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Impact of Former Mining Activity to Soil Contamination by Risk Elements

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Abstract

The level of contamination of the environment in the past few decades is largely influenced by the anthropogenic activity. The tailing pond, where is stored landfilled flotation sludge with the high content of mercury (more than 46 mg/kg), is located about 500 metres from the main processing plant. In the present work, we have focused on the assessment of the level of contamination of the upper horizon (0-0.1 m) of agricultural and forest land in the cadastral territory of Markušovce, where is located the emission source and the pond. We determined 28 sampling points by GPS method on areas of our interest. All soil samples have been subjected to the analyses for the detection of active and exchange soil reaction, the content of humus (%), total concentration of Hg and the concentrations of Cd, Pb, Cu and Zn (mg/kg) in the solution of the aqua regia. The results confirmed an extremely high level of soil contamination by the studied elements. Mercury concentration was in the range of 0.69-90.7 mg/kg, while the median value exceeding the standard established by applicable legislation over 30 times. The content of other heavy metals closely correlated with contaminant concentration, which varied in wide intervals as evidenced by high relative standard deviations (Cd: 1.62 ± 34.2 mg/kg; Pb: 37.6 ± 60.7 mg/kg; Cu: 23.5 ± 124 mg/kg Zn: 108 ± 40.4 mg/kg). Even in the case of these two elements (Cd and Pb) we have in excess of the limit value at the level of the averages. The results observed that heavy metal concentrations indicate a high level of soil contamination in the study area, which will be reflected to the contamination of other components of environment and food chain.

1. Introduction

The level of environmental contamination with risk elements represents a significant risk in relation to the quality of the food chain. In the last decades a significant increase in the concentration of contaminating elements such as cadmium, mercury and arsenic in all components of the environment was reported (**Granero & Domingo, 2002**; **Li *et al.*, 2008**). It is mainly due to continuous increase of industrialization of human society (**Govil *et al.*, 2008**; **Huang *et al.*, 2007**; **Li *et al.*, 2008**). Increasing demands for raw materials result in adverse interventions in nature. The level of environmental contamination on the local scale is also amplified by metallurgical industry, which increases the level of contamination of topsoil in forest and agricultural ecosystems mainly through atmospheric deposition (**Biester *et al.*, 2002**; **Jiang *et al.*, 2006**). Heavy metals are ubiquitous part of the environment as a result of mutual natural and anthropogenic activities causing increased exposure of human populations to their effects through various channels (**Póty *et al.* 2012**). Increasing concentrations of certain risk elements, especially their mobile forms can cause serious environmental concern about contamination and accumulation in soil, vegetation, animals, respectively, surface and ground waters (**Chopin & Alloway, 2007**). The primary source of environmental contamination is mainly metals, whose main part is particularly lead, zinc or copper, in addition, antimony, arsenic, mercury, cadmium, thallium, gallium, and others. In addition to the production of metals is a very important source of environmental contamination with metals and burning of fossil fuels, especially coal. Fly ash from the incineration of atmospheric leakage through polluted soil (**Steinnes *et al.*, 2005**). Increasingly important source of environmental pollution with heavy metals is becoming a burning municipal waste streams and pollution effluents containing elevated levels of toxic metals (**Bencko *et al.*, 1995**). Soil contamination reveals itself as overreaching the limit value of at least one risky substance. In case of the risk elements we talk about their overall content. The available data regarding the total content (after the soil decomposition using potent mineral acids, especially Aqua regia used in the Slovak Republic since 2004) may give us the necessary information in accordance with the valid legislation (including the approved maximum content of risk elements and the level of contamination). Relationships between the risk element contents in the soil and their concentrations in plants have been observed only in case of overreaching their established total concentrations in soils. Analyses the transport of the risk elements to the plants, the interactions soil - plant are

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especially crucial and studied by a variety of authors (**Castaldi *et al.*, 2009**). On the other hand, low concentrations of risk elements in soils, e.g. in acid soils, are related to their exceeded limits in plants (**Kobza *et al.*, 2007**).

2. Material and methods

2.1 Study area

In the present work we have focused on determination the level of contamination of top soil in the cadastral territory of Markušovce, which is located about 10 kilometres south-east from the district of Spišská Nová Ves. In the southern part of the territory is an old environmental burden (tailing pond, areal of the former mercury smelter and heaps of pyrites). The history of mining and processing iron ores containing Hg industry dates to the 2nd half of the 19th century. About 52 small mercury smelters were worked and were processed mined cinnabar and *tetraedrite* till the year 1963. According to estimates several hundred tons of elemental Hg had been issued to the environment during this period. New plant on the surface was launched into operation in 1963. The major sources of mercury were the heat of operation, agglomerations, mercury smelter, tailing pond and heaps of roasted material. The main mercury smelter was launched into operation in 1969. The summary of Hg emissions from all sources was 142 tonnes for the years 1963 - 1993 (**Svoboda *et al.*, 2000**). The mercury smelter emitted to the atmosphere 2.60–4.64 tons of mercury per year, during the period of 1980–1988 (**Hančuľák *et al.*, 2006**; **Hronec *et al.*, 1992**). The emitted mercury subsequently contaminated the area located in the direction of prevailing winds (north-south), which is strongly reflected in the level of pollution of all elements of the environment within 5 kilometres from the mercury smelter. The study area is of volcanic origin, characterized by frequent occurrence of geochemical anomalies, which penetrate up to surface. The substrate consists of naturally high concentration of mercury, copper, lead, zinc and cadmium, which sometimes occurs in the elemental form (**Angelovičová & Fazekašová, 2014**). The study area, indicating the emission source and sampling points is shown in Figure 1.

Figure 1. The study area, indicating the emission source (sampling point No. 1) and sampling points.

2.2 Sampling

Sampling and pre-analytical procedure. Soil samples (n = 28) of studied area were taken by randomly using pedological probe of depth 0.0 - 0.1 m in 2013. Sampling points were determined using GPS navigation device at regular intervals of 1 km from the emission source (sampling point 1). Each sample consisted of a mixed sample of soil from the sampling point (5 samples were taken from each sampling point and mixed together).

2.3 Pre-analytical procedure

The samples were dried at room temperature to constant weight and sieved through a sieve with a mesh diameter of 2 mm before all analyses. We carried out an analysis to determine the active (pH – H_2O) and exchange (pH - $CaCl_2$) soil reaction, humus content (%), total concentration of mercury and total concentration of monitored heavy metals (Cd, Cu, Pb, and Zn) in extract of *Aqua regia* (ISO 11466). High purity chemicals for all operations were used. Mineralization of soil samples was done by 10 cm^3 of *Aqua regia* (2.5 mL HNO_3 and 7.5 mL HCl, Merck, Germany) using microwave digestion unit Mars X-press 5 (*CEM Corp*., USA) in closed PTFE vessels. Digestion conditions for the applied microwave system were as follows: the heat was run up to 180°C for 15 minutes and kept constant for 15 minutes. A blank sample was carried out in the same way. The digests were subsequently filtered through a quantitative filter paper Filtrak 390 (*Munktell*, Germany) and filled up with deionized water to a volume of 100 mL (**Árvay *et al.*, 2013**).

2.4 Analytical procedure

The element determinations were performed in a Varian AA240Z (*Varian*, Australia) atomic absorption spectrometer with Zeeman background correction. The content of copper and zinc were determined by Atomic Absorption Spectrometry device with flame AA240FS Varian (Varian, Australia). The graphite furnace technique was used for the determination of Cd and Pb. The total mercury concentration was determined in the homogenized dried soil samples (0.005-0.01 g) using a cold-vapour AAS analyser AMA 254 (*Altec*, Czech Republic) with a detection limit of 0.5 ng/g. Mean difference between duplicates were up to 5% (**Árvay *et* al., 2015**; **Svoboda *et al.*, 2006**).

2.5 Statistical analysis and risk assessment

All statistical analyses were carried out using the statistical software Statistica 12.0 *(Statsoft,* USA). Descriptive data analysis included a minimum value, maximum value, median and standard deviation. The obtained data on the concentration of risk elements we have compared with the limit values that define the legislative norm Act No. 220/2004. Graphical outputs of spatial interpolation of monitored parameters are isoline maps that were created by the program ArcView 9.1 environment, which are depicted the concentration of monitored contaminants.

3. Results

According to our findings relatively small study area of cadastral territory Markušovce (19.2 km^2) is in all parameters significantly heterogeneous. Active but also exchange soil reaction as the basic chemical parameters, which have a significant influence on the behaviour of contaminants in the soil environment, varied in wide intervals. Active soil reaction varied in the range 6.40±1.34 (median±st.dv). Exchange soil reaction in $CaCl_2$ solution with c=0.01 Mol/L was at the level of the median value in the range 6.07±1.31. A wide range of both parameters is

probably due to the different morphology of the territory, the nature of the samples and the long-term effects of immission fallout, which correlates with the long-term prevailing winds (north-westerly and westerly).

Our findings show that 3.51 km² of area (18.3%) is strongly acidified (pH<4.5), which can affect the which can affect the mobility and subsequent bioavailability of monitored contaminants. The content of humus (%) varied in a wide range (2.72±3.41), which is caused by the different nature of the samples (Table 1).

Statistically significant effect of soil parameters on the level of contamination of topsoil with heavy metals is also confirmed by correlation coefficients (P≤0.05, P≤0.01) and these results are shown in Table 2.

Table 1. Basic characteristics of sampling points and monitoring parameters of soil samples

SP – sampling point; D-ES – distance from emission source; SC – sample characteristic; ES – emission source; AS – agricultural soil; F – forest; UA – urban area; **XXX** – exceed the limit value that is defined by law; *Act No. 220/2004.

SP	D-ES (km)	SC	Soil parameters							
			pH H_2O	pH $CaCl_2$	Humus (%)	$Hg_{tot.}$ (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
SP-01	0	ES	6.81	5.93	3.75	**63.2**	**3.52**	54.0	300	**126**
SP-02	1	AS	6.58	5.16	2.06	**9.13**	**1.69**	33.2	40.6	82.2
SP-03	1	AS	6.05	4.83	2.42	**7.25**	**1.16**	23.4	36.5	99.3
SP-04	1	AS	6.01	5.21	1.45	**7.03**	**1.56**	26.4	29.5	71.5
SP-05	1	AS	5.61	4.93	2.72	**11.3**	**0.97**	25.1	36.5	**104**
SP-06	1	F	7.06	6.48	11.2	**10.4**	**3.73**	125	**136**	**253**
SP-07	1	F	3.82	2.85	14.8	**73.1**	**1.14**	47.6	7.40	40.7
SP-08	5	F	4.30	3.26	5.63	**6.74**	**1.29**	35.8	33.7	38.8
SP-09	6	F	4.47	3.60	6.29	**15.7**	**1.85**	44.3	37.5	68.7
SP-10	1	AS	8.02	7.16	1.57	**3.08**	**3.85**	64.0	48.2	**173**
SP-11	2	F	4.91	4.01	3.15	**6.65**	**1.74**	43.6	38.1	57.2
SP-12	3	AS	6.17	5.41	4.05	**1.26**	**3.00**	56.2	26.9	151
SP-13	1	AS	5.94	7.04	1.45	**1.35**	**1.02**	24.9	52.5	78.7
SP-14	2	AS	5.08	5.91	1.82	**1.05**	**1.34**	27.9	36.6	84.5
SP-15	3	AS	6.67	7.35	3.81	**1.81**	**5.15**	43.7	39.1	88.6
SP-16	1	AS	8.13	7.32	1.33	**2.74**	**3.01**	45.3	38.2	94.3
SP-17	2	UA	7.94	7.19	2.72	**0.70**	**3.17**	47.7	**105**	**387**
SP-18	3	UA	7.94	7.27	1.57	**0.72**	**1.68**	25.6	24.0	86.2
SP-19	4	AS	8.13	7.16	3.27	**0.69**	**1.80**	25.9	18.9	79.3
SP-20	1	AS	7.22	7.34	4.30	**90.7**	**1.94**	45.8	331	**189**
SP-21	2	AS	5.40	6.33	1.88	**1.64**	**1.29**	27.1	47.9	**112**
SP-22	4	F	7.61	6.82	12.3	**9.36**	**3.24**	133	46.1	**364**
SP-23	5	AS	6.85	6.05	3.51	**1.45**	**0.94**	29.2	19.7	**127**
SP-24	6	F	6.38	5.34	1.82	**2.27**	**1.07**	27.4	20.3	**282**
SP-25	5	F	7.78	6.91	1.94	**16.3**	**1.06**	25.2	52.2	**125**
SP-26	5	AS	4.33	6.08	2.54	**1.01**	**1.06**	39.4	35.0	**152**
SP-27	6	AS	6.41	6.84	3.93	**0.70**	**1.51**	41.2	55.8	**270**
SP-28	7	AS	4.10	5.48	1.51	0.45	**1.52**	32.7	29.6	**191**
Limits value and descriptive statistics										
Limit value*			-	-	-	*0.50*	*0.70*	*70.0*	*60.0*	*100*
Minimum			3.82	2.85	1.33	0.45	0.96	23.4	7.40	38.8
Maximum			8.13	7.35	14.8	90.7	5.15	133	331	387
Median			6.40	6.07	2.72	**2.91**	**1.62**	37.6	37.8	**108**
Standard deviation			1.34	1.31	3.41	23.1	1.11	26.5	76.1	92.3

The highest levels of humus content were detected in the southern part of the territory. There is located forest, which is characterized by a higher content of organic matter in the top layer of soil compared with agricultural land. There is a significantly higher level of oxidation and the consequent degradation of organic matter occurs due to periodic aeration of topsoil. Data of the pH and humus in the study area are shown in Table 1 and their graphical interpolation on Figure 2.

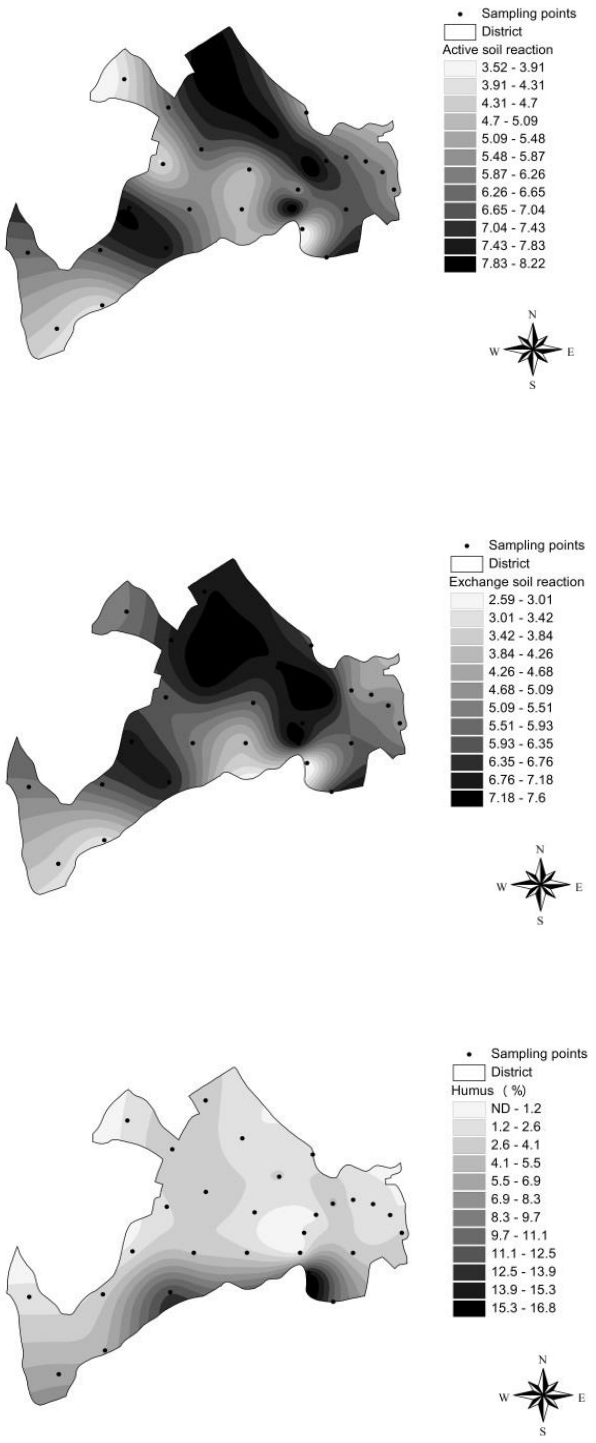

Figure 2. Spatial interpolation of active, exchange soil reaction and humus content of soil samples from study area

The total mercury concentration in the study area varied in a wide range. The level of the median value was 2.91±23.1 mg/kg. Figure 3 shows strong local contamination of soil within a radius of 1 km from the emission source in the direction of the prevailing winds. From the hygienic point of view, it is necessary to emphasize that in an area of more than 15 km^2 (78.5%) was detected an excess of the mercury limit value (0.5 mg/kg). The results of mercury content in the soil closely correlate with the results of other authors, who pointed to the high level of contamination of all environmental elements (**Árvay *et al.*, 2017; Bobro *et al.*, 2006; Demková *et al.*, 2017; Dombianová, 2005; Takáč *et al.*, 2008**). It represents a significant risk of possible contamination of agricultural production and food and feed chains (**Árvay *et al.*, 2014**).

The cadmium content of study area varied in the range 1.62 ± 34.2 mg/kg. Exceeding a limit value of 0.70 mg/kg was recorded at the level of the mean more than two times. High level of topsoil contamination is shown by isoline map (Figure 3). We can state that study area is contaminated with risk elements. The highest concentration of the contaminant was ascertained at the sampling point SP-15 and in the north-eastern part of the study area. The limit values were exceeded for almost 96% of the study area. The lead content exceeds the limit value of 75 mg/kg at two sampling sites (6 and 22). These points were located at the northern part of the area. The median values of the lead content varied in the range 23.4 ± 60.7 mg/kg. The concentration of heavy metals exceeded the limit value by 10.3 % of total area. It does not pose any risk to contamination of the agricultural products, because it is a forest soil. Between the lead content of the topsoil and humus content is a high positive correlation (Table 2) and it demonstrates a high level of affinity of the monitored parameters, what is confirmed by the results of other authors **Árvay *et al.* (2017)**.

The concentration of copper and zinc in the topsoil was compared to the level of mercury. It is not represent a significant risk. The level of contamination of study area is showed in Figure 3. Detected concentration of copper (37.8±76.1 mg/kg) and zinc (108±92.3 mg/kg) point to a high variability of monitored contaminants in the study area (Figure 3). In the case of all contaminants, we have seen local pollution in the vicinity of the emission source, which is confirmed by the findings of the authors **Angelovičová & Fazekašová (2014)**. Two sampling points (17 and 18) are an exception, where was measured higher concentration of zinc compared to the other sampling points. Our findings show that it is contaminated with copper 19.8% and zinc 69.9% of the study area. Obtained data with descriptive statistical evaluation are shown in Table 1.

Table 2. Correlation between risk elements and chemical parameters

	Hg	Cd	Pb	Zn	Cu
pH – H_2O	-0.08	0.46*	0.24	0.39*	0.25
pH – $CaCl_2$	-0.17	0.41*	0.15	0.42*	0.27
Humus(%)	0.42*	0.20	0.70**	0.19	0.07
Hg		0.02	0.11	-0.09	0.72**
Cd			0.61**	0.31	0.30
Pb				0.55**	0.24
Zn					0.23

*/** correlations are significant at P≤0.05, P≤0.01 level, respectively

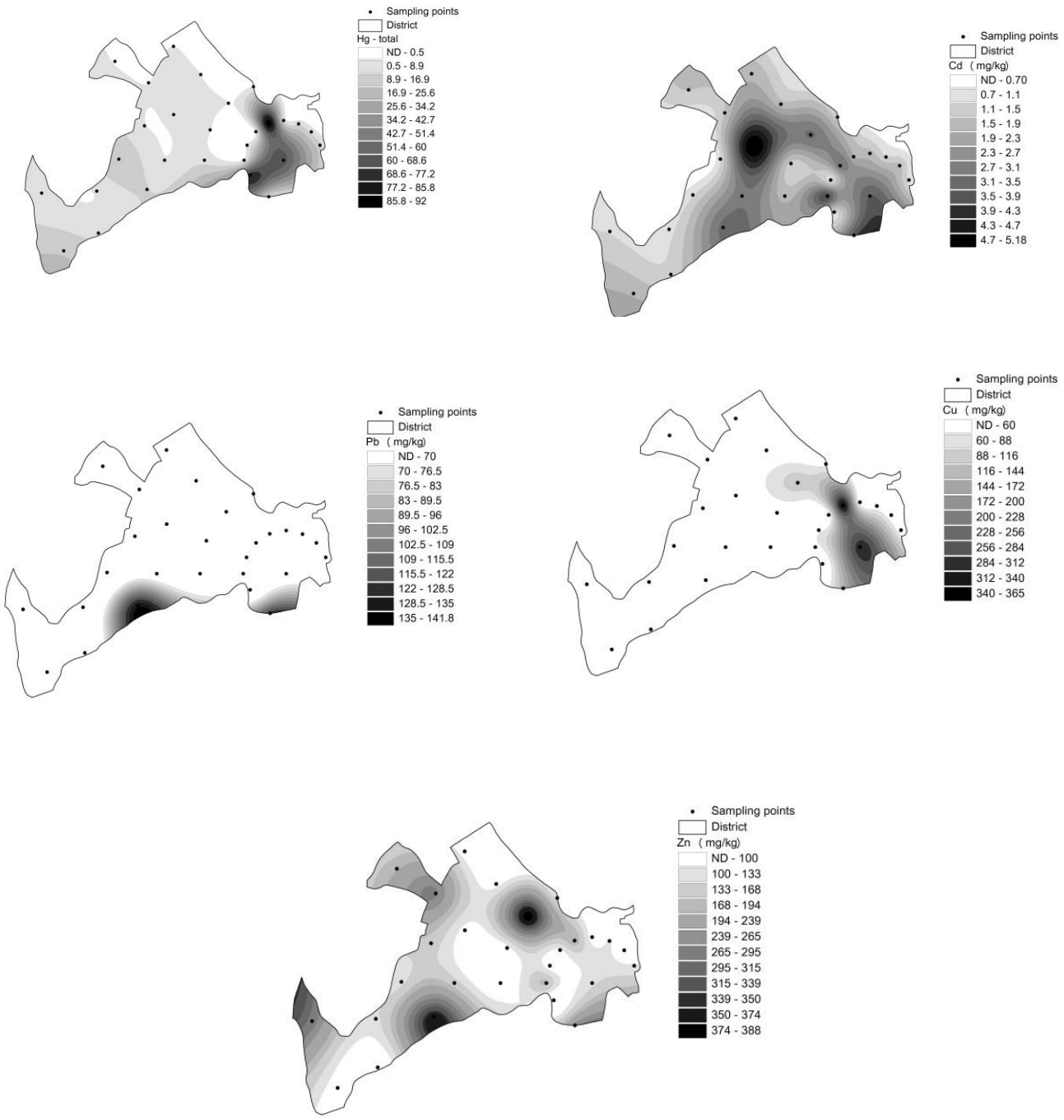

Figure 3. Spatial interpolation of total concentrations Hg, Cd, Cu, Pb, and Zn (mg/kg).

4. Conclusion

In the present paper, we aimed at detection of the level of burdened topsoil at the area of the former metallurgical company, which in 1993 was processing ore with a high content of Hg and other monitored elements. According to the results, it can be concluded that the area within 5 km from the emission source is heavily contaminated with monitored heavy metals, thus permitting passage of contaminants from the abiotic environment to the food chain, which in turn reflected the deterioration of health of the local population and thus to increased mortality.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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The Evaluation of Evidence Bisphenol A Exposure and Human Reproductive Health: A review

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Abstract

Infertility is widespread problem defined as the inability to conceive after one year of unprotected intercourse. One of the most notable factors causing this status is the exposure to environmental contaminants. It is now recognized that many contaminants present in the environment have the ability to interfere in the action of hormones and therefore are termed endocrine disruptors (EDs). Some of these compounds are present in nature, but the majority are artificial and released into the environment by the human activities without any prior knowledge of their impact on ecosystems, animal welfare, or wildlife and human health. Many epidemiological studies have reported a radical growth in the incidence of male infertility, accompanied by decreasing sperm quality, decline in spermatozoa motility, defect in Leydig cell morphology, insufficient activity of steroidogenesis and spermatogenesis. The similar situation was observed in female, when the increased risk for endometriosis, reproductive and other endocrine-related cancer, impaired oocytes, ovarian dysfunction, or irregular menstrual cycle was confirmed. All mentioned consequences have been associated with increasing concentration of bisphenol A (BPA) in the environment. Humans are exposed to BPA not only through specific occupational circumstance, but nowadays more generally also from the ordinary day-to-day domestic and workplace lifestyles. Almost 3.4 million tons per year of BPA is used in a variety common product such as food packaging, household products, epoxy resins, dental sealants and many others. Under these endpoints, apprehensions about the reproductive dysfunctions associated with BPA action are unquestionable. In this review, we address the topic of BPA effects on reproductive function in males and females and emphasize its effects on overall health. A considerably more detailed and systematic research in EDs toxicology is required for a better understanding of risks associated with exposure to environmental toxicants.

1. Introduction

Infertility is a major problem for many couples of reproductive age and currently affects around 10% of couples worldwide. In approximately 50% of the affected couples, infertility is due to male reproductive dysfunction **(Quah and Cockerham, 2017)**. In addition, increasing prevalence of congenital abnormalities such as hypospadias and cryptorchidism has also been confirmed by many studies during the last decades. Substantial part of the problem is the disruption of essential cellular processes responsible for normal reproductive functions **(Boisen et al., 2004; Boivin et al., 2007)**. Given the short time, genetic changes cannot explain it. We may assume that they only reflect persistently adverse changes in the environment. During the last decades, an increased occurrence of hazardous chemical present in human or wildlife environment was confirmed. Enormous production and release of industrial chemicals into the ecosystem has led the scientific community to hypothesize that current pollutants may irrefutably disrupt health conditions, leading to extensive damages of physiological functions **(Jambor et al., 2018; Kovacik et al., 2018)**. A huge number of different hazardous substances have been found to interact with the endocrine system of many animals, and there are increasing reports of endocrine disruption in wildlife **(Tyler et al., 1998; Svechnikov et al., 2010)**. The group of chemicals, that may alter the hormonal and homeostatic system is called endocrine disruptors (EDs). They are able to alter functions of the endocrine system, inhibit critical cellular processes, increase the risk of the hormone-dependent cancers and may result in many other adverse health effects. According to final report of **Darbre (2015)**, EDs are an exogenous chemical substances or mixtures that alter the structure or functions of the endocrine system and cause adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence and the precautionary principle. Several reports of declines in quality and decreases in the quantity of sperm production in humans over last four decades and reported increases in incidence of certain cancers (breast, prostate, testicular) that may have an

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endocrine-related basis have led to speculation about environmental etiologies **(Rochester, 2013)**.

Nowadays, questions are raising about mechanism of action or potential toxic effects of EDs. Given the complexity of the endocrine system, there are many ways in which EDs may affect physiological functions, and this makes the mechanisms of action difficult to unravel. A large number of EDs have been shown to mimic, block or modulate the actions of hormones such as estrogens, anti-estrogenes, androgens and anti-androgens. All of them act through interfering with the estrogen receptors (ER) or the androgen receptor (AR). Estrogens are able to regulate processes such as development and function of the reproductive system and protection against cardiovascular diseases. The estrogenic effects are mediated through ER of which two variants (α, β) regulate these physiological processes. Endogenous estrogens are primarily recognized for their role in the differentiation and growth of secondary sex-tissues. However, the endocrine system is especially vulnerable to perturbation by EDs with estrogenic properties. They can interfere with normal endogenous hormone action by binding themselves to receptors or by blocking the steroid receptor binding **(Welshons et al., 2006; Swedenborg et al., 2009)**.

2. Sources of EDs

Humans are exposed to environmental chemicals with endocrine-disrupting properties not only through specific occupational circumstance, but nowadays more generally also from the ordinary day-to-day domestic and workplace lifestyles of the twentieth and twenty-first centuries. Occupational exposures, such as of agrochemicals on farms or of plastics in manufacturing factories, can cause specific high exposures, but the general population also uses pesticides, herbicides, paints, personal care products, solvents, detergents and industrial lubricants. Another source of EDs exposure is through pharmaceuticals, nutraceuticals and food products that are promoted as providing health or medical benefits through the prevention of treatment of disease **(Darbre et al., 2015)**. Many textiles also contain contaminants, such as flame-retardants, including tetrabromobisphenol A and polybrominated diphenyl ethers **(Younglai et al., 2002)**. Although there is chronic exposure to EDs through the skin contact or inhalation, food products are the major source. **Wagner and Oehlmann (2011)** are convinced that plastic packaging is the largest source of EDs in the human diet. Repeated exposure of food – contact materials to UV light, acid and alkaline contents and heat may cause polymers to breakdown into monomers as phthalates, which then leach into the food or beverages. Phthalate esters are weakly estrogenic plasticisers used to soften plastics destined for material such as cling film and plastic wrappers. Bisphenol A (BPA) is a component of polycarbonate plastics and epoxy resins used in resins linings of food cans and water pipes. Perfluorinated compounds can be found in non-stick Teflon cookware and are also used in fast-food packaging tor their stain-resistant properties. While relatively low concentrations of EDs result in harmful effect, some kinds of EDs must be consumed at relatively high concentrations to produce corresponding effect on the human endocrine system because their hormonal activities are usually five or six times lower than 17β-estradiol. However, a combination of EDs at lower concentrations may result in additive, enhanced or low-level cocktail effect, thus posing a greater risk to the consumer. Other high-risk groups called alkylphenols persist in the environment for a long time. They are used in the production of agrochemicals, industrial and household detergents, paints or plastics **(Nimrod and Benson, 1996; Jambor et al., 2017)**. Detection of ED residues in human serum, seminal plasma and follicular fluid has supported the concern that environmental exposure to ED is affecting human fertility. Nowadays, some of them were banned or otherwise were removed from the industrial process years ago. The long-debated question remains - whether such effects might also occur in the human population in response to the EDs, and therefore whether the wildlife effects might be a forewarning of consequences for human health. A number of studies have suggested that hormone alterations in females resulting from environmental EDs exposure may represent an increased risk for endometriosis, reproductive and other endocrine-related cancer, impaired oocytes, competence or changes of ovarian function and menstrual cycle **(Toft et al., 2004; Dhooge et al., 2007)**. In males, environmental or occupational exposures to EDs may be associated with declining reproductive capacity or increasing the risk of testicular or prostate cancer, as well as poor semen quality and alterations in testosterone levels **(Uhler et al., 2003; Meeker et al., 2006; Jambor et al., 2017)**. In this context, possible adverse effects of EDs have been taken into focus, both regarding the effects of EDs on the male and female reproductive system and with respect to its differential susceptibility towards these compounds. Although there has been an effort to list and rank all possible EDs, the number of evaluated chemicals remains limited **(Vandenberg et al., 2007)**.

3. Bisphenol A and "safe" alternatives

Bisphenols are one of the most studied EDs in the field of male and female reproductive system. It has been shown that BPA alter the normal function of the endocrine system, cause adverse effects on male reproductive system and irrefutably affect essential processes responsible for functional health in humans and numerous animal species **(Welshons et al., 2006)**. A survey of the Pubmed database provides more than 10,000 articles on the topic, including epidemiological as well as experimental studies. The overwhelming majority of bisphenols are used as stable components of household products, epoxy resins, inner surface of food metallic cans, plastics packaging, dental sealants, and for myriad additional synthetic products **(Calafat et al., 2008)**. BPA is industrially produced by condensation of phenol and acetone in the presence of an acid catalyst (hydrogen chloride) and usually a promoter such as methyl mercaptan. After reaction and recovery of acid and phenol, the BPA is washed with water, neutralized with milk or lime and distilled under vacuum. Newer processes employ distillation and extractive crystallization under pressure to purify the BPA. Two grades are produced: one for epoxy resins production and a higher purity grade for polycarbonate manufacture. Almost 70% of BPA production is primarily used to produce polycarbonate plastics used in a variety of common products **(Vandenberg et al., 2007)**. BPA is ubiquitous in the environment, and humans are exposed to this chemical via dietary and nondietary sources (Figure 1). Based on the analysis of blood samples from adults found that the concertation of unconjugated BPA, which is the biologically active form, ranged from 0.2 to 10 ng/mL, with an average concentration from 1 to 3 ng/mL **(Vandenberg et al., 2012)**.

Exposure to BPA has been associated with several human diseases, such as diabetes, obesity, cardiovascular, chronic respiratory and kidney diseases, breast cancer, behavioral troubles, tooth developmental defect, and reproductive disorders in both sexes **(Geens et al., 2012; Vandenberg et al., 2012; Rochester, 2013)**. As we mentioned before, many studies have been shown to affect many endpoints of fertility. Exposure to high or low doses of BPA may induce adverse health effects in testis, including reduced sperm motility and viability, DNA damage or decreased sperm count. Several cohort studies examined individual undergoing infertility treatments and measured BPA in relation to various reproductive endpoints,

such as ovarian response, fertilization success, embryo quality and many others **(Ehrlich et al., 2012)**. On the other hand, there are plenty of studies focused on the ability of BPA to affect the brain even at very low doses. **Palanza et al. (2016)** showed, that the epigenetic action of BPA on the hippocampus and hypothalamus, may disrupt normal steroid programming in the brain and subsequently affect sex-specific behavior. Several animal studies report BPA to affect synaptogenesis and neurogenesis processes which are known as the brain self-regeneration against trauma and disease. In a study of the associations between BPA and cancer in humans, **Yang et al. (2009)** analyzed total serum BPA of women with and without breast cancer. There was a no-significant elevation of BPA in the cancer patients. However, there exists evidence from rodent **(Markey et al., 2001; Li et al., 2009)** and primate **(Tharp et al., 2012)** studies that prenatal exposure to BPA cause disruption of the mammary tissue and increases the susceptibility of the tissue to environmental carcinogens. Diabetes – type II has been also associated with BPA in many human studies. **Lang et al. (2008)** found that higher total urinary BPA was significantly associated with increased diagnosis of type-II diabetes (defined as fasting serum glucose greater than 126 mg/dL, non-fasting greater than 200 mg/dL, glycosylated hemoglobin greater than 6.5%). This positive association between BPA and diabetes was present among normal weight and overweight patients, and smokers as well as non-smokers. In another study, **Ning et al. (2011)** assessed Chinese adults. Higher urinary BPA was non-significantly associated with increased diabetes and measured by blood glucose. Cardiovascular disorders and hypertension are other adult onset diseases that have been associated with adult BPA exposure. **Lang et al. (2008)** reported that higher urinary BPA was associated with a more frequent diagnosis of cardiovascular disease (coronary heart attack, angina). Meanwhile, **Melzer et al. (2010)** assessed individuals 18-74 years of age and found a significant increase in myocardial infarction, coronary heart disease together with increased urinary BPA. The decomposition of BPA takes place in the liver by uridine5´-diphospho-glucoronyl transferase **(Yokota et al., 1999)** and the half-time of BPA is about 5.4 hours **(Stahlhut et al., 2009)**.

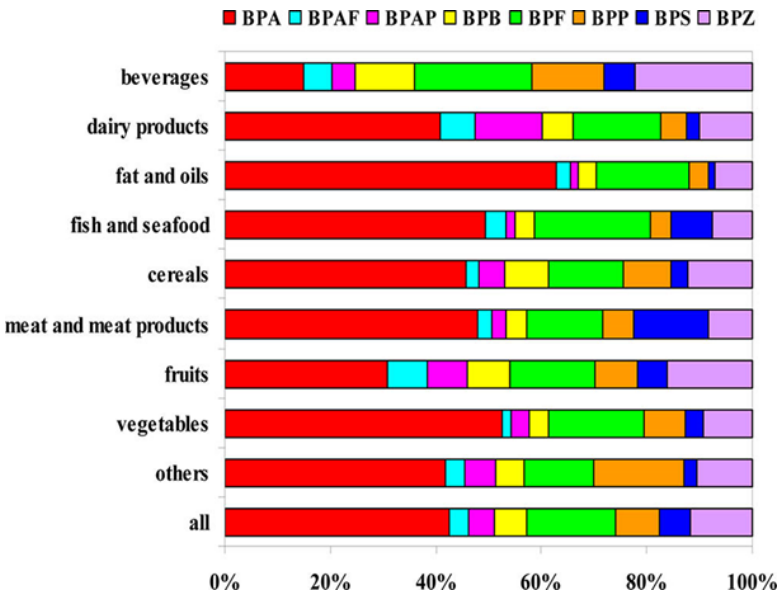

Figure 1. Composition profiles of bisphenols in foodstuffs **(Liao and Kannan, 2013)**

Due to many negative effects, toxicity and widespread exposure, the general public has drawn considerable attention to BPA. Based on many negative effects on human health restrictions on the use of certain consumer products have been suggested. For example, BPA has been prohibited from manufacture, sale, or distribution in some consumer products, such as reusable food or beverage containers, infant formula containers, and thermal receipt paper. The U.S. Food and Drug Administration (FDA) also banned the use of BPA in baby bottles and children´s drinking cups in July 2012 **(Eladak et al., 2015)**. At this point, alternatives to BPA have started to develop which can be used to replace it in food plastics packaging or epoxy resins. However, we are convinced that common analogues of BPA are not entirely "safe" as indicated by industrial companies. Nowadays, there are recognized several "safe" analogues to BPA such as bisphenol S (BPS; 4,4´-sulfonyldiphenol), bisphenol F (BPF; 4,4´-dihydroexydiphenylmethane), bisphenol B (BPB; 2,2-bis(4-hydroxyphenyl)butane), bisphenol AF (BPAF; 4,4´-(hexafluoroisopropylidene)diphenol) and many others. A total of 16 bisphenol analogues have been documented for industrial application. These chemicals share a common structure of two hydroxyphenol functionalities and are collectively referred to as bisphenol analogues **(Kuroto-Niwa et al., 2005)**. BPF, BPS and BPAF are among the main substitutes of BPA in the manufacturing of polycarbonate plastics and epoxy resins. In the case of BPS and BPF, these substances are structural analogue to BPA (Figure 2), thus their effect in physiological systems may be similar. BPF has a broad range of applications such as lacquers, dental sealants, oral prosthetic devices and adhesives plastics **(Cabaton et al., 2009)**. BPS is commonly used as an additive in dyes, epoxy glues and tanning agents, while BPAF is used in electronics plastics and optical fibres **(Konno et al., 2004; Naderi et al., 2014)**. Estrogenic and antiandrogenic potencies of bisphenol analogues have been the subject of intense investigations. BPF, BPS, BPAF and BPB exhibit estrogenic potencies similar to or greater than that of BPA. **Rosenmai et al. (2014)** determined the effects of these analogues on estrogen and androgen receptor activities and revealed that most of them exhibited strong potencies within the same magnitude as that of BPA. BPS was less estrogenic and antiandrogenic, but the former study showed the largest effect on 17α-hydroxyprogesterone. The inhibitory effect on the androgenic activity of 5α-dihydrostestosterone in mouse fibroblast cell line were the highest in BPS, BPF and BPA **(Kitamura et al., 2005)**. **Audebert et al. (2011)** reported similar ranges of cytotoxicity for BPA and BPF in human hepatoma cell line (HepG2). **Cabaton et al. (2009)** observed that BPF was effective on HepG2 cell DNA fragmentation, while concentrations of BPS ranging from 0.1 to 10 µmol/L also induced significant DNA damage after 24 h exposure. In comparison with BPA, BPF and BPAF enhanced the formation for reactive oxygen species, which damages the lipid and proteins in human peripheral blood mononuclear cells (PBMCs) and definitely decreased the viability of PBMCs **(Michalowicz et al., 2015)**. **Rosenmai et al. (2014)** used several assays to assess steroidogenic activity, as well as teratogenicity and metabolic effects. They found out that BPS and BPF had estrogen receptor binding, estrogenic activity and antiandrogenic activity similar to those of BPA, with BPS being the least potent. However, BPS and BPF exhibited the greatest steroidogenic (i.e., progesterone) activity, increasing levels of 17α-hydroxyprogesterone and progesterone levels, whereas BPA did not. **Zhang et al. (2011)** suggest a direct inhibition of the CYP17 (cytochrome P450 17A1) lyase reaction, independent from estrogen receptor action. Thus, BPA analogues may have additional disruptive effects that have not been detected with BPA. Toxicological data on BPS, BPF, BPB etc. are scarce and experimental studies evaluated their effect are unclear. Therefore, it is necessary to bring novel and original data to help solve this deficiency.

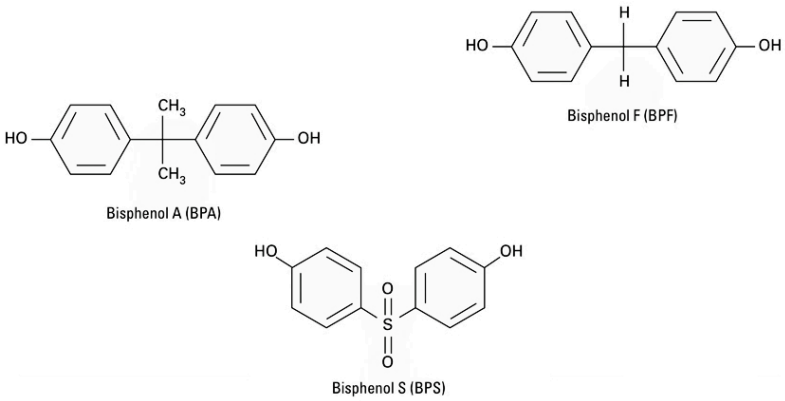

Figure 2. Chemical structure of bisphenol A, bisphenol S and bisphenol F **(Eladak et al., 2015)**

There is an evidence that BPA and their analogues have adverse effect on animal health and it is also suspected of having negative effect on human health although available data are limited. In the present review, we would like to present results from human studies, where significant changes in reproductive processes in men and women were observed.

4. Women reproductive health and BPA

There is accumulating evidence that fertility is decreasing among male and female. The reproductive health of female depends on maintaining coordinated responses of a network of endocrine signals that function primarily to ensure successful procreation but also have other wide-ranging influences on the female body. Consequences of any disrupting effects can be expected, therefore, not only to influence fertility, but to have wide repercussions for female health more generally **(McLachlan et al., 2006)**. One of the most important considerations for endocrine disruption and female reproductive health is the timing of exposure. While exposure to EDs may be expected to affect adult life, there is increasing evidence that some adult female reproductive health problems may be programmed by exposures in the early embryo of fetus *in utero* **(Jacob-Dickman and Lee, 2009)**. Sexual differentiation and congenital malformations of the developing fetus are extremely dependent on EDs action. Changes in hormone levels or the ration of hormones can disrupt sexual differentiation – clearly EDs that mimic important hormones like 17β-estradiol will perturb the levels that are required for normal development **(Toppari and Skakkeebaek, 1998)**. The placenta protects the developing embryo and fetus from hazardous substances, what could negatively affect sex-hormone mediated biochemistry. In terms of potential impact on the developing child EDs or estrogen mimics are the issue, because many of them have molecular structures very different from 17β-estradiol even though they still bind to and activate the estrogen receptor. The latter are likely to interfere with fetal development and therefore we should be aware of the implications of exposure during pregnancy **(Jin and Audus, 2005)**.

Some EDs like BPA decreases the production of progesterone in placental cells. It can be explained by reduced levels of mitochondrial enzyme (CYP450scc) that convert cholesterol to pregnenolone or progesterone. BPA is also able to inhibit aromatase activity by interacting directly with the aromatase enzymatic complex **(Nativelle-Serpentiny et al., 2003)**. **Gould et al. (1998)** found out that higher urinary BPA significantly correlated with lower serum of estradiol and oocyte yield. They also found that higher urinary BPA corresponded to reduced maturation of the oocytes, as measured by the number and percentage of mature oocytes at metaphase II on the day of egg retrieval. There were also fewer normal fertilized oocytes in women with higher urinary BPA, measured by the number and percentage of oocytes with two pronuclei. **Takeuchi and Tsutsumi (2002)** tested healthy women and women with polycystic ovary syndrome (PCOS) for serum BPA and hormone concentrations. The PCOS women had significantly higher testosterone, estradiol, LH and androstenedione concentration than in healthy women, and the total amount of BPA was higher in PCOS women. The endometrial disorders in adult women have been also associated with BPA exposure, although the evidence in humans is not strong. **Cobellis et al. (2009)** tested women with and without endometriosis for total BPA exposure. Serum BPA was not detectable in any of the controls but was detected in 52.7% of the individuals with endometriosis, when the limit of quantification was 0,5 μg/L. **Itoh et al. (2007)** also examined connection between urinary BPA and the severity of endometriosis. There was a trend for higher BPA correlation with more severe endometriosis. An accidental observation by **Sugiura-Ogasawara et al. (2005)** showed, that women with recurrent spontaneous abortion had significantly higher levels of serum BPA than healthy women from the same city in Japan. There are physiological and biochemical reason that might explain the observed effects. BPA increase progesterone receptor expression in the hypothalamus, which in turn alter hypothalamic mechanism and affects the onset of estrus and the receptivity of the uterus.

5. Men reproductive health and BPA

The sexual differentiation and formation of the penis, scrotum and accessory sex gland during the fetal development is also extremely sensitive to EDs action. These formations are under the influence of steroid hormones secreted during the hormonal phase of testicular development. Especially, testosterone and dihydrotestosterone are two major hormones involved in the above process **(Basrur, 2006)**. It has been reported that 20.8% of the males exposed to diethylstilbestrol (DES) *in utero* had epididymal cysts, 4.4% had hypospadias, 11.4% presented with cryptorchidism **(Sultan, 2001)**. Among the factors responsible for the increase in male infertility, BPA have been studied extensively. It has been shown that BPA may affect many endpoints of male fertility. Several studies have focused on the effects of BPA, but the experimental data are rather controversial, and there in no general agreement about the effects of BPA on essential reproductive processes such as steroidogenesis and spermatogenesis, and sperm parameters (e.g., sperm mobility, viability, morphology) **(Wetherill et al., 2007)**. According to the previous study performed on rodents, it was confirmed that exposure to BPA decrease free plasma testosterone and 17β-estradiol level and BPA had also negative effect on histomorphology of testis or sperm parameters. There were also alterations in daily sperm production, suppression in testosterone and follicle-stimulating hormone production, interruption of the hypothalamic-pituitary-testicular axis and progressive apoptosis in sperm, Leydig and Sertoli cells **(Sakaue et al., 2001; Akingbemi et al., 2004; Li et al., 2009; Alves et al., 2013)**. Detrimental effects of BPA on the male reproductive system in experimental animal models have been confirmed by some studies conducted among groups of BPA exposed human males. Data from the study of **Xiao et al. (2009)**, when man workers were exposed to high levels of BPA, showed that BPA causes erectile and ejaculatory problems, reduction of sexual desire and affects sperm morphology. **Li et al. (2010)** also declare the negative effects of BPA on men working in epoxy resins manufacturing companies in China. Participants took a general health survey but were not told that the effects of BPA were the targets of the study. Exposed workers had significantly

lower sexual functions (orgasmic function, sexual desire) than non-exposed men in the study. Decreased sexual function was related to BPA exposure in dose-dependent manner and higher urinary BPA concentration was confirmed. However, men who were exposed environmentally but not occupationally, showed a weak but significant negative correlation in a few parameters. It indicates, that BPA exposure could reduce male sexual function in the general population. In a cross-sectional study with 215 healthy men, it was investigated that urinary BPA concentrations are associated with a reduction in Leydig cell function, increased serum luteinizing hormone levels and decreased sperm count **(Adoamnei et al., 2018)**. **Meeker et al. (2010)** tested sperm quality parameters of sub-fertile couples. When analyzing urine samples, there was a significant correlation with higher urinary BPA and lower sperm count, sperm morphology, and DNA damage. According to another prospective cohort study, **Goldstone et al. (2015)** investigated the relationship of urinary BPA concentrations and sperm parameters in 418 male partners of couples trying to become pregnant in Michigan and Texas. They found out that urinary BPA concentrations were associated with lower percentile of sperm DNA fragmentation and no association with semen parameters was observed. Using the FeTA system (Fetal Testis Assay**) Eladak et al. (2015)** confirmed that 1000 nmol/L BPA significantly reduced basal testosterone secretion by human testis. This method allows the precise study of the kinetics and duration of specific controlled concentrations of a given compound or mixture of compounds in a defined medium. They also evaluated the effect of BPA on LH-stimulated testosterone secretion. In the presence of 100 ng/mL LH, a concentration that induced the maximum steroidogenic response, the inhibitory effect of BPA in human testis was much less pronounced than in basal conditions. Indeed, only 10 000 nmol/L BPA significantly reduced testosterone secretion. In the next part of the study they found out that BPF and BPS reduced the basal testosterone production in cultured human testis and showed antiandrogenic effects already at 10 nmol/L.

6. Conclusion

Our review demonstrates that BPA and their analogues exhibit a range of toxic effects similar to those observed for BPA and that similar modes of action may be expected between BPA and other analogues. It is clear that BPA definitely affect reproductive processes in males and females with irreversible consequences for overall health. Some analogues exhibited toxic potencies similar to or even greater than that of BPA, raising safety concerns on their applications as BPA replacements. To date, toxicity studies remain remarkably limited in the determination of modes of action and quantitative toxic end points or benchmarks, both *in vitro* and *in vivo*, for a variety of analogues. Additional research is urgently needed to fill in knowledge gaps and deepen toxicity evaluations, given that the production and applications of bisphenol analogues are on the rise and that many of them have already been present in environmental compartments, foods and humans.

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Declaration of interest

The authors declare no conflicts of interest.

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Biogenic Amines as Risk Factors of Food Chain

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Abstract

Biogenic amines at higher doses have negative effects on the human organism. Some biogenic amines (e.g. putrescine, spermine, spermidine, cadaverine, histamine) are an essential component of living cells because they are involved in the regulation of nucleic acid and protein synthesis and membrane stabilization. Amines are produced by the decarboxylation of natural free amino acids. Decarboxylases are not common in bacteria but occur in species of many genera, particularly in *Bacillus, Citrobacter, Clostridium, Escherichia, Klebsiella, Photobacterium, Proteus, Pseudomonas, Salmonella, Shigella* and lactic bacteria of the *Lactobacillus genera, Pediococcus* and *Streptococcus*. Main factors influencing the biogenic amines formation are pH, water activity, storage time, temperature and salt content. Typical levels of biogenic amines in foods range from 10 mg/kg to 100 mg/kg. Occasionally, the amount of biogenic amines in food can exceed 1000 mg/kg.

1. Introduction

Biogenic amines (BA) are low molecular weight aliphatic organic compounds derived from amino acids that commonly participate in metabolic processes in living tissues with different biological, pharmacological and physiological effects. At higher doses they have negative effect on the human organism **(Komprda, 2005; Ordóñez *et al.*, 2016).**
Amines are produced by natural free amino acids decarboxylation by the action of living organisms, or amination and transamination of aldehydes and ketones are referred to as biogenic amines **(Kohajdová and Karovičová, 2001)**.
According to the chemical structure, biogenic amines are divided into aromatic (tyramine, phenylethylamine), heterocyclic (histamine, tryptamine), aliphatic (putrescine, cadaverine) and polyamines (spermidine, spermine, or agmatine). Diamines can be also classified as polyamines, while the heterocyclic amines are assigned to a group of aromatic amines **(Velíšek, 2002; Čuboň *et al.*, 2017)**.
Some biogenic amines (e.g. putrescine, spermine, spermidine, cadaverine, histamine) are an essential component of living cells because they are involved in the regulation of nucleic acid and protein synthesis and membrane stabilization **(Halász *et al.*, 1994)**.

1.1 Amine production in food

The basic condition for biogenic amines formation is the presence of free amino acids in substrate, presence of microorganisms with decarboxylase activity and conditions for growth and multiplication of microorganisms. The process of forming biogenic amines is catalysed by microbiological enzymes (carboxylase, transaminases). Formation of these substances proceeds from proteins through peptides to amino acids that are decarboxylated. Other enzymes such as oxygenase or methyltransferase may be applied to their transformation into other biologically active products **(Halász *et al.*, 1994)**.
Histamine is the product of histidine decarboxylation in the presence of histidine decarboxylase (Figure 1). From lysine, the carboxyl group is cleaved by lysine decarboxylase and amine cadaverine is formed. Putrescine may be created by several biochemical pathways. By decarboxylation of arginine by arginine decarboxylase, agmatine and then putrescine is produced. Putrescine can also be formed by direct decarboxylation of ornithine by ornithine decarboxylase. Putrescine can be methylated by S-adenosylmethionine produced spermidine and further spermine. The tryptophan decarboxylation product by tryptophan decarboxylase activity is tryptamine, and by tyrosine decarboxylase activity is produced tyramine. Decarboxylation of phenylalanine with phenylalanine decarboxylase gives 2-phenylethylamine **(Velíšek *et al.*, 1999; Buňka *et al.*, 2012; Pachlová *et al.*, 2015)**.
Some conditions must be met for the formation of biogenic amines in food. It is the amino acid content, the presence of microorganisms with decarboxylate activity and the appropriate conditions. Most commonly, biogenic amines are formed in fermentation processes **(Miliotis *et al.*, 2003; Buňková *et al.*, 2013)**.

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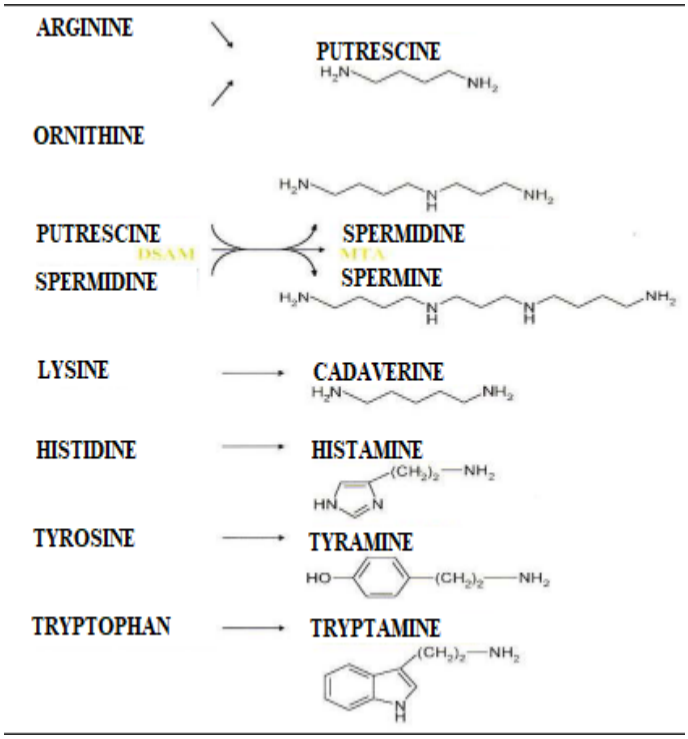

Figure 1. The formation of biogenic amines (according to http://www.vetweb.cz).

1.2 Factors influencing biogenic amines formation

Many factors influence the biogenic amines formation, such as pH, water activity, storage time, temperature and salt content **(Loizzo *et al.*, 2013)**. An optimal pH for decarboxylation of amino acids ranges from 2.5 to 6.5. Growth of bacteria in the acid foods stimulates the formation of decarboxylase enzymes.
Temperature is the most important factor that prevents the formation of biogenic amines. It significantly affects the enzymatic activity of microorganisms, and thus the formation of biogenic amines. Their production is related with temperature and storage time. Biogenic amines are thermostable, therefore heat treatment has a little influence on their contents **(Lorencová *et al.*, 2012; Benkerroum, 2016; Ladero *et al.*, 2017)**.
Salts generally have an inhibitory effect on biogenic amines formation. However, it depends on the salt mixture used. It was found that the addition of nitrite salt mixture decreases growth of biogenic amines more than the same amount of standard salt **(Buňková, 2010)**.
Of course, there are other factors influencing the formation of biogenic amines, such as starter cultures use, production hygiene, additives, and others.
Biogenic amines can also be produced by strains of LAB (lactic acid bacteria) which are commonly used for technological purposes (starter cultures) and therefore it is appropriate to test these strains for decarboxylase activity prior to use in the dairy industry. It would also be appropriate for the technological purposes to test the kinetics of biogenic amines production under similar environmental conditions that may occur during the technological process of producing fermented dairy products **(Pachlová et al., 2018).**

1.3 Microorganisms producing biogenic amines

Decarboxylases are not common in bacteria but occur in species of many genera **(Lorencová *et al.*, 2012)**, particularly in *Bacillus, Citrobacter, Clostridium, Escherichia, Klebsiella, Photobacterium, Proteus, Pseudomonas, Salmonella, Shigella* and lactic bacteria of *Lactobacillus* genera, *Pediococcus* and *Streptococcus*. Some bacteria producing biogenic amines are listed in Tab. 1 **(Kohajdová and Karovičová, 2001)**.
Another group of bacteria that is capable of producing larger amounts of biogenic amines is a group of enterococci. Enterococci have been isolated from foods that have caused poisoning by the action of biogenic amines (mainly tyramine) at high concentrations and are therefore considered to be the origin of their formation **(Suková, 2003)**.

Table 1. Some bacteria producing biogenic amines (according to Kohajdová and Karovičová, 2001)

Food	Bacteria	Produced BA
fish	*Morganella morganii, Klebsiella pneumonia, Hafnia alvei, Proteus mirabilis, P. vulgaris, Clostridium perfringens, Enterobacter aerogenes, Bacillus* spp., *Staphylococcus xylosus*	histamine, tyramine, cadaverine, putrescine, agmatine, spermidine, spermine
cheese	*Lactobacillus buchneri, L. bulgaricus, L. plantarum, L. casei, L. acidophilus, Streptococcus faecium, S. mitos ,Bacillus macerans, Propionibacterium* spp.	histamine, cadaverine, putrescine, tyramine, 2-phenylethylamine, tryptamine
meat and meat products	*Pediococcus, Lactobacillus, Pseudomonas, Streptococcus, Micrococcus, Enterobacteriaceae*	histamine, cadaverine, putrescine, tyramine, 2-phenylethylamine, tryptamine
fermented vegetables	*Lactobacillus plantarum, Leuconostoc mesenteroides, Pediococcus* spp.	histamine, cadaverine, putrescine, tyramine, tryptamine

1.4 Content of biogenic amines in cheese

Content of biogenic amines in certain foods may be very different. Typical levels of biogenic amines in foods range from 10 mg/kg to 100 mg/kg. Occasionally, the amount of biogenic amines in food can exceed 1000 mg/kg. It is very difficult to remove already formed biogenic amines from food. However, the most suitable way of producing food containing small amounts of biogenic amines is to adhere to such technological processes and hygienic conditions of production that prevent their creating **(Buňková, 2010)**.
Natural cheeses also belong to frequent sources of biogenic amines, in particular histamine, tyramine, putrescine and cadaverine. Starter cultures, non-starter lactic acid bacteria, or other spontaneous microflora may be the source of decarboxylase **(Halász et al., 1994; Buňková et al., 2013)**.
The BA (biogenic amines) concentration in fresh milk is less than 1 mg/kg. These are histamine and tyramine primarily. The histamine content in milk is 0.5 to 0.8 mg/kg, the histamine content in dried milk is 131 mg/kg, tyramine content 42 mg/kg. The BA content in cheese may be higher than 10 g/kg **(Greif and Greifová, 2006)**.
In cheese production technology, BA can be formed in several technological operations. The quality of milk determines their presence in the cheese. Milk with a high content of decarboxylation bacteria contains a larger amount of BA. This can be avoided by heat treatment of milk.

During cheese maturation, the proteins are enzymatically degraded to free amino acids. These may be precursors to the formation of BA. Current technologies use starter cultures that do not produce BA. Non-starter bacterial contaminants are a potential risk for their formation. These are for example, heterofermentative lactobacilli, *Enterobacteriaceae, Hafnia alvei* and others **(Benkerroum, 2016; Ladero *et al.*, 2017)**. During maturation process the BA content of all cheeses increases. Their kinetics also depend on the type of cheese and the technology used. Hard cheeses contain less BA than soft cheeses.
Fresh unfermented cheeses also undergo proteolysis during storage. The production of BA is affected mainly by content of salt, protein and pH value. The content of different BA in cheese is different, each cheese has a characteristic spectrum of BA. The most occurring BA are tyramine (up to 146 mg/kg) and histamine up to 85 mg/kg. Tryptamine, phenylethylamine, putrescine, cadaverine, spermine, spermidine, adrenaline and noradrenaline were also identified in the cheeses **(Kolesarova, 1995)**.

1.5 Effects of biogenic amines

Biogenic amines are natural antinutrients and are important in food hygiene. They have been indicated as one of the causes of many food poisons. They are able to initiate various undesirable biochemical reactions. Analyses of biogenic amines are particularly important for their use as indicators of freshness degree or food degradation **(Önal, 2007).**
Symptoms of high dose consumption of biogenic amines are vomiting, breathing difficulties, sweating, heart failure, hypo- or hypertension (histamine), and migraine (phenylethylamine, tyramine).
The major enzymes that degrade biogenic amines are monoamine oxidase and diaminooxidase. The activity of these enzymes is strongly influenced by the toxic effect of biogenic amines **(Velíšek, 2002).**
In practice, all biogenic amines are not specified, but mostly histamine is determined and limited in food. Histamine values at which signs of poisoning begin to show are above 100 mg per 100 g of food. It should be noted that there is an individual sensitivity to biogenic amines, other factors such as the amount of food consumed, the presence of other toxic substances, and so on. Therefore, it is very difficult to determine the level of toxicity of biogenic amines.
The food legislative of the Slovak Republic sets maximum limits for two biogenic amines. These are histamine (20 mg/kg in beer and 200 mg/kg in fish and fish products) and tyramine (200 mg/kg in hard cheeses).

1.6 Histamine and its effects

Under normal conditions, histamine that gets into the human gut is inactivated and does not produce any clinical signs of the disease. When large amounts of histamine are ingested, the inactivation mechanisms are broken down and histamine goes out of the digestive tract. There are two major enzymes known to metabolize histamine. It is histaminase and histamine-N-methyltransferase. The presence of other biogenic amines or the use of some drugs may inhibit the effect of these enzymes and potentiate the effect of biogenic amines. The effect of consumed biogenic amines is determined by their quantity but also by other factors **(Maintz and Novak, 2007; Buňková, 2010).**
Some people are hypersensitive to biogenic amines and have histamine intolerance. Histamine intolerance is manifested in individuals lacking the diaminoxidase enzyme which degrades histamine and other biogenic amines. Poisoning occurs within a few minutes to three hours after ingestion of a contaminated diet. Symptoms include headache, nausea, stomach cramps, skin reddening, feeling sick, breathing difficulties, blood flow and seizures, feelings of hot flushes, general discomfort **(Buňková, 2010; Alvarez and Moreno-Arribas, 2014)**.

1.7 Tyramine and its effects

Tyramine is a local tissue hormone and acts as a dopamine precursor. It causes severe headaches accompanied by frequent vomiting and elevated temperature. It rapidly increases blood pressure and acts irritant to the smooth muscles **(Velíšek *et al.*, 1999; Alvarez and Moreno-Arribas, 2014)**.

1.8 Putrescine and its effects

Putrescine is formed by the decarboxylation of the amino acid lysine or the amino acid ornithine. It also occur during the proteolysis of meat. It is therefore a degradation product of proteins and its toxic effects are almost the same as for ammonia. This effect is cumulative with other amines and ptomaine. The major functions of putrescine include stabilizing macromolecules (nucleic acids), subcellular structures (ribosomes), and stimulating cell differentiation. Putrescine synergistically enhances the effect of histamine and tyramine **(Buňková, 2010; Ruiz-Capillas and Herrero, 2019).**

1.9 Cadaverine and its effects

Similarly to putrescine, cadaverine originates in decarboxylation of the amino acids lysine and ornithine, and by meat degradation. Its toxic effects are similar to those of ammonia. Cadaverine belongs to the group of polyamines (like spermine, spermidine and putrescine). Their biological functions include participation in cell growth and proliferation. Polyamines are also considered as potential precursors of carcinogenic N-nitrosocompounds and aromatic heterocycles **(Buňková, 2010; Ruiz-Capillas and Herrero, 2019)**.

2. Conclusion

Biogenic amines are low molecular weight aliphatic organic compounds derived from amino acids that are commonly involved in metabolic processes in living tissues and exhibit different biological, pharmacological and physiological effects.
Amines are produced by the decarboxylation of natural amino acids by the action of living organisms. At higher doses they have a negative effect on the human organism. Some biogenic amines are an essential component of living cells because they are involved in the regulation of nucleic acid and protein synthesis as well as in membrane stabilization.
The content of biogenic amines in food can vary greatly. Commonly, biogenic amines in food are in tens of milligrams per kilogram. Values in hundreds of mg/kg are considered to be high.
Under normal circumstances, biogenic amines in the digestive system are inactivated and there are no clinical signs of the disease. In the case of large intake of biogenic amines the inactivation mechanisms are broken, they get out of the digestive system. The presence of higher levels of multiple biogenic amines or the use of certain drugs may inhibit the effect of enzymes and potentiate the effect of biogenic amines. The effect of biogenic amines is determined by their quantity but also by other factors. Under normal conditions, with common consumption of foods, the concentration of biogenic amines is low and does not result into allergic reactions.

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Declaration of interest

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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