assess the biological/zoological specimens which are the most suitable for contaminants monitoring thus to get information on the relationship between exposure to environmental chemical contaminants and human health, in order that in future to could provide guidelines for setting useful exposure limits;
- to develop guidelines for sampling, sample preparation, analytical requirements and storage relative to biomonitoring;
- to draw up recommendation for further research and development;
- to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results (WHO, 2006).

Nowadays, in medicine and ecotoxicology fields, it is widely accepted that contaminants effects testing must involve animals because in most cases researches performed on animals

helped scientists to discover and developed lifesaving treatments and medications, and to observe which chemical compounds might be dangerous to humans if they get contact with it through ingestion, inhalation and/or dermal contact. But even if we consider all benefits that were obtained in humans health science through animal testing, not everyone consider animal testing as the best method for toxicology. There are two major reasons for that they are not totally accepted, once due to the fact that toxicological study performed on animal subjects induce terrible pain on them, most of the time resulting in the animal subjects death, and secondly due to the fact that not always the approximation between the selected testing animal species physiology and their anatomical profile is similar enough with that of humans (Watson, 2009).

Leaving aside whether if the chosen animal subjects in the study correspond entirely in term of physiological and anatomical structure to humans, when we want to have certainty that a chemical compounds is able to leave some imprint on a living things, study on any kind of animal subject becomes priority. Based on the presence of a chemical compounds on a animal subject, scientist can made analogy about the possibility of this chemical compound presence in human body.

Taking into account all that were presented before as well the controversies that have arisen in the recent years regarding testing on animal subjects, today more and more researchers are trying to develop novel testing methods that are not invasive to the subjects in question.

Development of such of kind methods is beneficial even for human biomonitoring, permitting a larger number of studies on a wider range of subjects as age, gender and geographical living position.

2.1 "Old sampling methods" in monitoring of living things exposure to organic contaminants

Human biomonitoring used for determination of chemical substances in body fluids was for the first time introduced in the early of 1930's by occupational medicine for health protection of exposed workers (Angerer et al, 2007). The first biological sample matrixes that were used was blood and urine, (blood being the first biological sample collected through invasive mode while urine was the first biological sample matrix that was sampled through non-invasive ways). The benefits of using biological samples in order to get information regarding human exposure made that this mode of humans health evaluation in terms of exposure to chemical contaminants to expand, so today it is used in areas like public health politics, environmental medicine, toxicology and other science that relate to the impact of chemical compounds on human health.

Usually, testing the qualitative as well quantitative presence of any kind of chemical compounds in living organisms, involve the use of sample matrixes as blood, tissues or bones. Although in terms of pollutants biomonitoring from living things it is considered that blood samples offer the most accurate information regarding the quantitative presence of a pollutants or its metabolites (due to the fact that it's get contact with the whole organism), this test specimen is done by venipuncture (piercing artery or vein) which cause pain and discomfort to subjects (Beebee, 2008). As regards bone or tissue sampling methods, these are more painful causing in most cases severe injuries.

Moreover, in terms of ecotoxicological studies performed on animal subjects in most situations the performed studies were completed by their sacrifice. Considering these, in the recent years at world wide it has brought up repeatedly in discussions the ethical aspects throughout these studies are conducted (Watson, 2009). But considering the importance of such kind of study (mainly as regards environmental contaminants biomonitoring or their toxicological evaluation on living things) by which obtained information make possible to take measures (pollutants presence regulation through law, contaminated environment remediation) or get treatment measures (in case of diseases) made them until now acceptable and necessary even in conditions of sacrifice of studied animal subjects.

Today a large number of countries introduce regulation regarding the way through that are performed these studies, imposing clear conditions as limiting the number of animal subjects subjected for study as well regarding the mode through that they are treated during the experiment. Unfortunately, considering these requirements in most cases the necessity of a large number of data (which obviously involve uses of a large number of subjects) is increasingly difficult to be fulfilled in researcher studies.

2.2 Current trends in living things exposure monitoring

Taking into account the requirements imposed by legislation but also by the need of a large number of subjects submitted for study so that to could draw realistic and convincing conclusions induced the need to seek new ways for ecotoxicological evaluation of environmental contaminants.

In an attempt to meet these ideas has emerged and formed a new path in ecotoxicological assessment of chemical pollutants, namely qualitative and quantitative evaluation of chemical contaminants from biological matrices from living beings using noninvasive sampling ways. This new direction in ecotoxicological biomonitoring of environmental contaminants not only allows the monitoring of animal subjects exposed but make also possible to assess the impact of those chemical contaminants on humans that leave in contaminated areas. Also this way of sampling of biological matrix from living beings allows an extension of studied subjects in terms of both as species, age, gender and living environment.

3. Choice of sample matrix type collected non-invasively

As in any case of chemical pollutants monitoring a sample must to render an accurate picture about chemical compounds composition present in the environmental media from which the sample was taken at the sampling moment. In order of eliminating the problems of invasive sampling method of biological matrix from living beings, now sample matrixes were chosen on the following principles:

- primarily to not cause any kind of discomfort, pain or sacrifice to subjects submitted for study
- to be stable in time without altering the sample and the chemical structure, physicochemical properties and quantities of the monitored chemical contaminants
- to be a representative "mirror of the footprint" leaved by the chemical contaminants on the studied organisms

- to allow to be sampled:
  - in sufficient quantities to could perform the chemical analysis in a reliable, accurate and sensitive mode
  - to allow sampling on a more extensive field as number of samples, subject species, geographical position, duration, etc.

3.1 Noninvasively collected biological sample matrices from inhabitants

In case of human biomonitoring as regards their exposure levels to environmental contaminants as matrices sampled in a non-invasive way may be taken into accounts these types of samples: hair, nail, saliva, urine, milk, etc.

From these, the most often used sample in toxicological and environmental medicine studies was *urine* – these type of samples being used for different analytical assessment (medical, toxicological, ecotoxicological) with years ago, especially when water-soluble chemicals and environmental contaminants were the targets of study. Choosing of this type of matrices from very old times is because urine provides good information about the chemical content uptake by the human body and not in the last because it was easy to be collected and the available amount was sufficient to perform the analysis in good conditions. Also it is available at large scale as subject's geographical position, age and gender.

Hair, nowadays is another well used biological matrix presenting as major advantage its stability as matrix (comparing with other biological matrix), and easy collection, transport and storage. Hair as biomonitoring matrix is able to provide information both about short or long time exposure to contaminants of the studied subject.

Human breast milk is also o biological matrix that is collected through noninvasive ways. This sample type is used extensively when it is required information from both the mother as well her child. This sample is extremely suitable specially in monitoring of lipophilic chemicals considering its increased content in fat.

Nails and *saliva* are also noninvasively collected matrices that could be used in biomonitoring considering its low cost and the ease way through that they could be collected permitting the surveillance of a large number of subjects.

Once with the extension and evolution of this way of ecotoxicological and environmental epidemiology studies new biological sample matrixes collected also without cause any discomfort or damage on the studied subjects started to be introduced. Such of matrixes are sweat, faeces, semen, placenta and breath.

3.2 Noninvasively collected biological sample matrices from animal subjects

In case of animals monitoring previous studies has showed that uses of hair and feathers are useful in environmental contaminants impact evaluation on them. Also milk analysis could be used in term to evaluate the contaminants transfer from mammals to their offspring from the first days of their early life. Such kind of analysis helps to conclude the exposure pathways and its impact even on newborn subjects, giving important information for the future, which will help to understand subjects' development and pathological profile from their maturity period.

4. Sample preparation and analytical methods selection for analyzing organic contaminants in biological samples collected non-invasively

Through these studies two sample matrixes were chosen in order to evaluate the surrounding environmental contaminants on inhabitants, namely hair – in order to get a general overview about organic contaminants impact on humans exposed to them, and milk – throughout to could form an image regarding the infants' exposure from early age life. As regards animal exposure biomonitoring hair was chosen for mammalian animal subjects and feather in case of poultries biomonitoring studies. Therefore in our biomonitoring study we were able to collect high enough samples on an extended range of subjects (as species, age and geographical position) without causing any harm even when we talk about human biomonitoring or animal biomonitoring studies.

4.1 Sample collection and its preparation for analysis

In case of human biomonitoring as was suggested two sample matrixes were used, once – hair samples which made possible to be applied for any kind of subjects as age, gender, etc. and secondly – breast milk samples which as it matrix nature shows it was possible to be applied just for a special class of subjects - namely nursing mothers, way throughout we were able to found information about mother exposure as well about the infant exposure. Both methods don't involve any kind of physical or physiological trauma on the involved subjects.

Subjects number and key information regarding them	**Gender**		**Age**	**Working space exposure**	**Living place exposure**	**Smoker or diet habit**
	Female	*Male*				
Cluj district						
Rural sites	16	13	5-62	2	-	17
Urban sites	15	18	9-71	1	10	20
Industrial sites	9	10	22-49	6	19	13
Salaj district						
Rural sites	6	10	10-66	1	-	9
Urban sites	22	26	15-73	2	-	35
Industrial sites	5	4	33-43	1	9	5
Bistrita Nasaud district						
Rural sites	15	12	3-60	-	-	13
Urban sites	10	16	15-45	-	-	18
Industrial sites	-	1	39	-	1	1

Table 1. General information about the studied inhabitants from the three selected regions.

As regards animal subjects that were implied in our biomonitoring programs, hair was used in case of mammalians and feather in case of poultry subjects subjected for study.

Totally 208 number of human subjects were involved in these biomonitoring studies. Details regarding the human biomonitors are given in table 1. Shortly, 98 females were involved in this study from which 18 were child and 26 were nursing mother from which milk samples were collected also. As regards male, 110 subjects were monitored from which 20 persons were child. From this 208 subjects 131 persons declared smoking and diet pattern from

which 68 are smokers and 24 are involved in diet programs due to health problem and 39 persons are involved also in diet programs owing to their weight (in this case 78 % being females between 14 – 35 years).

Animal subjects were also chosen from both rural and urban sites as well from industrial sites. Just animals and poultries grown for consumption purposes in farms of the monitored inhabitants were considered in this study. As animal species were considered pig, cow and chicken, species that are the most popular in these three studied district. They are basically the most important source of meat for any humans from this part of Romania; they didn't missing from any rural farms and any plates of inhabitants from these regions.

Collected hair samples – 5 mg amount/subject – including the entire length of the hair were cut with uncontaminated scissors. These collected samples were placed in plastic bags (without adding of any stabilizing agent) and transported to laboratory. There washing process was done in order to avoid external contamination of the matrixes. After washing processes the samples were cute in small species (1-2 mm) from that 2.5 mg were placed in a 10 mL glass vials. This procedure was applied for both human and animal subjects' hair samples. Similarly was done in case of feather samples. In case of human scalp hair was used as sampling zone of human body while in case of animal subjects dorsal hair were used for biomonitoring.

As regards human breast milk analysis, 40 mL of samples were collected from every nursing mom in sterile glass vials to which prior was added $K_2Cr_2O_7$ as stabilizing reagent after that the vials were closed with Teflon lined screw cap and put in a freezer at 4 °C until analysis. No milk samples were stored more than three days from the sampling moment.

4.2 Organic chemical contaminants extraction

Considering the previous research studies performed in these areas three main pollutants category were the targets in these biomonitoring studies, namely organochlorine compounds, mono- and polycyclic aromatic hydrocarbons and organometallic compounds.

Mono- and polycyclic aromatic hydrocarbon compounds from hair samples were extracted using headspace-solid phase microextraction technique (HS-SPME). To the 2.5 mg of hair samples that was placed in the 10 mL glass vials were added 15 μL of HCl and 100 μL of aqueous sodium dodecylsulphate and 5 mL of hexane. This aliquot it was subjected to ultrasonication at 50 °C for 20 minutes after that the vials was subjected to centrifugation at 7 500 rpm for 10 minutes after that the hexane was separated and the remained aliquot was subjected again to centrifugation with hexane in the same condition as before. The two phases were put together finally and subjected to HS-SPME extraction.

Extraction method performance was increased through optimization of several extraction parameters as: selection of suitable fiber coating type, using or not of a derivatizing agent, desorption time and temperature, extraction time, applied agitation mode and salt effects. In this case using of a 100 μm thickness polydimethyl siloxane (PDMS) fiber increased the extraction method capacity comparing with extraction performance parameters obtained through using other types of fiber or different film thickness of the fibers.

As regards desorption time and gas chromatograph injector temperature optimization in order to increase the sensitivity of extraction procedure five different desorption times were

studied, namely 30 seconds and one, two, three and five minutes, respectively. In case of all monoaromatic hydrocarbon compounds it was observed that short time of desorption as 30 seconds or one minutes is not enough to reach a complete desorption therefore the time that is required for a good desorption of these compounds was established at two minutes. In case of polycyclic aromatic hydrocarbons the optimal desorption time was established at 5 minutes. As gas chromatograph injector temperature significant results were obtained when it was set at 230 °C.

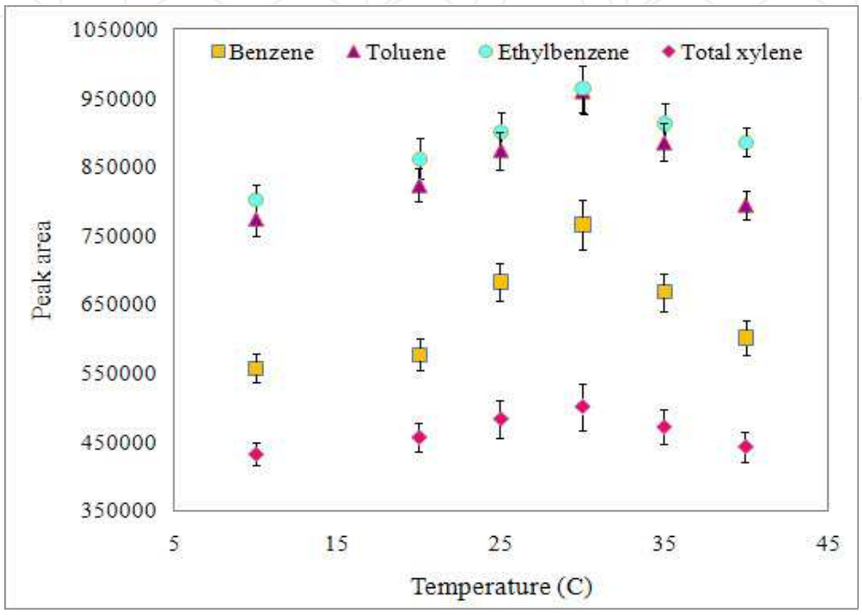


Fig. 2. Monoaromatic hydrocarbon analytes response after HS-SPME extraction method performance under different extraction temperature.

Considering the extraction temperature, a lots of study demonstrated that extraction temperature has multiple impact on the HS-SPME procedures. Liu et al, 2005, asserted that the high temperature increase the diffusion coefficients of the analytes in any kind of sample matrix and from it to the headspace, which reduce significantly the extraction time but in the same time have a negative influence on the partition coefficients of analytes in the fiber coating, reducing it, thus the efficiency of the extraction being reduced well. In this case the experiment was conducted for six different temperatures: 10, 20, 25, 30, 35 and 40 ^{o}C – see figure 2. In this figure it is observed that a good response for all mono aromatic hydrocarbon compounds is obtained at 30 ^{o}C, decreasing for higher or lower temperature.

Extraction time represents another factor that could chasten or impair the extraction method sensitivity, so we take it in consideration also. In order to establish the most suitable extraction time for the mono- and polycyclic aromatic hydrocarbon compounds ten different time were examined as follows: 3, 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 minutes, respectively. Compounds response increased up to 15 minutes extraction in case of monoaromatic hydrocarbon compounds after that was registered a continue decreasing. In case of polycyclic aromatic hydrocarbon compounds the maximum response was achieved at 20 minutes extraction, after that presented the same pattern of

decreasing as the monoaromatic hydrocarbon compounds Also the equilibrium was obtained at different time in case of all aromatic hydrocarbon compounds, therefore this equilibrium was approached more rapidly in case of monoaromatic hydrocarbon compounds (10 – 15 minutes) and after longer periods in case of polycyclic aromatic hydrocarbon compounds. According with these results 25 minutes was established as the most suitable extraction time for these compounds and as equilibration time 20 minutes was established as being satisfying.

The extraction method performance was evaluated based on the determination of recovery factor which was between 82-109 %b in case of monoaromatic hydrocarbon compounds and between 78-119 % in case of polycyclic aromatic hydrocarbon compounds.

Organochlorine compounds presence from *hair samples* were extracted as follows: to the 2.5 mg of hair samples that was placed in the 10 mL glass vials were added 15 µL of HCl and 100 µL of aqueous sodium dodecylsulphate and 5 mL of isooctane. This aliquot it was subjected to ultrasonication at 40 °C for 10 minutes after that the vials was subjected to centrifugation at 3 500 rpm for 10 minutes after that the isooctane was separated and the remained aliquot was subjected again to centrifugation with isooctane in the same condition as before. The two phases were put together finally and subjected to rotary evaporation at in a water bath at 40 °C until 1 mL aliquot it was obtained. This obtained aliquot then was subjected to HS-SPME extraction procedure followed finally by instrumental analysis. Shortly the optimal HS-SPME parameters were 2 and 5 minutes as desorption time respectively as extraction time. The optimal extraction temperature was found at 30 °C.

The extraction method performance in case of organochlorine compounds was evaluated based on the determination of recovery factor which was between 92-121 %, considering all studied compounds.

In case of *organometallic compounds* also HS-SPME extraction method was used with almost similar condition as in case of mono- and polycyclic aromatic hydrocarbon compounds, but in this case a dervatization step using sodium tetraethyl borate as derivatization agent was introduced before instrumental analysis. After derivatization process the following parameters were considered as optimum for HS-SMPE extraction process where desorption time and the extraction time were set at 7 and 12 minutes, respectively. The extraction temperature was set at 75 °C.

Target chemical compounds extractions from breast milk analysis were performed through HS-SPME extraction procedure using a PDMS fiber coating with 100 µm film thickness. The lipids from milk were separated according to Johansen et al, 2004 and Behrooz et al, 2009 without any significant modification in methods. Extraction method used for organochlorine compounds as well for mono- and polycyclic aromatic hydrocarbon compounds was the same as that was described by Skrbic et al in 2010.

4.3 Instrumental analysis of organic chemical contaminants from samples collected through non-invasive procedures

All sample extracts were analyzed through gas chromatographic method. In case of organochlorine compounds analysis electron capture detector was used while in case of organometallic compounds quadrupole mass spectrometer was used as detector. In case of

mono- and polycyclic aromatic hydrocarbons flame ionization detector was used in order to perform the analysis. Used working parameters are presented shortly in the follows.

In case of *mono- and polycyclic aromatic hydrocarbon analysis* these were performed on Trace GC Ultra gas chromatographic apparatus (Thermo Electron Corporation) equipped with a flame ionization detector (FID) and a split/splitless injector. After desorption of target analytes the SPME fiber was injected in the injector with a constant 230 °C temperature. As column was used TR 5 % phenyl methylsiloxane column, provided by Thermo Electron Corporation, having as characteristics 0.53 mm ID x 0.50 µm film thickness x 30 m length.

The oven temperature program has set as follows: 40 C for 5 minutes, followed by increases with 5 °C·min^{-1} at 150 °C and held at this temperature for 3 minutes, subsequent by an increases of temperature with 10 °C·min^{-1} at 220 °C and kept at this temperature for 5 minutes. The FID detector temperature was set at 300 °C.

As regards *organochlorine compounds* previous researches has demonstrated that electron capture detector in gas chromatographic analysis has an increased sensibility to halogenated organic compounds detection (ca. 10^{-13} g·mL^{-1}), this detector being considered probably the most sensitive gas chromatographic detector that is available (Quan et al, 2002; Chen et al, 2004; Khajeh et al, 2006). Considering these the analysis of extracts was performed in the followings through Trace GC Ultra gas chromatography (Thermo Electron Corporation) equipped with a ^{63}Ni electron capture detection system (Thermo Electron Corporation).

Good response were obtained for all target chlorinated organic compounds in case of TR-V1 Trace GC capillary column with cyanopropylphenyl polysiloxane phase type and with 0.53 mm I.D. x 3.0 µm film thickness x 30 m column length using the following working conditions: nitrogen was used as carrier gas with 30 mL·min^{-1} flow. Split ratio was set at 1:2 while the injector temperature and detector temperature were set at 220 and 250 °C, respectively. Oven temperature programe through out were done the analysis was as follows: 40 °C (with 3 minutes hold time) increased with 7 °C·min^{-1} at 100 °C (hold for 3 minutes time period) after that increased with 10 °C·min^{-1} at 220 °C, where this temperature was mantained constant for 7 minutes.

Organometallic compounds were detected also through gas chromatographyc technique but this time a quadrupole mass spectrometer was used as detector. Therefore a Focus GC engaged to a DSQ II quadrupole mass spectrometer provided by Thermo Electron Corporation was used as analytical instrument for organometallic compounds separation and quantification. A split/splitless injector in the splitless mode was used. Its temperature was set at 260 °C. The target analytes were separated using a TR 5-MS 5 % phenyl polysilphenylene-siloxane capillary column with the following characteristics: 0.25 mm ID x 0.25 µm film thickness x 30 m length. This column was inserted into the mass spectrometer and the interface temperature between GC and MS was set at 270 °C. For the GC oven the next temperature program was found to be optimum in case of organometallic compounds analysis: 35 °C (hold for 2 minutes), raised with 5 °C·min^{-1} at 90 °C and kept at this temperature for one minute after that continue the temperature increasing with 12 °C·min^{-1} until 220 °C, temperature that once achieved was maintained constant for five minutes. As carrier gas high purity helium was used with 0.8 mL·min^{-1} flow rate. The analysis through mass spectrometer detector was done in electron impact mode at 70 eV and through full scan monitoring mode.

5. Case studies

It is well known that a large scale of chemical pollutants as well their incaution management and damaging land uses make in menace the quality of our surrounding environment at world wide scale. Usually the environmental contaminants (having as main source anthropogenic activities) have great effects on the entire environmental system as draw down loss of habitats, diminish of biodiversity or adverse effects on humans that are exposed to these contaminant through food web chain, health care and/or recreational/working activities.

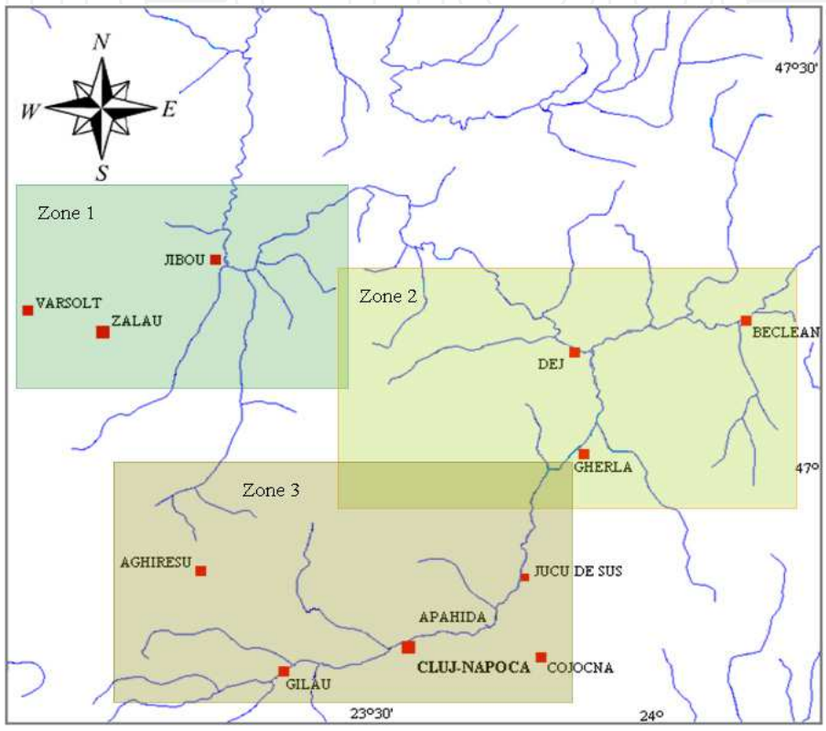


Fig. 3. Map of the studied regions during period of 2008 - 2010.

The damage of environment quality and living things health status has as result the increasing of necessity to monitor these environmental contaminants. More than that becomes a necessity to monitor their qualitative and quantitative presence even in organisms of living subjects whether it is humans or animals in order to could get regulatory, remedial and treatment decisions.

These studies were conducted in north western part of Transylvania including rural, urban and industrial zones – see map presented in figure 3. In figure 3 zone 1 represent a part of Salaj district which include both rural as well urban sites, zone 2 represent the north part of Cluj district and the western part of Bistrita-Nasaud district, this region including industrial, rural and urban sites while zone 3 represent the south part of Cluj district including urban as well rural areas. In idea to get a clear and realistic image regarding environmental contaminants presence and the degree of exposure of living beings to those contaminants environmental (soil, water) and biota samples (vegetables, biological samples from animal and human origins) were collected monthly in period of 2008-2010.

5.1 Living things exposure to organochlorine compounds – Case study of paper mills

During previous studies it was observed an increased presence of organochlorine compounds in regions near Dej city (zone 2 from figure 3). Main organochlorine compounds that were detected were chlorophenols (dichlorophenol, trichlorophenol and pentachlorophenol) and chlorinated solvents (trichloroethylene, tetrachloroethylene and carbon tetrachloride).

Presence of these compounds in environmental samples as soil and water were between range of 0.3 – 32 $\mu g \cdot kg^{-1}$ and 0.5 – 25.1 $\mu g \cdot L^{-1}$, respectively, usually with higher levels in case of soil samples.

Major amounts being detected in case of dichlorophenol and trichloroethylene compounds, usually with higher amounts in case of soil samples – see table 2 and figure 4.a. and 4.b.

Period	Chlorophenols average values					
	Monochlorophenols		Dichlorophenols		Trichlorophenols	
	Soil [$\mu g \cdot kg^{-1}$]	Water [$\mu g \cdot L^{-1}$]	Soil [$\mu g \cdot kg^{-1}$]	Water [$\mu g \cdot L^{-1}$]	Soil [$\mu g \cdot kg^{-1}$]	Water [$\mu g \cdot L^{-1}$]
2008	0.5 – 12.5	0.3 – 8.9	0.8 – 19.2	0.5 – 14.5	0.9 – 31.9	0.4 – 17.5
2009	0.3 – 18.5	0.5 – 17.2	1.8 – 22.5	0.4 – 16.8	0.5 – 21.9	0.3 – 19.5
2010	0.4 -16.2	0.4 – 10.2	0.5 – 12.5	0.6 – 8.59	0.3 – 9.2	0.7 – 3.2

Table 2. Chlorophenols average amounts in soil and water samples.

Increased presence of these compounds was attributed to the presence of paper mills that discharge its waste water in Somes River (one of the main river that cross northern part of Transylvania which is used as water source both for drinking water purposes and for irrigation networks of agricultural lands). This was demonstrated by the results of environmental monitoring before and after the closing of factory in 2009, when the factory was closed for a period of two months for rehabilitation – see figure 4a and 4b, when it was observed a decreasing tendency of organochlorine compounds presence in environmental samples (as soil and surface water) from the moment when the factory was closed.

In order to evaluate these pollutants impact on living beings it was observed that are quite strong correlation between the amount of these chlorinated compounds in surrounding environment and the amount of organochlorine compounds detected in animals and humans hair samples collected from subjects that live in the corresponding environment – see figure 5. But such dependence was obtained just in this special case, for other regions such correlation (in case or organochlorine compounds) wasn't applicable.

Consulting the obtained results it was observed that the amount of these compounds were lower in case of poultries feathers and higher in the case of cow hair. Also in case of human hair subjects their levels were higher even 30 – 50 percent than in other living beings. This could be explained by the cumulative effects of these pollutants, therefore once with age increases are possible to increase also the levels of environmental contaminants from animals and humans body.

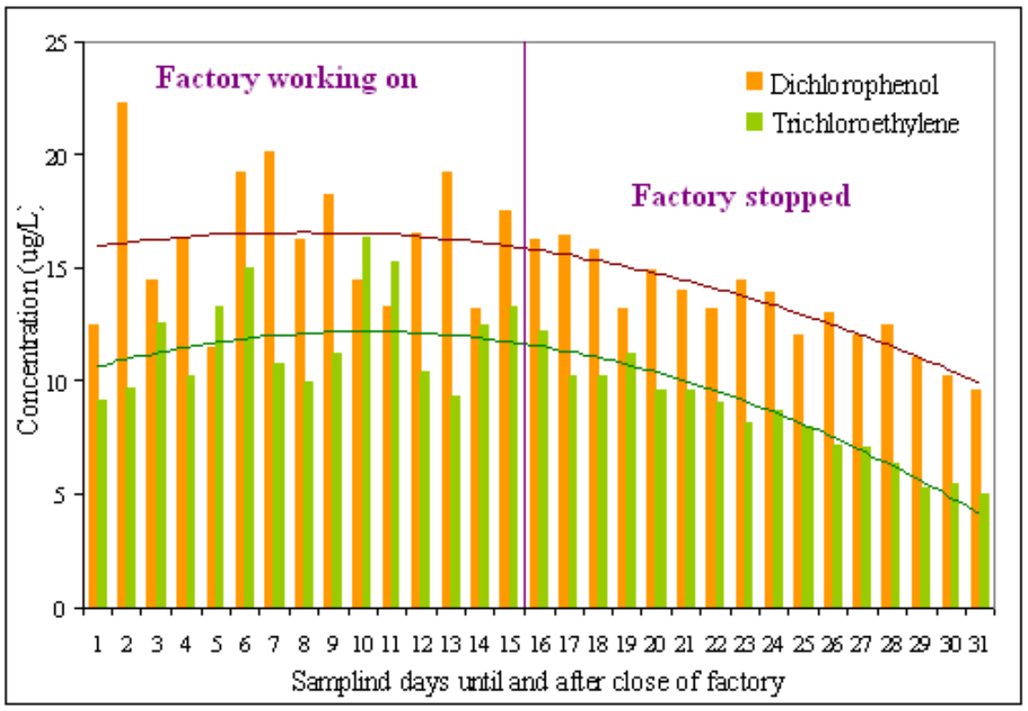


Fig. 4a. Organochlorine compounds amount in surface water while paper mills operating on and off.

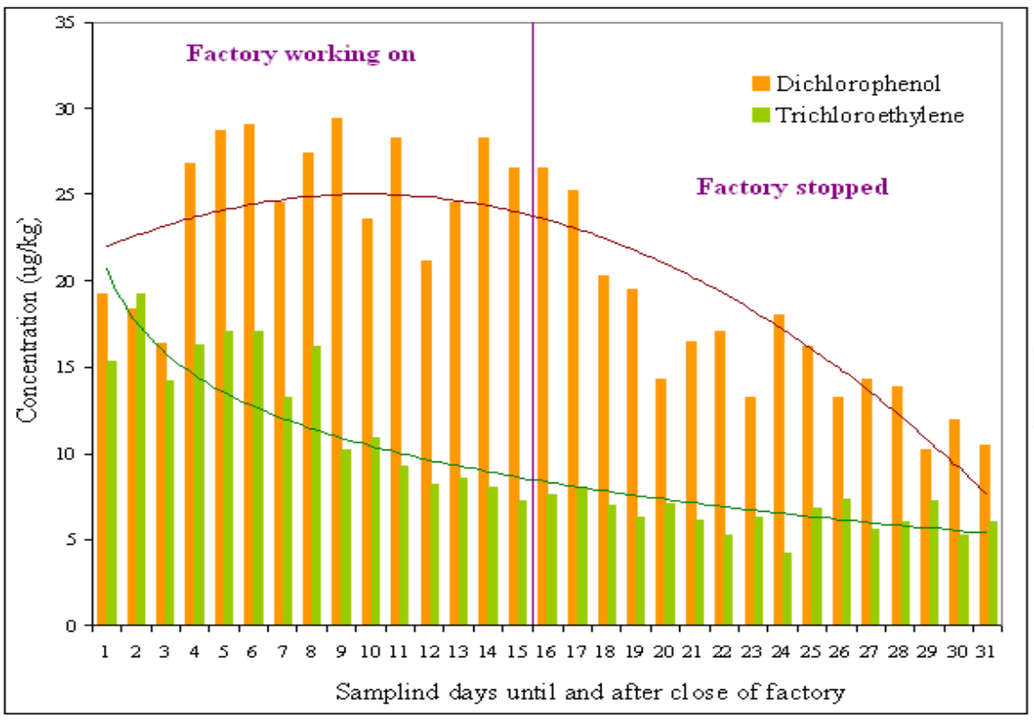


Fig. 4b. Organochlorine compounds amount in soil samples while paper mills operating on and off.

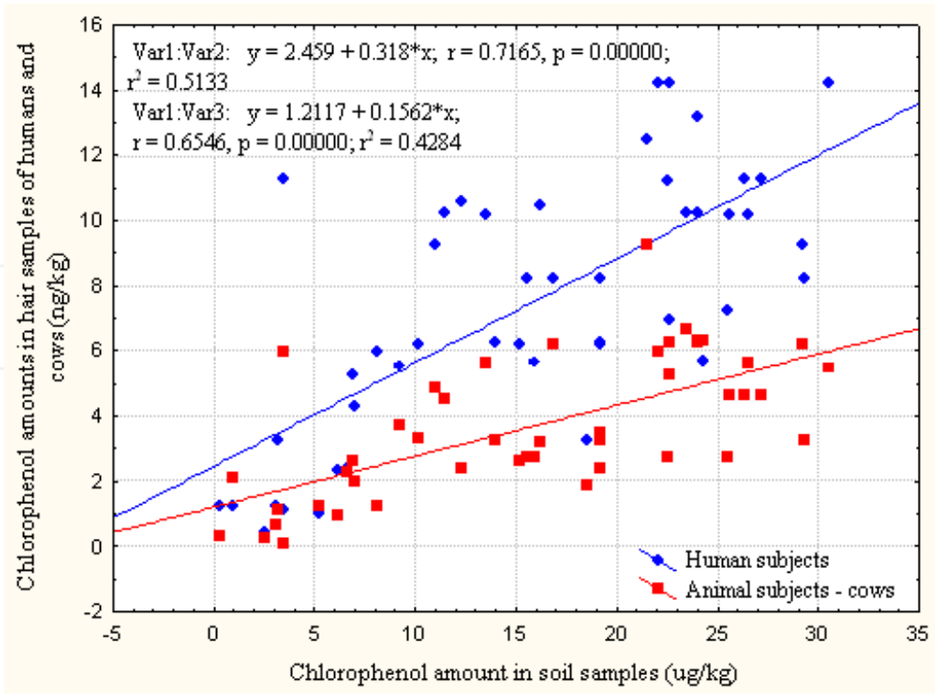


Fig. 5. Organochlorine amount dependence from humans and animal body by the surrounding environment.

As regards the amounts detected in human breast milk samples, the amount of chlorinated compounds were higher than in case of hair samples of the same subjects with almost 10 – 15 %. This fact could be attributed to the increased lipid content of breast milk, much more than exist in hair samples, therefore chlorophenol compounds being more easily bounded to the fat from the organisms.

5.2 Inhabitants and home grown animals' exposure to mono- and polycyclic aromatic hydrocarbon compounds

As regards monoaromatic hydrocarbon compounds the most detected compounds in the most increased amounts were benzene and toluene, if we take in consideration the fact that their uses was restricted in the last decades. Average values of mono- and polycyclic aromatic hydrocarbons detected in soil and water samples collected from this studied regions are presented in table 3. Higher amounts in case of anthracene and benzo[a]pyrene were detected usually.

Environmental contaminant	Environmental compartment	Average value during time period	
		2009	2010
Monoaromatic hydrocarbon	Soil	0.3 – 19.2 $\mu g \cdot kg^{-1}$	0.2 – 22.3 $\mu g \cdot kg^{-1}$
	Water	0.4 – 16.7 $\mu g \cdot L^{-1}$	0.8 – 15.9 $\mu g \cdot L^{-1}$
Polycyclic aromatic hydrocarbon	Soil	0.2 – 22.9 $\mu g \cdot kg^{-1}$	0.4 – 16.9 $\mu g \cdot kg^{-1}$
	Water	0.4 – 15.2 $\mu g \cdot kg^{-1}$	0.5 – 13.8 $\mu g \cdot kg^{-1}$

Table 3. Mono- and polycyclic aromatic hydrocarbons average amounts in soil and water samples.

In order to compare the results obtained after human hair analysis and the amount of these compounds detected in vegetables samples it was observed a quite good correlation as regards the humans' uptake values to hydrocarbons and the amounts of these compounds which are present in vegetables samples – see figure 6.

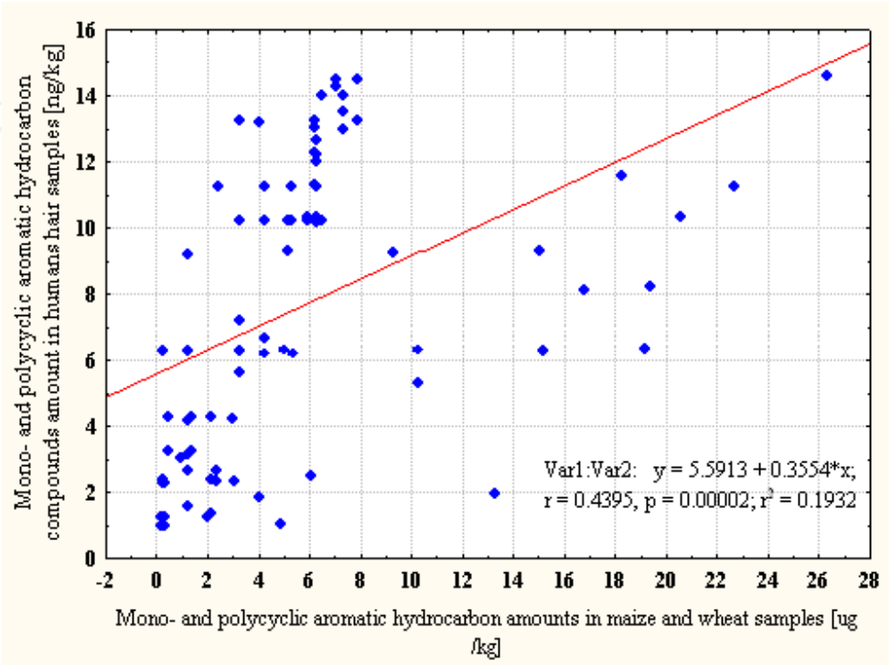


Fig. 6. Mono- and polycyclic aromatic hydrocarbons amount from human hair samples dependence by the amount of these compounds in the consumed foodstuffs.

Comparing the uptake levels of mono- and polycyclic aromatic hydrocarbons it was observed that polycyclic aromatic hydrocarbons are better retained in living organisms than monoaromatic hydrocarbons.

5.3 Organometallic compounds fingerprint on biological samples

Methylmercury was the most prevalent organometallic compound that was detected in environmental samples. This was a special case which is attributed to banned and improper managed old chemical factory. The amount of this compound founded in soil and water samples are listed through table 4.

Environmental contaminant	Environmental compartment	Average value during time period	
		2009	2010
Organometallic compounds	Soil	0.5 – 13.5 $\mu g \cdot kg^{-1}$	0.5 – 15.1 $\mu g \cdot kg^{-1}$
	Water	0.6 – 10.8 $\mu g \cdot L^{-1}$	0.6 – 13.9 $\mu g \cdot L^{-1}$

Table 4. Methylmercury range values in soil and water samples in the last two years.

Methylmercury amounts in vegetables samples grown in this region was between 0.5 – 12.1 $\mu g \cdot kg^{-1}$ while in human and animal hair samples it's amount was between 0.2 – 19.1 and 0.5 – 10.3 $ng \cdot kg^{-1}$, respectively. Once again higher values being detected in case of human subjects.

6. Advantages and limitation in eco-toxicological profiling based on biological samples collected non-invasively

Main disadvantages of these techniques are the difficulty in making difference between external and internal contamination – as regards hair samples. Also in case of hair samples the results vary intensively once with color, age and gender without existing a good correlation between the same parameters. The lack of a standardized procedure of analysis make more difficult to establish the reliability of results in order to estimate the method uncertainty and making real laboratory intercomparisons. Also a large number of studies showed that there are no correlation between the contaminant level from hair samples and blood samples in case of the same person.

As regards the study performed by us using of such sampling matrixes made possible to evaluate the impact of certain environmental contaminants on humans and animals (grown for consumption purposes) which are living in such contaminated areas. Also through the significant number of subjects that were monitored we was able to conclude that the environmental contaminants are uptake by humans mainly through food web chains (as a result of consumption of contaminated vegetables and animals). This is the same in case of home grown animals which are similarly exposed to these environmental contaminants mainly through the consumed contaminated food.

7. Conclusions

Major environmental pollutants that were detected in different environmental compartments of the studied regions were monoaryomatic hydrocarbons and chlorinated solvents, which were also the most prevalent as presence as well as amount. Methylmercury presence in environment is considered as a special case – environmental contamination in this case coming from the old banned chemical factory on which sites no remediation was applied. In almost all environmental contaminants cases, their presences are attributed to the industrial activities through these regions. All these contaminants leave an impact on the living things that live in the surrounded environment. Vegetables growing on a soil contaminated by former industrial activities may contain chlorinated or hydrocarbon pollutants in their tissues at significant concentrations.

Uses of noninvasive matrices in both animals as well humans' biomonitoring purposes have been showed to be suitable.

These compounds were detected in all humans and animals' hair samples. Compounds with higher molecular weight masses were easily accumulated by human bodies than the easier compounds.

8. References

Bhandari, A.; Surampalli, Y.P.; Adams, D.G.; Champagne, P.; Ong, S.K.; Tyagi, R.D.; Zhang, T.Ch. (2009). *Contaminants of emerging environmental concern*. pp. 1-6, American Society of Civil Engineers (ASCE) ISBN 978-0-7844-1014-1, Reston, Virginia USA.

Hansen, A. (1993). *The mass media and environmental issues – Studies in communication and society*. pp. 12-28, ISBN 978-0718520533 Leichesters University Press, UK.

Neuzil, M.; Kovaric, W. (1996). *Mass media & environmental conflict – America's green crusades*. pp. 105-125, ISBN 076190333X Sage Publication Inc., USA.

Harrison, M.R. (2001). *Pollution: causes, effects and control*. 4th edition, pp. 12-28, ISBN 0-85404-621-6 The Royal Society of Chemistry, UK.

Pierce, J.J.; Weiner, F.R.; Vesilind, P.A. (1998). *Environmental pollution and control*. 4th edition, pp. 15-29, ISBN 978-0-7506-9899-3 Butterworth-Heinemann, Wobum USA.

Byers, P.J. (2006). *Metalworking fluids*. 2nd edition, pp. 386, ISBN 978-1-57444-689-0 Taylor & Francis Group, CRC Press, USA.

Walker, C.H. (2009). *Organic pollutants: an ecotoxicological perspective*. 2nd edition, pp. 94-98, ISBN 978-1-4200-6258-8 Taylor & Francis Group, CRC Press, USA.

World Health Organization. (2006). *Principles for evaluating health risks in children associated with exposure to chemicals*. pp 7-15, in Environmental Health Criteria 237, IPCS – International Programme on Chemical Safety, ISBN 92-4-157-237-X Geneva, Switzerland.

Watson, S. (2009). *Animal Testing: Issues and Ethics*. 5th edition, pp. 5-12, ISBN 978-1-4358-5022-4 Rosen Publishing Group, Inc., New York, USA.

Beebee, T.J.C., Rowe, G. (2008). *An introduction to molecular ecology*. 2nd edition, pp. 92-101, ISBN 978-0-19-929205-9 Oxford University Press Inc., New York, USA.

Angerer, J., Ewers, U., Wilhelm, M. (2007). Human biomonitoring: state of the art. International Journal of Hygiene and Environmental Health, vol. 210, pp. 201-228, ISSN 1438-4639.

Liu, J.F., Li, N., Jiang, G.B., Liu, J.M., Jonssin, A.J., Wen, M.J. (2005). *Disposable ionic liquid coating for headspace solid-phase microextraction of benzene, toluene, and xylenes in paints followed by gas chromatography-flame ionization detection*. Journal of chromatography A, Vol. 1066 (1-2), Pag. 27-32, ISSN 0021-9673.

Johansen, P., Muir, d., Asmund, G., Riget, F. (2004). *Human exposure to contaminants in the traditional Greenland diet*. Science of the total environment, Vol. 331 (1-3), pp. 189-206, ISSN 0048-9697.

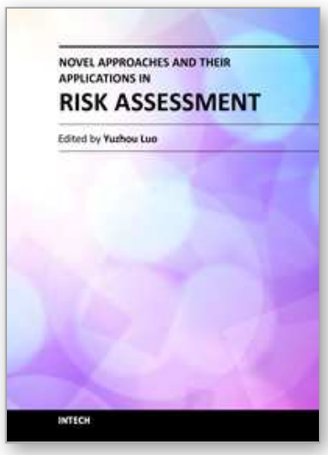
Behrooz, R.D., Sari, A.S., Bahramifar, N., Ghasempouri, S.M., (2009). *Organochlorine pesticide and polychlorinated biphenyl residues in human milk from the Southern Coast of Caspian Sea, Iran.* Chemosphere, Vol. 74 (7), pp. 931-937, ISSN 0045-6535.

Skrbic, B., Szrwinska, K., Durisic-Mladenovic, N., Nowicki, P., Lulek, J. (2010). *Principal component analysis of indicator PCB profiles in breast milk from Poland*. Environmental international, Vol. 36 (8). pp. 862-872, 0160-4120.

Quan, X., Chen, S., Platzer., Chen, J., GFrerer, M. (2002). *Simultaneous determination of chlorinated organic compounds from environmental samples using gas chromatography coupled with a micro electron capture detector and micro plasma atomic emission detector*. Spectrochimica acta part B: Atomic spectroscopy, Vol. 57 (1), Pag. 189-199, ISSN 0584-8547.

Chen, E.C.M., Chen, E.S. (2004). *Molecular electron affinities and the calculation of the temperature dependence of the electron-capture detector response*. Journal of chromatography A, Vol. 1037 (1-2), Pag. 83-106, ISSN 0021-9673.

Khajeh, M., Yamini, Y., Hassan, J. (2006). *Trace analysis of chlorobenzenes in water samples using headspace solvent microextraction and gas chromatography/electron capture detection.* Talanta, Vol. 69 (5), Pag. 1088-1094, ISSN 0039-9140.



Novel Approaches and Their Applications in Risk Assessment

Edited by Dr. Yuzhou Luo

ISBN 978-953-51-0519-0
Hard cover, 344 pages
Publisher InTech
Published online 20, April, 2012
Published in print edition April, 2012

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Melinda Haydee Kovacs, Dumitru Ristoiu and Cezara Voica (2012). Non-Invasive Matrices Use in Pollution Evaluation at Nanoscale Levels – A Way Forward in Ecotoxicological Studies, Novel Approaches and Their Applications in Risk Assessment, Dr. Yuzhou Luo (Ed.), ISBN: 978-953-51-0519-0, InTech, Available from: http://www.intechopen.com/books/novel-approaches-and-their-applications-in-risk-assessment/non-invasive-matrices-use-in-pollution-evaluation-at-nanoscale-levels-a-way-forward-in-ecotoxicologi

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