JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

SAFETY OF BLACK SOLDIER FLY LARVAE: MICROBIAL AND HEAVY METAL RISKS

Zuzana Barboráková[1], Jana Urminská[2], Silvia Jakabová[2], Eduard Kolesár[3], Dana Tančinová[1], Dana Urminská[1], Eva Ivanišová[2], Marián Tomka[1], Lukáš Hleba[1], Monika Mrvová[1], Juraj Medo[1], Zuzana Mašková[1]*

Address(es): Ing. Zuzana Barboráková, PhD.
[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, phone number: +421376414494.
[2] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[3] ecol Trade, s.r.o., J. Haška 1, 94901 Nitra, Slovakia.

*Corresponding author: zuzana.barborakova@uniag.sk

https://doi.org/10.55251/jmbfs.12069

ARTICLE INFO

Received 13. 8. 2024
Revised 18. 11. 2024
Accepted 25. 11. 2024
Published 1. 12. 2024

Regular article

OPEN ACCESS

ABSTRACT

Black soldier fly larvae (BSFL) are considered a sustainable protein source and an effective means of recycling organic waste. They are rich in nutrients, including proteins and lipids, making them suitable for animal feed and potentially for human consumption. In addition to their nutritional benefits, BSFL also have environmental benefits, i.e., reducing waste and greenhouse gas emissions. However, concerns about their safety persist, particularly regarding contamination by pathogenic microorganisms, toxins, and heavy metals. The aim of our study was to investigate the effect of different feedstuffs on heavy metal accumulation and on the microcenosis (Enterobacteriaceae, aerobic bacterial endospores, microscopic filamentous fungi) of BSFL. BSFL were fed four feed treatments (I: egg pasta cooked in whole milk; II: cooked rice with peas; III: poultry feed; and IV: cooked couscous, boiled eggs, and raw carrot peels). The results of our study showed that feed variants significantly affect the microbial safety of BSFL. Using MALDI-TOF, we identified 13 species of family Enterobacteriaceae in BSFL, including facultative pathogenic species *Enterobacter cloacae*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Morganella morganii*, and *Proteus mirabilis*. Our research confirmed that BSFL fed different feed variants under experimental laboratory conditions contained heavy metals, including cadmium, copper, manganese, molybdenum, nickel, lead, and zinc. Cadmium concentrations in BSFL ranged from 0.12 to 0.18 mg.kg^{-1}, with the highest values measured in BSFL in variant IV.

Keywords: *Hermetia illucens*, Microorganisms, Pathogens, Polyalloxides, Hazards

INTRODUCTION

Today, untreated waste represents one of the key problems for hygiene, the economy, and the environment. They represent a diverse mixture of chemicals, often foreign chemicals. Therefore, the European Union's Environmental Action Programme lists radical waste prevention as one of the highest priorities to achieve a significant and overall reduction in biodegradable, non-biodegradable, organic, or inorganic waste **(Östblom *et al*., 2010)**. Human life inherently generates waste. It is important to make maximum use of the Best Available Technique (BAT) or to promote new innovative technologies that consider the environment's revitalizing capacity, the zero waste goal, or the reduction of the carbon footprint in human life and consequently in waste management. One promising approach is the use of so-called biodegradable waste recovery technology by means of Diptera, a genus of two-winged insects, e.g., the black soldier fly (*Hermetia illucens* L.; Diptera: Stratiomyidae). Since 2017, thanks to the European Commission **(Regulation 2017/893/EC, 2017)**, it is allowed to use the larvae of double-winged insects for feed production as a substitute for conventional compound feed or biodiesel. This fact has led to an increased research intensity in the use of Diptera also for remediation purposes, with the possibility of reducing the concentrations of hazardous chemical elements in the food consumed by the larvae or increasing the nutritional value of the resulting substrate in trophically treated waste.
BSFL (Fig 1) originated in the South American savannah and are widely distributed in temperate, subtropical, and tropical regions, with an optimum temperature range of 25 °C to 30 °C **(Shumo *et al*., 2019)**, due to their low resistance to cold, they cannot survive in northwestern Europe and regions where temperatures drop below 5 °C **(Woodley, 2011)**. BSFL has ability to convert a wide variety of waste streams into high-quality proteins, fats, and minerals while demonstrating their potential for scalable and efficient production in the animal feed industry **(Woods *et al*., 2019)**. This ability is attributed to the selection of enzymes in their gut that allow them to consume a wide range of substrates, and this ability is useful, as they can reduce agricultural waste streams by feeding on them and converting it into their nutrient dense body mass **(de SouzaVilela *et al*., 2019)**. As most research has focused on BSFL development and consumption issues, relatively little is known about their safety or security as a food source. While few insects appear to be harmful when eaten **(Blum, 1994)**, allergens aside **(Barre *et al*., 2014)**, issues such as microbial contamination of insects should not be ignored **(EFSA, 2015)**. BSFL has been noted to reduce the microbial load of substrates, with processed composts showing lower concentrations of bacteriophages and bacteria, including *Salmonella enteritidis* and *Escherichia coli* **(Erickson *et al*., 2004; Liu *et al*., 2008)**. However, the larvae themselves can become contaminated if kept on contaminated substrates for too long **(Erickson *et al*., 2004)**. BSFL can contain 42% crude protein and 29% fat, although they are higher in saturated fats than most insects. They do not concentrate pesticides or mycotoxins **(Wang & Shelomi, 2017)**.

Figure 1 Black soldier fly (URL1) and larvae (orig. Barboráková)

Currently, due to increased anthropogenic activity and waste generation, the concentration of chemicals in the environment is rising disproportionately. This issue is alarming when it comes to hazardous, often at their concentration exceeding the limit in a given environment, toxic substances that are part of non-degradable wastes **(Khun *et al*., 2008; Urminská, 2018)**. Among the pollutants that have received scientific attention are heavy metals/polyalloxides potentially toxic, even toxic at trace concentrations, such as arsenic (As), cobalt (Cd), mercury (Hg), and lead (Pb) (**Koren *et al*., 2003a; Li *et al*., 2006; Oyewale *et al*., 2006)**. The risk of exposure for many of these is in their persistence and, consequently, depending on the chemical, physical, and biological properties of the environment, their mobility, bioavailability, and effects on organisms **(Andrews *et al*., 2004; Hegedűsová *et al*., 2006)**. These elements have a density greater than 5 g.cm^{-3} (except for titanium /Ti/ and selenium /Se/), and belong to transition metals and groups 3A, 4A, 5A, and 6A **(Fergusson, 1990; Urminská *et al*., 2022)**.

The objective of our study was to determine whether BSFL fed on available biodegradable waste under the conditions of a model experimental laboratory simulation contain hazardous heavy metals and to determine the total concentrations of selected hazardous heavy metals in the fed BSFL. Additionally, we also focused on assessing the microbial risks associated with the potential use of BSFL as animal feed or as a potential food source for humans.

MATERIAL AND METHODS

Rearing of BSFL

In the study, we worked with BSFL that were fed four feed variants (I: egg pasta cooked in whole milk; II: cooked rice with peas; III: poultry feed; IV: mixture of cooked couscous, boiled eggs, fresh spinach, and carrot peels). In each variant, 100 g of BSFL were fed at the beginning of the experiment. The experiment was carried out in 4 replicates for each variant. BSFL were reared at 27 ± 1 °C and 50 – 60 % relative humidity. BSFL were fed the feed in each variant for 6 days, and the feed ration in each replicate of the respective variant was 380 g.day^{-1}. After a one-day break in feeding, BSFL were killed by freezing at -80 °C. To weigh the larvae, we used laboratory balances KERN 440-43N (Kern & Sohn, Germany).

Microbiological analysis

Among the microbiological parameters, we monitored the number of Enterobacteriaceae (EB), aerobic bacterial endospores (ABES), and the number of microscopic filamentous fungi (MFF) in the BSFL (before experimental feeding and after feeding process in variant I, II, III, IV), according to relevant ISO standards (4833-1; 21528; 21527-1).
For microbiological analyses, we used the plate dilution method. We conducted each analysis in duplicate. We homogenised the BSFL, added 5 g to 45 ml of sterile saline, and shaken on a shaker for 30 minutes. The basic dilution (10^{-1}) was then used to prepare serial dilutions used in the study. The characteristics of the assays used are shown in table 1. For the determination of aerobic bacterial endospores, the inoculum was heated to 80 °C for 10 minutes, cooled, and then inoculated onto the medium. The final results of the number of each group of microorganisms were expressed in log CFU.g^{-1}.

Table 1 Characteristics of the microbiological analysis used

Group of microorganisms	Medium	Inoculation	Inoculum [mL]	Dillutions	Cultivation	
					T [°C]	t [hours/*days]
EB	VRBG	Spread	0.1	10^{-4}, 10^{-5}, 10^{-6}	37 ± 1	24
ABES	NA	Pour	1	10^{-3}, 10^{-4}, 10^{-5}	30 ± 1	24
MFF	DRBC	Spread	0.1	10^{-2}, 10^{-3}, 10^{-4}	25 ± 1	*5

Legend: EB - Enterobacteriaceae; ABES – aerobic bacterial endospores; MFF – microscopic filamentous fungi; VRBL – violet red bile agar with glucose, Biokar diagnostics, France; NA – Nutrient agar no. 2, Biokar diagnostics, France; DRBC – dichloran rose bengal chloramphenicol agar, Biokar diagnostics, France; T – temperature; t – time

MALDI-TOF identification

The identification of coliform bacterial isolates was carried out using the MALDI-TOF MS system (Bruker Daltonik GmbH, Bremen, Germany), a widely recognized tool for microbial analysis. The procedure was supported by the proprietary Biotyper software (Bruker Daltonik GmbH, Bremen, Germany), which facilitates accurate classification based on protein fingerprinting. Detailed information about the specific steps involved in protein extraction, sample preparation, and the identification workflow can be found in a previously published study **(Hleba *et al*., 2020)**.

Heavy metals detection

The study was conducted experimentally under laboratory conditions. For the purpose of the research, BSFL was used. BSFL were frozen, lyophilized and ground to the required analytical ICP-OES (Inductively Coupled Plasma – Optical Emission Spectroscopy) fineness. The ICP-OES method were used to determine selected heavy metals of concern in the following samples: BE (40 g BSFL - begining of experiment), I (BSFL-fed cooked egg pasta in whole milk), II (BSFL-fed cooked rice with peas), III (BSFL-fed poultry feed), and IV (BSFL-fed a mixture of couscous, cooked eggs, fresh spinach, and carrot peels).
Inductively coupled plasma emission spectrometry is an analytically suitable method for the determination of the concentrations of significant trace elements in samples. The sample solution was converted into a mist and the resulting mist was carried by an argon stream into a torch in which an argon plasma was maintained in the temperature range of 6 000 to 10 000 K by an alternating high-frequency field. Under such conditions, the solvent evaporated immediately, and the chemical bonds in the molecules of the compounds present were destroyed. The energy in the plasma was sufficient to excite electrons in the atoms to higher energy levels. The excited state of the atom is unstable; the excited electrons return to their original energy levels, emitting light with a precisely defined wavelength that is determined by the energy difference of the two levels. The emitted light created an electrical signal. The intensity of the signal, corresponding to the characteristic wavelength of the light produced by the transition of the energy states of the analysed element, indicates the concentration of the element under investigation **(Bajčan *et al*., 2018)**.

Statistical analysis

The SAS 9.4 (Northern California, USA) "Spearman's correlation coefficient" method was used for statistical treatment of dependencies. Spearman's correlation coefficient ρ_s is a measure of dependence based on the measurement of dependence between orders **(Stehlíková, 1999)**.
For additional statistical analysis, the software Past4.03 **(Hammer *et al*., 2001)** was utilised. The normality of the data was assessed using the Shapiro-Wilk normality test. Based on these results, ANOVA and Dunn´s post hoc tests were conducted to examine differences between experimental variants. The normality test indicated a normal distribution for most of variants.

RESULTS AND DISCUSSION

Weight gain of black soldier fly larvae

For each feeding variant, we determined the weight of 100 individual dead larvae at the beginning of the experiment and at the end of the experiment (Tab 2). The highest weight gain was observed in variant III, in which larvae were fed poultry feed (weight gain 253.1%). The lowest weight gain was recorded in variant IV, in which BSFL were fed cooked couscous with boiled eggs, fresh spinach, and carrot peels (132.6%).

Table 2 Weight of black soldier fly larvae (mean ± standard deviation)

Variant	Weight of 100 larvae BF [g]	Weight of 100 larvae AF [g]*	Weight gain [%]	CV [%]
I	4.9	13.7 ± 1.14	180.9	8.35
II	3.2	9.7 ± 0.10	201.6	1.04
III	3.2	11.3 ± 0.84	253.1	7.43
IV	4.3	10.0 ± 0.81	132.6	8.10

Legend: BF – before experimental feeding; AF – after feeding process; variant I - egg pasta cooked in whole milk; variant II – cooked rice with peas; variant III - poultry feed; variant IV - cooked couscous, boiled eggs, fresh spinach, and carrot peels; CV - coefficient of variation; *n = 4

Microbiology of black soldier fly larvae

Because of their positive nutritional properties and low environmental impact, edible insects could be considered the „food of the future". However, there are safety concerns associated with the consumption of insects, such as chemical and biological contaminants. The possible presence of pathogenic and toxigenic microorganisms is one of the main biological risks associated with edible insects **(Garofalo *et al.*, 2019)**. The safety and security of BSFL as a foodstuff is not well understood because the majority of research has concentrated on problems related to their production. In the study of **Garofalo *et al.* (2019)**, high microbial contents were identified across edible insect species, with pathogenic bacteria such as *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* being of particular concern. Other studies report that BSFL has been noted to reduce the microbial load of substrates, with processed composts showing lower concentrations of bacteriophages and bacteria, e.g., *Salmonella enteritidis* and *Escherichia coli* **(Liu *et al.*, 2008)**. Data from other studies on plant-feeding insects suggests that Enterobacteriaceae are a problem with raw insects **(EFSA, 2015)**. Powdering the insects and heating, drying, UV treatment, high-energy microwave treatment, pasteurization, acidification, or other treatment of the food against microbes, parasites, and bacterial spores would reduce the chance of microbial contamination compared to whole, unprocessed insects **(Klunder *et al*., 2012). Bessa *et al.* (2021)** mention that the killing method had a significant effect on the microbial load and the heavy metal content of the BSFL; as expected, blanching significantly reduced the microbial contamination of the BSFL and resulted in slightly lower heavy metal concentrations in the larvae compared to freezing the BSFL. Raw edible insects generally contain high numbers of mesophilic aerobes, bacterial endospores or spore-forming bacteria, Enterobacteriaceae, lactic acid bacteria, psychrotrophic aerobes and fungi, and potentially harmful species (i.e., pathogenic, mycotoxigenic, and spoilage microbes) may be present **(Garofalo *et al.*, 2019)**.

Table 3 Microbial counts in black soldier fly larvae

Variant		Group of microorganisms	M [log CFU.g^{-1}]*	CV [%]
BF		EB	5.85 ± 0.15	2.61
		ABES	5.66 ± 1.23	21.80
		MFF	3.62 ± 0.07	1.93
AF	**I**	EB	7.02 ± 0.11	1.57
		ABES	5.04 ± 0.62	12.30
		MFF	2.15 ± 0.30	13.95
	II	EB	7.84 ± 0.05	0.64
		ABES	3.60 ± 0.00	0.00
		MFF	<2.00 ± 0.00	0.00
	III	EB	7.47 ± 0.19	2.54
		ABES	6.16 ± 0.09	1.46
		MFF	3.24 ± 2.09	64.50
	IV	EB	6.44 ± 0.38	5.48
		ABES	4.93 ± 0.10	2.03
		MFF	<2.00 ± 0.00	0.00

Legend: BF – before experimental feeding; AF – after feeding process; variant I – egg pasta cooked in whole milk; variant II – cooked rice with peas; variant III – pultry feed; variant IV – cooked couscous, boiled eggs, fresh spinach, and carrot peels; EB - Enterobacteriaceae; ABES – aerobic bacterial endospores; MFF – microscopic filamentous fungi; SD – standard deviation; CV – coefficient of variation; M - mean [log CFU.g^{-1}] ± standard deviation; ***n** = 4

In our study (Tab 3), the highest Enterobacteriaceae counts were found in BSFL fed variant II (cooked rice with peas, 7.84 ± 0.05 log CFU.g^{-1}). Enterobacteriaceae are markers of hygienic food quality and faecal contamination. Therefore, lower levels of Enterobacteriaceae are desirable. However, the lowest counts of aerobic bacterial endospores (3.60 ± 0.00 log CFU.g^{-1}) and microscopic filamentous fungi (<2.00 log CFU.g^{-1}) were found in the BSFL fed this variant. The lowest Enterobacteriaceae counts were found in the BSFL before experimental feeding (5.85 ± 0.15 log CFU.g^{-1}), respectively higher were in each feeding variant compared to the counts before experimental feeding. Thus, each feeding variant influenced the rise in these bacteria counts in the BSFL.
Evaluation of statistical differences between each variant was performed using ANOVA test followed by Dunn´s post hoc test. The results are shown in table 4. The ANOVA test showed significant differences in the data set, therefore a Dunn´s post hoc test was applied. The results showed the following findings. The values for Enterobacteriaceae bacteria were significantly different in variants II and III compared to the initial experimental variant. Variant I had significantly different results for Enterobacteriaceae bacteria with variant II, and variant II was significantly different compared to variant IV. Aerobic bacterial endospores had significantly different values between variants before feeding and II and between variants II and III. Results for microscopic filamentous fungi were significantly different between the initial variant and variants I, II, and IV.

Table 4 Dunn´s post hoc with raw *p*-values

Variant		BF	I	II	III	IV
EB	**BF**		0.1597	0.0001286	0.004924	0.1271
	I	-		0.01539	0.1597	0.9048
	II	***	**		0.3091	0.02126
	III	*	-	-		0.1983
	IV	-	-	*	-	
ABES	**BE**		0.401	0.01175	0.2802	0.4716
	I	-		0.09303	0.05491	0.9045
	II	*	-		0.0003191	0.07192
	III	-	-	***		0.07192
	IV	-	-	-	-	
MFF	**BE**		0.0155	0.003212	0.1314	0.003212
	I	*		0.5988	0.3617	0.5988
	II	**	-		0.1504	1
	III	-	-	-		0.1504
	IV	**	-	-	-	

Legend: BF – before experimental feeding; variant I – egg pasta cooked in whole milk; variant II – cooked rice with peas; variant III – pultry feed; variant IV – cooked couscous, boiled eggs, fresh spinach, and carrot peels; EB - Enterobacteriaceae; ABES – aerobic bacterial endospores; MFF – microscopic filamentous fungi; significant differences: - no significant difference; * significant difference with $p < 0.05$ (yellow cells); ** significant difference with $p < 0.01$ (orange cells); *** significant difference with $p < 0.001$ (red cells)

Table 5 Overview of Enterobacteriaceae species in black soldier fly larvae (MALDI-TOF identification)

Species	Variant					Species	Variant				
	BF	**I**	**II**	**III**	**IV**		**BF**	**I**	**II**	**III**	**IV**
*Enterobacter cloaceae**	+					*Morganella morganii**	+	+	+	+	+
Enterobacter putida	+					*Proteus mirabilis**	+	+		+	+
Hafnia alvei		+			+	*Pseudomonas fluorescens*					+
*Klebsiella aerogenes**		+				*Pseudomonas putida*					+
*Klebsiella pneumoniae**	+	+	+	+		*Kluyvera georgiana*			+		
Klebsiella vavicola		+				*Serratia marcescens*	+				
Moellerella wisconsensis		+			+						

Legend: BF – before experimental feeding; variant; I – egg pasta cooked in whole milk; variant II – cooked rice with peas; variant III – pultry feed; variant IV – cooked couscous, boiled eggs, raw carrot peels; * facultatively pathogenic bacterial species

The species spectrum of Enterobacteriaceae in the BSFL of each variant based on MALDI-TOF identification is shown in table 5. We isolated representatives of 13 Enterobacteriaceae species, including facultatively pathogenic species *Enterobacter cloaceae*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Morganella morganii*, and *Proteus mirabilis*. It was *Morganella morganii* that was present in all variants of BSFL. The presence of this bacterium in BSFL has been reported by several authors **(Kim *et al*., 2014; Shumo *et al*., 2021; Cifuentes *et al*., 2022; Ijdema *et al*., 2022)**. *Morganella morganii* belongs to the tribe Proteeae of the Enterobacteriaceae family. This species is considered an uncommon opportunistic pathogen that mainly causes postoperative wound and urinary tract infections **(Liu *et al*., 2016)**. In a study by **Shi *et al.* (2022)**, multidrug-resistant *Klebsiella pneumoniae* strains were isolated from BSFL. Typical *Klebsiella pneumoniae* is an opportunistic pathogen that mostly affects people with weakened immune systems and tends to cause nosocomial infections **(Li *et al*., 2014)**. **Tegtmeier *et al.* (2021)** and **Cifuentes *et al.* (2022)** also isolated representatives of the genera *Morganella*, *Enterococcus*, and *Proteus*, as well as *Providencia*.
Aerobic endospore formers present a challenge for edible insects **(Vandeweyer *et al*., 2017; Campbell *et al*., 2020)**, therefore it is suggested to adopt strategies to use feeds with low aerobic spore counts to limit possible contamination **(Osimani & Awuilanti, 2021)**. The presence of spore-forming bacteria in insect-based foods poses a serious threat to consumer health, as these microorganisms include well-known foodborne pathogens **(Osimani & Awuilanti, 2021)**. In our study, the BSFL fed variant II (cooked rice with peas) had the lowest microbiological load in terms of aerobic bacterial endospores (3.60 ± 0.00 log CFU.g^{-1}). In all other feeding variants and in the BSFL before feeding, aerobic bacterial endospore counts were higher, with the highest counts in the BSFL variant IV (6.44 ± 0.38 log CFU.g^{-1}).
Microscopic filamentous fungi are important producers of toxic secondary metabolites (mycotoxins). **Purschke *et al.* (2017)** mention that in their study, there was no accumulation of mycotoxins and pesticides in the larval tissue, nor did they significantly affect the growth determinants of BSFL. In our study, the highest counts of microscopic filamentous fungi were found in the BSFL before feeding (3.62 ± 0.07 log CFU.g^{-1}). The filamentous fungi counts were lower in BSFL in all feeding treatments, with the lowest counts in variants II and III (<2.00 ± 0.00 log CFU.g^{-1}).

Heavy metals in black soldier fly larvae

The accumulation of heavy metal in the larvae of insects raise concerns, several studies have been conducted on the accumulation of heavy metals in BSFL. Our results (Tab 6) suggest that diet composition may influence the concentration of some heavy metals in BSFL. Considering the use of larvae as a food supplement for humans or animals, it is important to consider these differences, especially for contaminants such as cadmium and lead, which have potential health risks.

Table 6 Heavy metals concentrations (mg.kg^{-1}) ± standard deviation in black soldier fly larvae

Heavy metal	Variant				
	BF	I	II	III	IV
Cd *214.438	<LOD	<LOD	0.13 ± 6.84	0.12 ± 4.87	0.18 ± 1.22
Co 238.892	<LOD	<LOD	<LOD	<LOD	<LOD
Cr 284.325	<LOD	<LOD	<LOD	<LOD	<LOD
Cu 324.754	33.77 ± 0.29	17.54 ± 0.83	6.67 ± 1.79	26.45 ± 0.44	6.53 ± 0.98
Mn 257.610	123.43 ± 0.71	106.01 ± 0.69	69.51 ± 0.58	118.71 ± 0.21	63.38 ± 0.39
Mo 204.598	<LOD	<LOD	<LOD	2.45 ± 5.82	<LOD
Ni 221.647	9.92 ± 0.34	3.89 ± 1.06	6.63 ± 0.54	8.86 ± 0.64	6.32 ± 0.85
Pb 182.205	3.07 ± 2.12	<LOD	<LOD	<LOD	<LOD
Zn 213.856	114.49 ± 1.57	110.57 ± 1.27	48.97 ± 0.81	98.62 ± 0.68	39.42 ± 0.54

Legend: BF – before experimental feeding; variant I – egg pasta cooked in whole milk; variant II – cooked rice with peas; variant III – poultry feed; variant IV – cooked couscous, boiled eggs, fresh spinach, and carrot peels; Cd – cadmium; Co – cobalt; Cr – chromium; Cu - cuprum; Mn - manganese; Mo - molybdenum; Ni - nickel; Pb – lead; Zn - zinc; LOD - detection limit: Cd 0.0004 mg.kg^{-1}, Co 0.002 mg.kg^{-1}, Cr 0.011 mg.kg^{-1}, Cu 0.003 mg.kg^{-1}, Mn 0.0003 mg.kg^{-1}, Mo 0.004 mg.kg^{-1}, Ni 0.002 mg.kg^{-1}, Pb 0.008 mg.kg^{-1}, Zn 0.691 mg.kg^{-}

Heavy metals/polyalloxides are not subject to degradation in the environment, and their concentrations can increase through continuous "replenishment of daily doses" by anthropogenic activities, including waste, trophic, and biomagnification (many are so-called cumulative poisons). The biological effects of heavy metals/polyalloxides depend on many specific chemical-physical and physiological factors **(Pandey *et al*., 2006; Peng *et al*., 2009)**. Some elements (e.g., Se, zinc /Zn/), up to a certain concentration in the environment, are essential for the organism and its functions; on the other hand, at elevated concentrations they are toxic **(Rapant *et al*., 2002; Makovnikova *et al*., 2006)**. There are also elements whose minimal content produces immediate toxic effects, e.g., Hg, berillium /Be/, As, Pb, and Cd. Organometallic compounds of heavy metals are among the most toxic substances; they are lipophilic and cross cell membranes **(Kafka *et al*., 2002)**. In many cases, a correlation has been found between organism damage, disease, and abnormal concentrations of a particular trace element in organisms **(Genuis, 2008; Inhorn *et al*., 2008; Kosnet, 2010)**. The development of damage to the organism can be immediate if these chemicals act suddenly, in high concentrations, acutely. The development of chronic damage is a relatively lengthy process; the damage, the disease, only becomes apparent in the organism after prolonged exposure to a metal **(Kampa *et al*., 2008)**. Potentially toxic are those elements of geogenic or anthropogenic origin that may be toxic to organisms if they are ingested in a sufficiently high dose, over a long period of time, can bioaccumulate and transform in the system, and occur in forms that are mobile under specific conditions **(Koren *et al*., 2003b, Khun *et al*., 2008)**.
The question of the toxicity of chemical elements in relation to the environment is complex. Today it is no longer sufficient to point out that a toxic element is present in a particular region. It is necessary to assess the realistic conditions under which it may become actively toxic to living organisms. Years of research have shown that heavy metals/polyoxides significantly affect the whole environment through their properties **(Nguyen *et al*., 2005; Peng *et al*., 2009; Enfeng *et al*., 2010; Hiller *at al*., 2010)**. They are ubiquitous. In addition to acute accidental geogenic or anthropogenic environmental emergencies, these impacts do not manifest immediately or visually. Rather, they occur gradually through indirect changes that result in long-term environmental degradation **(Khun *et al*., 2008)**. The nature of the toxic effects of heavy metals/polymetals is determined by two critical factors **(Babčan *et al*., 1999; Urminská *et al.*, 2022)**. The primary one is the existence of a reactive form of the heavy metal/semimetal in relation to a living organism. A heavy metal that is inactive in a given environment, e.g., present in an insoluble form or in a bond that does not affect the organism, cannot have a toxic effect on a living organism. A secondary factor in the toxic effect of a heavy metal/semi-metal is its binding to various organs, causing damage and disease changes in the organism **(Babčan *et al*., 1999; Urminská *et al*., 2022)**.

Our results showed that the measured Cd concentrations in BSFL ranged from 0.12 to 0.18 mg.kg^{-1}. Cd concentrations were below the limit of detection (<LOD: 0.0004 mg.kg^{-1}) in some variants (before feeding, I), with Cd reaching 0.12 ± 4.87 mg.kg^{-1} in variant III (poultry feed), 0.13 ± 6.84 mg.kg^{-1} in variant II (rice, peas), and 0.18 ± 1.22 mg.kg^{-1} in variant IV (couscous, eggs, spinach, carrot peels). Cd is a chemical element that is abundant in every compound of the environment and, as a highly toxic heavy metal, it does not exhibit any essential function. The toxicity of Cd to each environmental compartment is significant and alarming because it affects parts of both the abiotic and biotic realms. As described by several authors, it is a cumulative poison. The risk of its action lies in its bioavailability and consequently its toxic effect on living organisms **(Khun *et al*., 2008)**. **Purschke *et al*. (2017)** and **Diener *et al*. (2015)** report that BSFL concentrate Cd from their diet (having higher concentrations of Cd in their bodies than their food substrate did), while concentrations of Cr, As, Ni, and Hg in BSFL do not exceed those of the substrate. In our study, the concentrations of Co and Cr in BSFL were below the LOD of the ICP-OES method (Co 0.002 mg.kg^{-1}, Cr 0.011 mg.kg^{-1}). The measured concentrations of Cu in BSFL varied from 6.53 to 33.77 mg.kg^{-1}, Mn from 63.38 to 123.43 mg.kg^{-1}. Cu showed different concentrations between treatments, with the highest value at the beginning of the experiment (33.77 ± 0.29 mg.kg^{-1}). Variant III and variant I had higher concentrations (26.45 ± 0.44 mg.kg^{-1} and 17.54 ± 0.83 mg.kg^{-1}), while variants II and IV have lower concentrations (6.67 ± 1.79 mg.kg^{-1} and 6.53 ± 0.98 mg.kg^{-1}). This difference may be influenced by the Cu content of the individual feedstocks. Mn is present in all variants, and its concentration is relatively high. The highest concentration was observed at the beginning of the experiment (123.43 ± 0.71 mg.kg^{-1}), with variants I and III showing only slightly lower concentrations (106.0 ± 0.69 mg.kg^{-1} and 118.71 ± 0.21 mg.kg^{-1}). Mo was detected in a single feed variant, namely in variant III (poultry feed), where it reached a concentration of 2.4 ± 5.82 mg.kg^{-1}. In all other variants, its concentration was below the limit of detection (<LOD: 0.004 mg.kg^{-1}). This finding may indicate that only certain ingredients in the chicken feed contained sufficient amounts of Mo, which were subsequently absorbed by the larvae. The presense of Pb was only detected in BSFL before experimental feeding, where it reached 3.07 ± 2.12 mg.kg^{-1}, indicating a low presence or reduction of Pb in other feed types. While **Gao *et al.* (2017)** report that BSFL accumulate Pb well above the substrate concentration, **Diener *et al.* (2015)** claim the opposite. Although Ni concentrations were relatively low, variant I (egg pasta in milk) showed the lowest concentration (3.89 ± 1.06 mg.kg^{-1}), while the other variants ranged between 6.32 and 9.92 mg.kg^{-1}. Zn was present at the highest concentration in BSFL before experimental feeding (114.49 ± 1.57 mg.kg^{-1}). Variants I and III also had higher concentrations (110.57 ± 1.27 mg.kg^{-1} and 98.62 ± 0.68 mg.kg^{-1}), while variants II and IV contained much lower amounts (48.97 ± 0.81 mg.kg^{-1} and 39.42 42 ± 0.54

mg.kg^{-1}). **Diener *et al.* (2015)** report that Pb and Zn concentrations in larvae remain lower than the original dietary levels. Nevertheless, as Cd accumulates in BSFL, it could potentially limit the use of larvae in animal feed production. In the case of Pb and Zn, concerns about the use of BSFL in animal feed are less critical **(Diener *et al.*, 2015)**.

In the case of metals, the roles of BSFL as waste manager and food source are in conflict. The removal of heavy metals from wastes, where the unconverted portion is composted, would be an environmental benefit of processing waste through BSFL, however, this would also reduce the edibility of the larvae **(Wang & Shelomi, 2017)**.

Confirmation of the scientifically established facts by statistical complementation showed that there were very obvious statistical correlations between BSFL-fed variant I and variants III, IV; between variant II and variants III, IV; further between variant III and variants I, II, IV; and also between variant IV and variants I, II, III. At the 0.05 level of significance, i.e., with a 95% confidence level, it can be stated that the Spearman's coefficient was highly significant for these variables as the p-value for all of them was $0.0001 < 0.05$. This was a very strong positive correlation, and this dependence means that the variables mentioned above, which represent different feeding variants, play a significant role in correlation with each other. The diversity of the input biodegradable waste plays a highly positive role in the processing of the BSFL input material.

CONCLUSION

The new waste legislation obliges municipalities to ensure the collection of biodegradable municipal waste (BMW), a large part of which consists of kitchen scraps from households and catering establishments. These are secondarily used in biogas or composting processes, but there is also the possibility of using new recovery alternatives, e.g., through BSFL. Each technology used in waste management has its positives and negatives. The use of larvae technology for the treatment of municipal solid waste (MSW) is logistically and technologically challenging, but it has and will continue to have its advantages in the future, as larvae fed on treated bio-waste can be used as feed in dried or fresh form. They are a suitable substitute for classically used feed mixtures for poultry or pigs and in aquaculture. However, the waste used to feed BSFL contains substances that are undesirable and pose a risk of accumulation in the BSFL and there are associated risks of accumulation of these substances in BSFL. Our research confirmed that BSFL fed different feed variants under experimental laboratory simulation conditions contained heavy metals - cadmium, copper, manganese, molybdenum, nickel, lead, and zinc - which are ubiquitous in the environment. It can be concluded that in addition to the alternative use as a substitute or supplement to conventional pig and poultry feed, there is a possibility of using BSFL in the effective recovery of MSW and also as a potential remediation medium in the problem of the occurrence of some of the risky heavy metals. After additional statistical analyses, it can be concluded that the diversity of biodegradable waste among the different feeding options plays a significant, positive role. The results of our study show that feeding variants significantly affect the microbial safety of BSFL. Due to the presence of pathogenic species such as *Morganella morganii* and *Klebsiella pneumoniae* in BSFL, it is important to pay attention to feed material selection and sanitation practices. Other factors affecting microbial contamination of BSFL should be investigated in the future to ensure their edibility and safety for human consumption. In view of the potential health risks as well as the ability of BSFL to accumulate toxic substances, strict hygiene measures and feed quality monitoring for BSFL are necessary. It is also important to continue further research on the toxicity and microbial safety of BSFL to ensure their safety as a sustainable source of protein for animals and potentially for humans.

Acknowledgments: The VEGA 1/0512/22 project provided financial support for the study.

REFERENCES

Andrews, J. E., Brimblecombe, P., Jickells, T. D., Liss, P. S., & Reid, B. (2004). *An Introduction to Environmental Chemistry*. Oxford: Blackwell Science. 209 p. ISBN 0-632-05905-2.

Babčan, J., Kuhn, M., & Ševc, J. (1999). Špecifiká pohybu ťažkých kovov v životnom prostredí. *Životné prostredie*, č. 5, Ústav krajinnej ekológie SAV Bratislava

Bajčan, D., Hegedüs, O., & Vollmannová, A. (2018). *Analytická chémia,* Nitra: SPU, 82 s., ISBN 978-80-552-1788-8.

Barre, A., Caze-Subra, S., Gironde, C., Bienvenu, F., Bienvenu, J., & Rougé, P. (2014). Entomophagie et risque allergique. *Revue Française dÁllergologie. 54*(4), 315-321. https://doi.org/10.1016/j.reval.2014.02.181

Bessa, L. W., Pieterse, E., Marais, J., Dhanani, K., & Hoffman, L. C. (2021). Food safety of consuming black soldier fly (*Hermetia illucens*) larvae: Microbial, heavy metal and cross-reactive allergen risks. *Foods*, *10*(8), 1934. https://doi.org/10.3390/foods10081934

Blum, M. S. (1994). The limits of entomophagy: A discretionary gourmand in a world of toxic insects. *Food Insects Newsl.*, *7*, 1-6.

Campbell, M., Ortuño, J., Stratakos, A. C., Linton, M., Corcionivoschi, N., Elliott, T., Koidis, A., & Theodoridou, K. (2020). Impact of thermal and high-pressure treatments on the microbiological quality and in vitro digestibility of Black soldier fly (*Hermetia illucens*) Larvae. *Animals*, *10*(4), 682, https://doi.org/10.3390/ani10040682

Cifuentes, Y., Vilcinskas, A., Kämpfer, P., & Glaeser, S. P. (2022). Isolation of *Hermetia illucens* larvae core gut microbiota by two different cultivation strategies. *Antonie Van Leeuwenhoek*, *115*(6), 821-837. https://doi.org/10.1007/s10482-022-01735-7

Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. http://data.europa.eu/eli/reg/2017/893/oj

de Souza-Vilela, J., Andrew, N. R., & Ruhnke, I. (2019). Insect protein in animal nutrition. *Animal Production Science*, 59(11), 2029-2036. https://doi.org/10.1071/AN19255

Diener, S., Zurbrügg, C., & Tockner, K. (2015). Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. Journal of Insects as Food and Feed, *1*(4), 261–270. https://doi.org/10.3920/JIFF2015.0030

Gao, Q., Wang, X. Y., Wang, W. Q., Lei, C. L., & Zhu, F. (2017). Influences of chromium and cadmium on the development of black soldier fly larvae. Environmental Science and Pollution Research, *24*(9), 8637-8644. https://doi.org/10.1007/s11356-017-8550-3

Garofalo, C., Milanović, V., Cardinali, F., Aquilanti, L., Clementi, F., & Osimani, A. (2019). Current knowledge on the microbiota of edible insects intended for human consumption: A state-of-the-art review. *Food Research International*, *125*, 108527. https://doi.org/10.1016/j.foodres.2019.108527

EFSA Scientific Committee. (2015). Risk profile related to production and consumption of insects as food and feed. *EFSA Journal*. *13*(10), 4257. https://doi.org/10.2903/j.efsa.2015.4257

Enfeng, L., Ji, S., Liyuan, Y., Enlou, Z., Xianghua, M., & Jianjun, W. (2010). Assessment of heavy metals contamination in the sediments of Nansihu Lake catchment, China. *Environmental Monitoring & Assessment*, *161*(1-4), 217-227. http://dx.doi.org/10.1007/s10661-008-0739-y

Fergusson, J. E. (1990). *The Heavy Elements, Chemistry, Environmental Impact and Health Effects.* New Zeland: Pergamon Press. 614 p. ISBN 0080402755.

Erickson, M. C., Islam, M., Sheppard, C., Liao, J., & Doyle, M. P. (2004). Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar enteritidis in chicken manure by larvae of the black soldier fly. *Journal of Food Protecton*, *67*(4), 685-690. https://doi.org/10.4315/0362-028X-67.4.685

Genuis, S. J. (2008). Toxic causes of mental illness are overlooked. *Neuro Toxicology*, *29*(6), 1147-1149. https://doi.org/10.1016/j.neuro.2008.06.005

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4(*9), 1-9. http://palaeo-electronica.org

Hegedüsová, A., Hegedüs, O., & Musilová, J. (2006). *Riziká kontaminácie pôd kadmiom*. Nitra: UKF, č. 222, 89 s., ISBN 80-8094-047-9.

Hiller, E., Jurkovič, Ľ., & Šutriepka, M. (2010). Metals in the surface sediments of selected water reservoirs, Slovakia. *Bulletin of Enironmental Contamination and Toxicology*. 84, 635-640. http://dx.doi.org/10.1007/s00128-010-0008-y

Hleba, L., Hlebová, M., Kováčik, A., Šmehýl, P., Hricáková, N., Petrová, J., … Čuboň, J. (2020). *Escherichia coli* as a carrier of tetracyclines and penicillins resistance in wild pheasant (*Phasianus colchicus*). *Journal of Environmental Science and Health, Part A*, *55*(10), 1201–1209. https://doi.org/10.1080/10934529.2020.1777050

IJdema, F., De Smet, J., Crauwels, S., Lievens, B., & Van Campenhout, L. (2022). Meta-analysis of larvae of the black soldier fly *(Hermetia illucens*) microbiota based on 16S rRNA gene amplicon sequencing. *FEMS Microbiology Ecology*, *98*(9), fiac094. https://doi.org/10.1093/femsec/fiac094

Inhorn, M. C., King, L., Nriagu, J. O., Kobeissi, L., Hammoud, N., Awwad, J., Abu-Musa, A. A., & Hannoun, B. (2008). Occupational and environmental exposures to heavy metals: Risk factors for male infertility in Lebanon? *Reproductive Toxicology*, *25*(2), 203-212. https://doi.org/10.1016/j.reprotox.2007.10.011 ISSN 0890-6238

Khun, M., Ďurža, O., Milička, J., Dlapa, P. (2008). *Environmentálna geochémia*. Bratislava: Geo-grafika Bratislava, 278 s., ISBN 978-80-89317-03-5.

Kafka, Z., & Punčochářová, J. (2002). Těžké kovy v přírodě a jejich toxicita. *Chemické listy*, *96*(7). 611-617. ISSN: 0009-2770.

Kampa, M., & Castanas, E. (2008). Human health effects and air pollution. *Environmental Pollution*, *151*(2), 362-367. ISSN 0269-7491.

Kim, E., Park, J., Lee, S., & Kim, Y. (2014). Identification and physiological characters of intestinal bacteria of the black soldier fly, *Hermetia illucens*. *Korean journal of applied entomology*, *53*(1), 15-26. http://dx.doi.org/10.5656/KSAE.2013.09.0.049

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. J. R. (2012). Microbiological aspects of processing andstorage of edible insects. *Food Control*, *26*(2), 628-631. https://doi.org/10.1016/j.foodcont.2012.02.013

Koren, H., & Bisesi, M. (2003a). *Handbook of Environmental health, Pollutant Interactions in Air, Water and Soil* (Vol. 2). USA: CRC Press LLC. 876 p. ISBN 1-56670-547-9.
Koren, H., & Bisesi, M. (2003b). *Handbook of Environmental health, Biological, Chemical and Physical Agents of Environmentally Related Disease* (Vol. 2). USA: CRC Press LLC. 792 p. ISBN 1-56670-536-3.
Kosnett, M. J. (2010). Chelation for heavy metals (arsenic, lead and mercury): protective or perilous? *Clinical Pharmacology & Therapeutics*, *88*(3), 412-415. https://doi.org/10.1038/clpt.2010.132
Kuhn, M. (1992). *Lekárska geochémia*. Bratislava: Manuskript Katedry geochémie Prírodovedeckej fakulty UK, 66 s.
Li, Z., Wang, Q., & Luo, Y. (2006). Exposure of the urban population to mercury in Changchun city, Northeast China. *Environmental Geochemistry and Health*, *28*(1-2), 61-66. https://doi.org/10.1007/s10653-005-9012-2
Li, B., Zhao, Y., Liu, C., Chen, Z., & Zhou, D. (2014). Molecular pathogenesis of *Klebsiella pneumoniae*. *Future microbiology*, *9*(9), 1071-1081. https://doi.org/10.2217/fmb.14.48
Liu, Q. L., Tomberlin, J. K., Brady, J. A., Sanford, M. R, & Yu, Z. N. (2008). Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. *Environmental Entomology*, *37*(6), 1525-1530. https://doi.org/10.1603/0046-225X-37.6.1525
Liu, H., Zhu, J., Hu, Q., & Rao, X. (2016). *Morganella morganii*, a non-negligent opportunistic pathogen. *International Journal of Infectious Diseases*, *50*, 10-17. https://doi.org/10.1016/j.ijid.2016.07.006
Makovníková, J., Barančíková, G., Dlapa, P., & Dercová, K. (2006). Anorganické kontaminanty v pôdnom ekosystéme. *Chemické listy*, *100*. 424-432.
Nguyen, H. L., Leermakers, M., Osán, J., Török, S., & Baeyens, W. (2005). Heavy metals in lake Balaton: water column, suspended mater, sediment and biota. *Science of the Total Environment*, *340*(1-3), s. 213-230. https://doi.org/10.1016/j.scitotenv.2004.07.032
Oyewale, A. O., & Musa, I. (2006). Pollution assessment of the lower basin of lakes Kainji/Jebba, Nigeria: heavy metal status of the waters, sediments and fishers. *Environmental Geochemistry and Health*, *28*(3), 273-281. http://dx.doi.org/10.1007/s10653-006-9043-3
Osimani, A., & Aquilanti, L. (2021). Spore-forming bacteria in insect-based foods. *Current Opinion in Food Science, 37*, 112-117. https://doi.org/10.1016/j.cofs.2020.10.011
Östblom, G., Ljunggren Söderman M., & Sjöström, M. (2010). Analysing future solid waste generation - Soft linking a model of waste management with a CGE-model for Sweden, 54 p. ISSN 1100-7818.
Pandey, P. K., Sharma, R., Roy, M., Roy, S., & Pandey, M. (2006). Arsenic contamination in the Kanker district of Central-East India: geology and health effects. *Environmental Geochemistry and Health*, *28*(5), 409-420. http://dx.doi.org/10.1007/s10653-005-9039-4
Peng, J. F., Song, Y. H., Peng, Y., Cui, X., & Qiu, G. (2009). The remediation of heavy metals contaminated sediment. *Journal of Hazardous Materials*, *161* (2/3), 633-640. http://dx.doi.org/10.1016/j.jhazmat.2008.04.061
Purschke, B., Scheibelberger, R., Axmann, S., Adler, A., & Jäger, H. (2017). Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. *Food Additives & Contaminants: Part A*, *34*(8), 1410-1420. https://doi.org/10.1080/19440049.2017.1299946
Rapant, S., Kuhn, M., Jurkovič, Ľ., & Letkovičová, M. (2002). Potentional influence of geochemical background on the health state of population of the Slovak republic. *Slovak Geological Magazine*, *8*(2), 137-145.
Shi, Z., Zhang, J., Jiang, Y., Wen, Y., Gao, Z., Deng, W., Zhenghui, G., Wenhui, D., Yumeng, Y., & Zhu, F. (2022). Two low-toxic *Klebsiella pneumoniae* strains from gut of black soldier fly *Hermetia illucens* are multi-resistance to sulfonamides and cadmium. *Environmental Pollution*, *312*, 120062. https://doi.org/10.1016/j.envpol.2022.120062
Shumo, M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K., Subramanian, S., Ekesi, S., Van Huis, A., & Borgemeister, C. (2019). Influence of temperature on selected life-history traits of black soldier fly (*Hermetia illucens*) reared on two common urban organic waste streams in Kenya. *Animals*, *9*(3), 79. https://doi.org/10.3390/ani9030079
Shumo, M., Khamis, F. M., Ombura, F. L., Tanga, C. M., Fiaboe, K. K., Subramanian, S., Sunday, E., Schlüter, O. K., van Huis, A., & Borgemeister, C. (2021). A molecular survey of bacterial species in the guts of black soldier fly larvae (*Hermetia illucens*) reared on two urban organic waste streams in Kenya. *Frontiers in Microbiology*, *12*, 687103. https://doi.org/10.3389/fmicb.2021.687103
Stehlíková, B. (1999). *Biometrika (Terminologický slovník): Učebné texty pre dištančné štúdium a ostatné formy vzdelávania*. Nitra: SPU, 1999, 95 s.
Tegtmeier, D., Hurka, S., Mihajlovic, S., Bodenschatz, M., Schlimbach, S., & Vilcinskas, A. (2021). Culture-independent and culture-dependent characterization of the black soldier fly gut microbiome reveals a large proportion of culturable bacteria with potential for industrial applications. *Microorganisms*, *9*(8), 1642. https://doi.org/10.3390/microorganisms9081642
URL1: Q&A: What is the ideal food to feed Black Soldier Fly larvae? - Insect School
Urminská, J. (2018). *Odpady a životné prostredie*. Odborná knižná publikácia. SPU Nitra, 155 s., ISBN 978-80-552-1893-9.
Urminská, J., Vollmannová, A., Tóth, T., Šnirc, M., & Musilová, J. (2022). *Environmentálna chémia Environmentálne problémy v biosfére*. Nitra: SPU, 156 s. ISBN 978-80-552-2494-7.
Vandeweyer, D., Lenaerts, S., Callens, A., & Van Campenhout, L. (2017). Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). *Food Control*, *71*, 311-314. https://doi.org/10.1016/j.foodcont.2016.07.011
Wang, Y. S., Shelomi, M. (2017). Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods*, *6*(91), 1-23. https://doi.org/10.3390/foods6100091
Woodley, N. E. (2011). A world catalog of the Stratiomyidae (Insecta: Diptera): A supplement with revisionary notes and errata. *Myia*, *12*, 379-415.
Woods, M. J., Hoffman, L. C., & Pieterse, E. (2019). Artificial diets for neonatal black soldier fly (*Hermetia illucens*) larvae. *Journal of Insects as Food and Feed*, 5(2), 99–105. https://doi.org/10.3920/JIFF2018.0028