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XENOBIOTICS IN AUTUMN WHEAT GRAINS

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Abstract

Controlling the contamination of crop plants with pollutants has raised increasing interest in recent years; among the investigated pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs) are of particular interest because some are toxic and/ or carcinogenic. The main purpose of this paper is to establish the contamination of autumn wheat grains (Arieşan variety) with the xenobiotics lead, cadmium, copper, zinc and 15 priority PAHs in the conditions of experimental cultures carried out during three years in three locations with different pollution patterns: a reference field, a contaminated surface from diffuse sources and a site with historical contamination. PAHs' determinations were performed by high performance liquid chromatography on an Agilent 1100 system with both photodiode array and fluorescence detection, while the content of heavy metals was determined by atomic absorption spectrometry using a Shimadzu AA6300 spectrophotometer. The obtained results revealed a higher share of low molecular weight PAHs, mainly naphthalene, fluorene and acenaphthene, while the contamination of analyzed wheat samples with high molecular weight PAHs was caused by benzo(a,h)anthracene, benzo(b)fluoranthene and dibenzo(a,h)anthracene. The content of both PAHs and heavy metals was highest in the samples from Cluj-Napoca, with average contents of 8.67 μg total PAHs/kg, 0.03 μg Pb/kg, 2.26 mg Cu/kg and 43.31 mg Zn/kg, in this urban location the contamination being mainly caused by the atmospheric depositions loaded with combustion products. Overall, the obtained results highlighted relatively low concentrations of the studied xenobiotics in autumn wheat grains; these data may be useful in future studies dealing with human exposure on these pollutants.

Key words*: heavy metals, polycyclic aromatic hydrocarbons, contamination, analysis, chromatography.*

INTRODUCTION

Xenobiotics are chemical substances that are foreign to a biological system; the classes of xenobiotics include pesticides, polycyclic aromatic hydrocarbons (PAHs), heavy metals, polychlorinated aromatics, solvents, flame retardants etc. (Jagtap and Bapat, 2017; Qadir et al., 2017).

Controlling the contamination of crop plants with pollutants has raised increasing interest in recent years, especially in developed countries; among the investigated pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs) are of particular interest because some are remarkable for their toxicity and/ or carcinogenicity (Islas-Espinoza and de las Heras, 2015; Luch, 2005; Muntean et al., 2016).

Contamination of crop plants with PAHs and heavy metals is the point of their entrance into the food chain, creating risks for the consumers' health (Goran et al., 2019; Muntean et al., 2013; Muntean et al., 2015).

PAHs are a large class of hydrocarbon compounds containing two or more fused aromatic rings, formed during incomplete combustion processes; they are ubiquitous in the environment, originating from both natural sources (such as forest fires or volcanic eruptions) and anthropogenic sources (power plants, motor vehicle exhausts, oil spills, incinerators etc.); they occur as complex mixtures and are usually associated with particulate matter, soil and sediments (Kim et

al., 2013). Although PAHs concentrations are higher in the environment near the sources, they are capable of long distance transport. Excluding the occupational exposure and the smoker's exposure, the highest human exposure was established to be due to the consumption of contaminated food (Zelinkova and Wenzl, 2015). Since certain PAHs have mutagenic and/or carcinogenic properties (Alexander et al., 2008; Kim et al., 2013), international regulations were designed to protect human health and the environment (EC1881, 2006; EC1933, 2015).

Heavy metals are natural components of the Earth's crust, as common components of various matrices. The concentration of several heavy metals in most natural products have increased in recent years as a result of anthropogenic activities, many of them posing a toxicological risk on human health at higher concentrations (Elekes et al., 2010). Crop plant contamination with heavy metals can occur in all stages of their development as a result of inputs from the environment (soil, water, air, rainfall, atmospheric dust, sewage sludge, plant protective agents and fertilizers) and also later, during the manufacturing processes (Nagajyoti et al., 2010; Pivić et al., 2017; Tănase et al., 2017).

The main purpose of this paper is to establish the contamination degree of autumn wheat grains (Arieşan variety) with the xenobiotics lead, cadmium, copper, zinc and 15 priority PAHs in the conditions of experimental cultures carried out during three years in three locations with different pollution patterns: a reference field situated in the west of Jucu de Jos (experimental field of USAMV Cluj-Napoca), a contaminated surface from diffuse sources (an area with heavy car traffic located in Cluj-Napoca) and a site with historical contamination caused by SC Sometra SA Copşa Mică, located in Şeica Mare.

MATERIALS AND METHODS

HPLC mobile phase was prepared from high purity acetonitrile (Merck, Darmstadt, Germany) and ultrapure water with a specific resistance of 18.2 MΩ/cm, obtained from Direct Q 3UV Smart (Millipore), being filtered through a 0.45 μm membrane (Millipore) then degassed using an Elmasonic S30 H ultrasonic bath (Elma, Germany). Solvents used for extraction (acetone, hexane) were of HPLC grade (Merck, Darmstadt, Germany). Standard working calibration solutions for PAHs analysis were prepared by dilution with acetonitrile from an Agilent PN 8500-6035 certified multi-standard solution (Agilent Technologies) containing the 16 PAHs specified by US Environmental Protection Agency's as priority pollutants (Nieva-Cano et al., 2001): acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno (1,2,3-c,d) pyrene, naphthalene, phenanthrene and pyrene. The standard solutions for atomic absorption spectroscopy containing 1000 mg/ L metal in HNO_3 were from Fluka; all solutions were prepared using ultrapure water (specific resistance of 18.2 MΩ/cm, obtained from DirectQ 3UV Smart -Millipore).

The plant material used was the *Triticum aestivum* L. - autumn wheat, Arieşan variety, harvested from three culture locations, each exposed to different degrees of pollution: a reference, non-polluted site - experimental field of USAMV Cluj-Napoca (at 46°52'16"N/23° 45'27"E); a site contaminated by diffuse sources, close to a high traffic road in Cluj-Napoca (at 46°45'57"N/23°34'01"E); a site with historical contamination caused by SC Sometra SA Copsa Mica, located in Şeica Mare (at 46°01'52"N/24°09'39 "E). Field trials were based on a randomized block design with three replications with the harvested plots of 4 m^2; the experimental plots were not fertilized or chemically treated neither during the research period, nor two years earlier. From each plot, three samples were collected each year in polyethylene bags, being transported to the laboratory.

For PAHs analysis, representative samples of wheat grains were grounded using a WZ-1 laboratory mill (Sadkiewicz Instruments, Bydgoszcz, Poland), then accurately weighed on a Kern ABT-220-5DM analytical balance (Kern & Sohn GmbH, Bahlingen-Frommern, Germany). PAHs' extraction was performed using ~5 g accurately weight grounded grains,

with 50 ml mixture hexane: acetone (2: 1), by sonication for 30 minutes in an ultrasonic bath S 30H Elmasonic (Elma, Germany), at room temperature. The resulted suspension was filtered under vacuum, then dried over anhydrous sodium sulfate and evaporated to dryness in a rotary evaporator Laborotta 4000 Efficient under vacuum (Heidolph, Germany); the obtained residue was dissolved in 2 ml acetonitrile and injected in the chromatographic system. PAHs' determinations were performed by high performance liquid chromatography (HPLC) on an Agilent 1100 system (Agilent Technologies Inc., Palo Alto, USA) equipped with an Envirosep PP column, using a mixture of acetonitrile: water (45:55 v/v) as mobile phase (Muntean et al, 2013). Using this instrumental setup, 15 from the 16 considered PAHs have good responses using fluorescence detection, but no acenaphthylene, which was excepted (Figure 1); separations were thus accomplished in less than 30 minutes.

For heavy metal analysis, around 0.5 g milled grains were accurately weighed and transferred in Teflon reaction vessels, then 5 ml of HNO_3 65% (ultrapure grade, Merck, Germany) and 3 ml of H_2O_2 (Fluka) were added; wet digestion was accomplished with a Berghoff Microwave Digestion System MWS-3+ (Eningen, Germany) at 190^0C for 2 hours. Lead, cadmium, copper and zinc were determined by atomic absorption spectrometry (AAS) using a Shimadzu AA-6300 double beam spectrophotometer (Shimadzu Corporation, Japan) with both flame and graphite furnace atomization, equipped with hollow-cathode lamps for each of the studied elements; the operating conditions were those recommended for each metal in the instrument's method. Calibration curves were prepared using five concentrations of each element, the obtained linear correlation coefficients ranging between 0.985 - 0.996.

Instrument control, data acquisition and data analysis were completed using Chemstation 08.03 software (for HPLC analysis) and WizAArd software (for AAS analysis); experimental data were finally processed in Excel 2003 (Microsoft) and mean values of 3 determinations for each analyte were reported.

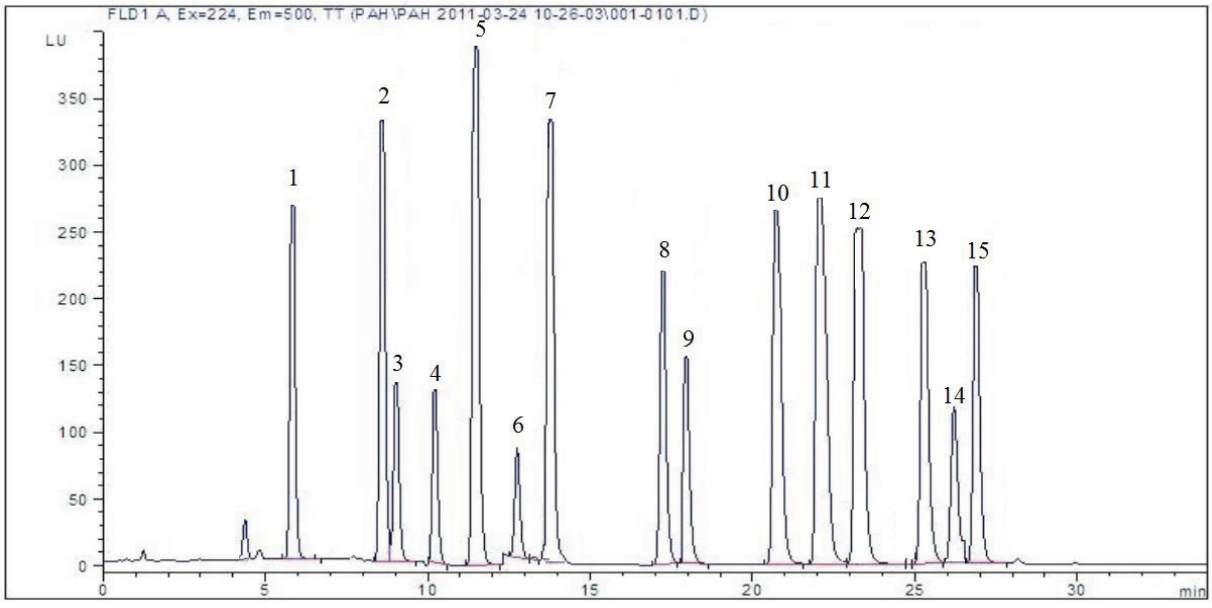

Figure 1. HPLC chromatogram for the standard mixture of PAHs: 1 - naphthalene, 2 - acenaphthene, 3 - fluorene, 4 - phenanthrene, 5 - anthracene, 6 - fluoranthene, 7 - pyrene, 8 - benzo(a)anthracene, 9 - chrysene, 10 - benzo(b)fluoranthene, 11 - benzo(k)fluoranthene, 12 - benzo(a)pyrene, 13 - dibenzo(a,h)anthracene, 14 - benzo(g,h,i)perylene, 15 - indeno(1,2,3-c,d)pyrene

RESULTS AND DISCUSSIONS

The heavy metal content was maximum in the samples from Cluj-Napoca, with the highest values for the contents of lead, copper and zinc (with an average of 0.03 mg/kg, 2.26 mg/kg and 43.31 mg/kg respectively - Table 1), being minimal in the wheat samples from Jucu (Table 2). The cadmium content was low, this being below the detection limit for wheat grown in

Cluj-Napoca and Jucu (Tables 1 and 2). The wheat samples from Seica Mare (Table 3) have the highest cadmium and lead concentrations (historical contamination).

The trend of decreasing heavy metals' content in the samples analyzed in all locations can be observed during the three experimental years.

Table 1. Average contents of heavy metals and PAHs from wheat grains originating from Cluj-Napoca

Xenobiotics	2012	2013	2014	Average
Pb [μg/kg]	0.05	0.04	0.01	0.03
Cd [μg/kg]	N.D.	N.D.	N.D.	-
Cu [mg/kg]	2.97	2.58	2.31	2.62
Zn [mg /kg]	49.25	42.09	38.05	43.13
Naphthalene [μg/kg]	3.48	3.95	3.36	3.60
Acenaphthene [μg/kg]	0.87	1.02	0.99	0.96
Fluorene [μg/kg]	2.55	2.83	3.42	2.93
Anthracene [μg/kg]	0.31	0.28	0.17	0.25
Benzo(a)anthracene [μg/kg]	0.12	0.16	0.19	0.16
Dibenzo(a,h)anthracene [μg/kg]	0.5	0.43	0.31	0.41
TOTAL PAHs	7.83	8.67	8.44	8.31

*N.D.: not detected

The obtained results revealed the higher share of low molecular weight PAHs, mainly naphthalene, fluorene and acenaphthene; from high molecular weight PAHs, only benzo(a, h)anthracene, benzo(b)fluoranthene and dibenzo(a, h)anthracene were quantifiable.

From the monitored PAHs, the following were not detected: benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i) perylene, benzo(a)pyrene, chrysene, fluoranthene, indeno(1,2,3-c,d)pyrene, phenanthrene and pyrene.

The location of the most contaminated samples is Cluj-Napoca (7.83 - 8.67 μg total PAHs/kg - Table 1), in the contamination mechanism predominantly being involved the atmospheric depositions loaded with fuel combustion products (car traffic).

Table 2. Average contents of heavy metals and PAHs from wheat grains originating from Jucu

Xenobiotics	2012	2013	2014	Average
Pb [μg/kg]	0.01	0.01	N.D.	-
Cd [μg/kg]	N.D.	N.D.	N.D.	-
Cu [mg/kg]	2.46	2.31	1.95	2.24
Zn [mg /kg]	45.57	40.15	37.92	41.21
Naphthalene [μg/kg]	2.15	2.03	2.36	2.18
Acenaphthene [μg/kg]	0.61	0.32	0.49	0.47
Fluorene [μg/kg]	1.97	2.19	2.34	2.17
Anthracene [μg/kg]	0.25	0.21	0.18	0.21
Benzo(a)anthracene [μg/kg]	0.07	0.03	N.D.	-
Dibenzo(a,h)anthracene [μg/kg]	0.04	0.02	N.D.	-
TOTAL PAHs	5.09	4.8	5.37	5.09

*N.D.: not detected

The chromatographic profile of PAHs from wheat samples from Jucu is similar to that of samples from Cluj-Napoca, with maximum contents of naphthalene and fluorene, but in lower concentrations (Table 2).

Table 3. Average contents of heavy metals and PAHs from wheat grains originating from Seica Mare

Xenobiotics	2012	2013	2014	Average
Pb [μg/kg]	0.02	0.03	0.03	0.03
Cd [μg/kg]	0.04	0.07	0.03	0.05
Cu [mg/kg]	1.29	1.31	1.06	1.22
Zn [mg /kg]	21.43	30.61	25.37	25.80
Naphthalene [μg/kg]	1.53	1.42	1.23	1.39
Acenaphthene [μg/kg]	0.40	0.31	0.44	0.38
Fluorene [μg/kg]	1.46	1.10	1.41	1.32
Anthracene [μg/kg]	0.12	0.06	0.08	0.09
Dibenzo(a,h) anthracene[μg/kg]	0.26	0.12	0.15	0.18
TOTAL PAHs	3.80	3.06	3.31	3.39

*N.D.: not detected

The PAHs' content in the wheat samples from Seica Mare was the lowest, while the most dominant PAHs determined in them were naphthalene and fluorene (Table 3); the potential sources of PAHs in the studied area are individual households (burning of waste, burning of wood for food preparation and heating purposes), while traffic is very low (on a national road located about 200 m away).

The obtained values for PAHs are in the lower end of the range corresponding to those obtained in other similar studies (Kobayashi et al., 2008; Li and Ma, 2016; Liu et al., 2017) - with certain differences mainly due to the type of cultivars studied and environmental conditions. Most of the data dealing with the heavy metal content in wheat grains were published for samples from contaminated sites, hence the reported data are much higher than the current ones (Bermudez et al., 2011; Huang et al., 2008; Jamali et al., 2009).

CONCLUSIONS

Overall, the obtained results highlighted relatively low concentrations of the studied xenobiotics in autumn wheat grains, below the maximum permitted limits established by the European Commission Regulation no. 1881/2006 (0.2 mg Pb/kg, 0.1 mg Cd/kg, for PAHs no limits being established for unprocessed cereals).

The main contributors to these are the historical pollution of the soil, the car traffic and the burning of fossil fuels.

The obtained data may be useful in future studies regarding the determination of human exposure on these pollutants.

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