TECHNOLOGICA ACTA

JOURNAL OF SCIENCE PROFESSIONAL FROM CHEMISTRY AND TECHNOLOGY - FACULTY OF TECHNOLOGY TUZLA

ISSN 1840-0426
ISSN 2232-7568

Vol. 9
Number 2,
page 1-74,
Tuzla,december 2016. year

TECHNOLOGICA ACTA

JOURNAL OF SCIENCE-PROFESSIONAL FROM CHEMISTRY AND TECHNOLOGY - FACULTY OF TECHNOLOGY TUZLA

Vol. 9 Number 2, page 1 - 74, Tuzla, december 2016. year

TECHNOLOGICA ACTA

JOURNAL OF SCIENCE-PROFESSIONAL FROM CHEMISTRY AND TECHNOLOGY FACULTY OF TECHNOLOGY TUZLA

Vol. 9 Number 2, page 1 – 74, Tuzla, december 2016. year

Publisher / Izdavač
Faculty of Technology University in Tuzla

Reader / Lektor
Milja Joguncić, teacher of english language

Technical Editor / Tehnički urednik
«OFF-SET» Tuzla

Printing / Štampa
«OFF-SET» Tuzla

Journal prints two times of year

Technologica Acta is indexed in the following database: CAB Abstracts, COBISS, Index Copernicus Journal Master List, EBSCO

This number of Technologica acta is supported by the Federal Ministry of Education, Science and Culture of Bosnia and Herzegovina

Edition / Tiraž: 150

Editorial Office / Uredništvo

Secretary / Sekretar: Nermina Jahić
Faculty of Technology, University in Tuzla
Univerzitetska 8, 75000 TUZLA
Tel/fax: +387 35 320 740

CONTENT

DETERMINATION OF BENZENE CONCENTRATIONS AS AN INDICATOR OF AIR QUALITY NEAR COKE PLANTS ARCELLOR MITTAL ZENICA AND GLOBAL ISPAT COKE INDUSTRY LUKAVAC

ORIGINAL SCIENTIC PAPER

A. Đozić[1], V. Selimbašić[1], V. Stuhli[1], M. Zohorović[1], N. Alić[2]

[1] *Faculty of Technology, University of Tuzla, 75000 Tuzla, Bosnia and Herzegovina*
[2] Institute for quality, standardization and ecology 75300 Lukavac, *Bosnia and Herzegovina*

ABSTRACT

Determinations of immission concentration of benzene were carried out at 4 sites in area near the coke plant in Arcellor Mittal Zenica (AMZ) and 4 sites in the industrial complex of Global Ispat Coke Industry Lukavac (GIKIL). Measurements were performed in AMZ in order to determine the air quality and to determine a possible cause of the high concentration of benzene at the automatic measuring station in Tetovo (AMS Tetovo), in GIKIL to determine the possible source of emissions and to compare the results. To measure the concentration of benzene GASMET DX4030 portable gas analyzer was used with a Fourier Transform Infrared (FTIR) spectrometer, the measurement range of 0,01 ppm to 200 ppm. Positions of sampling sites were selected in relation to the nearest populated area, and based on the wind rose. On location AMZ at the sampling site SZ1 an average concentration of benzene was 44,47 $\mu g/m^3$ and the maximum measured 139,2 $\mu g/m^3$. The sampling site SZ2 was the nearest to the coke plant, the average benzene concentration was 117,28 $\mu g/m^3$ and the maximum measured 243,60 $\mu g/m^3$. At the sampling site SZ3 the average concentration of benzene was 99,69 $\mu g/m^3$ and the maximum 313,20 $\mu g/m^3$. The sampling site SZ4 was located north-east of the coke plant, the average measured concentration of benzene was 108,13 $\mu g/m^3$ while the highest concentration was 278,40 $\mu g/m^3$. On location GIKIL, at the sampling site SL4 the highest concentration of benzene of 5000 $\mu g/m^3$ and the lowest of 1450,10 $\mu g/m^3$ at the SL4 were measured. At the AMS Tetovo, in the period of measurement, the average benzene concentration was 1,62 $\mu g/m^3$, and the highest value was 2,50 $\mu g/m^3$. On the basis of the measurements it can be concluded that air quality in the immediate vicinity of the coke plant does not meet clean air quality with regard to the content of benzene.

Keywords: air quality, immission, benzene, coke ovens, wind rose

INTRODUCTION

Air pollution is a major environmental health risk; low air quality reduces human life expectancy by more than eight months on average and by more than two years in the most polluted cities and regions[1]. Benzene is a natural component in fossil fuels and its emission is associated with the course of natural and anthropogenic activities such as combustion of coal and petroleum products. As an unavoidable component of unleaded gasoline, benzene is added in concentration of 1-2% by volume for its octane-enhancing and anti-knock properties and 80–85 % of benzene emissions are due to vehicular traffic in Europe[2,3]. In air, benzene exists predominately in the vapour phase, with residence times varying between a few hours and a few days, depending on the environment, the climate and the concentration of other pollutants[4]. Benzene as a volatile organic compound (VOC) can have serious adverse effects on human health[5]. The primary use of benzene today in Bosnia and Herzegovina in GIKIL Lukavac is in the manufacture of maleic anhydride. In Europe, benzene is mainly used in the manufacture of organic chemicals such as phenol, aniline, cyclohexane and alkyl benzenes. Exposure to benzene can be divided into two groups occupational and non-occupational. Occupational exposure occurs via dermal or inhalation absorption of solvents contained in paint, rubber and lacquer. Workers such as taxi and bus drivers are often exposed to benzene from exhaust gases and petrol stations. The primary source of exposure to benzene for the general population are ambient air containing tobacco smoke, air contaminated with benzene (for example, in areas with heavy traffic around gasoline filling-stations, drinking contaminated water, or eating contaminated food)[6]. Acute (short term) inhalation exposure of humans to benzene may cause drowsiness, dizziness, headaches as well as eye, skin and respiratory tract irritation and, at high levels, unconsciousness[7]. Chronic (long term) inhalation exposure has caused various disorders in the blood, including reduced numbers of red blood cells and aplastic anemia, in occupational settings[7]. Increased incidences of leukaemia have been observed in humans occupationally exposed to benzene, although this level of exposure is unlikely in ambient air[7]. Ambient (outdoor air pollution) in cities and rural areas was estimated to cause 3,7 million premature deaths worldwide per year in 2012; this mortality is due to exposure to small particulate matter of 10 microns or less in diameter (PM_{10}), which cause cardiovascular and respiratory diseases, and cancers[8]. Benzene is recognised as a carcinogenic pollutant. The most significant adverse effects from prolonged exposure are haematotoxicity, genotoxicity and carcinogenicity. Chronic exposure to benzene can depress bone marrow and cause haematological effects such as decreased red and white blood cell counts[3]. Also, benzene has an acute toxic effect on aquatic organisms, it bioaccumulates in invertebrates. High concentration in air can damage leaves of agricultural crops and lead to death in plants[3]. In many developed countries monitoring networks that measure concentrations of VOC have been established, these networks consist of fixed site monitoring stations[9]. In the Federation of Bosnia and Herzegovina stations for benzene monitoring are installed in Zenica at three sites. Outdoor concentrations of benzene were estimated by one monitoring campaign (March 30, 2015) at four sampling sites near the coke plant Arcellor Mittal Zenica and results were compared with the data obtained from the monitoring stations. The measurement was also performed as one monitoring campaign (August 8, 2015) at four sampling sites near the coke plant GIKIL Lukavac. The monitoring of benzene near sources is an important part in protecting human health.

MATERIALS AND METHODS

Study area: Measurements of benzene concentration were performed near the coke plants in the industrial areas of Zenica and Lukavac. These towns are the largest industrial areas in Bosnia and Herzegovina. The town of Zenica has a population of 13000 inhabitants, and has the largest iron-stell plant in the region with a coal carbonization department that yields metallurgical coke for use in other departments like an iron-making blast furnace. Lukavac is a town with 15000 inhabitants and numerous industrial plants, such as the cement and soda factory and the large plant for coke production GIKIL Lukavac. Coke production technology used in Arcellor Mittal Zenica and GIKIL Lukavac is from slot-type by product coke ovens. In coke ovens coal is heated to 1300 ^0C in the absence of air to distill out tars and light oils. At high temperature a gaseous by-product occurs, as coke oven gas, together with sulphur compounds, water, ammonia and many other components removed thermally from the coal.

Sampling sites and methodology: The direct measurement was carried out at four different sampling sites around the coke plant in Arcellor Mittal Zenica and with the same number of sampling sites around the coke plant in GIKIL Lukavac. In all sampling sites the sampler was placed at typical heights of breathing of an adult 1,5 m. The temperature, relative humidity, atmospheric pressure, wind speed and direction recordings during sampling operations, data for meteorology parameters were obtained from the Federal Hydrometeorology Agency. Meteorology parameters for Lukavac were obtained from an automatic measuring station of air quality located about 3150 m east from the coke plant GIKIL. The sampling sites in Zenica were marked as SZ1, SZ2, SZ3 and SZ4, and in Lukavac SL1, SL2, SL3 and SL4. The first sampling site (SZ1) was located at the southwest from the coke plant at the distance of approximately 240 m. The sampling site (SZ2) was the nearest to the coke plant at the distance of about 190 m, SZ3 was at distance of 350 m north from the coke plant. The fourth sampling site (SZ4) was situated about 290 m southeast. The first sampling site in GIKIL (SL1) was located at the southwest from the coke plant at the distance of about 190 m. The sampling site (SL2) was the nearest to the coke plant at the distance of about 130 m, SL3 was at distance of 170 m east from the coke plant. The fourth sampling site (SL4) was situated about 300 m southeast. The sampling sites were chosen according to the wind rose and the nearest settlement to the factories Arcellor Mittal and GIKIL; the locations are marked and shown in Fig. 1 and 2. The Gasmet DX-4030 was used to analyze benzene in all the investigated sites. The Gasmet DX – 4030 analyzer module houses a Fourier Transform Infrared (FTIR) spectrometer, Rhodium-Gold coated sample cell and signal processing electronics. The sample gas is extracted into the sample cell via a probe with built in a particle filter. The instrument is designed for on-site measurements of different compounds (both organic and inorganic) at low concentrations in ambient air. Measurement range for benzene is 0,01 to 200 ppm. Duration of measurement at each sampling site was one hour.

Figure 1. Locations of sampling site in Arcellor Mittal Zenica

Figure 2. Locations of sampling site in GIKIL Lukavac

RESULTS AND DISCUSSION

The coke oven is a major source of fugitive emissions into the air, the coking process emits sulphur oxides (SOx), nitrogen oxides (NOx), volatile organic compounds (non-methane VOC and methane CH_4), carbon dioxide (CO_2), carbon monoxide (CO), ammonia (NH_3), particulate matter, heavy metals and polycyclic aromatic hydrocarbons (PAH)[10]. The amount of such fugitive emissions depends on numerous factors such as the design, age and condition of the coke battery and the operating and maintenance practices employed[11]. Coke oven emissions are a yellowish-brown gas which contains literally several thousand compounds, several of which are known carcinogens and/or cocarcinogens (including polycyclic organic matter from coal tar pitch volatiles, beta-naphthylamine, benzene, arsenic, beryllium, cadmium, chromate, lead, nickelsubsulfide, nitric oxide and sulphur oxide)[12]. Imission concentration were highest in breathing zones lidman (3220 $\mu g/m^3$), tar chaser (3140 $\mu g/m^3$), larry-car operator (3050 $\mu g/m^3$), and were lowest for pusher-machine operator (390 $\mu g/m^3$)[13]. About 60% of total coke oven emissions occur during charging, 30% during pushing and 10% during quenching of coke[14]. The average concentrations of benzene at sampling sites in AMZ and GIKIL are reported in Table 1. The meteorology data obtained from the nearest AMS Tetovo (about 600 m) and AMZ Lukavac (about 3150 m) are presented in the same table as well. The highest concentration of benzene was observed at SZ2 (117,28 $\mu g/m^3$), this site was the nearest to the coke plant, and there are not any buildings between SZ1 and the coke oven plant. The lowest concentration of benzene in AMZ was at SZ1 (44,47 $\mu g/m^3$), this site was nearest to the torch where generated coke gas is combusted. There is no significant difference in measured concentrations between sampling sites SZ3 (99,69 $\mu g/m^3$) and SZ4 (108,13 $\mu g/m^3$). A slightly higher concentration at SZ4 is probably due to less air flow between buildings, reservoirs and other industrial infrastructural objects. The concentrations of benzene in GIKIL sampling sites were much higher than in AMZ. The highest concentration of benzene was recorded at site SL1 (5000 μg/m^3), this site was next to the aeration-flocculation wastewater basin, which is located within the biological wastewater treatment plant as its first step. The wastewater basin is used for aeration and flocculation of wastewater and to adjust temperature, pH value and O_2 content concentration to the optimal level. For the purpose of O_2 content basin is occupied with an air chamber where air flows through the pipeline at the bottom of basin. The volume of basin is 50 m^3, depth 3,4 m, level of water in the basin is 2,56 m. Considering that SL1 was the second furthest site away from the coke plant, and that other sites which were closer to the coke plant had the lowest concentrations, it can be concluded that this basin of raw wastewater is the source of fugitive emission of benzene; this result can be explained by the fact that the wastewater contained dissolved benzene and that in the process of aeration at an elevated temperature (about 50 ^{0}C) benzene evaporated in the air. It has a relatively low boiling point (80.1 ^{o}C) and a high vapour pressure (9.95kPa at 20 ^{o}C), causing it to evaporate rapidly even at room temperature. At site SL2, near an electrostatic precipitator which is used for additional tar removal from coke oven gas after its passing spray towers, the concentration of benzene was measured (2450,15 μg/m^3). For this site, it can be concluded that other sources of benzene emissions exist around the electrostatic precipitator, because it is unlikely that only one source - the coke battery which is 130 m away - contributed to the content of benzene at SL2 site. The average concentration of benzene at SL3 was (450,10 μg/m^3), this is the lowest concentration of benzene recorded during campaign, the site was 170 m away from the coke oven battery opposite to the nearest inhabited place. The content of (2650,00 μg/m^3) benzene in air was recorded at SL4. This site was next to the spray tower where coke is drenched with water and cooled for transport and size screening. This water contains suspended particles of coke which settled in the basin near the tower. Significantly higher concentrations of benzene in GIKIL Lukavac are results of fugitive emissions from other departments in GIKIL where benzene is produced as the raw material for other process such as production of maleic anhydride. The compiled part of GIKIL is a department called condensation, where benzene from coke oven gas mixture is recovered in the liquid state, stored in reservoirs and with pumps sent through pipelines to other departments for further processing. The highest concentration of the benzene at sampling sites GIKIL can be associated with the fact that coke oven battery in GIKIL consists of 100 ovens in regard to AMZ where 65 ovens are installed. In AMZ there is not benzene recovery from coke oven gas. The lowest content of benzene in air at AMZ can be explained by the presence of OH^- radicals in atmosphere where benzene can be removed by OH^- radicals; this is the main removal mechanism of atmospheric higher molecular weight aromatic compounds. The National Institute for Occupational Safety and Health (NIOSH) recommends that benzene be controlled and handled as a potential human carcinogen in the workplace and that exposure be reduced to the lowest feasible limit. The NIOSH recommended exposure limit (REL) is 320 μg/m^3 as an 8-hour time-weighted average (TWA) is 3200 μg/m^3. According to directive 2004/37/EC-carcinogens or mutagens at work, the limit value for occupational exposure is 3250 μg/m^3, this limit value was exceeded at site SL1. Average concentration of the benzene at AMS Tetovo during measuring campaign was 1,62 μg/m^3. In Zenica there are three automatic measuring stations: stations AMS Centar, AMS Radakovo and AMS Tetovo. The AMS Tetovo is about 200 m away from the first industrial objects in AMZ and about 780 m away from the coke oven battery. Its location is in the backyard of the family home, about 50 m away from the nearest road. The AMS Centar is located on the roof of the shopping mall (about 2170 m away from the coke oven battery) at the height of about 15 m, which is not the standard for AMS. The AMS Radakovo is also located on the roof of the elementary school (about 4200 m away from the coke oven battery) at the height of about 5 m, and 30 m away from a road. In this study, data of benzene concentrations from AMS Centar, AMS Radakovo and AMS Tetovo are presented (Fig.3, Fig.4, Fig.5, Fig.6, Fig.7, Fig.8, Fig.9 and Fig.10) for the period from March 23, 2015 to March 31, 2015. In this period the highest benzene levels were recorded which caused anxiety among the general public in Zenica. The increase of benzene was recorded at 11 a.m. on March 24, 2015 at the AMS Tetovo, 61,4 µg/m^3. In the same period at other two AMS the concentration of benzene was significantly lowest, 1,23 µg/m^3 and 1,01 µg/m^3. It can be concluded that the increase of benzene level in Tetovo is not accompanied by the increase in Centar and Radakovo. The highest concentration of benzene was recorded between 10 a.m. and 16 p.m., on March 25 2015 at AMS Tetovo, 104,78 µg/m^3. In this case the concentration of benzene at the other AMS showed correlation with AMS Tetovo. This fact can be associated with the increase of traffic and industrial emissions. On March 26 2015 the maximum concentration was observed at 2 a.m., 37,99 µg/m^3 at AMS Tetovo, between 5 a.m. and 8 a.m. there were no data from the AMS Tetovo and between 1 a.m. and 8 a.m. from AMS Radakovo. For this period there was no significant correlation between metering stations until 5 p.m. From March 27 to March 31 2015 there was no significant increase of benzene concentration and there were some correlations between measured levels of benzene in all three AMS.

Table 1. Average concentrations of benzene (µg/m^3) and meteorological data

	Benzene		Meteorology parameters				
Sites	Average	Average*	Temperature, ^{0}C	Atmospheric pressure, hPa	Relative humidity, %	Wind speed, m/s	Wind direction,^{0}
SZ1	44,47	1,23	11,09	971,67	60,12	1,59	147,28
SZ2	117,28	1,83	13,27	970,13	53,25	2,13	143,71
SZ3	99,69	2,02	15,61	968,09	47,10	2,56	137,30
SZ4	108,13	1,42	17,84	965,94	41,35	3,19	149,10
SL1	5000,00	ND	28,21	989,47	45,30	0,91	66,20
SL2	2450,15	ND	29,50	987,65	43,72	1,12	70,52
SL3	2650,28	ND	30,62	988,17	40,30	1,13	75,30
SL4	1450,10	ND	31,27	986,22	39,59	0,95	78,45

*data obtained from automatic measuring station Tetovo
ND – no available data

Table 2. Linear correlation coefficients between average concentrations of benzene and meteorological data in AMZ

	Average SZ1-SZ4	Average AMS-Tetovo	Temperature, ^{0}C	Atmospheric pressure, hPa	Relative humidity, %	Wind speed, m/s	Wind direction,^{0}
Average SZ1-SZ4	1						
Average AMS-Tetovo	0,664	1					
Temperature, ^{0}C	0,675	0,267	1				
Atmospheric pressure, hPa	-0,632	-0,205	-0,998	1			
Relative humidity, %	-0,708	-0,307	-0,999	0,994	1		
Wind speed, m/s	0,687	0,225	0,997	-0,996	-0,995	1	
Wind direction,^{0}	-0,260	-0,890	-0,026	-0,026	0,055	0,040	1

Table 3. Linear correlation coefficients between average concentrations of benzene and meteorological data in GIKIL

	Average SL1-SL4	Temperature, ^{0}C	Atmospheric pressure, hPa	Relative humidity, %	Wind speed, m/s	Wind direction,^{0}
Average SL1-SL4	1					
Temperature, ^{0}C	-0,914	1				
Atmospheric pressure, hPa	0,953	-0,861	1			
Relative humidity, %	0,820	-0,981	0,760	1		
Wind speed, m/s	-0,393	0,282	-0,108	-0,232	1	
Wind direction,^{0}	-0,893	0,997	-0,859	-0,986	0,208	1

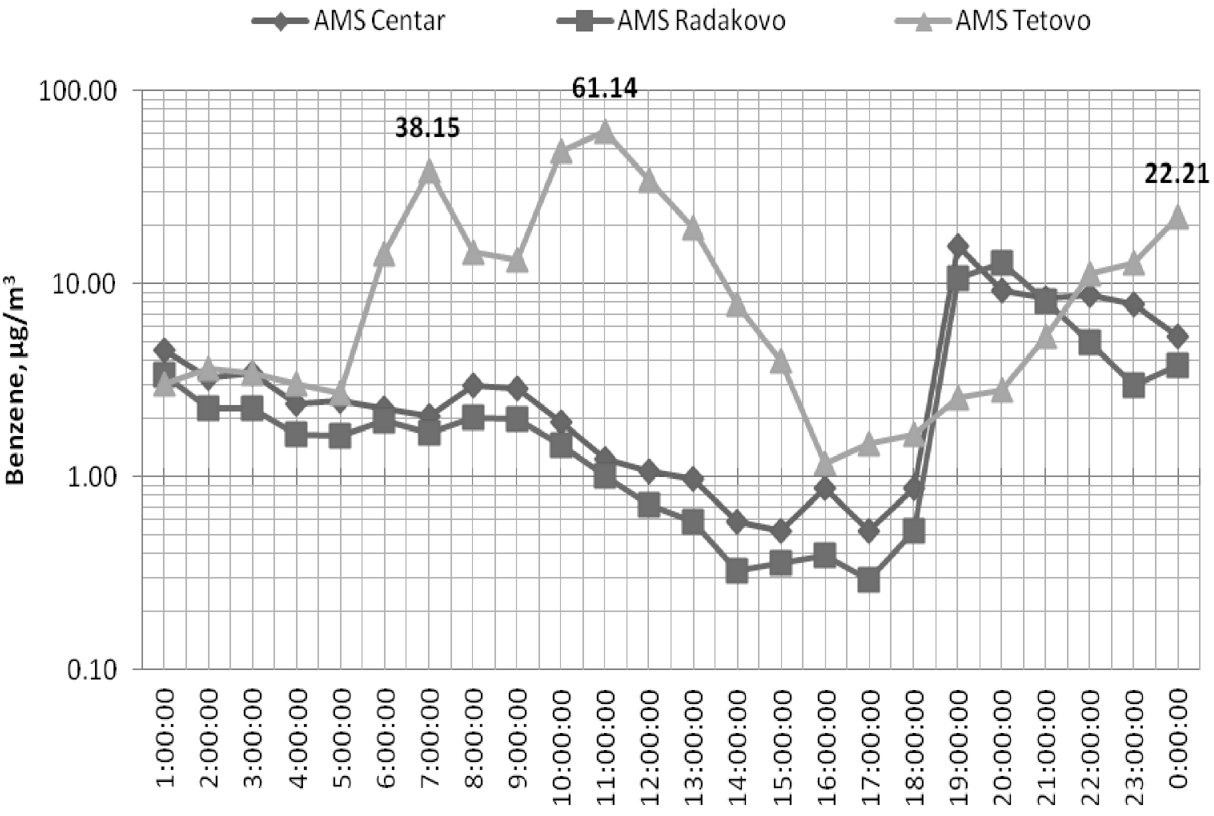


Figure 3. Benzene concentration levels, 24.03.2015.

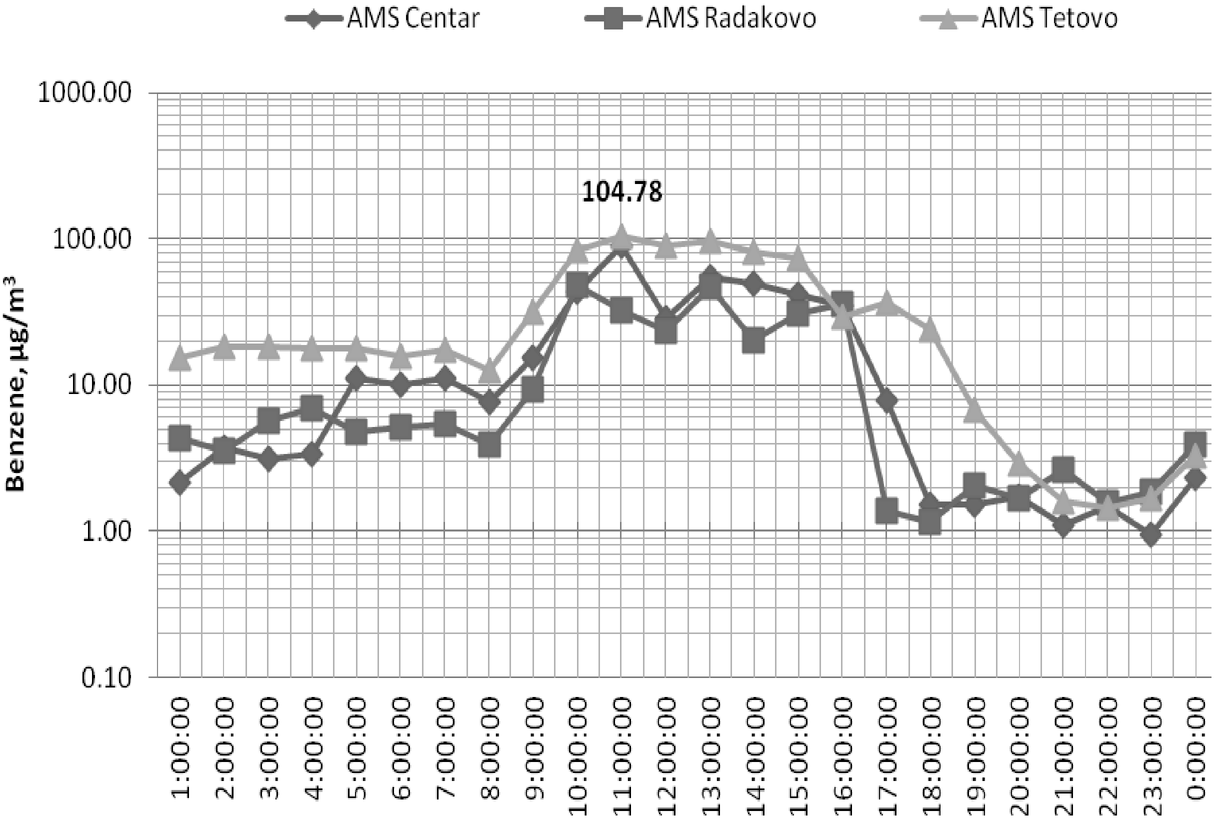


Figure 4. Benzene concentration levels, 25.03.2015.

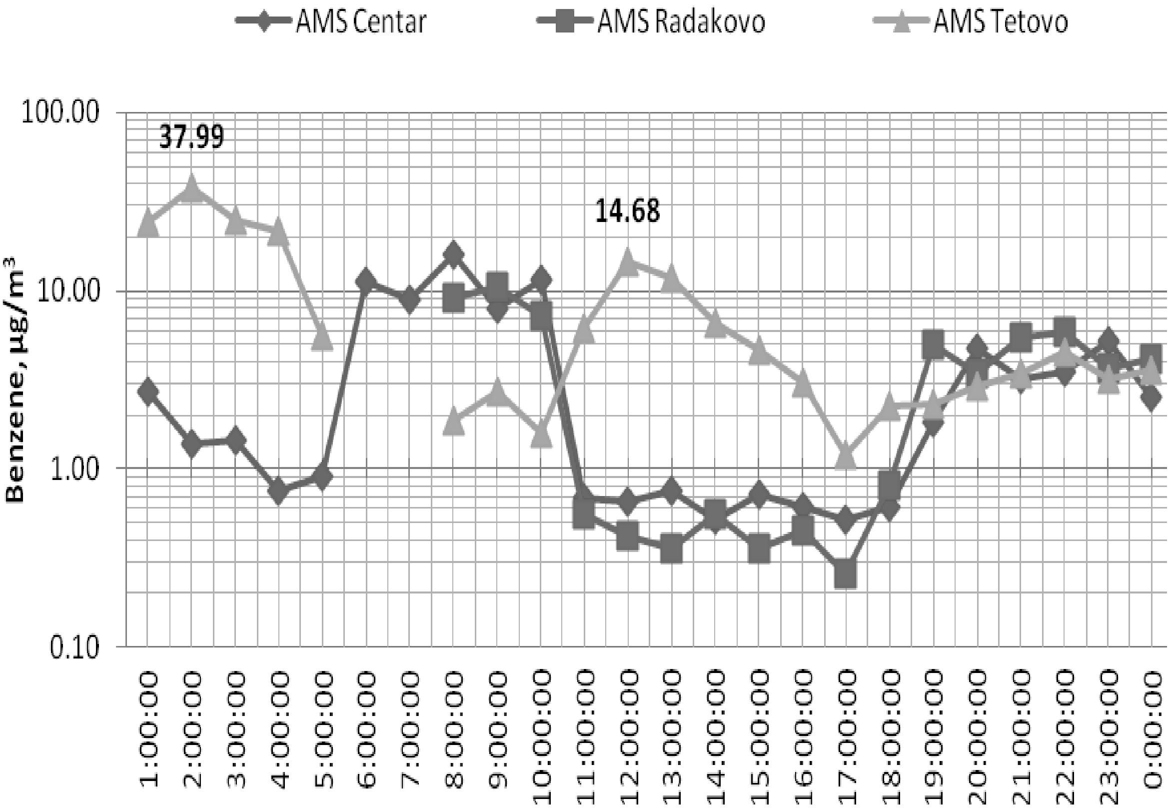


Figure 5. Benzene concentration levels, 26.03.2015.

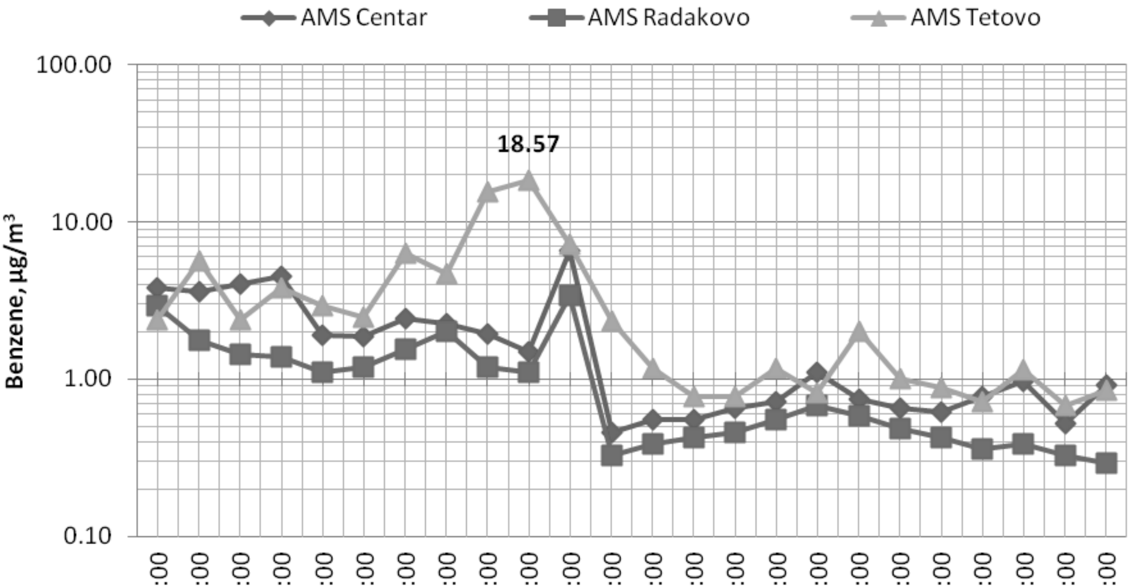


Figure 6. Benzene concentration levels, 27.03.2015.

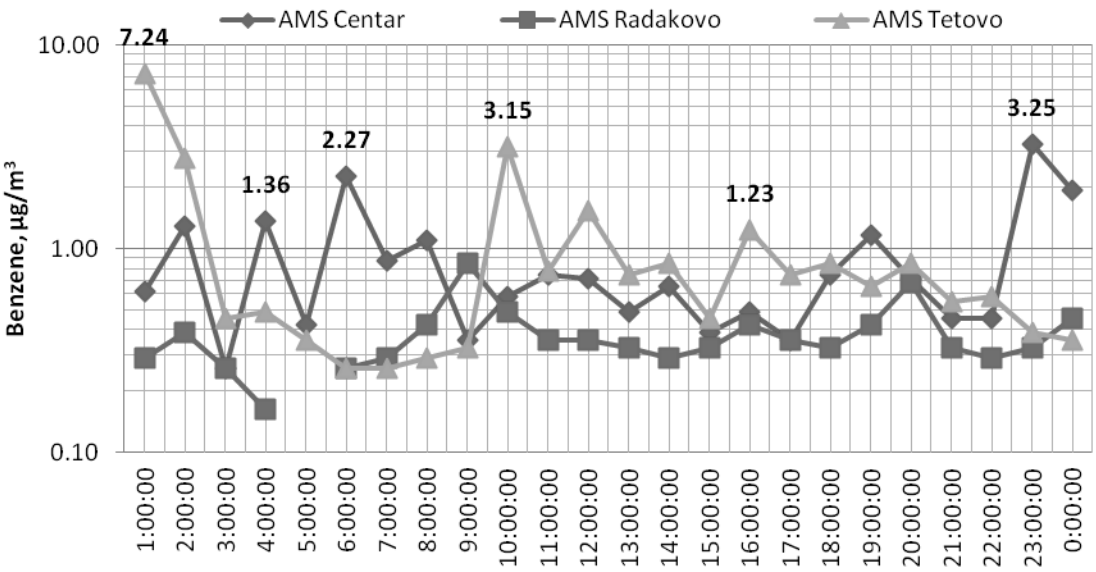


Figure 7. Benzene concentration levels, 28.03.2015.

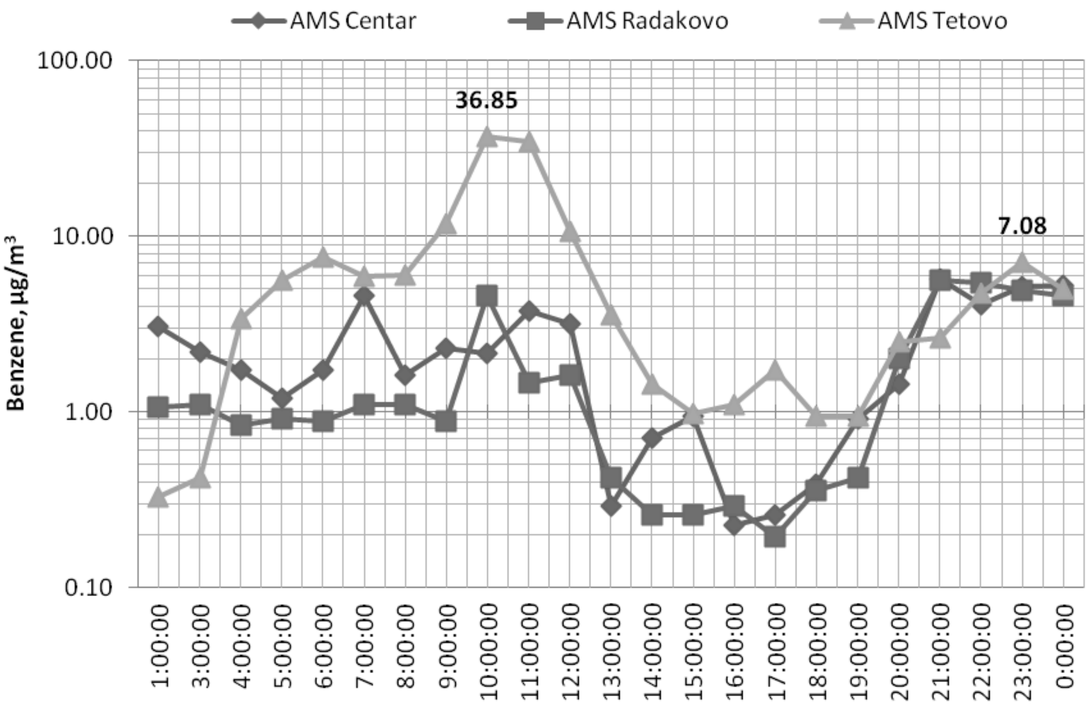


Figure 8. Benzene concentration levels, 29.03.2015.

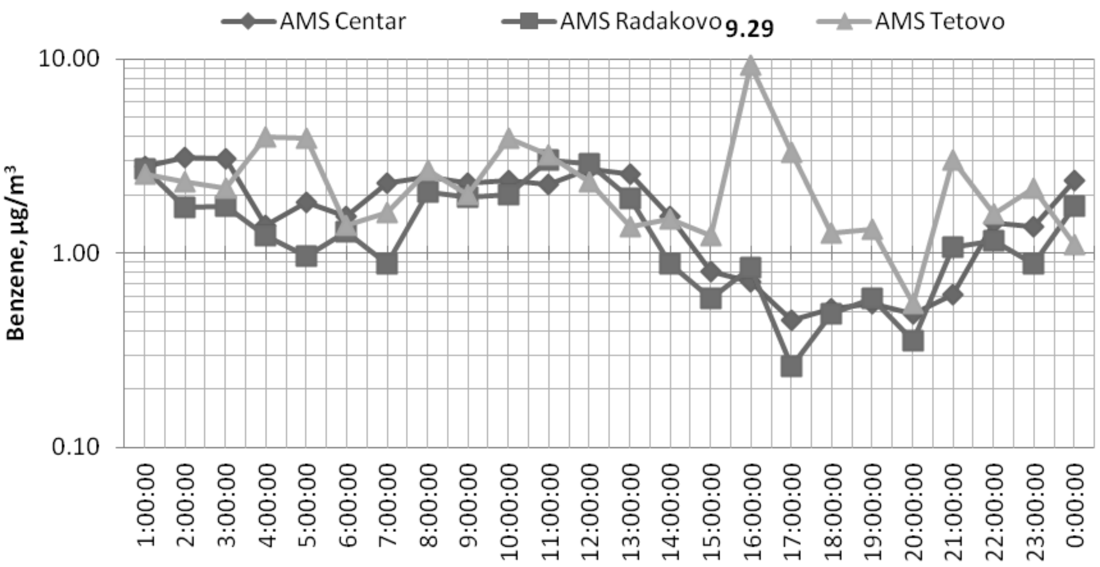


Figure 9. Benzene concentration levels, 30.03.2015.

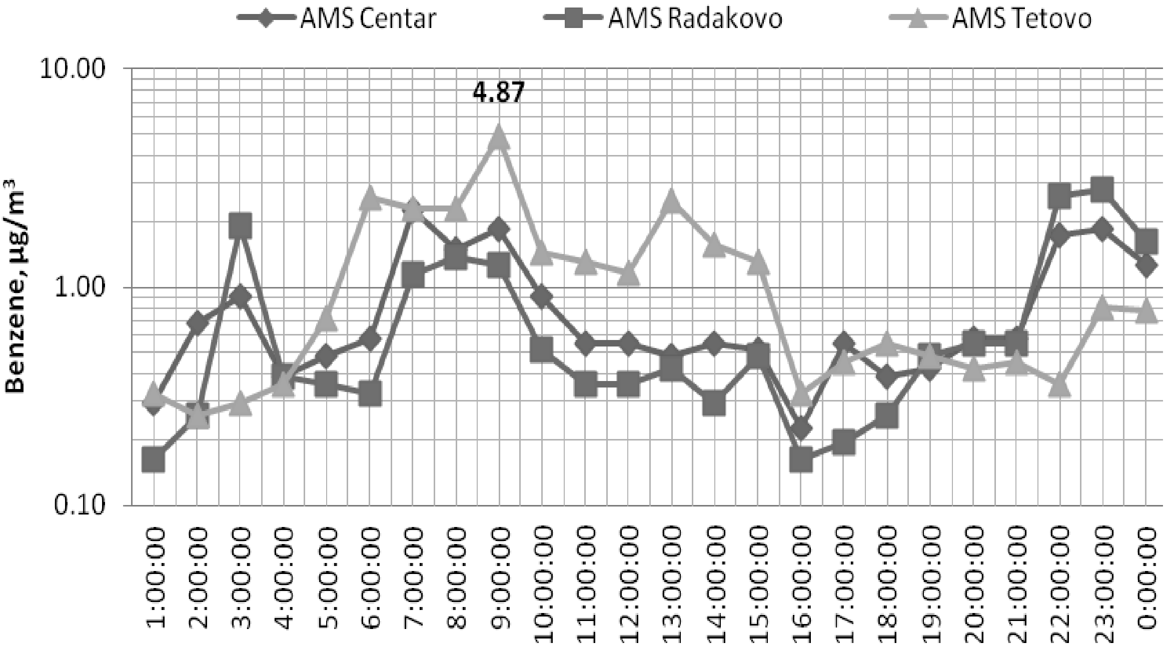


Figure 10. Benzene concentration levels, 31.03.2015.

Table 4. Benzene concentrations in ambient air of various cities worldwide,

Cities	Benzene, µg/m^3
Zenica*	1,62
Zenica**	8,90
Algiers[16]	1,94
Rome[17]	35,5
Berlin[18]	6,90
London[19]	2,70
Athens[20]	13,3-26,0

*AMS Tetovo, March 31 2015 (11 a.m. to 15 a.m.)
** From 24 to 31 March 2015

A benzene level in urban area without industry is associated with traffic intensity, solar radiation, relative humidity, air temperature, wind speed and direction. On 1 January 2000 the EU limit for benzene in petrol reduced from 5% to 1% and the average content of benzene in petrol in the EU is 0,7%. This has significantly reduced the levels of benzene across Europe. Although in the period from March 24 to 27 2015 the relatively high concentrations of benzene were observed, its average value at AMS Tetovo was 8,90 µg/m^3 which is in the range of ambient air quality of various cities (Table 4). For the purpose of comparison and interpretation of obtained results we have taken into account the indoor benzene concentrations. The main pathway of human exposure to benzene is inhalation; therefore, the benzene concentration in indoor air is an important source in determination of its impact on general public. Indoor concentrations of benzene are normally higher than those in outdoor air as a consequence of the existence in many indoor sources, infiltration and accumulation of benzene present in outdoor air. Viewed across published studies, indoor concentrations of benzene ranged from 0,6 to 3,4 (arithmetic mean 1,8) times than the outdoor concentrations and are greatly influenced by those outdoors[20]. This occurs in part because there are numerous indoor sources of benzene and because the relatively low rates of ventilation typically used in residences and offices prevent the rapid dispersal of airborne contaminants[21]. Environmental tobacco smoke (ETS) is considered one of the main indoor sources of benzene[20]. Benzene emissions from cigarette smoking range from 430 to 590 µg per cigarette[22]. Studies in humans showed that 50% of the quantity of inhaled benzene is absorbed[23,24]. The benzene concentration in the blood of 14 smokers was significantly higher (median 493 ng/l) than that in 13 non-smokers (median 190 ng/l)[25]. Indoor benzene levels measured in the United States showed arithmetic values of 5,54–10,5 µg/m^3 in homes exposed to ETS compared to 3,86–7,0 µg/m^3 in ETS-free homes[26,27]. A similar situation was reported in Italy, with levels of 32,2 and 18,9 µg/m^3 in ETS and ETS-free homes, respectively and in Germany, with levels of 11,0 and 6,5 µg/m^3, respectively[28,29]. Many published studies showed increasing indoor concentrations in European cities from north to south. The lowest indoor concentrations (2 µg/m^3) were measured in Finnish homes, while they ranged from 2 to12 µg/m^3 in central European cities and from 10 to 13 µg/m^3 in southern cities such as Milan and Athens[30-38]. Average concentration of benzene in children's bedrooms determined by passive sampling in households in Germany showed an average concentration 9,5 µg/m^3 compared to 1.8 µg/m^3 in outdoor air[39]. Studies of benzene concentrations in the interior of vehicles while driving have shown values of 10–120 µg/m^3 in Germany, 37–57 µg/m^3 in Sweden, 30–115 µg/m^3 in the Netherlands, and mean values of 12–50 µg/m^3 in the USA[40]. According to the results reported in Table 2 the correlation coefficients (R^2) between benzene concentrations levels at AMS Tetovo and sampling sites (SZ1-SZ2), temperature and wind speed are high. The results reported in Table 3 show that temperature, wind speed and direction have no effect on the measured benzene concentration at sampling sites (SL1-SL4).

CONCLUSION

The benzene concentration levels were measured near two coke oven battery in the industrial area of Zenica and Lukavac as two largest industrial zones in Bosnia and Herzegovina. Measurements were performed in two campaigns in spring for AMZ and summer for GIKIL. In both cases sampling sites were located around the coke oven battery. With respect to air quality standard the limit value in regard with EC legislation as an annual average value of 5 μg/m^3 exceeds at all measurements sites. Measured benzene concentrations near the coke oven batteries in AMZ and GIKIL can affect the assessment of the impact of air quality in Zenica and Lukavac.

Average measured benzene concentrations at sampling sites in AMS Tetovo are in the range of similar ones in most European cities. At one sampling site in GIKIL the concentration of benzene exceeds the limit values established by NIOSH which can lead to the increase of occupational exposure at workplace. At this regard, further investigations are necessary to estimate levels of benzene, toluene, ethylbenzene and *o,m.p* xylene.

References

1. European Commission (EC), Press release IP/10/687, 2010.
2. Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Benzene. Atlanta, (2007) pp. 438.
3. European Environment Agency (EEA), Air Quality in Europe-2011. EEA Technical Report No. 12/2011. pp. 65.
4. World Health Organisation (WHO), Air Quality for Europe. 2nd Edn. (2000) ISBN: 92 890 13583. pp. 62.
5. K. Victorin, Health effects of urban air pollutants, guideline values and conditions in Sweden, Chemosphere, 27 (1993) 1691-1706.
6. International Agency for Research on Cancer (IARC), A review of human carcinogens. 100F (2012) pp. 255.
7. Air Quality in Ireland 2013, Key Indicators of Ambient Air Quality. Environmental Protection Agency (2014) pp. 22.
8. World Health Organisation (WHO), Ambient (outdoor) air quality and health available online at http://www.who.int/mediacentre/factsheets/fs313/en/
9. International Agency for Research on Cancer (IARC), Coke production. Monographs 100F (2012) pp. 174. available online at http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-18.pdf
10. European Environmental Agency (EEA), EMEP/CORINAIR Emission Inventory Guidebook (2006) pp.B146-4
11. J.D. Graham, D.R. Holtgrave, Coke Oven Emissions: A Case Study of Technology-Based Regulation. available online at http://ipmall.info/risk/vol1/summer/graham.htm
12. U.S. Environmental Protection Agency (USEPA), Carcinogen Assesment of Coke Oven Emissions, (1984) Final Report of OHEA.
13. National Toxicology Program (NTP) Report on Carcinogens, 13th Edition. (2014) U.S. Department of Health and Human Services. available online at http://ntp.niehs.nih.gov/pubhealth/roc/roc13/
14. D. Kaegi, V. Addes, H. Valia, M. Grant, Coal conversion processes carbonization. In Kirk-Othmar Encyclopedia of Chemical Technology. (2000) pp.1-17.
15. H.B. Singh, J.L. Salas, B.K. Cantrell, and R.M. Redmond. Distribution of aromatic hydrocarbons in the ambient air. Atmos. Environ. 19 (1985) 1911–1919.
16. D. Brocco, R. Fratarcangeli, R. Petricca, I. Ventrone. Determination of aromatic hydrocarbons in

urban air of Rome. 31 (1997) 557-566.

17. A. Monod, B.C. Sive, P. Avino, T. Chen, D.R. Blake, F.S. Rowland. Monoaromatic compounds in ambient air of various cities: A focus on correlations between the xyylenes and ethylbenzene. Atmos. Environ. 35 (2001) 135-149.

18. C. Chatzis, E.C. Alexopoulos, A. Linos. Indoor and outdoor personal exposure to benzene in Athens, Greece. Sci. Total Environ. 349 (2005) 72-80.

19. S. Kirchner. National dwellings survey: report on air quality in French dwellings, Final Report. Paris, Indoor Air Quality Observatory, (2006).

20. World Health Organisation (WHO), Guidelines for Indoor Air Quality: selected pollutants (2010) pp. 19.

21. A.T. Hodgson, H. Levin. Volatile organic compounds in indoor air: a rewiev of concentrations measured in North America since 1990. Lawrence Berkeley National Laboratory, (2003).

22. B.C. Singer, A.T. Hodgson, W.W. Nazaroff. Gas-phase organics in environmental tobacco smoke: 2. Exposure-relevant emission factors and indirect exposures from habitual smoking. Atmospheric Environment, 37 (2003) 5551.

23. K. Nomiyama, H. Nomiyama, Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Internationales Archiv fur Arbeitsmedizin. 32 (1974) 75–83.

24. K. Pekari. Biological monitoring of occupational exposure to low levels of benzene. Scandinavian Journal of Work, Environment & Health, 18 (1992) 317–322.

25. H. Hajimiragha. Levels of benzene and other volatile aromatic compounds in the blood of non-smokers and smokers. International Archives of Occupational and Environmental Health, 61 (1989) 513–518.

26. D.L. Heavner, W.T. Morgan, M.W. Ogden. Determination of volatile organic-compounds and ETS apportionment in 49 Homes. Environment International, 21 (1995) 3–21.

27. L.A. Wallace. Major sources of benzene exposure. Environmental Health Perspectives, 82 (1989) 165–169.

28. H. Fromme. Gesundheitliche Bedeutung der verkehrsbedingten Benzolbelastung der allgemeinen Bevolkerung. Zentralblatt fur Hygiene. 196 (1995) 481–494.

29. P. Carrer et al. Assessment through environmental and biological measurements of total daily exposure to volatile organic compounds of office workers in Milan, Italy. Indoor Air,10 (2000) 258–268.

30. R.D. Edwards. VOC concentrations measured in personal samples and residential indoor, outdoor and workplace microenvironments in EXPOLIS, Helsinki, Finland. Atmospheric Environment, 35 (2001) 4531–4543.

31. E. Ilgen. Aromatic hydrocarbons in the atmospheric environment. Part II: univariate and multivariate analysis and case studies of indoor concentrations. Atmospheric Environment, 35 (2001) 1253–1264.

32. P. Schneider . Indoor and outdoor BTX levels in German cities. Science of the Total Environment, 267 (2001) 41–51.

33. M.J. Jantunen. Air pollution exposure in European cities: the "EXPOLIS" study. Journal of Exposure Analysis and Environmental Epidemiology, 8 (1998) 495–518.

34. V. Cocheo. Urban benzene and population exposure. Nature, 404 (2000) 141–142.

35. P.H. Fischer. Traffic-related differences in outdoor and indoor concentrations of particles and volatile organic compounds in Amsterdam. Atmospheric Environment, 34 (2000) 3713–3722.

36. R.M. Harrison Measurement and modeling of exposure to selected air toxics for health effects studies and verification by biomarkers. Boston, Health Effects Institute, (2009).

37. L. Mosqueron, V. Nedellec. Inventaire des donnees francaises sur la qualite de l'air a l'interieur des batiments: actualisation des donnees sur la periode 2001–2004. Lyon, Observatoire de la Qualite de l'Air Interieur, 2004 (available online at http://www.airinterieur.org/userdata/documents/Document_16.pdf.

38. Critical appraisal of the setting and implementation of indoor exposure limits in the EU. Brussels, European Commission, Joint Research Centre, 2005.

39. F. Porstmann. Benzene and toluene in children's rooms. Staub-Reinhaltung der Luft, 54 (1994) 147–153.

40. H. Fromme. The significance of traffic-related benzene exposure for the general public. Zentralblatt für Hygiene, 196 (1995) 481–494

BIOACCUMULATION OF METALS FROM THE ENVIRONMENT AS A FACTOR OF FOOD SAFETY

PROFESSIONAL PAPER

Z. Knezović[1*], A. Stipišić[1], M. Trgo[2], D. Sutlović[3,4]

[1]Public Health Institute of Split Dalmatian County, Vukovarska 44, 21000 Split, Croatia
[2] Faculty of Chemistry and Technology, University of Split, Teslina 10/V, 21000 Split, Croatia
[3] Department of Pathology and Forensic Medicine, University Hospital Centre Split, Spinčićeva 1, 21 000 Split
[4]Department of Forensic Medicine, University of Split School Medicine, 21000 Split, Croatia

ABSTRACT

Results of numerous studies show the constant presence of heavy metals in the environment. Metals are present in the air, water and soil due to natural processes, but mostly as a result of human activities such as industrial and agricultural processes.

Fruits and vegetables accumulate soil metals during growth while different animal species bioaccumulate metals either from their environment or through food. Since metals are not biodegradable they can accumulate towards the top of the food chain presenting a risk to human health.

This paper presents lead, cadmium and mercury concentrations in food sampled in the area of Split-Dalmatia County in the period 2001 - 2013. The study included food that is most prevalent in the daily diet such as fruits, vegetables, grains, milk, meat, fish and their products. Concentrations above LOD were determined in 83% of analyzed samples for lead,78% of analyzed samples for cadmium and 61% of analyzed samples for mercury. In certain food groups, more than 30% of the samples contained 75% of the maximum allowed concentration.

Therefore, simultaneously with measures for reducing emissions of heavy metals into the environment, it is necessary to conduct constant monitoring of heavy metals presence in food which is an important route of human exposure to metals.

Keywords:metal pollution, bioaccumulation, environment, food

INTRODUCTION

Metals are ubiquitous in the biosphere. They are an integral part of the earth’s crust, ore and geological strata discharged intotheenvironment from natural sources such as volcanic eruptions, forest fires, rocks and soil leaching. More significant contribution are different human activities, such as coal burning power plants, industrial plants, foundries, smelters, car exhaust, uncontrolled disposal of solid waste, industrial and municipal waste waters,[1,2].

Metals are redistributed in the environment through geological and biological cycles participating in biochemical exchanges between the environment and living beings. They are not biodegradable, and most of them are bioaccumulative. They accumulate in plants and animals and thus enter into the food chain affecting humans as well as other living organisms [3].

Air inhalation and ingestion of contaminated water and foodare the most important route of human exposure to metals.Considering that children are more sensitive to absorption and to the toxic effects of some metals, food safety must be given special attention [4].Lead, cadmium and mercury are metals whose presence in the environment is of particular concern, because of their adverse effects. The main sources of lead emissions are coal combustion, transport, industrial production of steel, iron, ferrous metals and cement [5,6]. The chronic lead exposure is associated with deficiencies in mental development and occurrence of mental illness of children. Cadmium is released into the environment from industrial production of non-ferrous metals, iron,steeland cement.Waste incineration plants as well as the application of phosphate fertilizerscan also be significant sources of itsemissions [7]. Cadmium is primarily toxic to the liver and kidneys, but according to recent studiesitincreasesarisk of cancer;interferes with calcium metabolism in bone formation leading to softening and deformation of bones [3].Mercury,considered as one of the most toxic pollutants, is widespread throughout the environment as elemental, inorganic and organic mercury. All three forms are toxic to living organisms. In addition to substantialnatural sources of mercury emissions into the environment, 5207 t/year, anthropogenic sources of mercury are combustion of fossil-fuel in power plants, industrial and municipals sewage treatments, ferrous and non-ferrous metals manufacturing facilities, caustic soda and vinyl chloride production plants, ore processing facilities, incinerators for urban, medical and industrial wastes, cement plants and chemicals production facilities [8,9].Since in the organic form passes the brain barrier, a primary toxic effectofmercuryis on the nervous system. The chronic exposure to low doses of mercury is associated with the development of chronic neurodegenerative diseases.

Considering that oral ingestion is the principal pathof human exposure to lead, cadmium and mercury,the aim of this study was to investigate the content of these metals in types of food that are most prevalent in the daily diet of inhabitants in Split-Dalmatia County.

MATERIALS AND METHODS

Samples

Food samples most commonly used in the diet of the local population were sampled in the area of Split-Dalmatia County in period 2001 -2013. The total number of samples 3528,were colected in local markets as well as in small stores and supermarkets, regardless of the origin of the sample, i.e. imports or domestic production. The lead, cadmium and total mercury content were determined in 3497, 3528 and 1605 samplesrespectively. The samples were analyzed individually and the results were classified into major groups; fruit and fruit products, vegetables and products, cereals and cereal products, milk and dairy products, fish and fish products, meat and products.

Methods

For the purpose of lead and cadmium determination,the samples were digested with concentrated HNO_3/HCl and H_2O_2mixture in an automated microwave digestion unit, (CEM Mars 5).

Quantitative determination of lead and cadmium were carried out on a graphite furnace atomic absorption spectrometer (AAS Vario6, Analytik Jena). Measurements were performed with the hollow cathode lamps 283,3 for lead and 228,8 nm for cadmium, with deuterium background correction. Graphite furnace with pin platform and palladium modifier were used for interferences reduction.

Determination of the total mercury (THg) concentrations was performed on Advanced Mercury Analyser AMA 254 (Altec, Prague, Czech Republic),directly from the homogenized sample, without previous preparation.

The limits of the detection (LOD)were calculated from the standard deviations of the blanks and were 1 mg L^{-1} for lead and cadmium and 0,0005mg kg^{-1} for mercury.

The accuracy of measurements was examined with known concentrations of standard solutions, which were run as a sample.

RESULTS AND DISCUSSION

The results for each metal are sorted into six groups by type of food and depending on vegetable or animal origin. The experimental results of lead, cadmium and mercury concentrations expressed by the mean, median, standard deviation (SD), and range (minimum, maximum) are shown in Table 1.

Table 1. Presentation of the experimental results for Pb, Cd and THg determination in food samples.

Type of food Metal		Fruit & products	Vegetables, mushrooms & products	Cereals & products	Milk & products	Fish, shellfish, cephalopods & products	Meat & products
Pb (mg kg^{-1})	No. of samples	636	1374	462	288	415	323
	Mean	0,054	0,079	0,063	0,072	0,337	0,108
	SD	0,090	0,114	0,089	0,115	0,358	0,121
	Median	0,019	0,034	0,027	0,027	0,228	0,069
	Min	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Max	0,500	0,707	0,400	0,500	1,98	0,500
Cd (mg kg^{-1})	No. of samples	629	1381	463	284	435	336
	Mean	0,009	0,017	0,022	0,010	0,062	0,017
	SD	0,015	0,030	0,023	0,014	0,085	0,022
	Median	0,003	0,009	0,015	0,005	0,040	0,010
	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Max	0,184	0,479	0,159	0,075	1,00	0,147
Hg (mg kg^{-1})	No. of samples	306	425	105	65	567	138
	Mean	0,002	0,003	0,001	0,000	0,193	0,002
	SD	0,005	0,010	0,003	0,002	0,241	0,006
	Median	0,001	< LOD	< LOD	< LOD	0,115	< LOD
	Min	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Max	0,050	0,164	0,017	0,017	1,5	0,050

The regulations regarding maximum allowable concentrations (MAC) of heavy metals in food in Croatia werechanged several times during the examined period. However, the individual results were interpreted in relation to the regulations that were applicableat the time of analysis [10,11,12,13]. The concentration of all heavy metals in all analyzed samples wasunder MAC.

Each group was further divided into three subgroups. The samples in which metal content wasbelow the limit of detection were in the first subgroup. The second subgroup was related to a number of samples where the heavy metal content was between LOD and 75% of MAC., while the third subgroup included samples containing more than 75% of MAC. (Fig.1).

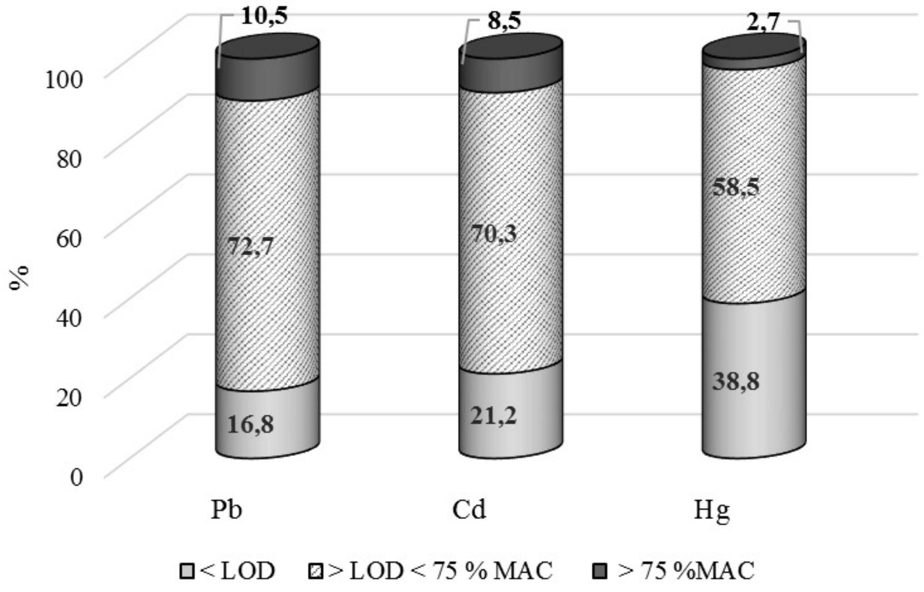
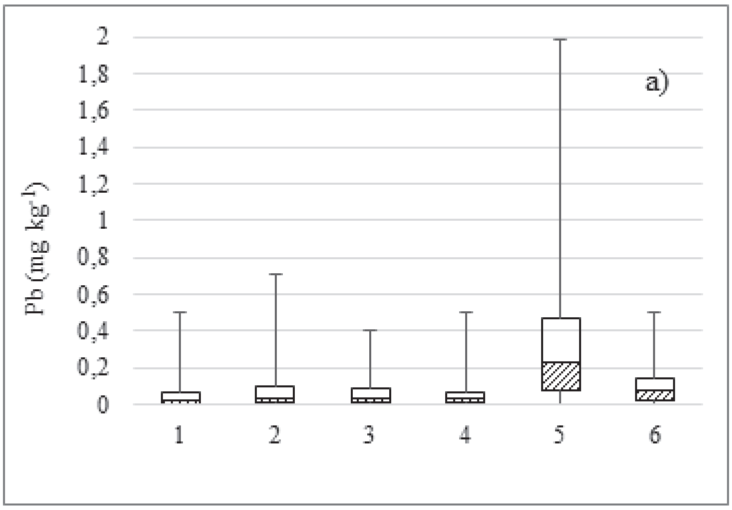


Figure 1. Concentrations of lead, cadmium and mercury inall analysed samples in relation to the maximum allowable values

Statistical analysis confirmed that the concentrations of lead, cadmium and THg were above LOD inthemajority of analyzedsamples,more precisely, lead was found in 83,2%, cadmium in 78,8%, and mercury in 61,2% of all analyzedsamples. Furthermore, concentrations above 75% of MAC were determined in 10,5%, 8,5% and 2,7% of all samples for lead, cadmium and THgrespectively. In Fig. 2 the distribution of lead, cadmium and mercury within each food groupis displayed.



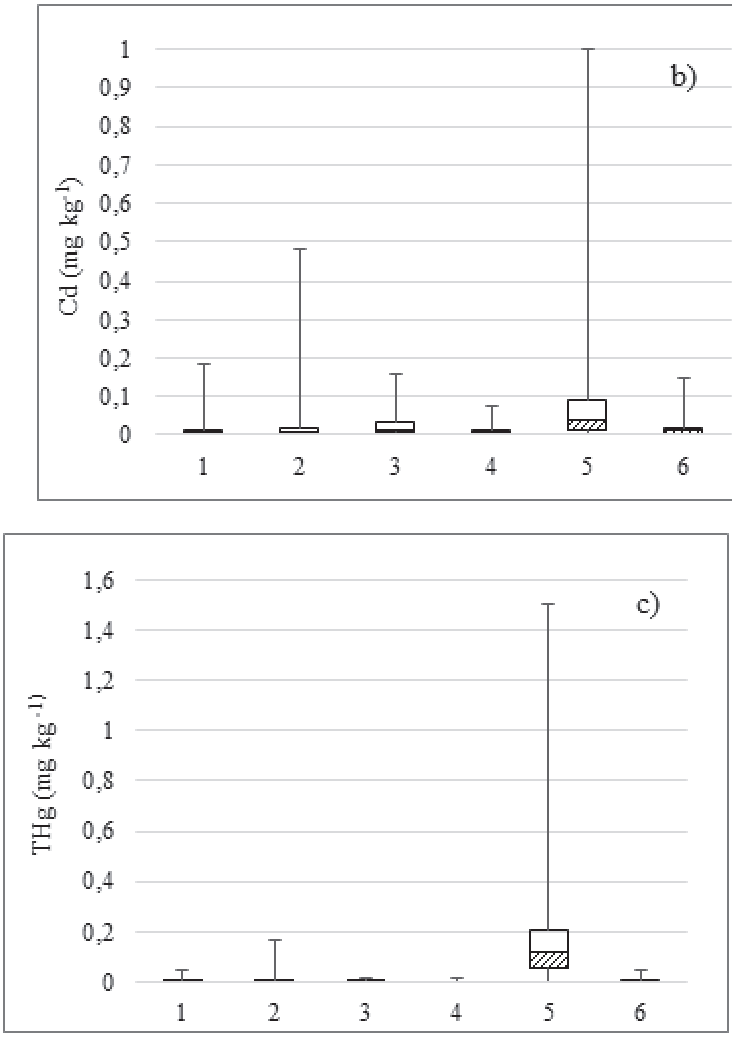


Figure.2. Concentrations of metal in certain food; a) lead, b) cadmium, c) total mercury; (1=fruit and products, 2=vegetables, mushrooms and products, 3= cereals and products,4=milk and products, 5=fish and products, 6=meat and products).

Lead wasfoundin a great number of analyzed samples, more accurately, in all food groups lead was above LOD in 80% samples. The main sources were fish, meat and vegetables with group median values 0,228; 0,069 and 0,034 mgkg^{-1}respectively.For the cadmium, the main contributors were fish and cereals (mean concentrations 0,040 and 0,015 mgkg$^{-1)}$. Fish and product were the main source of mercury (mean concentration 0,115 mg kg $^{-1}$) as well as lead and cadmium. Aquatic environment, especially marine, due to its large surface is the largest recipient of pollutants from the environment coming from wet and dry deposition as well as industrial and municipal wastewater. Living organismsbioaccumulate metals fromthe aquatic environmentand thus they enter the food chain. The results of our study are consistent with results of similar studiesindicating that the food is one of the important routes of human exposure to metals [14,15].

CONCLUSION

In most samples, the content of lead, cadmium and total mercury was above the LOD affirming that people are exposed to a constant intake of low concentrations of metals through food. From our results it is evident that fish and fish products contribute significantly to the body burden with lead, cadmium as well as mercuryconsidering the dietary patterns of local inhabitants where fish and fish products are frequently consumed foods. In all samples concentrations were below MACand in most samplesmay be evaluated as low-content. Nevertheless,it is necessary to emphasize that the study covered foods consumed on a daily basis, and thatinover 80% of analyzedsamples content metals was above LOD.Therefore,it is advisable to implement monitoring systems to track temporal trends in human exposures to metals through food consumption.

References

1. P.C. Naagajyoti, K.D. Lee, T.V.M. Sreekanth, Environ. Chem. Lett. 8 (2010) 199.

2. UNEP, van der Voet E., Salminen R., Eckelman M., Mudd G., Norgate T. Hischier R., (2013): Environmental Risks and Challenges of Anthropogenic Metals Flows and Cycles, A Report of the Working Group on the Global Metal Flows to the International Resource Panel.

3. A. R. Goyer, T. W. Clarkson, Toxic effects of metals, in: C.D. Klaasen (ed.), Toxicology - The basic science of poisons, McGraw-Hill, 2001, pp. 812-815.

4. W.S. Beckett, G. F. Nordberg, T.W. Clarkson, Routes of Exposure, Dose, and metabolism of Metals, in: G. F. Nordberg, B. Afowler, M. Nordberg, L. Friberg (ed.), Handbook on the Toxicology of Metals, 3rd, Academic Press, 2007, pp. 41-45.

5. J. Liang, J. Mao, Environ. Sci. Pollut. Res. Int. 22 (2015) 7129.

6. G. Oudijk, Environ. Forensics. 11 (2010) 14.

7.UNEP Chemicals Branch, DTIE**.**Fnal review of scientific information on cadmium. 2010.

8. N. Pirrone, S. Cinnirella, X. Feng, R.B. Finkelman, H.R. Friedli, J. Leaner, R. Mason, A.B. Mukherjee, G.B. Stracher, D.G. Streets, K.Telmer, Atmos. Chem. Phys. 10 (2010) 5951.

9. E. G. Pacyna, J.M. Pacyna, K. Sundseth, J. Munthe, K. Kindbom, S. Wilson, F. Steenhuisen, P. Maxson, Atmos. Environ. 44 (2010) 2487.

10. Amendments to the Regulation on the amount of pesticides, toxins, mycotoxins, metals and histamine and similar substances that may Occur in Food, and the other conditions regarding the health safety of food and general use (Official Gazette 011/2001).

11. Regulation amending the Regulation on quantities of pesticides, toxins, mycotoxins, metals, histamine and similar substances that may be found in food, and other conditions regarding the health safety of food and general use (Official Gazette 039/2003).

12. Regulations on toxins, metals, metalloids and other harmful substances in foodstuffs (Official Gazette 016/2005.

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

14. I. Martorelli, G. Perello, R. Marti-Cid, J. M. Llobet, V. Castell, J. L. Domingo, Biol. Trace Elem. Res. 142 (2011) 309.

15.G. Falco**,** J. M. Llobet, A. Bocio, J. L. Domingo,J. Agric. Food Chem.54 (2006) 6106.

EVALUATION OF WATER MANAGEMENT, SOCIETY AND SUSTAINABILITY FOCUSING ON SAMSUN CITY/TURKEY

PROFESSIONAL PAPER

H. Büyükgüngör

Ondokuz Mayis University, Environmental Engineering Department 55200 Samsun/Turkey E-mail: hbuyukg@omu.edu.tr

ABSTRACT

Sustainable Water Management (SWM) attempts to deal with water in a holistic fashion, taking into account the various sectors affecting water use, including political, economic, social, technological and environmental considerations. Where sustainability is meeting the needs of the present generation without compromising the ability of future generations to meet their needs. Samsun is a big densely populated city situated in the middle of the Black Sea region with a population of one million 250 thousand which generate a lot of domestic and industrial wastewater. Reuse of wastewater to irrigate crops is not a recent practice in Turkey though this practice has been applied for many years in mostly the rural regions of the country, where municipal sewer systems do not exist. There is a need of development of well-planned integrated urban water management system that will be able to identify criteria for decentralized and centralized system components which can accelerate the implementation of such systems. Present study is the feature of evaluation for sustainable development of Samsun city wastewater as an aspect of wastewater project. So far, it is observed that direct application of treated effluent on agricultural land is the mostly a viable, convenient and indirect use of treated effluent in agriculture.

Keywords: Sustainability, water management, grey water, reuse, irrigation, wastewater treatment plants

INTRODUCTION

Water is an essential resource for all life on the planet. Much effort in water resource management is directed at optimizing the use of water and in minimizing the environmental impact of water use on the natural environment.

For sustainability, potential options are first screened based on social acceptability to identify both implementation issues and system features that must be developed to enable social institutions. Second, systems are screened based on affordability, evaluated in comparison to the most expensive water supply and wastewater management options currently in use within the existing urban area. Then, a complete sustainability analysis can be completed using multi-criteria analysis for the viable options [1, 2].

The population of coastal areas with significant tourism activities has increased even faster than other areas. Same is the case with Samsun where two of the major rivers of Turkey, the Kızılırmak and the Yeşilırmak flow through the borders of this city and finally reach the Black Sea. Also the Abdal, Mert, Kürtün and Engiz rivers, run through Samsun city and reach the Black Sea. Where the Abdal and Kürtün rivers are contaminated by industrial waste water, the others are exposed to pollution that comes from domestic and industrial wastewater. Besides these problems, Samsun city is also exposed to other environmental problems like industrialization with the intense population growth, intense agricultural activity involving use of fertilizer, insecticide and herbicide, the rapid growth of the population, urbanization without conforming to city plans that requires waste water treatment on large scale.

Water security has been defined as “the reliable availability of an acceptable quantity and quality of water for health, livelihoods and production, coupled with an acceptable level of water-related risks.” Sustainable development will not be achieved without a water secure world. On the other hand, water security is under threat from many sources: population growth, urbanization, increasing water pollution, abstraction of groundwater, water-related disasters, climate change etc. It seems that current planning and management are insufficient to address the challenges of society's needs for water [3].

For instance, existing wastewater and storm water collection systems are insufficient and faulty. With extensive Industrialization, numbers of inhabitants are quickly increasing in the city center, but the planning and construction of sewers and cleaning equipment are not increasing at the same pace in parallel. A waste water treatment plant project which is a large deficiency for a planned city like Samsun is still a deficiency. The plant, which was planned to be installed in the area of 350000 m^2 at first stage, will be able to treat 120.000 m^3 wastewater per day and then the capacity will be raised to 180000 m^3/day. After the plant comes into operation, it will be able to treat organic fertilizers that come in contact with water as the result of agricultural runoff. Thus, it will not contribute to the economy but also the untreated wastewater discharge to the Black Sea will also be prevented [4, 5].

Reuse of wastewater for irrigational purposes in agriculture has been a widely applied practice all around the world compared to such applications in industries. In Turkey, reuse applications in agriculture have been in use by indirect application by means of withdrawing water from the downstream end of treatment plants. Such practices have affected the deterioration of surface water resources due to the lack of water quality monitoring and control. The reuse of water for irrigation allows partly the reuse of the wastewater's nutrients (N and P). Outside the irrigation period the water must be treated for disposal, thus nutrient removal is often required in order to avoid detrimental effects on the receiving surface water body. Only wastewater treatment plants with different operation modes for different seasons can realize these requirements. The nitrification is the most sensitive biological process in the aerobic wastewater treatment process [6, 7].

In this study, the concept of society and environmental sustainability, integrated water and wastewater management systems will be evaluated with the individual technologies along with some institutional arrangements in the frame of sustainable water management in Samsun, Turkey. It seems to be the most convenient alternative of reuse applications in Samsun compared to industrial reuse, because irrigational reuse of biologically treated urban treatment plants increasing in number every year.

Project

Declining water quality has become a global issue of concern as human populations grow, industrial and agricultural activities expand, and climate change threatens to cause major alterations to the hydrological cycle.

Aspects of water security and sustainability will be examined through three thematic areas: Basin Water Management, Urban Water Services and Rural Water Services. Successful management of any resource requires an accurate knowledge of the resource available. Generally, water quality is determined by comparing the physical and chemical characteristics of a water sample with water quality guidelines or standards. These are usually based on scientifically assessed acceptable levels of toxicity to either humans or aquatic organisms.

Samsun Water and Sewerage Administration, SASKI General Directorate, invites internationally experienced companies, consortia or joint-ventures for pre-qualification to tender for Samsun Waste Water Project "Waste Water Treatment Plant (WWTP) and Deep Sea Outfall (DSO) Construction Works". The mentioned tender also includes operation of the constructed plant for a period of 60 months. The Project covers design (during tender process different alternatives will have to be considered by Applicants) and construction of mechanical and biological Waste Water Treatment Plant for the year 2015, including electrical and mechanical works, pumping station for the treated waste water and deep sea outfall as well as operation of this plant [8].

Requirements for discharges from the Waste Water Treatment Plant in accordance with the "Urban Wastewater Treatment Regulation published 08.01.2006 in the Official Gazette of Turkey numbered "26047" are as follows (Table 1).

Table 1. Parameters for discharges from the Waste Water Treatment Plant

Parameters	Concentration (mg/l)l	Minimum percentage of reduction (%)
Biochemical oxygen demand (BOD5 at 20 °C) without nitrification	25	90
Chemical oxygen demand (COD)	125 mg/l	75
Total suspended solids	35 mg/l	90
Total phosphorus	1 mg/l P	80
Total nitrogen	10 mg/l N	75

DISCUSSION

Sustainable, efficient and equitable management of water in cities has never been as important as in today's world. The ultimate goals of treated wastewater reuse are Irrigation, injection to groundwater for feeding, reuse in Double Distribution System and recreational areas formation. There could be other uses like construction, prevention of salt-water intrusion. Capacities to make change happen in water are typically diffused between many different stakeholders including the different publics in our cities. Therefore, increasingly coming to the forefront are the holistic approaches, methods and skills needed to enable successful cooperation and collaboration, including those communication techniques which enable stakeholders to improve their performance, exchange knowledge, views and preferences and act collectively with a feasible vision of the future, promoting effective implementation [9].

To this end, Samsun Waste Water Treatment Plant (WWTP) and Deep Sea Outfall (DSO) design construction works as well as operation. SASKI invites all national and international companies, consortia or joint-ventures which have the required experience, to apply for pre-qualification for the above works. International foreign applicant shall form a consortium/joint venture with qualified contractors in Turkey [10]. Experience and resources of subcontractors shall be taken into consideration in Applicant's pre-qualification evaluation. Subcontractors will not be changed by the contractor before end of works. Companies, consortia or joint-ventures applying for the above works must have design, construction and operation experience relating to the Project and must prove that they meet the requirements described in the pre-qualification documents for expertise, experience and financial capacity. Experience and resources of subcontractors shall be taken into consideration in applicant's pre-qualification evaluation. Subcontractors will not be changed by the contractor before end of work.

References

1.http://www.sustainablecitiesinstitute.org/topics/water-and-green-infrastructure/water-101/water-sustainability-principles , (November , 2015)

2.http://water.epa.gov/infrastructure/sustain/sustainable_infrastructure.cfm, (November, 2015)

3. H. Büyükgüngör, "Watewater Reuse Options for Rural Areas", Turkish-German Workshop on Sustainable Waste Water Management, (2010), Konya/Turkey

4. Project, "GEF-BSEP National Black Sea Land-Based Pollution Routine Monitoring Project",(1995-1996-1997), Ministry Of Environment and Environmental Problems Research Center of Ondokuz Mayis University, Project Leader Büyükgüngör, H. (1997)

5. F. Geyikci, H. Büyükgüngör, "Monitoring of organochlorine pesticides in the surface waters from Mid-Black Sea Region, Turkey", Environmental

Monitoring Assessment, 173:127–137, (2011)
6. F. Akbal, L. Gürel, T. Bahadir, I. Güler, G. Bakan, H. Büyükgüngör, “Water and sediment quality assessment in the mid-Black Sea coast of Turkey using multivariate statistical techniques”, Environmental Earth Sciences, Environ Earth Sci. 64:1387–1395, (2011)
7. H. Büyükgüngör, „Umweltverunreinigung in der Stadt Samsun“, Seminar Umweltschutz, 47-55, (1990), Stuttgart, Germany.
8. http://www.saski.gov.tr/ (November , 2015)
9. http://www.waterworld.com/ (October, 2015)
10. Iller Bank (Bank of Provinces),” Turkey-Italy Bilateral Cooperation Project Policies and Institutions for Regional Development in Turkey, “Local Administrations in Turkey and Iller Bank. (2007).

EFFICIENCY OF NONIONIC TENSIDES AS TRANSPORT AGENTS FOR METAL CATIONS

ORIGINAL SCIENTIFIC PAPER

M. Suljkanović[1], E. Nurković[2*], Ž. Nikolić[1]

[1]Faculty of Natural Sciences and Mathematics, University of Tuzla
[2]Faculty of Technology, University of Tuzla

ABSTRACT

In this paper, interactions between cations and non-ionic tensides were investigated. Spectrometric measurements on model-systems (cation+counter ion+tenside) were applied. Interactions were explained based on the position and intensity of absorption maxima. Investigated model-systems were also applied in liquid membrane preparation. Nonionic tensides dissolved in nonpolar organic solvents were tested as possible carriers in liquid membranes for metal cation transport. The effect of their application reflects in decreasing the level of cation transport, caused by strong metal-tenside interactions within the membrane.

Nonionic tensides: TX-100, TX-45, Brij 35, Brij 58 and Brij 78 were used in experiments. Interactions with metal cations Pb(II) and Cd(II) and competitive ligands: 18-crown-6 and dibenzo-18-crown-6 dissolved in organic solvents (1,2-dichloroethane, dichloromethane and chloroform) were investigated.

The results showed that the strongest interactions occurred between cations and tensides with longer chains, i.e. a higher number of electron-donor centers (TX-100 compared to TX-45). Atomic absorption spectrometry was applied for metal cation concentration measurements, after 3 hours of experiment: lower transport efficiency actually proved the efficiency of nonionic tensides as carriers in liquid membranes.

Key words: metal cations, nonionic tensides, liquid membrane transport

INTRODUCTION

It is known that tensides as surface active agents find wide application in different analytical procedures (″cloud-point″ extraction, membrane ultrafiltration, bioremediation techniques, etc.), due to their amphiphilic properties and possibility of micellar aggregation. However, application of tensides as carriers for metal cations has become the subject of many investigations, recently. Actually, the presence of repeating oxyethylene units (contains electron-donor oxygen atoms) in their structure explains the interactions with cations. These kinds of interactions are not explained enough, so the principle of ″molecular recognition″ and formation of coordination bond cannot be confirmed (compared to typical macrocyclic ligands).

Coexistence of hydrophilic and lipophilic part in the same tenside molecule (polar ″head″and non-polar ″tail″) enables their aggregation in formations known as micelles [1]. Organisation and aggregation of monomeric tenside molecules into polymeric micellar structures begins at a specific concentration value: ″critical micellar concentration″ (CMC). Below the CMC, only monomeric molecules exist in solution. Above the CMC, added tenside molecules incorporate in already formed micellar structures [2]. There are numerous advantages of micellar systems. Their homogeneity and transparency allows their application in many spectroscopic techniques. Common techniques used for separation and quantitative determination of metal ions which also include micellar systems are micellar extraction ultrafiltration (MEUF) and ″cloud-point″ extraction (CPE). Tondre [3] described certain improvements in the field of application of micelles as transport agents for cations. This specific role implies the existence of cation-tenside interactions, which have not been explained enough so far. It can be assumed that interactions occur between polioxyethylene chains as electron-donors and metal cations as electron acceptors [4]. Stevens *et al* [5] explained interactions based on the principle of molecular recognition (″host-guest″ relations), resulting in formation of complex compounds. However, in most cases, solubilisation of cations by their incorporation in micellar structures is the main explanation for metal-tenside interactions. Characteristics of tensides: the structure of their molecules (charge type at polar ″head″ and length of nonpolar ″tail″), as well as values of: hydrophilic-lipophilic balance (HLB) and critical micellar concentration (CMC) are important for cation-tenside interactions (parameters for some tensides are given in Table 1.).

Table 1. The critical micellar concentration (CMC) and hydrophilic-lipophilic balance number (HLB) of some tensides [6]

Tensides	**Molar Weight (g mol^{-1})**	**Charge Type**	**Hydrophilic-lipophilic balance (HLB)**	**Critical micellar concentration CMC (mM)**
SDS	288	Anionic	40	8,10
Igepal CA-720	75	Anionic	14,6	0,23
Tween 80	1310	nonionic	15	0,01
Triton X-100	628	nonionic	13,5	0,31
Lecitine	773	zwitterionic	3-4	610 mg/L

HLB values for many compounds are between 1 and 40. Lower HLB means higher lipophilicity. Classification of tensides is based also on this parameter: very lipophilic (1 to 9), amphiphilic (10 to 15) and hydrophilic (> 15). For example: anionic tenside Sodium dodecyl sulphate, SDS (HLB = 40) have the best solubilisation properties among other tensides (Table 1). At concentrations higher than CMC, tensides can provide a certain level of solubilisation for hydrophobic organic compounds [7].

Surface activity and hydrophilicity of nonionic tensides are based on the polarity and size of the ″head″ parts of their molecules. pH values are insignificant for their surface activity, which is their advantage among other types of tensides. To apply tensides as possible carriers for interactions with metal ions, they should have adequate values of stability constants (provide complex formation), appropriate solubility within the organic phase or organic membrane (lipophilicity) which prevents ″leakage″ outside of a membrane in the analyte solution. Semnani [8] also highlights the importance of interactions between tensides and membrane solvent. Hydrophilic and lipophilic parts of nonionic tensides are not clearly separated in their molecular structure, so it is hard to define the limits between them. Every oxygen atom contributes to hydrophilic character and every alkyl group to lipophilic nature of tenside molecule. Lower values of CMC require application in low concentrations.

In earlier investigations, authors [9,10] proposed the mechanism of transport (Figure 1). Based on this mechanism, interactions in these systems can be explained.

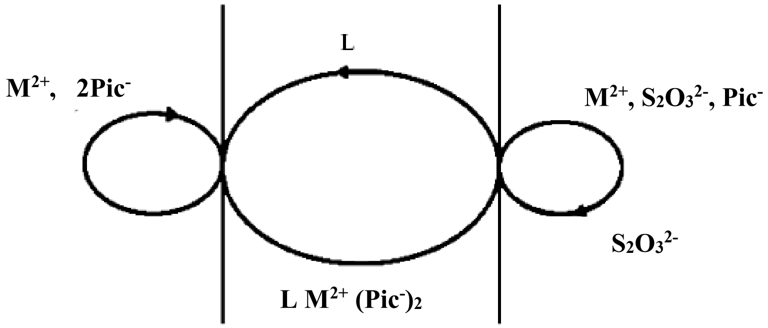


Figure 1. Proposed mechanism of metal ion transport[9] through organic liquid membrane containing dissolved ligand (L), from the source aqueous phase (contains: M^{2+} cations and counterions picrate Pic$^-$) to the receiving aqueous phase (contains thiosulphate ions)

In this paper, ″model-systems″ consisted of known amounts of metal ions (Cd(II) and Pb(II)), nonionic tensides as carriers (Triton X-100, Triton X-45, Brij 35, Brij 58, Brij 78), counter-ions (picrate-PA) and competitive macrocyclic ligands, crown ethers-CE (18-crown-6, 18C6 and dibenzo-18-crown-6, DB18C6) were used for investigation. The goal was to emphasize some tensides among others as more suitable for interactions with metal ions, based on the results of transport experiments. Efficiency of metal ion transport was monitored by measuring the changes in their concentration in time using AAS.

MATERIALS AND METHODS

Materials

Chemicals:

- Standard solution of Cd(II) (1000 mg/L), ($Cd(NO_3)_2$ in 0,5M HNO_3), Merck
- Standard solution of Pb(II) (1000 mg/L), ($Pb(NO_3)_2$ in0,5M HNO_3), Merck
- $C_{12}H_{24}O_6$ (18-crown-6); 99%, ACROS OGANICS
- $C_{20}H_{24}O_6$ (dibenzo-18-crown-6); >99%, TCI
- $C_6H_3N_3O_7$ (picric acid), p.a. Sigma-Aldrich
- $C_{14}H_{22}O(C_2H_4O)_{9,5}$ (Triton X-100); p.a. Sigma-Aldrich
- $C_{14}H_{22}O(C_2H_4O)_{4,5}$ (Triton X-45); p.a. Sigma-Aldrich
- $C_{12}H_{25}(OCH_2CH_2)_{23}OH$(Brij 35); p.a. Sigma-Aldrich
- $C_{16}H_{33}(OCH_2CH_2)_{20}OH$(Brij 58);p.a. Sigma-Aldrich
- $C_{18}H_{37}(OCH_2CH_2)$(Brij 78);p.a. Sigma-Aldrich
- CH_2Cl_2, dichloromethane; p.a. Kemika
- $C_2H_4Cl_2$, 1,2-dichloroethane; p.a. Kemika
- $CHCl_3$, chloroform; p.a. Kemika
- CH_3COOH, acetic acid; purris. p.a., Fluka
- NaOH, sodium hydroxide; g.r., Merck
- $Na_2S_2O_3$, sodium thiosulphate; purrum.p.a. Sigma-Aldrich

Some characteristics of nonionic tenzides used in the experimental part of this paper are presented in Table 2. There are two groups of used nonionic tenzides: polyoxyethylene octylphenyl ethers (Triton X-100 and Triton X-45) and polyethoxylated alcohols (Brij 35, Brij 58 and Brij 78).

Table 2. The characteristics of nonionic tensides used for this paper

Nonionic tensides	**Molecular formula**	**Molar Weight**	**Hydrophilic-lipophilic balance HLB**	**Criticall micellar concentration CMC (mM)**
Triton X-100	$C_{14}H_{22}O(C_2H_4O)_{9,5}$	628	13,5	0,24
Triton X-45	$C_{14}H_{22}O(C_2H_4O)_{4,5}$	404	10,4	0,30
Brij 35	$C_{12}H_{25}(OCH_2CH_2)_{23}OH$	1198	16	0,06
Brij 58	$C_{16}H_{33}(OCH_2CH_2)_{20}OH$	1124	15,7	0,007
Brij 78	$C_{18}H_{37}(OCH_2CH_2)_{20}OH$	1151	15,3	0,006

Instruments:

- UV/VIS spectrophotometer (CECIL CE 2021)
- Atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200)
- pH-meter (GLP31 Crison Instruments)

Methods

Transport experiments

The cylindrical glass container, i.e. "transport cell", with an inner diameter of 5 cm and a central tube (2 cm in diameter), were used for investigations (Figure 2).

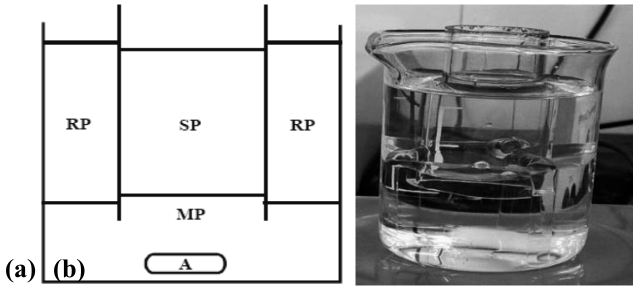


Figure 2. (a) Scheme of „transport cell"; (b) cell used in transport experiments SP-source phase; RP-receiving phase; MP-membrane phase; A-magnetic stirrer

The central tube provided physical separation of two aqueous phases: source phase (SP) and receiving phase (RP). SP contained 10 mL of mixture consisted of metal ions ($1 \cdot 10^{-3}$M) and counter-ions, picrates ($1 \cdot 10^{-3}$M). RP contained 30 mL of suitable stripping agent solution - thiosulphate (0,10M). Both aqueous phases were buffered at pH=5. The membrane phase (MP) contained macrocyclic ligand, L ($1 \cdot 10^{-3}$M) dissolved in 50 mL of organic solvent with added tenside as potentially competitive transport agent. The membrane phase lies under the aqueous phases and connects them. Constant stirring with a magnetic stirrer is essential to provide that contact surfaces: RP/MP/SP remain flat and well defined. Concentration of metal ions in RP was measured after 3h of transport, using AAS.

Spectrophotometric measurements

Absorption spectra (UV/VIS) of organic phases (MP), with different types of tensides within, as well as different type of the membrane solvent were recorded. The results showed (from qualitative aspect) the level of interactions occurred between metal ions and tensides.

AAS technique with flame atomization was implemented for the quantitative part of investigation: measurements of metal ion content during transport from source to receiving phase, after diffusion through liquid membrane. The determinations of Pb (II) and Cd(II) content were carried out by using Pb-hallow cathode lamp and Cd-hallow cathode lamp and were made under the recommended conditions for Pb (II) and Cd(II) ions. The procedure was carried out as described above in "transport experiments" section. The receiving phase was sampled and analyzed for the metal ion concentration by AAS, after 3 hours of transport using the method of calibration curve. Gained data points are averages of three replicate measurements. Obtained results showed percentage of transported cations, which represent the efficiency of nonionic tensides as possible carriers in liquid membrane.

RESULTS AND DISCUSSION

Efficiency of nonionic tensides as carriers for Pb(II) ions during their transport through two types of liquid membranes: dichloromethane (DCM) and 1,2-dichloroethane (DCE) were investigated. As transport agents for metal ions, crown ethers (CE): 18-crown-6 (18C6) and dibenzo-18-crown-6 (DB18C6), as well as the combinations: 18C6+TX-100, DB18C6+TX-100, 18C6+TX-45 and DB18C6+TX-45, were used. There is an obvious impact of tenside presence on Pb(II) transport (Figure 3): lower transport efficiency for all systems with tenside-crown ether combination within the membrane compared to systems without tensides is evident. Possible explanation here is that the complexation of Pb(II) ions with CE is a different process in the absence of tensides. It is possible that stronger interactions (Pb-CE-tenside) within the membrane cause the limited release of cations at MP/RP interface. Formation of micelles, which incorporate formed complexes, as well as direct interactions between cations and tensides as possible carriers actually explains tenside behavior in interactions with metal ions.

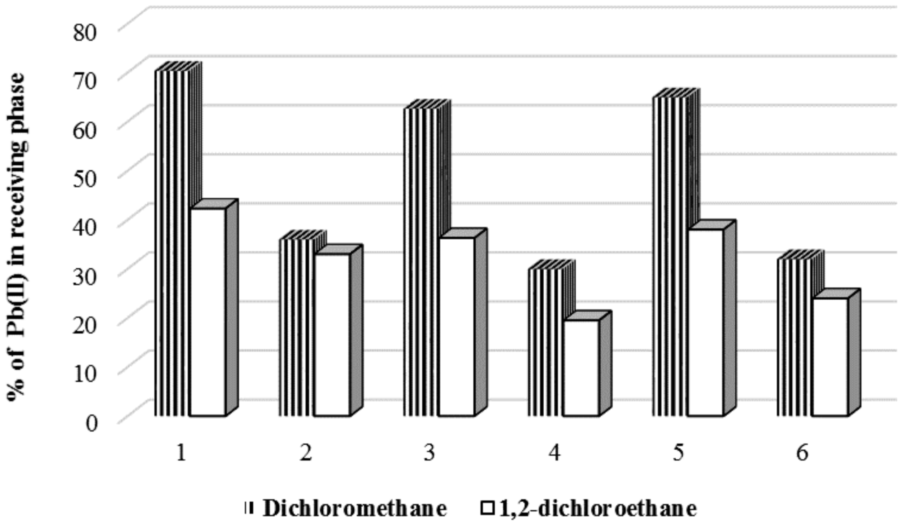


Figure 3. Comparison of Pb(II) transport results through DCM and 1,2-DCE membranes for different transport agents: 1–18C6; 2–DB18C6; 3–18C6+TX-100; 4–DB18C6+TX-100; 5-18C6+TX-45; 6–DB18C6+TX-45

Connection between the strength of cation-tenside interactions and the efficiency of transport can be observed by comparing the results gained for different tensides (Fig.3): the larger number of oxyethylene units for TX-100 (9,5) leads to stronger interactions with metal ions, compared to TX-45 (contains 4,5 oxyethylene units) and consequently causes lower percentage of Pb(II) ions transported in receiving phase.

Absorption spectra (Figure 4) for systems: PA+Pb(II)+18C6+T (T=TX-100 or TX-45) also show the influence of tenside structure (different lengths of polyoxyethylene chains).

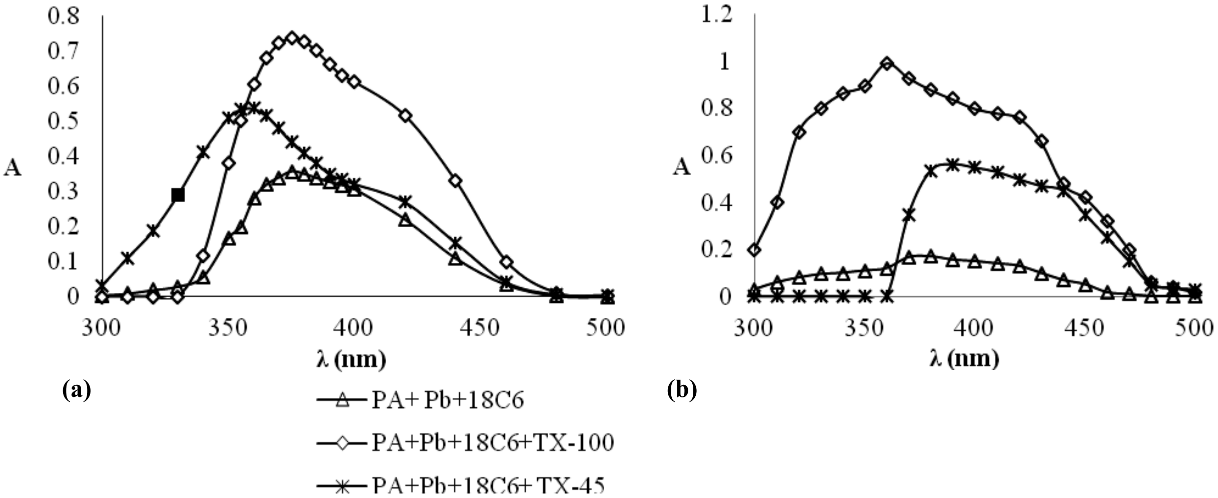


Figure 4. Influence of tenside chain-length on absorption: **(a)** in DCM membrane; **(b)** in 1,2-DCE membrane (PA = Picric Acid)

Different intensities of absorption contribute to the earlier assumption of different possibilities of interactions between TX-100 and Pb(II) compared to TX-45, in both solvents. A larger number of oxyethylene units (the number of oxygen atoms as electron-donors) means higher intensity of absorption for TX-100 compared to TX-45, and probably stronger interactions which lead to lower Pb(II) transport efficiency.

To investigate the efficiency of Cd(II) transport through the dichloromethane membrane (DCM), macrocyclic ligand 18-crown-6 (18C6) and ligand-tenside combinations: 18C6+TX-100; 18C6+Brij 78; 18C6+Brij 58, were used. As in the previous case for Pb(II) transport (Fig.3), gained results also show the higher percentage of transported cations in the absence of tensides within the membrane (Fig. 5, Chart 1). Charts: 2, 3 and 4 show different values of transported Cd(II) ions for different tensides. The structure of tensides determine the transport efficiency: the lowest value of 8% (Chart 4) for Brij58 indicates that the larger number of oxygen atoms and higher HLB value for this tenside (Table 2) enables stronger interactions with metal ions and consequently decrease their transport to the receiving phase.

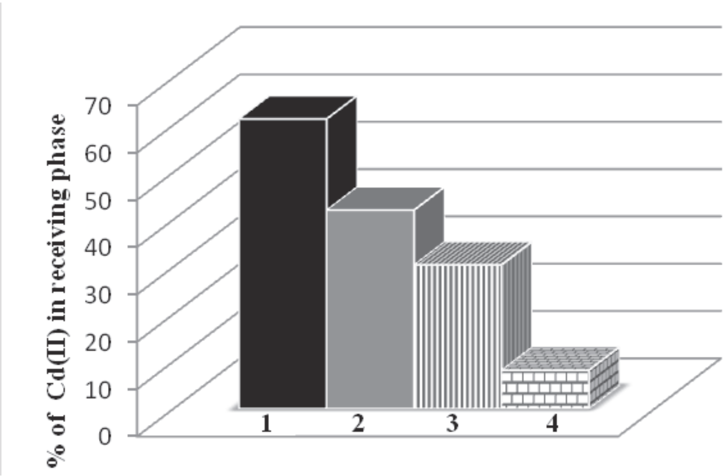


Figure 5. Comparison of Cd(II) transport results through DCM membrane for different transport agents: 1 – 18C6; 2 – 18C6+TX-100; 3 – 18C6+Brij 78; 4 - 18C6+Brij 58

To investigate the efficiency of Cd(II) transport through the chloroform membrane (CH), macrocyclic ligand 18-crown-6 (18C6) and ligand-tenside combinations: 18C6+TX-100; 18C6+Brij 35; 18C6+Brij 78 and 18C6+Brij 58, were used. The results (Fig.6) show the lowest percentage (30%) of transported Cd(II) ions in receiving phase for Brij 35 (Chart 3) compared to other tensides. The greatest intensity of absorption for system: PA+18C6+Cd+Brij35 (Figure 7) is in accordance with the lowest percentage of transported Cd(II) ions, probably due to the largest number of oxygen atoms as well as the highest HLB value for Brij 35 (Table 2) which enable the strongest interactions with Cd(II) ions and consequently the lowest percentage of their transport to receiving phase.

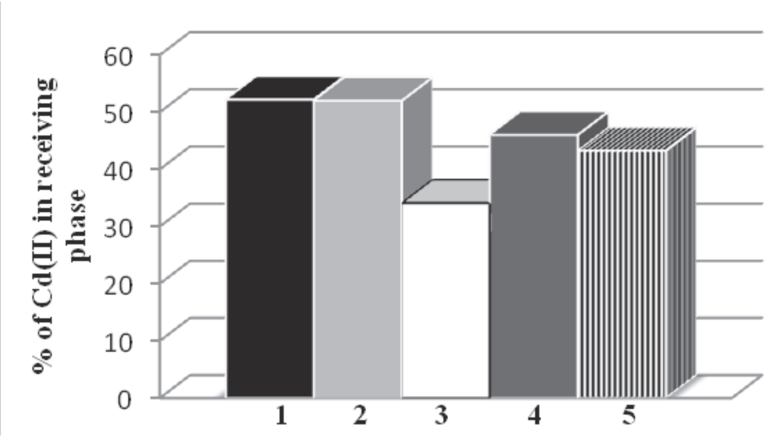


Figure 6. Comparison of Cd(II) transport results through the chloroform membrane for different transport agents: 1 – 18C6; 2 – 18C6+TX-100; 3 – 18C6+Brij 35; 4 – 18C6+Brij 78; 5- 18C6+Brij 58

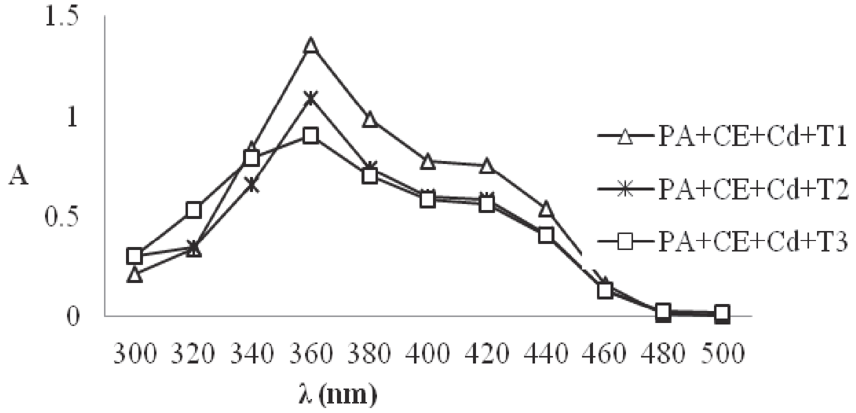


Figure 7. Influence of tenside chain-length on absorption in chloroform membrane: T1-Brij 35; T2 – Brij 78; T3 – Brij 58 (PA = Picric Acid; CE = Crown Ether, 18C6)

CONCLUSIONS

Nonionic tensides with oxygen atoms in hydrophilic segment of their molecules show electron-donor properties during interactions with metal ions and find their application in liquid membranes as the "carriers" of metal ions.

The presence of nonionic tensides in liquid membranes causes a decrease in the percentage of transported metal ions due to stronger interactions within the membrane.

If the number of oxygen atoms in the tenside structure is higher (longer oxyethylene chain), e.g. TX-100 compared to TX-45 and Brij 35 compared to Brij58 and Brij78, higher is the level of interactions with metal ions.

Brij 35 showed the highest absorption intensity and the lowest percentage of transported ions, due to the largest number of oxygen atoms on their structure and the highest HLB value, which leads to the greatest possibility for interactions with cations in liquid membranes.

The explanation of interactions within the membranes mainly lies in formation of micellar structures of tensides, which incorporate metal ions or formed metal-ligand complexes and limit their release into the receiving phase during the transport.

References

1. M.V. Flores, E.C. Voutsas, N. Spiliotis, G.M. Eccleston, G. Bell, D.P. Tasios and P.J. Halling, Journal of Colloid and Interface Science, 240 (2001) 277-283.
2. M.N. Jones and D. Chapman, Micelles, monolayers and biomembranes. WileyLiss, New York, 1995.
3. C. Tondre and M. Hebrant, Journal of Molecular Liquids 72 (1997) 279-294.
4. M. Suljkanović, Uticaj strukture polieterskih liganada na kompleksiranje Cd(II) i Pb(II) iona u organskim rastvaračima. Doctoral thesis, University of Tuzla, Faculty of Natural Sciences and Mathematics, Tuzla (2013).
5. G.W. Stevens, J.M. Perera and F. Grieser, Current Opinion in Colloid and Interface Science. 2 (1997) 629-634.
6. R.A. Doong and W.G. Lei, Journal of Hazardous Materials 96 (1) (2003) 15-27.
7. G.G. Ying, Distribution, Behavior, Fate and Effects of Surfactants and Their Degradation Products in the Environment, In (ed) Zoller U. Handbook of detergents – part B: Environmental Impact. Surfactant Science Series 121 (2004) 77-107.
8. A. Semnani and M. Shamsipur, Spectrochimica Acta Part A: Molecular Spectroscopy, 49 (3) (1993) 411-415.
9. D. Nipamanjari, B. Sanjib and A.K. Mukherjee, Molecular Physics 108 (11) (2010) 1505–1511.
10. F.A. Christy and P.S. Shrivastav, Critical Reviews in Analytical Chemistry, 41 (3) (2011) 236-269.

EVALUATION OF 5-(HYDROXYMETHYL)FURAN-2-CARBALDEHYDE (HMF) CONTENT IN HONEY: COMPARISON OF CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHOD

ORIGINAL SCIENTIFIC PAPER

I. Flanjak*, Lj. Primorac, B. Bilić, M. Novak, M. Cvijetić Stokanović, D. Kenjerić
Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek

ABSTRACT

Estimation of honey freshness and storage conditions usually includes determination of 5-(hydroxymethyl)furan-2-carbaldehyde (HMF) content. Maximal HMF content in honey is prescribed by the regulations dealing with honey quality. Therefore, it is important to use proper methods for its determination. The aim of this study was to compare two official methods prescribed by International Honey Commission for determination of HMF content in honey (chromatographic (HPLC) method and spectrophotometric method after White). Method performances were estimated through linearity, precision and accuracy. Later, comparison of methods was performed on 13 honey samples (5 black locust and 8 chestnut honey samples). The results showed that both methods are fit for purpose and comparable within the evaluated range.

Keywords: HMF content, honey, chromatographic method, spectrophotometric method, comparison

INTRODUCTION

When placed on the market honey, like every other food product, must comply with food safety and quality regulations. Croatian national regulation dealing with honey quality [1] which is harmonised with international regulations [2,3] prescribes that honey must not be heated in such a way that the natural enzymes are either destroyed or significantly inactivated. However, the only practical way for honey viscosity reduction, delaying/preventing the crystallisation and prevention of fermentation is processing. Processing of honey usually implies the controlled heating with the aim of packaging facilitation and insurance of storage stability [4]. On the other hand, uncontrolled heating results with quality degradation (e.g. enzyme activity reduction, increase of 5-(hydroxymethyl)furan-2-carbaldehyde (HMF) content, darkening of honey and formation of Maillard reaction products). HMF content and diastase activity are widely recognised as indicators of quality degradation and their limits are prescribed by regulations [1,2,3]. The maximal allowed HMF content in honey is generally 40 mg/kg, while the prescribed limit for honeys from regions with tropical climate is 80 mg/kg [1,2,3].

HMF is cyclic aldehyde which is formed during acid-catalysed dehydration of hexoses and/or as intermediate in the Maillard reactions. Fresh honey contains a negligible amount of HMF, but the concentration tends to rise after heating and storage of honey. The dynamic of HMF formation depends on the chemical composition of honey (primarily on pH value and acidity), processing and storage conditions [5,6,7]. Various studies showed that HMF formation follows the first order kinetics and that in honeys with a low initial pH value (e.g. black locust and sage honeys) HMF formation is faster that in honeys with a higher pH value (e.g. chestnut and honeydew honeys) [5,6,7].

International Honey Commission (IHC) recommended three methods for HMF determination. The spectrophotometric method after Winkler is abandoned in most of the laboratories, due to the toxicity of reagent used for formation of coloured product. Therefore, the routine analyses of HMF content determination include either the spectrophotometric method after White or the chromatographic (HPLC) method. The aim of this study was to compare and evaluate performance characteristics of two proposed methods.

MATERIALS AND METHODS

Honey samples and preliminary analyses

Thirteen honey samples (5 black locust (*Robinia pseudoaccacia* L.) and 8 chestnut (*Castanea sativa* Mill.)) were collected from different parts of Croatia. Preliminary analyses, which were performed within the month after the collection, included a qualitative orienting melissopalynological analysis according to the method prescribed by Louveaux et al. (1978) [8] and determination of basic physicochemical parameters (water content, electrical conductivity and diastase activity) according to the Harmonised methods of the International Honey Commission [9]. At the time of HMF content determination, all black locust (B1-B5) and four chestnut honey samples (C5-C8) were stored for 6 months while four chestnut honey samples (C1-C4) were stored for 48 months at the room temperature in the dark.

HMF content determination

High-performace liquid chromatography (HPLC) method

The analysis was performed according to the HPLC method described in the Harmonised methods of the International Honey Commission [9] using Varian instrument consisting of Solvent Delivery Module (ProStar 230), Column Valve Module (ProStar 500) and Photodiode Array Detector (ProStar 330) coupled to a computer with ProStar Chromatography Workstation 5. The samples were analysed using column LiChrospher 100 RP-18, dimensions 125x4 mm, 5 μm particle size (Merck, Germany). The following chromatographic conditions were used: isocratic elution with methanol/water (10:90, v/v), flow rate 1 mL/min, injection volume 20 μL. Methanol (Merck, Darmstadt, Germany) of HPLC gradient grade purity and ultrapure water were used to prepare the mobile phase solution. The monitoring wavelength range was 200-400 nm, while the detection wavelength was set at 285 nm. HMF identification was achieved based on the retention time and by comparison of the HMF absorbance spectrum of honey samples with spectrum of HMF standard. Quantification was obtained through external calibration using HMF (GC, >98%, Merck, Germany) standard solutions in the range from 0,1 mg/L to 10 mg/L. Each standard solution was analysed in triplicate. Before injection, sample solutions were filtered through a 0,45 μm membrane nylon filter. All analyses were done in duplicate, and each sample solution was injected twice. The results were expressed as mg/kg of honey.

Spectrophotometric method

The analysis was performed according to the method after White described in the Harmonised methods of the International Honey Commission [9]. Absorbance measurements of honey solutions at 284 nm and 336 nm were determined using CECIL UV/VIS spectrophotometer (CE 2040). Measurements were performed in square 10-mm path length quartz cuvettes. Each honey sample was prepared in duplicate, and each sample solution analysed twice. The calculation of HMF content was done using the formula reported in Harmonised methods of the International Honey Commission [9] and the results were expressed as mg/kg of honey.

Method performance characteristics

Method performance was estimated throughout linearity, accuracy and precision. Linearity of both methods was evaluated by preparation of HMF standard solutions (0,1-10 mg/L) and creation of the calibration curves. Each standard solution was analysed in triplicate. Methods precision was tested throughout sample preparation repeatability and measurement repeatability. For the sample preparation repeatability evaluation, the same honey sample was weighed 5 times and each solution analysed in triplicate. Measurement repeatability was performed by repeated measurement of the same honey solution 5 times. The method precision was expressed as the relative standard deviation (RSD, %) of obtained data set for each method. To test the accuracy of methods, a recovery test was performed. First, the HMF content was determined in a honey solution. Then, different concentrations of HMF solutions were added to honey solution in order to gain fortified samples. Each solution was analysed in triplicate. The accuracy of methods was expressed as % recovery of known, added amount of HMF [10].

Data analysis

Average values, standard deviations for each parameter and additionally relative standard deviations for precision were calculated using Microsoft Excel 2010 (Microsoft Corp.) software. The *t*-test for dependent samples was used to test the statistical differences between HMF content in honey samples obtained with compared methods at the 95% confidence level using StatSoft, Inc. (2013). STATISTICA software.

RESULTS AND DISCUSSION

The preliminary analyses included melissopalynological analysis and determination of water content. Additionally, electrical conductivity and diastase activity were performed in order to determine/confirm botanical origin of collected samples. Regarding the melissopalynological analysis, all analysed samples had specific pollen share above the prescribed limit, which is 20% for black locust honey and 85% for chestnut honey [11]. The average value of *R. pseudoaccacia* pollen share in black locust honey samples was 59±12%, while the average value of *C. sativa* in chestnut honey samples was 95±3%. Water content of all analysed samples was below maximally prescribed 20% [1]. The average value of electrical conductivity and diastase activity (diastase number, DN) were 0,112±0,007 mS/cm and 13,4±3,8 for black locust honey and 1,276±0,226 mS/cm and 24,0±5,5 for chestnut honey. The obtained results were also in compliance with literature data for analysed honey types [12].

Both methods for HMF content determination were evaluated using the same approach (concentration ranges, sample solutions, etc.) and under the same conditions (analyst, balances, etc.). Linearity was estimated in the range from 0,1 up to 10 mg/L and for both methods linearity was confirmed in the whole evaluated concentration range (Table 1.). Very high correlation coefficients were obtained also by other authors [13,14] even in a wider range of concentrations.

Sample preparation repeatability for both methods was expressed as the relative standard deviation (RSD, %) obtained from 15 measurements (5 honey solutions, each measured in triplicate) and the results (Table 1) showed that the spectrophotometric method (RSD 3,4%) was more precise than the HPLC method (RSD 6,4%). Regarding the measurement repeatability which was calculated from 5 repeated measurements of the same honey solution, the HPLC method showed better precision than the spectrophotometric method (Table 1). Better precision of the HPLC method compared to the spectrophotometric method was obtained also by Truzzi et al. [13], Spano et al. [14] and Zapala et al. [15] with slightly lower RSD values from those obtained in the present study.

Table 1. Method performance characteristics of compared methods for HMF content determination in honey

Method performance characteristic	HPLC method	Spectrophotometric method after White
Linearity		
Linearity range (mg/L)	0,1-10	0,1-10
Correlation coefficient (r)	1,000	0,9997
Precision		
Sample preparation repeatability RSD (%)	6,4	3,4
Measurement repeatability RSD (%)	6,4	9,1
Accuracy		
Recovery (%)	100-103	92-93

The results of methods accuracy are presented in the Table 1. For the HPLC method recoveries were from 100% to 103%. For the spectrophotometric method the values were slightly inferior but still acceptable (from 92% up to 93%). These results of recovery test were expected since it is the commonly known fact that the chromatographic methods are in general more accurate and sensitive than the spectrophotometric ones [13,14].

Though the HPLC method has been proven to be more appropriate for HMF content determination in our study as well as in the literature [15], Spano et al. [14] showed that the official HPLC method was not appropriate for determination of the HMF content in strawberry tree honey. The interfering substance, identified as homogentistic acid, prevents adequate HMF determination by official HPLC method. Spano et al. have proposed a new, improved method for HMF content determination [16], which showed better method performance characteristics than the official HPLC method [9], the old method is still used by most of the laboratories in a routine analysis.

After the method performance characteristics were determined, the methods comparison was performed on 13 honey samples. The HMF contents of honey samples determined by both methods are summarised in Table 2. The HMF content in honey samples was below 40 mg/kg which is the maximum allowed value prescribed by regulations [1,2,3]. According to the *t*-test for dependent samples, only values obtained by two methods on 3 samples (B1, B2 and C4) differ statistically at the confidence level of 95% while the results of other analysed honey samples are comparable.

Table 2. HMF content of analysed honey samples (B-black locust, C-chestnut) determined by HPLC and spectrophotometric (after White) methods (average±SD)

Sample	HPLC method (mg/kg)	Spectrophotometric method after White (mg/kg)
B1	11,9*±0,3	11,1*±0,1
B2	3,2*±0,2	1,5*±0,2
B3	4,6±0,4	4,7±0,7
B4	2,6±0,4	2,2±0,6
B5	9,0±0,2	8,9±0,6
C1	38,2±1,4	37,0±1,3
C2	22,7±4,5	21,3±1,1
C3	29,2±0,1	29,0±0,8
C4	30,7*±0,5	28,0*±0,9
C5	2,4±0,2	1,6±1,4
C6	1,8±0,5	2,9±0,6
C7	3,8±0,7	3,8±0,2
C8	5,5±1,0	3,4±1,9

Average values and SD were calculated from 4 measurements
Average values within the row marked with (*) differ statistically according to *t*-test for dependent samples

CONCLUSION

Although from the analytical point of view both methods were found to be fit for purpose, from the practical point of view, in case if laboratory is equipped with instruments for both methods, the HPLC method presents better choice due to its simplicity of sample solution preparation and automated analysis while the spectrophotometric method requires much more sample manipulation. On the other hand, the spectrophotometric method was the first choice when financial and safety aspects (considering chemicals used) are compared.

References

1. Croatian Official Gazette, Pravilnik o medu (NN 93/2009).
2. Alinorm, Revised Codex Standard for honey CODEX STAN 12-1981, Rev.1 (1987), Rev.2 (2001).
3. Official Journal of the European Communities, Council Directive 2001/110/EC of 20 December 2001 relating to honey.
4. R. Krell (1996). Value-added products from beekeeping. FAO Agricultural Services Bulletin 124 [Online]. Available: http://www.fao.org/docrep/w0076e/w0076e00.htm
5. E. Tosi, M. Ciappini, E. Ré, H. Lucero (2002) Food Chem. 77, 71.
6. B. Fallico, M. Zappala, E. Arena, A. Verzera (2004) Food Chem. 85, 305.
7. I. Turhan, N. Tetik, M. Karhan, F. Gurel, H. Reyhan Tavukcuoglu (2008) LWT-Food Sci. Techn. 41, 1396.
8. J. Louveaux, A. Maurizio, G. Vorwohl (1978) Bee World 59, 139.
9. S. Bogdanov (2009). Harmonised methods of the International Honey Commission. International Honey Commission [Online]. Available: http://www.bee-hexagon.net/en/network.htm
10. I. Taverniers, M. De Loose, E. Van Bockstaele (2004) Trends in Anal. Chem. 23, 535.
11. Croatian Official Gazette, Pravilnik o kakvoći uniflornog meda (NN 122/2009).
12. L. Persano Oddo, R. Piro (2004) Apidologie 35, S38.
13. C. Truzzi, A. Annibaldi, S. Illuminati, C. Finale, M. Rosseti, G. Scarponi (2012) J. Food Sci. 77, C784.
14. N. Spano, L. Casula, A. Panzanelli, M. I. Pilo, P. C. Piu, R. Scanu, A. Tapparo, G. Sanna (2006) Talanta 68, 1390.
15. M. Zappalà, B. Fallico, E. Arena, A. Verzera (2005) Food Control 16, 273.
16. N. Spano, M, Ciulu, i. Floris, A. Panzanelli, M. I. Pilo, P. C. Piu, S. Salis, G. Sana (2009) Talanta, 78, 310.

BIOSORPTION OF LEAD AND COPPER IONS FROM AQUEOUS SOLUTION USING BARLEY STRAW AS ADSORBENT

ORIGINAL SCIENTIFIC PAPER

A. Odobašić, I. Šestan, S. Ćatić, H. Keran, A.a Bratovčić, M. Ahmetović

Faculty of Technology, University of Tuzla

ABSTRACT

In the last few decades, different alternative materials are being tested for the adsorption treatment of waters polluted by heavy metals. Biosorption is particularly suitable for the treatment of polluted water in cases when the low heavy metal ion concentration is requested at the exit, and before pouring into the surrounding waterway, in order not to pollute the plant and animal life. Biosorbents are of significant importance in water purification processes due to their low cost, good performance and availability in large quantities. The paper presents the test results of lead and copper ion sorption from synthetic solutions in the barley straw as adsorbent. Biosorption of lead and copper in the barley straw is carried out by ion exchange mechanism, whereby the lead and copper ions are replaced by calcium and hydrogen content in the molecular structure of the straw. The obtained results show a significant influence of pH values on the removal efficiency of the metals in the process of biosorption. The results show that barley straw can be used as an inexpensive, natural adsorbent for lead and copper ion sorption from aqueous solutions.

Key words: biosorption; barley straw; lead, copper

INTRODUCTION

One of the most serious consequences of the rapid industrial development is the pollution of the environment. Many industries produce wastewater with relatively high content of heavy metals, whose influence on the ecosystem is of great significance [1]. Some metals from this group, such as Cu, allows normal functioning of the living organisms, and in higher doses it can have toxic effects, while the other metals, such as Pb, are highly toxic even in smaller concentrations [2-3]. Biosorption of heavy metal ions from aqueous solutions is a new process, still under development, that has been proved to be effective for the removal of heavy metal ions from aqueous solutions [4]. Biosorption is defined as the ability of certain bio-molecules to bind certain ions by its active groups or to concentrate them from aqueous solutions, whereby the removed pollutants are usually metal ions or organic compounds [5-8]. The prefix "bio" indicates the presence of a biological entity, for example a living organism, a component or product created or extracted from a living organism, as a term used in biotechnology, bioengineering and bioprocesses. Biosorbents have high affinity for heavy metal ions with which they create connections in the processes of chemisorption, complexation, adsorption on the surface and pores, ion exchange, helatisation, physical forces adsorption, diffusion, precipitation.

Compared to conventional methods, biosorption has certain advantages: it is selective, inexpensive and effective even at very low concentrations of pollutants, ecologically sustainable, because the renewable source – biomass is commonly used as a biosorbent, which unused, represents the waste in the environment [9]. As appropriate biomass (biosorbent), waste biomaterial is recommended, which is available in large quantities and cost-effective. The aim of the paper is to examine the efficiency of Pb(II) and Cu(II) ions removal by adsorption process using barley straw as agricultural waste.

MATERIALS AND METHODS

Chemicals and reagents

All reagents used were of analytical grade: Supra pur HNO_3 (Merck), supra purNaOH, standard solution $Cu(NO_3)$ in HNO_3, c(Cu) = 1 g/L, standard solution $Pb(NO_3)$ in HNO_3, c(Cu) = 1 g/L.

Biosorbent

Barley straw has been used as biomass/biosorbent, collected at the local fields. The straw first was milled, and then sieved using the appropriate laboratory sieves, where the fraction of 0,5 mm was used for the adsorption process. The concentrations of Cu(II) and Pb(II) ions in the solutions before and after adsorbtion were determined by the atomic absorption spectrophotometer “Perkin Elmer” ANALYST 200 (AAS method). The pH of the solution was measured with a WTW pH meter using a combined glass electrode. Biosorption processes were carried out at pH 2 and pH 5 wherein the appropriate pH value is adjusted using 0.1M HNO_3 and 0.1M NaOH. Barley straw, weighing 1g, was brought into contact with 100 ml of synthetic solution of the tested metals individually, for a different time, mixing speed of 300 rev/min. The moment of adding biosorbent represented the beginning of the treatment. Total time of the treatment was 120 minutes. At intervals of 20, 40, 60, 80, 100 and 120 minutes, samples were collected for analysis of metals and filtrated through a porous filter paper 0,45 µm in order to remove the absorbent particles. Thereafter, the filtrate was analyzed for the content of Pb(II) and Cu(II) ions. The experiments were performed at room temperature. The amount of absorbed Pb(II) and Cu(II) in the selected sorbents is calculated by sorption capacity using the 1 equation;

$$q = \frac{(C_o - C_R) \cdot V}{m} \quad (1)$$

here C_O and C_R are the initial and equilibrium concentrations (mg/L) of Cu^{2+} solution respectively; V is the volume (L); and m is the mass (g) of the adsorbent.

Removal efficiency, E (%), was calculated as a ratio between concentration Cu (II) or Pb(II) at time t (mg/L) and the initial metal ions concentration (mg/L):

$$R, \% = \frac{C_o - C_R}{C_o} x100 \quad (2)$$

RESULTS AND DISCUSSION

The effect of contact time was examined in the paper, as well as the effect of the pH values and initial concentration of lead and copper ions on the efficiency of the metals Pb and Cu removal process. During the adsorption tests all the experiments were performed in two trials, and as the official results we took the average values, wherein the initial concentration of lead solution was 50 mg/L, while the copper concentration was 10 mg/L. In order to have the adsorbent capacity fully used, it is necessary to provide a long enough contact between the adsorbent and water samples to be processed, where it is necessary to take into account the economical justification of the adsorption duration. Experimental results showed that the balance is achieved in the period of about 100 minutes. Barley straw in contact with the tested ions proved to be a material which can effectively remove lead and copper ions. After 100 minutes of contact time begins the maximum adsorption of the test metals per gram of adsorbent. From the results shown in Figures 1 and 2, it can be seen that barley straw has a great affinity for lead ions, and a smaller for copper ions.

When all other factors are constant, the efficiency of metal ions sorption may be a consequence of the differences in the ionic radii, i.e. the relation of electric charge and mass, ionization potential of these ions, electronegativity, hydrolysis constants. The smaller the ion radius, the greater the ion tendency to hydrolyze in water, which leads to the reduction of the sorption, due to the lower concentration of the free ions.

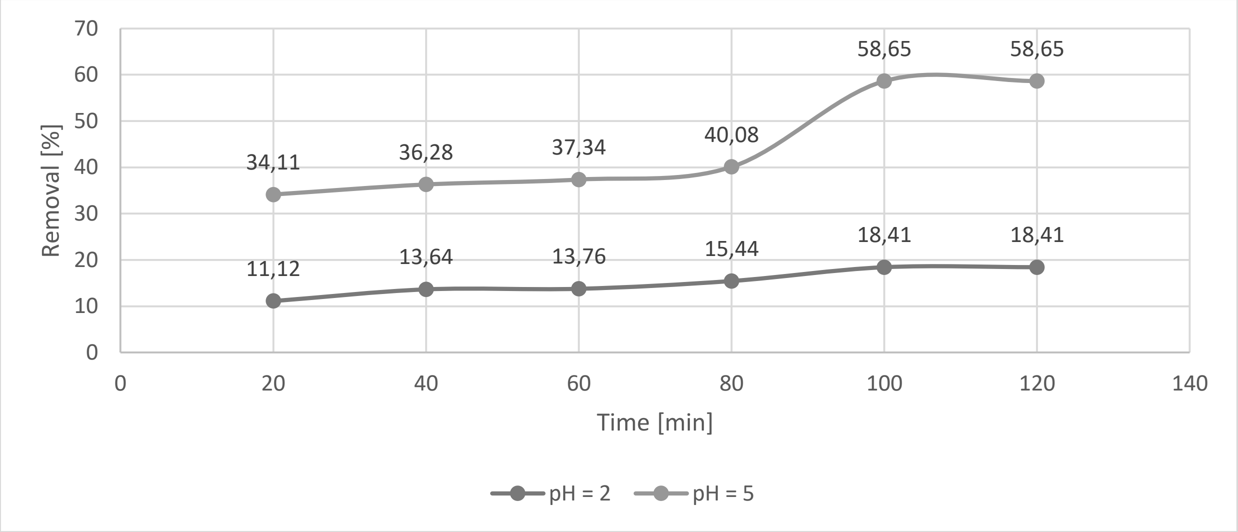


Figure 1. Removal of copper (II) ions from aqueous solution

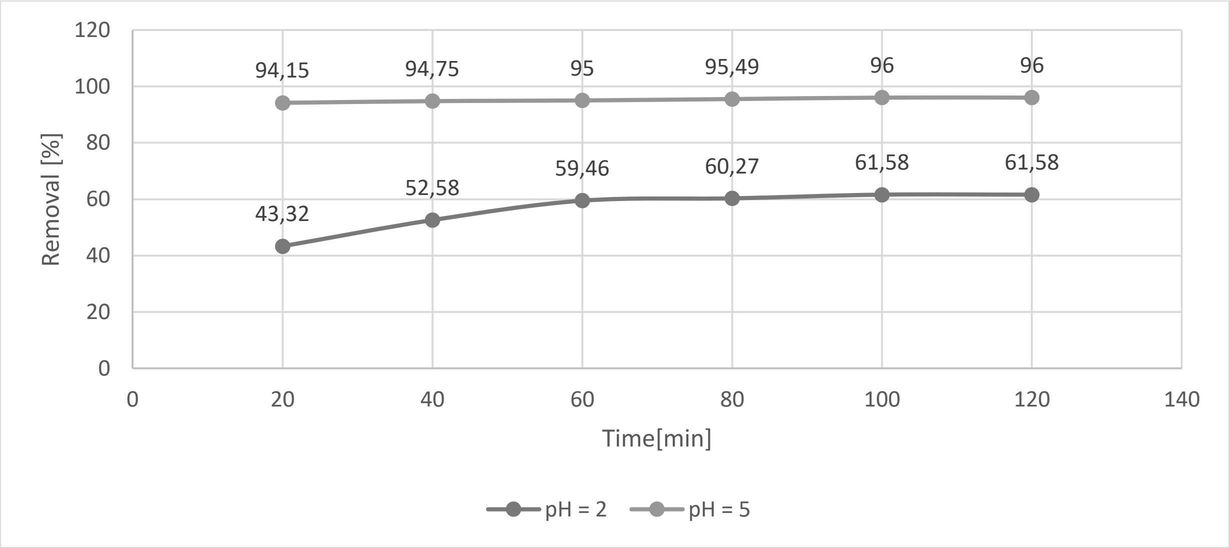


Figure 2. Removal of lead (II) ions from aqueous solution

The results show that the lead and copper removal efficiency using barley straw grows with increasing pH environment, where it is necessary to lead the process at a constant, predefined pH values, because, although the highest percentage of metals is removed relatively quickly, failure to maintain the pH value leads to reduce the removal percentage. Less adsorption efficiency of Pb^{2+} and Cu^{2+} ions at low pH values of the solution could be explained by a high H^+ ion concentration and its great mobility, which is primarily the reason that the hydrogen ions are adsorbed on the adsorption centers. With the reduction of the environment acidity, metal ions in solution enter into competition with the H^+ ions in position of adsorption, so the maximum efficiency of adsorption was achieved at pH 0,5, where about 96% of lead ions and 60% of copper ions were removed. The Figures 3 and 4 present the values of the adsorption capacity given in mg/g of adsorbent in the function of time for copper and lead.

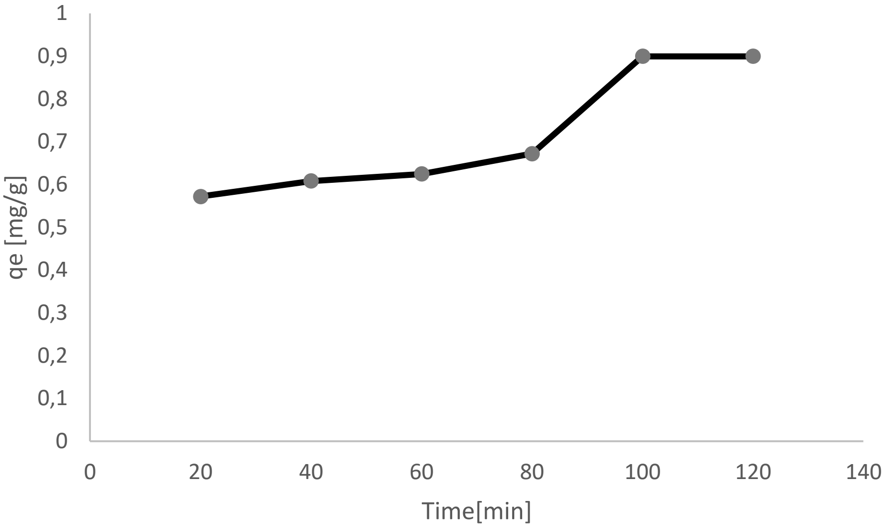


Figure 3. Effects of contact time on the adsorption capacity Cu^{2+} ion

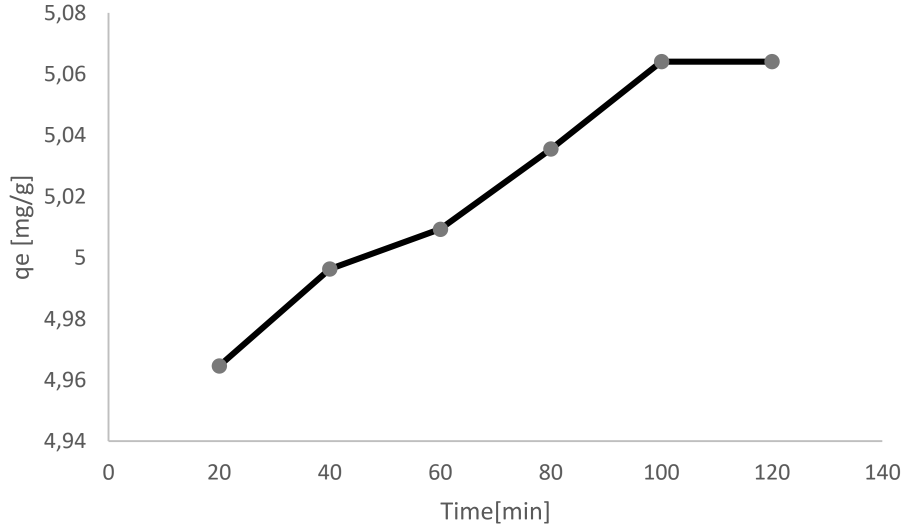


Figure 4. Effects of contact time on the adsorption capacity Pb^{2+} ion

The obtained results of the adsorption capacity of lead and copper ions at the time, indicate that the adsorption capacity of metal removal increases until the time of 100 minutes, when a balance is achieved, whereupon the value of the residual concentration is nearly constant. In the beginning of the treatment, the metal concentration in the solution is high, which has a positive effect on the speed of the process, which mostly takes place under the diffusion control. With the reduction of the metal concentration, the driving force of the heterogeneous sorption process decreases, whose speed depends on the diffusion of ions into the solution and through the sorbent pores. Also saturating the active centers on the biosorbent surface over time, reduces the probability of binding metal ions, and thus the overall process speed.

CONCLUSION

The results have shown that the biosorbents in barley straw base has a greater affinity for the lead ions removal compared to the copper ions. As a result, we can recommend the application of barley straw for the adsorption of copper ions from the water with a low ion concentration, and lead ions with the higher concentration.The re sults show a significant effect of pH values on the biosorption process, whereby it is necessary to keep a constant pH values in order to avoid the reduction in the removal percentage.

References

1. M. Gavrilescu. Removal of heavy metals from the environment by biosorption, Engineering in Life Science, (2004), 4,3, 219-232.
2. P.C. Nagajyoti, K.D. Lee,, T.V.M. Sreekanth. Heavy metals, occurrence and toxicity for plants: a review, Environmental Chemistry Letters, (2010), 8, 199–216.
3. D.W. O’Connell, C. Birkinshaw, T.F. O’Dwyer. Heavy metal adsorbents prepared from the modification of cellulose: A review, Bioresource Technology, (2008), 99, 6709–6724.
4. J. Febrianto, A. N. Kosasih, J. Sunarso, Y. Ju, N. Indraswati and S. Ismadji; Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: A summary of recent studies; Journal of Hazardous Materials 162 (2009) 616-645.
5. G.M. Gadd, Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment, J. Chem. Technol. Biotechnol. 84 (2009) 13–28.
6. R.H. Vieira, B. Volesky, Biosorption: a solution to pollu-tion, Int. Microbiol. 3 (2000) 17–24.
7. D. Sud, G. Mahajan, M.P. Kaur, Agricultural waste mate-rial as potential adsorbent for sequestering heavy metal ions from aqueous solutions – A review, Bioresour. Technol. 99 (2008) 6017–6027.
8. M. Rao, A.V. Parwate, Utilization of low-cost adsorbents for the removal of heavy metals from wastewater – A review, J. Environ. Pollut. Contr. 5 (2002) 12–23.
9. J. Wang, C. Chen, Biosorption of heavy metals by Sac-charomyces cerevisiae – A review, Biotechnol. Adv. 24 (2006) 427–451.

ANTIMICROBIAL ACTIVITY OF THE CELL FREE SUPERNATANTS OF THE LACTIC ACID BACTERIA ISOLATED FROM FRESH COW CHEESE PRODUCED IN TUZLA REGION

ORIGINAL SCIENTIFIC PAPER

D. Husejnagić[1], S. Hodžić[1], A. Avdić[1], S. Širanović[1], M. Vilušić[2]

[1]Universtity in Tuzla, Faculty of Science, Department of Biology
[2]Universtity in Tuzla, Faculty of Technology

ABSTRACT

Lactic acid bacteria (LAB) play essential role in production of fermented food, being a part of natural microbiota of milk. With their metabolic activity they influence the ripening process of milk, allowing the creation of desirable sensory qualities of the product, while inhibiting the growth of undesirable microorganisms. As a by-product of their metabolism, lactic acid bacteria produce a variety of substances with antimicrobial activity such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl and bacteriocins. Bacteriocins are antimicrobial peptides or proteins with bactericidal activity on certain microorganisms. In this study, cell free supernatant was isolated from lactic acid bacteria of domestically produced fresh cow cheese. This kind of cheese was produced in traditional way, in households of Tuzla Canton, without adding any starter cultures. Isolation and characterization of LAB found that natural microflora of domestically produced cheese was consisted of bacteria of *Lactobacillus* and *Lactococcus* species. Concentrated samples of cell free supernatant were isolated out of ten characterized strains, and their antimicrobial activity was tested against pathogenic bacteria *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes* and *Salmonella enteritidis.* The highest inhibitory activity on the growth of pathogenic bacteria showed cell free supernatant isolated from *Lactobacillus plantarum 1* and *Lactobacillus brevis 1*. Sensitivity of the supernatant to proteolytic enzyme was tested by using proteinase K. After exposure of the supernatants to proteinase K, the results showed a significant reduction of inhibitory effect. The results proved the protein structure of the cell free supernatant, or the possible presence of bacteriocins and their bacteriocinogenic activity.

Key words: Lactic acid bacteria (LAB), cell free supernatant, fresh cow cheese, bacteriocin

INTRODUCTION

Fermented dairy products are products that can be produced via fermentation of lactose by microorganisms especially by lactic acid bacteria (LAB). When examining worldwide, various dairy products which are different in name but similar in content can be found and those products are an important part of human diet [1]. LAB plays an essential role in the production of fermented products. They are an integral part of the natural microbita of milk, and its metabolic activity influences the ripening process, allowing creation of the desired sensory properties of the product [2].

Domestic fresh cow cheese in Tuzla Canton is produced in a traditional way, without addition of starter cultures, so that the fermentation process takes place under the influence of lactic acid bacteria that are naturally present in raw milk. However, precisely because of this kind of production, hygiene of these foods is often questionable. According to the study conducted in the period from June to November 2012, fresh cow cheese made in households in TK had very bad microbiological quality, where from 50 analyzed samples even 39 were contaminated, mainly due to the presence of *Escherichia coli* of faecal origin (isolated in 78% of samples). Coagulase positive *Staphylococcus* species and *Salmonella* species were also found in analyzed samples [3].

In fermented foods, LAB display numerous antimicrobial activities. This is mainly due to the production of organic acids, hydrogen peroxide, ethanol, diacetyl, acetaldehyde, but also of other compounds, such as antifungal peptides and bacteriocins [4]. Small proteins called bacteriocins enable LBA to compete against other bacteria in the environment. In traditionally made fresh cow cheese LAB should allow biological safety of these products through the production these specific metabolites [5]. Bacteriocins are extracellular peptides or protein molecules, which have certain bactericidal properties in relation to specific types of microorganisms. Today, bacteriocins, as natural antimicrobial peptides or proteins, are very interesting potential applications in the food industry, as bioprotectors [6]. They are generally recognized as safe natural substances. Their protective role in the food industry is based on detected desired properties, among which are particularly important: non-toxicity and non-action in eukaryotic cells, decomposition under the action of digestive enzymes, little impact on the intestinal microflora, tolerance to pH and increased temperature, and a relatively broad spectrum of antimicrobial activity in relation to potential pathogens in food [7].

The aims of this study were: to isolate LAB from fresh cow cheese produced in households in Tuzla Canton and to characterized them with API 50 CHL test; to isolate and concentrate methabolic substances produced by LAB in a form of cell free supernatant; to test the antimicrobial activity of the isolated supernatant on the pathogens most commonly transmitted by food (*Listeria monocytogenes, Staphylococcus aureus, Escherichia coli and Salmonella enteritidis*); and also to test the effect of proteolytic enzyme on concentrated supernatant.

MATERIAL AND METHODS

Methods for the isolation and characterization of lactic acid bacteria

Microbiological analysis was performed on 50 samples of fresh cow's milk cheese produced in the Tuzla Canton. Isolation and characterization of lactic acid bacteria is done by the method described by Lengkey [8]. From the primary dilution (20g foods / 180 ml saline) decimal dilutions were made and then inoculated on De Man, Rogosa, Sharpe (MRS) plates. The inoculated plates were incubated at 37 °C 48-72h in anaerobic conditions. After incubation, typical colonies (round milky white colonies) were isolated from the plates and tested for the catalase production with H_2O_2 and stained by Gram. Biochemical characteristics of lactic acid bacteria were determined by API 50 CHL test (Biomerieux SA).

The method for the isolation of cell free supernatant

Each isolated lactic acid bacteria from fresh cow cheese was inoculated in 100 ml MRS broth and cultured in anaerobic conditions at 37°C/48, for multiplication. At the end of the incubation period, the bacteria were removed by centrifugation at 3000 RPM for 10 min. Supernatant was filtered through a membrane filtration device for applying filters Millipore of 0,45 microns. The filtrate was then concentrated using a rotary evaporator. The obtained product was a mixture of antimicrobial substances produced by the LAB; hereafter referred as "cell free supernatant".

Determination of antimicrobial activity of cell free supernatants

Antimicrobial activity of cell free supernatant was tested on pathogenic bacteria which are usually transmitted by food (*Listeria monocytogenes, Escherichia coli, Staphylococcus aureus and Salmonella enteritidis*). The bacteria that were used for testing were transferred on tryptone soy agar (TSA + 0,6% yeast extract) and incubated aerobically at 37 °C / 24h. One colony from a pure culture was suspended in 4-5 ml of sterile saline solution to get a blurring corresponding to 0,5 McFarland scale. After adjusting the inoculums, the sterile swab soaked with bacterial suspension was inoculated on the surface of Mueller-Hinton agar (MH agar). Four wells (10mm) were cut into the MH agar plates. Two wells were filled with 50 µl of cell free supernatant derived from different isolated LAB, and two wells were filled with control (distillate water of which pH value is adjusted with concentrated HCl, to match the pH of the tested supernatant). The pH of the cell free supernatant was adjusted to 6 with sterile 1N NaOH to neutralize the effect of lactic acid. The plates were incubated aerobically at 37 °C / 24 h. After the incubation period, a diameter of inhibition zone was registered in mm [9,10].

Testing the effect of proteolytic enzyme on cell free supernatants

The effect of proteolytic enzyme on cell free supernatants was tested as described by Zdolec and Lazic [11]. Two of the tested cell free supernatant that showed the highest antimicrobial activity, were treated with proteolytic enzyme (proteinase K) in order to verify their protein structure. Proteinase K was prepared according to the manufacturer instructions. After treatment with proteinase K, supernatants were incubated for 1 hour at 37 °C. After the incubation period, the antimicrobial activity of the treated cell free supernatants was tested on pathogenic bacteria, using well diffusion method as described earlier.

RESULTS AND DISCUSION

From 50 analyzed samples of fresh cow cheese from the Tuzla Canton, lactic acid bacteria were isolated from 20 samples. Isolated lactic acid bacteria were characterized with standardized API test. The results are shown in Table 1.

Table 1. Lactobacteria strains characterzied by their biochemical characteristics

Sample mark	Lactobacteria strains
1	*Lactobacillus plantarum 1*
2	*Lactobacillus sp.*
5	*Lactobacillus plantarum 2*
9	*Lactobacillus sp.*
19	*Lactobacillus curvatus*
20	*Lactobacillus sp.*
23	*Lactobacillus sp.*
25	*Lactobacillus sp.*
26	*Lactobacillus paracasei 3*
29	*Lactococcus sp.*
32	*Lactobacillus fermentum 1*
33	*Lactobacillus plantarum 1*
35	*Lactobacillus sp.*
38	*Lactobacillus plantarum 2*
39	*Lactobacillus brevis 1*
40	*Lactobacilllus sp.*
46	*Lactococcus lactis ssp lactis 1*
47	*Lactococcus sp.*
49	*Lactobacillus sp.*
50	*Lactobacillus plantarum 1*

Following species were identified as the natural microflora of the fresh cow cheese: *Lactobacillus plantarum 1, Lactobacillus plantarum 2, Lactobacillus curvatus, Lactobacillus paracasei 3, Lactobacillus fermentum 1, Lactobacillus brevis 1, Lactococcus lactis ssp lactis 1.* Isolation and the identification of the yeasts was not included in this study. Because similar studies have not been carried out in this part of Bosnia and Herzegovina, this study gives us an indicative insight into the composition of the natural microflora of domestically produced fresh cow cheese, from this region.

The antimicrobial activity of the obtained cell free supernatant was tested on *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, and Salmonella enteritidis* species. Diameters of inhibition zone of pathogenic bacteria, formed after the activity of the cell free supernatant as well as the pH value of the cell free supernatant is presented in Table 2.

Table 2. Diameters of inhibition zone of *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enteritidis* and pH values of the cell free supernatant

Sample mark	**Identified species of lactic acid bacteria**	**Antimicrobial activity of the cell free supernatant (diameters of inhibition zone are presented in mm)**			
		Escherichia coli	***Salmonella enteritidis***	***Staphylococcus aureus***	***Listeria monocytogenes***
1	*Lactobacillus plantarum 1*	23	20	20	33
50	*Lactobacillus plantarum 1*	19	20	18	35
33	*Lactobacillus plantarum 1*	26	26	21	35
5	*Lactobacillus plantarum 2*	21	20	20	30
38	*Lactobacillus plantarum 2*	18	21	20	30
26	*Lactobacillus paracasei 3*	20	20	20	28
32	*Lactobacillus fermentum 1*	19	21	16	30
19	*Lactobacillus curvatus*	20	20	20	30
39	*Lactobacillus brevis 1*	24	22	23	30
46	*Lactococcus lactis ssp lactis 1*	15	19	18	26

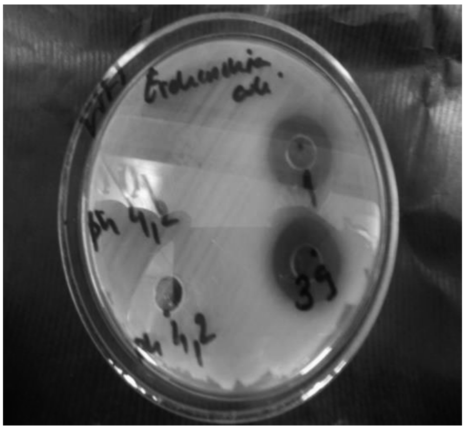


Figure 1. Antimicrobial activity of cell free supernatant obtained from *Lactobacillus plantarum* 1 (1) and *Lactobacillus brevis* 1 (39) on *E. coli*

Figure 2. Antimicrobial activity of cell free supernatant obtained from *Lactobacillus plantarum* 1 (1) and *Lactobacillus brevis* 1 (39) on *L. monocytogenes*

The highest inhibitory effect cell free supernatant had on the growth of *Listeria monocytogenes* , which had the biggest diameter of the inhibitory zone. The least significant effect was on *E. coli*, with the smallest diameter of the inhibition zone. It is known that the bacteriocins of LAB have a higher inhibitory effect on Gram positive bacteria, while the effect on Gram negative bacteria is less intense. The reason for this lies in the less complicated structure of the cell membrane compared to Gram-negative bacteria possessing an additional, outer membrane, which is impermeable to most macromolecules, and which provides the excellent protection against many external agents. The greatest antimicrobial potential on all pathogens had the supernatants isolated from *Lactobacillus plantarum* 1 and *Lactobacillus brevis* 1. The concentrated supernatants from these species were taken for further testing. The effect of proteolytic enzymes was tested with proteinase K.

Antimicrobial activity of isolated cell free supernatant from *Lactobacillus plantarum* 1 and *Lactobacillus brevis* 1 on the growth of *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus and Salmonella enteritidis*, before and after treatment with proteinase K, is shown in Table 3.

Table 3. Diameter of the inhibition zone (mm), before and after treatment, of the cell free supernatant isolated from *Lactobacillus plantarum* and *Lactobacillus brevis* 1, with proteinase K

	Antimicrobial activity of the cell free supernatant obtained from *Lactobacillus plantarum 1*	
	Before treatment with proteinase K	After treatment with proteinase K
Escherichia coli	23	13
Listeria monocytogenes	33	25
Staphylococcus aureus	20	16
Salmonella enteritidis	20	14
	Antimicrobial activity of the cell free supernatant obtained from *Lactobacillus brevis*	
	Before treatment with proteinase K	After treatment with proteinase K
Escherichia coli	24	15
Listeria monocytogenes	30	25
Staphylococcus aureus	23	15
Salmonella enteritidis	22	16

Figure 3. The antimicrobial effect of the cell free supernatant isolated from *Lactobacillus plantarum 1 (1) and Lactobacillus brevis 1* (39) on *Salmonella enteritidis,* before (left) and after (right) treatment with proteinase K

Significantly reduced inhibitory zone was observed in all pathogenic species, after the supernatant was exposed to the effect of the proteolytic enzyme – proteinase K. This way we proved the protein nature of the tested supernatant, and the fact that in the mixture the cell free supernatant are also present substances of protein nature that have a significant antimicrobial effect on the growth of pathogenic bacteria – bacteriocins. Therefore these results indicate that LAB, naturally present in domestically produced fresh cow cheese, produce bacteriocines and have possible bacteriocinogenic activity on food borne pathogens.

CONCLUSION

Producing a variety of metabolic products, LAB in a selective and competitive way, affect the local microflora in fresh cow cheese. That microflora may contain pathogenic bacteria or microorganisms that cause food spoilage. In our study, secondary metabolic products of LAB showed the lowest inhibitory effect on the growth of *Escherichia coli.* If we consider the fact that in the previous study [3] (the hygiene validity of fresh cow cheese) in the most of samples *E. coli* of faecal origin was isolated, we can conclude that LAB from domestic fresh cow cheese, have no significant effect on inhibiting the growth of *E. coli* in these foods.

From the analyzed foods *Staphylococcus aureus* and *Salmonella enteritidis* were also isolated but in less number of samples, than *Escherichia coli* (*Staphylococcus aureus* in two samples, *Salmonella enteritidis* in four samples) [3], which coincides with the results of testing the antimicrobial activity of the cell free supernatant. Metabolic products of LAB had inhibitory effect on growth of *Staphylococcus aureus* and *Salmonella enteritidis*, but not in such a ratio to fully prevent their presence in these foods.

Hodžić and Širanović [13] in their study examined the presence of *Listeria monocytogenes* in food in the area of Tuzla. After the analysis the authors have failed to isolate this pathogen from tested samples of fresh cheese. However, *Listeria monocytogenes* is commonly present in fresh dairy products and many cases of listeriosis are usually associated with the consumption of non pasteurized products such as fresh cow cheese [14]. The absence of this pathogen in fresh cow cheese from our region may be associated with distinctive influence of antimicrobial substances (such as bacteriocins) produced by LAB that constitute natural domestic cow cheese microflora. The results of our study also show that the greatest inhibitory effect, the concentrated supernatant had on this pathogen species.

Bacteriocins as natural antimicrobial peptides are now the subject of numerous studies. With an inhibitory effect on a large number of pathogenic bacteria, they represent very interesting potential for application in the food industry, as a natural biopresevatives [15].

REFERENCES

1. J. Šušković et al. Antimicrobial Activity of Lactic Acid Bacteria, Food Technol. Biotechnol. 2010; 48 (3): 296–307
2. N. Zdolec, S. Lazić, L. Kozačinski, M. Hadžiosmanović, I. Filipović, Inhibicijsko djelovanje bakterija mliječnih kiselina izolovanih iz svežeg kravljeg sira. Mljekarsto. 2007; 51: 5-13.
3. D. Kubiček, Produkcija bakteriocina mliječno-kiselinskih bakterija izolovanih iz svježeg kravljeg sira, Magistarski rad, Univerzitet u Tuzli, Prirodno-matematički fakultet, Odsjek za Biologiju. 2013; p 38-40.
4. B. Kos et al., Coculture-inducible bacteriocin biosynthesis of different probiotic strains by dairy starter culture *Lactococcus lactis.* Mljekarstvo. 2011; 61 (4), 273-282
5. Dj. Drider, F. Bendali, K. Naghmouchi, M. L. Chikindas. Bacteriocins: Not Only Antibacterial Agents. Probiotics and antimicrobial proteins. Springer, New York. 2016;
6. L. De Vuyst, E.J. Vandame, Bacteriocines of lactic acid bacteria. Blackie Academic and Profesional. UK. 1994.

7. J.L. Parada, C.C. Ricoy, P.M. Bianchi, Bacteriocines from Lactic Acid Bacteria: Purification, Properties and use as Biopreservatives. Brazilian archiver of Biology and Tehnology. 2007; 50: 521-542.

8. H.A.W. Lengkey, R.L. Balia, I. Togoe, B.A. Tasbac, M. Ludong, Isolation and identificaton of Lactic acid bacteria isolated from raw poutery meat. Biotehnology in Animal Husbendry. 2009; 25: 1071-1077.

9. U. Schillinger, F. Lucke, Antibacterial activity of Lactobacillus sake isolated from meat. Appl. Enviroment Mikrobiology. 1975; 55:1901-1906.

10. L. Uhlman, U. Schillinger, J.R. Rupnow, Holzapfel. Identification and characterization of two bacteriocin-producing strains of Lactococcus lactis isolated from vegetables. International Journal of Food Microbiology. 1992; 16: 141-151.

11. N. Zdolec, S. Lazić, L. Kozačinski, M. Hadžiosmanović, I. Filipović, Inhibicijsko djelovanje bakterija mliječnih kiselina izolovanih iz svežeg kravljeg sira. Mljekarsto. 2007; 51: 5-13.

12. R. W. Jack, J. R. Tagg, R. Bibbek, Bacteriocin of Garm pozitiv bacteria. Microbiological reviews. 1995; p171-200.

13. S. Hodžić, S. Širanović, Listeria monocytogenes in food and water enviroment of Tuzla. Technologica acta. 2012; 5: 29-41.

14. K. Markov i sar. Listeria monocytogenes i drugi kontaminanti u svježem siru i vrhnju domaće proizvodnje sa područja grada Zagreba. Mljekarstvo. 2009; 59 (3): 225-231.

15. S. Vesković-Moračanin Bakteriocini BMK kao prirodni protektori hrane-mogućnosti primjene u industriji mesa. Tehnologija mesa. Beograd. 2010; 51: 83-94.

SUSTAINABLE POME FRUIT PRODUCTION IN PATAGONIA, ARGENTINA

PROFESSIONAL PAPER

V. Sfara[1*], T. Fonovich[2], C. Pérez-Coll[1,2]

[1]Environmental Engineering and Research Institute; [2]School of Science and Technology.
National University of San Martín.
Av. 25 de Mayo y Francia (1650), San Martín, Prov. de Buenos Aires
*Corresponding author: vsfara@unsam.edu.ar

ABSTRACT

Apple and pear production in Argentina mostly takes place in the provinces of Río Negro and Neuquén, along a valley located in an area irrigated by the rivers Neuquén, Limay and Negro. Most commonly agrochemicals applied for these cultures protection are pesticides against mite and codling moth (*Cydiapomonella*, L). Carbaryl,azinphos-methyl and dimethoate have been recently detected and quantified in water samples from these rivers. Heptachlor, was also found sporadically, despite its use is forbidden in our country. Although these organophosphorus pesticides use is allowed, dimethoate and azinphos-methyl, as well as carbaryl are harmful substances that exert adverse effects through inhibition of cholinesterases and carboxylesterases. Also they are responsible of eliciting antioxidant responses in different organisms. Fruit production in this region became more sustainable in the last years as the consequence of government prohibition on the use of old generation pesticides and the introduction of new generation ones. A National Program designed to avoid codling moth damage, through a sexual confusion method, was implemented in 2006. The codling moth induced damage reached 6,1% of total production in 2003 in farms that were afterwards included in the Program, and 0,73% of it, after 3 years of program development; and at the same time there was a 385 ton/year decrease in azimphos-methyl use. Improvement in management techniques for pests as San José louse and European red mite, as well as government programs for their control promoting the use of non-toxic natural compounds, will certainly contribute to achieve sustainable fruit production in Patagonia.

Key words: fruit production, apple, pear, pesticides, sexual confusion control.

INTRODUCTION

The requirements of food in the last decades produced an expansion of the agricultural areas and an increase in the use of agrochemicals, such as pesticides and fertilizers, to reach greater crop yields. Pest control is a fundamental tool to increase crop productivity, since the economic loss due to pest damage is very high. It has been calculated that there would be a loss of the third part of the total global food production if agrochemicals were not used to control pests and crop diseases. However, the excessive use of pesticides can cause air, soil and water pollution. After application, pesticide formulations are distributed in all environmental compartments in relation to their physicochemical properties. In this way, the active principle as well as the excipients and the degradation products, interact with the soil matrix, eliciting changes in their structure and chemical composition. Weather conditions also influence the fate of pesticides, because they can be transported by the wind several kilometers away from the application site. Finally, pesticides and their degradation products can reach surface and underground waters, by means of transport phenomena such as leaching or run-off. Pesticide residues have also an impact on non-target species, including humans, causing acute and chronic intoxications that lead to different type of diseases.

On the other hand, taking into account the economic aspect of food production, the presence of residues of certain pesticides restrict or avoid the possibility to export the production of crops, such as fruits, from Latin American countries to United States and Europe. Thus, it became evident the need for development of novel strategies replace or decrease the use of synthetic pesticides. The purpose of the present paper is to describe one Argentinian program already implemented and compare it with other experiences.

Fruit production: Damages on pome fruits by insect pests and climate conditions in the Alto Valle

Agriculture in Argentina is centered on the production of wheat, corn, sunflower, soya, tobacco, cotton, potato, grape, stone fruits, pome fruits and others [1].1.8 million tons of pome fruits are produced annually in Argentina, distributed equally between apples and pears. 85% of the apples and 75% of the pears are produced in the Alto Valle of Río Negro and Neuquén provinces, in the north of the Patagonia region, among the basins of the rivers Neuquén, Limay and Negro.

There are several species of arthropods that cause damage to pome fruit plantations in the Alto Valle region. The most conspicuous are four species of mites, some homopterans such as mealybugs, aphids and leafhoppers, the lepidopterans*Cydiapomonella*, *Cydiamolesta* and *Oiketicusplatensis*, and, in a lower extent, thrips and pear psylla. Due to its distribution and to the magnitude of the damage it causes, *Cydiapomonella* is the most important pest of fruit cultures in the Alto Valle. This pest can elicit a loss of the 80 to 100% of the total pome fruit production, if it is not controlled.

The Alto Valle is characterized by a continental climate, which is temperate and arid.This region soil is mostly composed by sand and silt, with a relatively high rate of infiltration. It has a high salt content but a low content of organic matter. Rain is scarce in the whole area, reaching 200 mm/year. Annual media temperature is 14°C, reaching 40°C in summer and -13°C in winter [2,3]. All of these conditions are favorable for fruit production.

This zone is often affected by strong westerly winds, with gusts reaching velocities as high as 80 to 100 km/h, southern-west winds and by occasional hail in summer. Frost taking place during springtime, generally causes serious damage to the fruit cultures.These climate conditions demand extra economical and working efforts to farmers, in order to implement active protection measures. Poplar plantations are used to protect the fruit cultures against the winds. To avoid the damage caused by the frost, sprinkler irrigation systems are used, as well as heaters. In many cases farmers burn different materials to produce heat [4], but the smoke originated in this combustion is a source of pollutants that can affect people living in the neighboring towns. The only protection against hail available for farmers to count on is to pay insurance policies. Table 1 summarizes old and new generation control methods usually implemented by farmers in the recent past years to ameliorate damage caused by pests and climatic factors.

Table 1. Methods used to ensure acceptable levels of fruit production and quality in Patagonia, Argentina

Month/season of application	Pest/Frosts	Control method
September-October	Frost	Smoke (tire burning), heaters (fuel combustion), irrigation
October (1st generation) December (2nd generation)	*Cydiapomonella*(Codling moth)	SCT (Sexual confusión technique); Granulosis virus
November to May	*Quadraspidiotusperniciosus* (San Joselouse)	Insecticides (buprofezin, metomil, spirotetramat, clorantraniliprole + lambdacialotrina + suprathion, acetaminoprid) ; Calcium polysulfide (organic production);Oils
October to March	*Cydiamolesta* (Grapholite)	SCT;OrfamoneBaits
Spring and end of summer	*Panonychusulmi* (European red mite)*Psyllapyricola* (psyllids)	Oils; Abamectine
End of February	*Phitoptuspyri*	Sulfur-based products; Acaricides (endosulfan)

Data from INTA (2014) [8]

Pesticides in use for pest control and contamination of environmental compartments

Pome fruit production in Argentina occupies the fourth place of the agricultural activities requiring the use of great amounts of pesticides. Insecticides are widely used for pome fruit production and belong to group Ib (very toxic) according to toxicological risk classification by WHO. Loewy et al (2000) have made a complete report on some pesticides found in groundwater of the Alto Valle and their concentration, during 1996, 1997 and 1998 [5]. The list included dimetoate, metidathion, methylazimphos, fosmet, cypermethrin, carbaryl, propoxur, carbofuran, benomil and carbendazim. More recently, other authors have monitored pesticide concentrations at different locations and months of the year 2005, in water and sediments of the rivers Negro, Limay and Neuquén [6]. They found levels of the organophosphorus insecticide dimetoate (moderately toxic) between 0,085 and 0,0934 mg/l in superficial water of the Neuquén river, and traces of carbaryl (carbamate), methylazinphos (organophosphate) and heptachlor (organochlorinated compound). Though these concentrations were well below established limits by World Health Organization [7] for potable water, it is of concern that they were found in river waters after reaching groundwater.

Semiochemicals. Use of the sexual confusion technique (SCT) to control the codling moth in pome fruit production of the Alto Valle

Fruit producing countries are continuously looking for alternatives to old generation insecticides use. New integrated pest management programs are being implemented and include the use of more selective and non-pollutant products, such as botanical insecticides or repellents, insect growth regulators, or behavior modifiers, combined with cultural practices.

The use of semiochemicals (allelochemicals and pheromones) as behavior modifiers is one of the most widespread strategies to control agricultural pests.

Pheromones have many advantages to be used for pest control. They are very selective, because of their specificity; the manipulation of pheromones is safe because they are non-toxic, and as they are not persistent, they have a low potential as environmental pollutants.

Sex pheromones are the main tool of the sexual confusion technique (SCT). This method has been successfully implemented to control fruit pests, especially lepidopterans. The sexual confusion technique consists on the application in the field of formulations of a synthetic analog of the sex pheromone produced by females of the species to control, in order to avoid males to locate the females. Pheromones are formulated to permit a constant and prolonged release of the active substance. The concentration reached in the atmosphere is higher than the physiological concentration released by the female in natural conditions, eliciting confusion of males. In 2006 a National Program to eliminate the codling moth in the production of apple and pear in the Alto Valle region was implemented, based on the sexual confusion technique. The program consisted in the use of plastic pheromone emitters, containing the active principle codlemone, in apple and pear trees [9].

The principal goal achieved by the implementation of this program was the decrease in the percentage of fruit (apple and pear) damaged by codling moth from 6,1% to 0,73%in production areas under National Program [9]. Figure 1 shows reduction only in apple production damage both in farms under the program and those outside it (insecticide fumigation and SCT outside the program), along three years of application of this control strategy. The cited reduction percentage represents that 71713 tons of apples are recovered as commercial products, for being free of the damage caused by this pest. At the same time there was a 385 ton/year decrease in azimphos-methyl use [9].

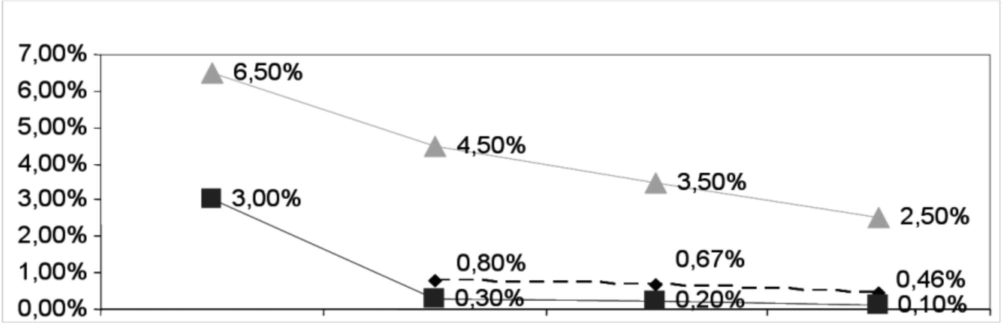


Figure 1. Decrease in the percentage of damage caused by Cydia pomonella in apples after the application of the Program; ▲ = conventional pesticides; ◆ National program SCT and ■ = SCT outside National Program. From http:// www.funbapa.org.ar

The use of this technique has also permitted that Argentinean fruit could overcome a number of restrictions of the international markets regarding the presence of quarantine pests and pesticides residues. The decrease in the use of conventional pesticides causes an increase in the abundance of beneficial species that can eventually act as biological controllers of the pest. Moreover, the development of insecticide resistance is delayed because the exposure of the pests to conventional active principles is lower. In the same way, health conditions of workers and people of the neighboring towns were improved, as the consequence of a decrease in their exposure to old-generation pesticides. Different pome fruit producer countries have implemented similar programs and have also had beneficial effects during pilot experiences. A small pilot test in Asturias has rendered beneficial effects, but only when pheromones were applied after three pretreatments with granulovirus, perhaps due to proximity of farms not participating in the test [10]. Similar results than the ones from the National Program presented here, were obtained in Spain and Italy and have been recently described by Damos et al. [11].

CONCLUSION

While the fight against spring frost and summer hail continue to demand high economical efforts, local farmers have found an important tool in the above mentioned National Program using SCT technique. The proof of Program success is evident as farmers have now to fulfill local government forms on SCT as a mandatory requirement to export their fruit production. The implementation of similar programs against other pests, like San José louse and European red mite, shall constitute other important tools to increase benefits of pome fruit production, together with environment and human health protection, in the Alto Valle of Río Negro and Neuquén.

REFERENCES

1. E.C. Villaamil Lepori, G. Bovi Mitre, M. Nassetta M Rev. Int. Contam. Ambie. 29 (Special Issue on Pesticides) (2013) 25-43.

2. L. Conticello, B. Cerazo, A. Bustamante. Gayana Bot. 59 (2002) 13-20. http://dx.doi.org/10.4067/S0717-6432002000100003

3. Autoridad interjurisdiccional de cuencas. Pronósticos meteorológicos (*Interjurisdictional Watershed Authority. Weather forecasts*) (2015). http://www.aic.gov.ar/aic/prono01.aspx

4. L. Digiacomo. Revista Agrovalle (*Agrovalle Journal*) (2015) http://www.revista-agrovalle.com.ar/info/

proyectos05.htm

5. R.M. Loewy, M. Novelli, L.G. Carvajal, H. Labollita, G. Neme, J. Ramos, C.Saenz , A.M. Pechen de D'Angelo (2000). 1st Joint World Congress on Groundwater. http://aguassubterraneas.abas.org/asubterraneas/article/viewFile/24298/16307

6. H.P. Boland, M.I. Gil, H.A. Labollita, B.Laurenzano, M.Novelli, J. Ramos, P. Reyes (2007). Congreso Nacional del agua (*National congress on water*). Tucumán, Argentina. http://www.ecopuerto.com/bicentenario/informes/MONITOREOAGROQUIMICOS.pdf

7. World Health Organization. Guías para la calidad del agua (*Guide for water quality*), Third Edition, Vol. 1, 2004. http://www.who.int/water_sanitation_health/dwq/gdwq3sp.pdf

8. INTA. Rev F y D (2014). http://inta.gob.ar/sites/default/files/script-tmp-inta_-_fyd_73.pdf

9. P. Villarreal, S. Mattei, M. Villegas Nigra and G. Forchetti. FunBaPa Ediciones, Buenos Aires, Argentina 2010, 81 pp. http://www.funbapa.org.ar/wp-content/uploads/2013/06/impacto-carpocapsa.pdf

10. M. Miñarro y E. Dapena. Bol. San. Veg Plagas, 26 (2000) 305-316. http://www.mapama.gob.es/ministerio/pags/biblioteca/plagas/BSVP-26-03-305-316.pdf

11. P. Damos, L. A. Escudero Colomar, C. Ioriatti. Insects 6(3) (2015) 626–657. Doi: 10.3390/insects6030626.

EFFECT OFF FAT ON TRACE ELEMENT CONTENT IN BROILER CHICKEN LIVER

ORIGINAL SCIENTIFIC PAPER

S. Dedić, A. Džaferović, H. Makić, J. Ibrahimpašić, A. Bećiraj

University of Bihać, Biotechnical Faculty, Kulina Bana 2, 77 000 Bihać, Bosnia and Herzegovina
The corresponding author: Dedić Samira, samira.dedic@yahoo.com

ABSTRACT

The aim of this research was to determine the trace element content (Pb, Cd, Cu, Cr, Zn and Fe) in liver, depending on the type of fat used in diets for broiler fattening. Four groups of Cobb 500 broiler chickens were used for the experiment. Each group had 60 broiler chickens. Broilers were fed with diets containing soybean oil, sunflower oil, beef tallow and lard. Broilers were fed with the starter mixture during the first sixteen days and with the finisher mixture from the 17th to 42nd day. The fat content in feed mixtures was 3% per treatment. Broilers were submitted to 12 hour pre-slaughter fasting time. After the slaughter, their carcasses were first cooled down to 4°C within 24 hours and stored at -18°C prior to the chemical analyses. Samples were taken randomly.

The highest lead (Pb) concentration was recorded in liver samples of broilers fed with feed containing soybean oil and it was 0,042 mg/kg. The concentration of lead in samples of broilers fed with feed containing sunflower oil was 0,036 mg/kg, followed by lard (0,022 mg/kg), and beef tallow (0,019 mg/kg).

Cadmium (Cd) content in broilers fed with feed containing soybean oil was 0,021 mg/kg, followed by sunflower oil (0,017 mg/kg), lard (0,013 mg/kg) and beef tallow (0,011 mg/kg). We can conclude that using animal and vegetable fat in broiler diets has affected the parameters of heavy metal content in broiler liver.

Key words: oil, beef tallow, lard, metals, broiler chicken.

INTRODUCTION

Vegetable and animal fat hold a special place in our diet, because of essential fatty acids and other components. Vegetable oil contains 84%-98% of fatty acids, mainly triacylglycerol, and minor components which belong to the unsaponifiable matter. Triacylglycerol properties depend on the properties of the fatty acids [1]. Each fatty acid has different properties which make it distinctive. It is constructed of hydrocarbon with a distinctive carboxylic acid group COOH. Fat consistency, as its physical and chemical properties, depends on the type of fatty acid. Depending on their degree of saturation, fatty acids can be divided into two classes: saturated and unsaturated. On the basis of the number of double bonds, they can be grouped into monounsaturated fatty acids (MUFA), if only one double bond is present, and polyunsaturated fatty acids (PUFA), if two to six double bonds are present [2].

In all vegetable oils, especially in sunflower oil, linoleic acid makes up about 70% of oil. Linolenic acid makes up 4-10% of soybean oil, whereas arachidonic acid is only found in animal fat in lower concentrations of about 0,3-1% [2]. Scientists are trying to change the level of certain matter in meat, especially fat content and composition, according to the newest studies on the effect of that nutrient on human health. New technologies are being developed for lowering the cholesterol levels and increasing the content of polyunsaturated fatty acids (PUFA) n-3 in lipids in muscle tissue, as demonstrated by numerous studies [3,4,5]. Animal feed clearly affects not only the quality of meat but the quality of animal products, which are the most nutritious food. Little attention has been given to mineral content in meat which, although in smaller amounts, has an important role in the tissue structure and the structure of biological systems, or as a functional part of many biochemical reactions found in the human body [6].

Heavy metals are divided into essential microelements which are vital for many functions in human body: copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), molybdenum (Mo), nickel (Ni), and nonessential elements which include all those toxic metals such as cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg) and astatine (At).

Although toxic metal contamination of animal feeding stuffs cannot be completely avoided due to the continuing presence of those pollutants in the environment, there is a clear need to reduce the amount of pollution to a minimum to lessen its direct impact on animal health and indirect impact on human health [7]. Lead (Pb), which presents a major problem in the world, is the most well-known heavy metal which pollutes the environment and can affect human health. Lead is stored in bones, partially in liver, kidneys and soft tissues. Lead poisoning affects the brain and nervous system, it can impair intellectual development, shorten attention span and memory, and in worse cases it can be lethal. Large amounts of cadmium (Cd) in kidneys cause kidney tissue damage, kidney stones, and high blood pressure. Cadmium affects the bone structure and can lead to bone deformity. It is a common cause of anaemia, and heart and kidney problems. Cadmium is also carcinogenic. Cadmium (Cd) enters the food chain from natural sources [8].

Poultry meat is high in B-complex vitamins (thiamine, riboflavin, niacin and pantothenic acid) and phosphorus (P), iron (Fe), zinc (Zn), but it is not a good source of liposoluble vitamins, vitamin C, calcium (Ca), potassium (K), magnesium (Mg) and manganese (Mn) [9]. Meat is a rich source of trace elements, while chicken entrails such as liver and kidneys often have high concentration of metals. [10] researched on the content of iron minerals (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in liver and red and white meat of broilers fed with feed containing minerals (metal salts) and medicinal plants (lemon balm, salvia, Saint John's wort and hoary willow herb). Under the influence of medicinal plants which are rich in polyphenols, accumulation of metals in broilers' liver and red and white meat is significantly different compared to the group which was fed with diets containing only metal salts. Each medicinal plant had a distinctive effect on metal accumulation. [11] reported that different types of iron in broiler feed did not have any influence on the amount of accumulated non-heme iron in liver in the period from the 21st to 42nd day, while significantly less iron was accumulated in liver of broilers fed with feed containing ferrous ascorbate from the 35th day.

MATERIALS AND METHODS

Experimental research was carried out in the commercial broiler processing plant „Koka-Sana" in Sanski Most. Four groups of Cobb 500 broiler hybrids (60 broilers) were used in this research. Broilers were fed with identical feeding mixtures containing: group I-soybean oil, group II-sunflower oil, group III –beef tallow, and group IV-lard. Percentage of fat in feed mixtures was 3% per treatment.

Broilers were fed with the starter mixture for the first sixteen days, and with the finisher feed mixture from the 17th to 42nd day.

Table 1 Ingredients of Starter Broiler Diets

Ingredients	**Starter diet (%)**			
Corn	53,5	53,5	53,5	53,5
Soybean meal	38,0	38,0	38,0	38,0
Sunflower meal	1,5	1,5	1,5	1,5
Soybean oil	3,0	-	-	-
Sunflower oil	-	3,0	-	-
Beef tallow	-	-	3,0	-
Pork fat	-	-	-	3,0
Premix	4,0	4,0	4,0	4,0
Total	100	100	100	100

Table 2. Ingredients of Finisher Broiler Diets

Ingredients	Finisher diet (%)			
Corn	58,5	58,5	58,5	58,5
Soybean meal	33,0	33,0	33,0	33,0
Sunflower meal	1,5	1,5	1,5	1,5
Soybean oil	3,0	-	-	-
Sunflower oil	-	3,0	-	-
Beef tallow	-	-	3,0	-
Pork fat	-	-	-	3,0
Premix	4,0	4,0	4,0	4,0
Total	100	100	100	100

Table 3. Chemical analyses of Starter Broiler Diets

Chemical analyses	Treatment 1	Treatment2	Treatment 3	Treatment 4
Water (%)	10,86	11,86	11,40	11,27
Ash (%)	6,73	6,01	6,36	5,45
Proteins (%)	23,55	24,14	23,51	23,49
Fat (%)	5,65	5,65	5,52	6,46
Cellulose (%)	3,19	2,93	2,60	2,77
Calcium Ca (%)	2,67	2,29	1,95	2,70
Phosphorus P (%)	1,10	0,77	0,88	0,93

Treatment 1 (broiler liver, feed containing soybean oil), Treatment 2 (broiler liver, feed containing sunflower oil), Treatment 3 (broiler liver, feed containing beef tallow), Treatment 4 (broiler liver, feed containing lard)

Table 4. Chemical analyses of Finisher Broiler Diets

Chemical analyses	Treatment 1	Treatment2	Treatment 3	Treatment 4
Water (%)	9,87	10,00	9,67	10,32
Ash (%)	6,50	6,03	6,29	6,56
Proteins (%)	21,29	22,12	19,79	19,63
Fat (%)	6,92	6,88	6,87	5,25
Cellulose (%)	3,19	2,93	2,60	2,77
Calcium Ca (%)	1,48	2,09	1,56	2,22
Phosphorus P (%)	0,18	1,24	0,76	0,66

After 42 days, broilers were submitted to 12 hour pre-slaughter fasting time. After the slaughter, internal organs and condemned viscera were separated, fat tissue was removed, and carcasses were stored at -18°C prior to the chemical analyses. After the samples were defrosted, the broiler liver was homogenised and used for the analysis in that way. The samples were taken randomly. The analyses were carried out at the Food quality control laboratory of Biotechnical Faculty, University of Bihać.

The following chemical methods were used to determine the chemical content of broiler liver: EN ISO 662:2000 [12] method was used to determine the water content in the samples, HRN ISO 1443:1999 [13] method was used to determine the fat content, and the protein content was determined by HRN ISO 937: 1999 [14].

Metals (Pb, Cd, Cu, Cr, Zn and Fe) were determined by Atomic absorption spectrophotometry (AAS) „Analytical Methods“FP-3 Analysis of Meat and Meat Products (2000), „Perkin Elmer“AAnalyst -800. The instrument measures every standard, i.e. sample, three times, performs statistical analysis, generates a curve display and calculates the concentration of the unknown sample. Sample preparation was done in a microwave oven for digestion (Multiwave PRO, ROTOR 8NXQ80, 0, 3 bar/s, 80 bar, 1500W). First, 8 ml of concentrated HNO_3 was added to 0,5 g of sample. Then, it was ignited according to the temperature programme. Heating, combustion and cooling lasted for 50 minutes. Afterwards, the samples were diluted. Finally, absorbance was calculated by flame AAS in such a sample.

RESULTS AND DISCUSSION

Water, fat, protein and ash content in broiler chicken liver samples were determined by chemical analysis, and the energy value was calculated based on these results. The average values of trace elements depending on feed treatments are shown in Table 5.

Table 5. Average values of chemical analyses of broiler liver per treatment

Parameters	**Broiler liver samples per treatment**			
(n = 30)	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Water (%)				
$\overline{X}$	78,26	71,89	76,17	77,05
SD	1,405	1,195	1,407	1,781
Sx	0,256	2,218	0,256	0,325
X min	76,21	69,98	72,91	73,18
X max	81,86	74,28	78,56	80,34
Fat (%)				
$\overline{X}$	5,16	5,08	5,29	5,33
SD	0,166	0,200	0,274	0,158
Sx	0,030	0,036	0,050	0,029
X min	4,76	4,56	4,85	5,05
X max	5,45	5,37	5,86	5,65
Proteins (%)				
$\overline{X}$	21,58	18,31	17,42	20,42
SD	0,428	0,372	0,333	0,551
Sx	0,078	0,068	0,060	0,101
X min	19,99	17,43	16,69	19,32
X max	22,32	19,27	18,24	22,02

Ash (%)				
$\overline{X}$	1,18	1,19	1,19	1,20
SD	0,035	0,043	0,047	0,067
Sx	0,006	0,007	0,008	0,012
X min	1,10	1,13	1,11	1,11
X max	1,25	1,30	1,32	1,37

Parametrs	Sum of sqrs	ANOVA df	Mean squere	F	P (same)
watter	65,2653	5,698	8,7048	7,498	0,03571**
fat	3508,06	3,001	548,30	0,9377	0,4043
protein	19,4699	6,032	3,2449	2,731	0,01528**
ash	0,0781	3,384	59,440	1,981	0,1289
KRUSKAL-WALLIS TEST					
	watter	**fat**	**protein**		**ash**
H (ch2)	4,83	0,75	1,33		5,33
Hc (tie correted):	4,83	0,76	1,33		5,389
P (same)	0,0133**	0,3865	0,0248**		0,2016

*n-The number of samples analysed; Treatment 1 (broiler liver, feed containing soybean oil), Treatment 2 (broiler liver, feed containing sunflower oil), Treatment 3 (broiler liver, feed containing beef tallow), Treatment 4 (broiler liver, feed containing lard) There is a significant difference between sample medians**, There is less significant difference between sample medians*, There is no significant difference between sample medians*

The liver samples of broilers which were fed with feed containing soybean oil had the slightly higher energy value than the liver samples of broilers fed with feed containing beef tallow and sunflower oil. The energy value was slightly higher due to higher protein and fat content. The energy value of the samples were as follows: Treatment 1 - 557.78 kJ/100g, Treatment 2 - 499.23 kJ/100g, Treatment 3 - 491.87 kJ/100g and Treatment 4 - 544.35 kJ/100g. The energy value results of broiler liver per treatment are shown in Chart 1.

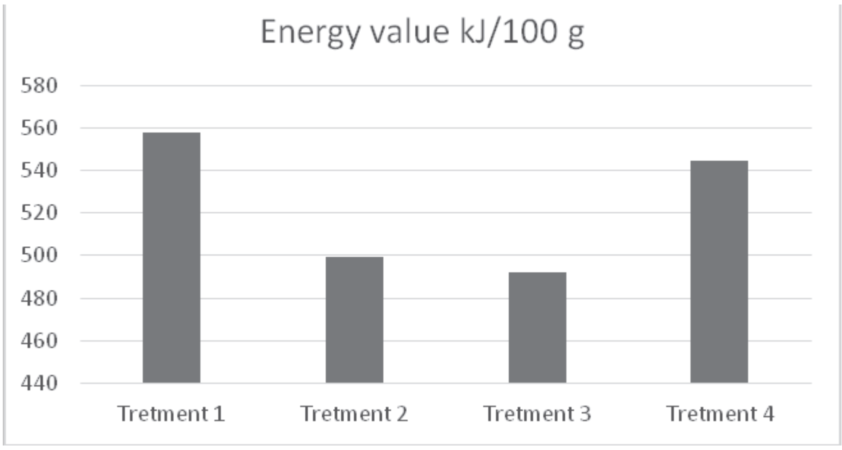


Chart 1. Graphical display of broiler liver energy values per treatment

Dry matter, ash content, and iron in liver of broilers fed with feed containing 30 mg of iron in 1 kg of feed has been studied by [15]. It has been determined that average values of dry matter, ash and iron are 27,50%, 1,36% and 169,35 mg/kg, respectively.

Chicken liver has the average energy value of 570 kJ, and the relative protein and fat content is 22,1% and 4,7%, respectively. Average saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids content is 1,48 g/100 g, 1,18 g/100 g, and 0,773 g/100 g, respectively.

The average cholesterol level is 555 mg/100 g [16]. According to *USDA 2012* [17], the average water content in broiler liver is 76,46%, and the average protein content is 16,92%; the average fat content is 4,83%, and the average ash content is 1,06%. The energy value in 100 g is 485 kJ. The average values of the trace element content depending on the feed treatment are shown in Table 6.

Table 6. Trace element content parameters in broiler liver (mg/kg) per treatment

Parameters	**Broiler liver samples per treatment**			
(n = 6)	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Pb	0,042	0,036	0,019	0,022
Cd	0,021	0,017	0,011	0,013
Cu	2,64	2,58	4,98	3,87
Cr	0,007	0,008	0,004	0,006
Zn	8,04	11,31	24,30	29,37
Fe	62,61	97,97	134,86	163,54

Parametrs	**Sum of sqrs**	**ANOVA df**	**Mean squere**	**F**	**P (same)**
Pb	25,9206	9,156	3,48625	1,898	0,1845
Cd	26,1639	9,009	3,52721	5,083	0,01524**
Cu	32,6663	9,324	0,608845	0,2743	0,9356
Cr	26,3196	9,043	3,55324	1,981	0,1709
Zn	2735,35	8,228	141,987	6,185	0,01019*
Fe	78292,5	8,014	7227,12	18,32	0,8346

KRUSKAL-WALLIS TEST						
	Pb	**Cd**	**Cu**	**Cr**	**Zn**	**Fe**
H (ch2)	11,19	15,45	2,25	10,87	10,16	10,02
Hc (tie correted):	11,02	15,49	2,25	10,97	10,16	10,02
P (same)	0,08244	0,01676**	0,8953	0,8943	0,01181*	0,1163

*n - number of samples analysed; Treatment 1 (broiler liver, feed containing soybean oil), Treatment 2 (broiler liver, feed containing sunflower oil), Treatment 3 (broiler liver, feed containing beef tallow), Treatment 4 (broiler liver, feed containing lard) There is a significant difference between sample medians** There is less significant difference between sample medians* There is no significant difference between sample medians*

The highest lead (Pb) content was found in liver samples of broilers fed with feed containing soybean oil (0,042 mg/kg). Lead content (Pb) in broilers fed with feed containing sunflower oil, lard and beef tallow was 0,036 mg/kg, 0,022 mg/kg and 0,019 mg/kg, respectively. Cadmium (Cd) content in broilers fed with soybean oil was 0,021 mg/kg, in broilers fed with sunflower oil it was 0,017 mg/kg, in broilers fed with lard it was 0,013 mg/kg, and in broilers fed with feed containing beef tallow it was 0,011 mg/kg. Lead (Pb) and cadmium (Cd) content were higher in samples of broilers fed with vegetable fat.

According to the Regulation of maximum levels for certain contaminants in foodstuffs [18], the maximum levels allowed for lead (Pb) and cadmium (Cd) in chicken liver are 0,50 mg/kg, and for copper (Cu) 80 mg/kg. Our results have shown that lead (Pb) and cadmium (Cd) levels are lower than those recommended by the Regulation [18].

A research on cadmium and lead content in broiler chicken has been done by Zorica Novaković et al., 2010 [19], who have determined that the cadmium content in two samples is 0,017 and 0,020 mg/kg, while the cadmium content in other three samples is below the limit of detection. Flame atomic absorption spectrophotometry was used for the lead content which was below the detection limit in broiler liver samples.

The highest copper content (Cu) was found in liver of broilers fed with feed containing animal fat. The copper (Cu) content in liver of broilers fed with feed containing beef tallow was 4,98 mg/kg, and in feed containing lard was 3,87 mg/kg, whereas adding vegetable fat resulted in values of 2,64 mg/kg for soybean oil and 2,58 mg/kg for sunflower oil. Our results are all according to the Regulations [18], by which samples analysed must not contain more than 80 mg/kg of copper (Cu). Higher amount was only found in broilers fed with feed containing animal fat.

The chromium (Cr) content in liver of broilers fed with sunflower oil was 0,007 mg/kg, while in liver of broilers fed with soybean oil, lard and beef tallow the content was 0,007 mg/kg, 0,006 mg/kg and 0,004 mg/kg, respectively. The chromium (Cr) content in broiler liver was not significantly affected by different feed treatments.

Certain differences were noted in treatments regarding Zinc (Zn) content in broiler liver. The highest value was determined in liver of broilers fed with lard, which was 29,37 mg/kg, followed by 24,30 mg/kg in broilers fed with beef tallow and 11,31 mg/kg in broilers fed with sunflower oil. The lowest value of 8.04 mg/kg was determined in liver of broilers fed with soybean oil.

The highest iron (Fe) content was found in liver of broilers fed with lard, which was 163,54 mg/kg. Content values for beef tallow and sunflower oil were 134,86 mg/kg and 97,97 mg/kg, respectively. The lowest iron (Fe) content, which was 62,61 mg/kg, was determined in broilers fed with soybean oil.

According to *USDA 2012,* the average content of Ca is 8 mg/100g, Fe 8,99 mg/100g, Mg 19 mg/100g, Zn 2,67 mg/100g, Cu 0,492 mg/100g, Mn 0,255 mg/100g and Se 54,6 µg/100g.

Oforka Nicolas et al. (2012) [20] have reported on average values for certain macro- and microelements, based on the analysis of liver, gizzard or muscle. The liver contained the highest concentration of metals Cd (0,046 mg/g), Pb (0,304 mg/g), Mn (0,415 mg/g), Zn (2,325 mg/g) and Ni (0,108 mg/g), followed by gizzard; Cd (0,024), Pb (0,287), Mn (0.127) Zn (1,940) and Ni (0,062 mg/g) while the muscle had the least concentration except for Mn; thus Cd (0,016 mg/g), Pb (0,215 mg/g), Mn (0,266 mg/g) Zn (1,570 mg/g) and Ni (0,062 mg/g).

Cieślik et al. (2011.) [21] have done a study research on metal content in raw liver in broilers and foie gras. The concentration of analysed microelements in broiler liver ranged between 63,09 and 146,47 mg/kg for iron (Fe), 3,46 to 5,34 mg/kg for copper (Cu); 2,99 to 4,99 mg/kg for manganese (Mn); 40,19 to 59,42 mg/kg for zinc (Zn) and 0,03 to 0,06 mg/kg for cadmium (Cd).

Mineral content in Foie Gras was much higher than in broiler liver. Their content ranged between 52,93 and 659,84 mg/kg for iron (Fe), copper (Cu) 39,32 to 64,45 mg/kg; manganese (Mn) 3,43 to 5,32 mg/kg; zinc (Zn) 50,87 to 67,20 mg/kg and cadmium (Cd) from 1,10 to 4,12 mg/kg. Cadmium concentration values found in broiler liver was below the limit values set by the European Union.

Perekhan Aljaff et al., (2014) [22] have done research on metal content in broiler liver and determined these values: Cr 0,08693 µg/g, Cu 0,1583 µg/g, Fe 6,482 µg/g, Mn 0,09071 µg/g, Pb ND, Zn 1,342 µg/g, Se 0,01742 µg/g, Co 0,005919 µg/g, Ni 0,0904 µg/g, and Cd 0,00509 µg/g.

According to the results, Oforka et al., (2012) [20] have reported that broilers from the oil rich Port-Harcourt capital have low concentration of heavy metals in muscles, liver and gizzard, but that the lead (Pb) concentration is much higher than the limit set by FAO/WHO, which is 0,2 mg/kg. Liver contained the highest concentration of metals: cadmium (Cd) 0,046 µg/g, lead (Pb) 0,304 µg/g, manganese (Mn) 0,415 µg/g, zinc (Zn) 2,325 µg/g and nickel (Ni) 0,108 µg/g; gizzard content is as following: cadmium (Cd) 0,024 µg/g, lead (Pb) 0,287 µg/g, manganese (Mn) 0,127 µg/g zinc (Zn) 1,940 µg/g and nickel (Ni) 0,062 µg/g, while the muscle had the lowest concentration except manganese (Mn): cadmium (Cd) 0,016 µg/g, lead (Pb) 0,215 µg/g, manganese (Mn) 0,266 µg/g zinc (Zn) 1,570 µg/g and nickel (Ni) 0,062 µg/g.

Reem Th. Hussain et al., (2012) [23] have conducted a study to determine the concentration of Cd, Pb and Zn (using Atomic absorptionspectrophotometer AAS) in nine brands of chicken liver, which are widely consumed from the local markets of Basrah city. The levels of metals in the chicken liver ranged between 0,004 µg/g and 0,124 µg/g for Cd; 0,171 µg/g and 3,269 µg/g for Pb; and 4,116 µg/g and 3,266 µg/g for Zn. The results have shown that the concentration of Pb in four brands exceed the permissible limits set by WHO/ FAO and ANZFA (Australia New Zealand Food Authority).

CONCLUSION

Based on the study we can conclude that in all broiler liver samples in all four diet treatments, the concentration of cadmium (Cd) and lead (Pb) was within permissible limits. Comparing these elements in analysed samples, it was clear that the cadmium (Cd) and lead (Pb) content was higher in the samples where broilers were fed with feed containing soybean or sunflower oil, while the lower content was found in the liver of broilers fed with beef tallow and lard. This was clearly affected by the type of diets and fat used in feed. The copper (Cu) content was according to the Regulations [18]*;* higher values were found in the liver of broilers feed with feed containing animal fat. The highest chromium (Cr) content was found in samples of broilers fed with feed containing vegetable fat, whereas zinc (Zn) and iron (Fe) were higher in liver samples of broilers fed with feed containing animal fat.

Based on the study, we can conclude that using animal and vegetable fat in broiler diets has affected the parameters of heavy metal content in broiler liver.

References

1. M. Bockisch (1993) Nahrungsfette und Öle. Handbuch der Lebensmittel technologie Ulmer Verlag, Stuttgart, Germany
2. S. Čorbo (2008) Tehnologija masti i ulja, Univerzitetski udžbenik Sarajevo.
3. Chanmugam, P., Boudreau, M., Boutte, T., Park, R.S., Hebert, J., Berrio, L., Hwang, D.W. (1992.): Incorporation of different types of n-3 fatty acids into tissue lipids of poultry. *Poultry Science,* 71, 516-521.
4. S. Lopez-Ferrer, M.D. Baucells, A.C. Barroeta, M.A. Grashorn, (1999) Influence of vegetable oil sources on quality parameters of broiler meat. Archiv fur Geflugelkunde 63(1):29-35.
5. T. Komprda, J. Zelenka, P. Bakaj, D. Kladroba, E. Blažková, E. Fajmonova, (2003) Effect of dietary sunflower, linseed or fish on cholesterol and fatty acid content in Turkey meat. Proceedings of XVI European Symposium on the Quality on Poultry Meat 04-08.09.1993, Tours, France, I. Quality of Poultry Meat, Vol. II, 185-189.
6. G. Kralik, Z. Škrtić, M. Galonja, S. Ivanković, (2001.): Meso pilića u prehrani ljudi za zdravlje. Poljoprivreda, 7 (1): 32-36.
7. SCAN. (2003) Scientific Committee on Animal Nutrition: Opinion of the Undesirable Substances in Feed. Retrieved from: http://europa.eu.int/comm/food/fs/sc/scan/out126_bis
8. M. Jašić Toksične tvari u hrani, 2009, www.tehnologijahrane.com/.../toksicne-tvari-hrani
9. G. Lombardi-Boccia, S. Lanzi, A. Aguzzi, (2004) Aspect of meatquality: trace elements and B vitamin in raw and cooked meats, *Journal of Food Composition and Analysis,* Volume 18, Issue 1, February, 39 – 46.
10. S. Ducu Sandu and G. Iosif (2012) Effect of mineral-enriched diet and medicinal herbs on Fe, Mn, Zn, and Cu uptake in chicken, *Chemistry Central Journal*, 6:19 http://journal.chemistrycentral.com/content/6/1/19.
11. S. Milanović, M. Lazarević, Ž. Jokić (2008) The influence of organic and inorganic Fe supplementation on red blood picture, immune response and quantity of iron in organs of broiler chickens. *Acta Veterinaria* 2008;58,(2-3):179-189.
12. EN ISSO 662:2000- Animal and vegetable fats and oils- Determination of moisture and volatile matter content

13. HRN ISO 1443:1999 – Meat and meat products- Determination of total fats content.
14. HRN ISO 937:1999 – Meat and meat products_ Determination of nitrogen content (Referent metod).
15. F. Alibegović - Zečić, A. Kavazović, A. Gagić, E. Rešidbegović, S. Piplica (2011) Efekt dijetalnog željeza na njegov sadržaj u jetri brojlera *Veterinaria* 60 (3-4),113-120, Sarajevo 2011.
16. Danish Food Composition Databank – ed. 7.01. National Food Institute – Technical University of Denmark (DTU), www.foodcomp.dk
17. USDA National Nutrient Database for Standard Reference, Release 25 (2012.).
18. Službeni list BiH, br.37/09. Pravilnik o najvećim dopuštenim količinama određenih kontaminanata u hrani, 2009.
19. Z. Novaković, T. Gerić, T. Bošković, J. Jovanović, S. Janković, S. Stefanović, (2010) Značaj kontinuiranog ispitivanja prisustva nekih hemijskih kontaminenata u jetri goveda, svinja i brojlera. Vet. glasnik 64 (5-6) 367 - 374
20. N.C. Oforka, L.C. Osuji, U.I. Onwuachu, (2012) Estimation of Dietary intake of Cadmium, Lead, Manganese, Zincand Nickel due to consumption of chicken meat by inhabitants of Port-Harcourt Metropolis, Nigeria, *Archives of Applied Science Research,* 2012, 4 (1):675-684.
21. E. Cieślik, I. Walkowska, J.M. Molina-Ruiz, I. Cieślik, W. Migdal, (2011) Comparison of content of selected minerals and cadmium in chicken a nd goose liver, Biotechnology in Animal Husbandry, Publisher: *Institute for Animal Husbandry,* Belgrade-Zemun, 27 (4), p 1857-1858.
22. A. Parekhan, O.R. Banaz, M.S. Dler, (2014) Assessment of heavy metals in livers of cattle and chicken by spectroscopic method. IOSR *Journal of Applied Physics* (IOSR-JAP) e-ISSN: 2278-4861. Volume 6, Issue 1 Ver. II (Feb. 2014), PP 23-26 www.iosrjournals.org
23. Th.H. Reem, Kh.E. Manal, M.M. Hanady (2012) Assessment of heavy metals (*Cd, Pb and Zn*) contents in livers of chicken available in the local markets of Basrah City, Iraq. Bas.J.Vet.Res. Vol.11,No.1,2012.
24. HRN ISO 937:1999 – Meat and meat products_ Determination of nitrogen content (Referent metod).

INSTRUCTIONS FOR AUTHORS OF PAPERS

1. The manuscript which is to be submitted to the Editorial Board should be written without columns with single spacing on one side of **A4 paper, with all margins of 2.54 cm (1 "), font Times New Roman 12 pt.** The work will be sent in electronic form, prepared solely using word processing program Microsoft Word, ending with the 2007 versions. The file should be named as follows: TA_last name of first author _first word of title. doc. The extension must match the image format (tif, pcx, jpg, png ....). Images should have a resolution of min. 300 dpi and should be prepared so that they can be printed well in B/W technique. Each individual image should not be greater than one third of A4 format. Image labels should be written below the picture.

2. The size of the article (text, along with summaries, pictures and drawings and with a list of literature references, not counting titles and signatures, as well as information about authors) should be limited to 6 pages (two illustrations correspond to approximately one page). An exception can be negotiated with the editorial board, and to receive a larger volume of work if the content and quality justifies it.

3. Abstracts should be attached to the manuscripts:
- Summary (synopsis) in the language of article of the maximum volume of a one printed site. It must explain the purpose of the paper, and must include the important data and conclusions, as well as keywords. This summary should be entered in the manuscript right after the header of the article.
- The same summary in English (summary) with keywords (descriptors **-** keywords).

4. The paper should contain the full official address, phone and e-mail address of all authors (on a separate sheet). Emphasize the correspondence author, with whom will the editorial board consult.

5. The title of the article should be specific and informative, in order to better determine the content of the paper. It is desirable to be as short as possible.

6. Text should be clear, concise, and grammatically correct without spelling errors, written in third person (impersonal).

7. Papers should be written with the assumption that readers know the discussed subject. Thus in (a short) introduction should briefly be stated only what is necessary for understanding of the text.

8. Experimental technique and device should be described in detail only if they deviate significantly from the description already published in the literature; for the known techniques and devices only source of necessary information is provided.

9. Tables and diagrams should be drawn and described so that they are understandable without reading the text**, font Times New Roman 10 pt**. in table. The same data should not be placed at the tables and diagrams, except in exceptional cases. The author will then give its reasons, and its validity is subject to final assessment of Editorial board and its reviewers.

10. Tables that contain a lot of data, where not all are necessary for understanding of the text should be reduced to the minimum. If desired by author, the editor will store complete tables in the archives and enable to interested readers an insight into the archive. This should be stated as a note with the abbreviated table.

11. Symbols of physical values should be written cursive, and unit of measure with vertical letters, eg. V, m, p, t, T, but: m^3, kg, Pa, °C, K.

12. Formulas and equations should be typed, if possible, in one line (use aoblique fractional line instead of horizontal). Indexes, at top and bottom, should be written clearly. Avoid upper indexes so that they would not replace with numerical exponents. All special characters (Greek letters, etc.) that can cause confusion, authors should explain separately.

13. In the paper should be used sizes and units of measurement in accordance with International System of Units (SI). For specific nomenclature a list of all used labels and definitions should be add in the language of the article and on the English language.

14. References cited should be selective rather than extensive, except when it comes to review article. Literature citations should be enclosed on a separate sheet of paper and they should be numbered in the order they appear in the text. The numbersof citations are written to the text as a superscript. If the original literature was not available to the authors, they should cite by the source from which the quotation was taken. Abbreviations for magazines must be in strict accordance with the abbreviations that are alleged by the Chemical Abstract.

Books

Basic Format:

[1] J. K. Author, “Title of chapter in the book,” in Title of His Published Book, x the d. City of Publisher, Country if not USA: Abbrev. of Publisher, year, ch. x, sec. x, pp. xxx–xxx.

Example:

[1] B. Klaus and P. Horn, Robot Vision. Cambridge, MA: MIT Press, 1986.

Handbooks

Basic Format:

[1] Name of Manual/Handbook, x ed., Abbrev. Name of Co., City of Co., Abbrev.State, year, pp. xx-xx.

Example:

[1] Transmission Systems for Communications, 3rd ed., Western Electric Co., Winston-Salem, NC, 1985, pp. 44–60.

Articles

Basic Format:

[1] J. K. Author, “Title of paper,” in Unabbreviated Name of Conf., City of Conf., Abbrev. State (if given), year, pp.

xxx-xxx.

www

Basic Format:

[1] J. K. Author. (year, month day). Title (edition) [Type of medium]. Available: http://www.(URL)

Example:

[1] J. Jones. (1991, May 10). Networks (2nd ed.) [Online]. Available: http://www.atm.com

15. In the corrective prints only the author can correct the error text. For possible changes in the text (additions, etc.), the author bears the cost.

FACULTY OF TECHNOLOGY

CONTENT

9 771840 042000