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Overall *D. melanogaster* Cohort Viability as A Pollution Indicator of the Atmospheric Air of Urban Landscapes

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ABSTRACT: The method of air pollution level evaluation of urban landscapes on the basis of *D. melanogaster* cohort analysis has been suggested. The method implies the binding to the landscape areas of the city. Within each landscape area traps and cultivators for *D. melanogaster* have been installed in sanitary-protective zones of various enterprises as well as on the background territory with the least level of anthropogenic load serving as the control. Based on specifically elaborated technique for field conditions, the amount of eggs, third instar larvae, pupae and imago has been calculated. Then, using the computer program ImageJ, the square under the curves of cohort survival has been determined which is considered overall cohort viability ($OCV_{D.m}$). The previously mentioned indicator considers cohort survival at all stages of ontogenesis. In addition, the expressed in percentage indicator of oppression (IO $OCV_{D.m}$) in relevance to the control $OCV_{D.\ m}$ affects the level of air pollution of urban landscapes by emissions of various enterprises. The relevance between these indicators is determined by a four-level scale elaborated specifically for the purpose. The method has been tested based on technogenic landscapes of Chernivtsi, Ukraine. The sensitivity of the suggested indicator for a wide range of pollutants has been proved and its ability to respond to different levels of greening of similar enterprises has been shown.

Keywords: air pollution, biomonitoring, *Drosophila melanogaster*, cohort viability.

INTRODUCTION

Biomonitoring, i.e. the use of biological indicators to evaluate the state of the environment, is drastically different from the classical chemical or physical methods and has a number of advantages (Markert et al., 2003; Holt & Miller, 2010). Notwithstanding the fact that biomonitoring studies have recently been focused on the ecosystem level, the evaluation of system general properties on the basis of trophic and nutrient flows has gained the ever-growing popularity recently (Buzhdygan et al., 2014; Buzhdygan et al., 2016), and the diagnostics with the use of species – bioindicators is still topical.

Ecological bioindicators are the instruments, which help to evaluate the probable presence and the level of damage of stressor-factors in the environment due to the sensitive receptors and population change. In the context of monitoring

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studies the bioindicators are represented by organisms or organism communities, which convey the information about the quality of the environment. Finally, the major objective of bioindicators usage lies in providing an empirical basis to evaluate the health state of the environment (Zillioux, 2009).

The use of species-bioindicators makes ecological monitoring biologically more important and economically more effective. Bioindication allows revealing quickly the presence of toxicants in the environment and taking adequate measures to minimize their harmful effect on a human being and environment (Kucera, 1993).

To obtain complete and true picture about the ecosystems it is necessary to compare the information given by various bioindicators (Gadzała-Kopciuch et al., 2004).

Drosophila melanogaster Meigen, 1831 has been gaining popularity as a bioindicator of atmospheric air pollution. With field and laboratory research, the sensitivity of *D. melanogaster* to hydrogen fluoride (Gerdnes et al., 1971), sulfur dioxide (Ginevan & Lane, 1978; Cargando & Real, 2013) and heavy metals (Al-Momani et al., 2005; Ternes et al., 2014; Mathew & Krishnamurthy, 2018; de Santana et al., 2018; Ben-Shahar, 2018) has been proved.

Rand et al., 2014 showed that *D. melanogaster* may be a convenient model to identify and find out the level of effect of a wide range of toxicants on a living organism. In contrast to our approach the abovementioned authors conducted the analysis of a fruit fly survival at the egg stage separately from the other stages of ontogenesis.

Ecological pollution is a serious threat that is why the biomonitoring methods should be continuously improved to foresee and control the potential ecological hazards (Gadzała-Kopciuch et al., 2004).

The search for the indicator species, which can perform the role of reliable and economically effective instruments for environmental quality monitoring, is most urgent nowadays (Asif et al., 2018).

This search for new methods of quality evaluation of the environment with the use of bioindicators has involved also *D. melanogaster*. Therefore, the Brazilian scholars headed by de Santana (2018) have proved the effectiveness of the usage of modern DNA-comet method while evaluating the genotoxical potential of urban ambient pollution by means of *D. melanogaster*.

According to Tsaryk (2011), the most effective diagnostic criteria while organizing ecosystem monitoring, certain species and change of the environment may be integral parameters of populations, which are to be developed thoroughly. The authors of this paper suggest one of such methods.

We have developed and patented the method of evaluation of ambient air pollution of urban landscapes by the inhibition indicator of overall cohort viability *D. melanogaster* (Rudenko & Leheta, 2007). The advantage of the method is the use of the integral indicator, which considers cohort viability *D. melanogaster* at different stages of ontogenesis: egg, third instar larva, pupa, and imago. To determine the overall cohort viability it is advisable to calculate the square under the curves of *D. melanogaster* cohort survival. The method has undergone several stages of improvement (Rudenko & Leheta, 2011; Rudenko et al., 2019a; Rudenko et al., 2019b). We have managed to optimize the procedure of determining the squares under survival curves by means of the open computer program ImageJ for analysis and processing of images.

The objective of the publication under consideration is the effectiveness evaluation of the usage of inhibition indicator of overall *D. melanogaster* cohort viability for bioindication of the level of atmospheric air pollution of urban landscapes.

MATERIALS & METHODS

The studies were conducted within the period of July 20, 2018 until August 20, 2018. It is this period when the temperature in Chernivtsi is approximated to 25ºC, i.e. temperature optimum for a fruit fly (Antonov, 2002). The relevance of air temperature of the period under study to the temperature preferendum is proved by the data of Chernivtsi weather archive (Weather Archive, 2018).

The traps and cultivators were modeled from two parts: the upper transparent container made of the cut off upper part of a 1,5 liter standard PET-bottle (1/3 of the height) and the lower part, Petri dish with 10 cm in diameter. Both constructs had two openings: the upper, which was the neck of the bottle and the side one cut in accordance with the diameter of the neck.

Fresh inoculated early ripe type of apples (Geneva Early) was used as bait in traps. Drosophilas are attracted by succulence, aroma and sweet sour taste with sweet wine overtones of these fruit. The apples were washed in the running water, after that immersed in the solution containing 4ml/l of propanoic acid and 25 ml/l 10% of nipagin in 95% ethanol for 5 minutes to inhibit the growth of fungi and dried with paper towels. Then the fruit together with peel were chopped into small pieces of about $1cm^3$ and inoculated with yeast in terms of 10-minute immersion into the Saccharomyces *cerevisiae* (18 g/l) suspension. The material was put on the sieve letting extra inoculate flow down.

The flies were kept in the cultivators under standard conditions which are used in Bateson Centre at Sheffield University (Great Britain) (Fly food, 2018): cold running water -1l, corn flour middle grind – 80 g, dry yeast – 18 g, soya flour – 10 g, dark malt extract – 80 g, molasses (blackstrap) – 40 g, agar – 8 g, 10% nipagin in 95% ethanol – 25 ml, propanoic acid – 4 ml. Due to the presence of molasses and dark malt the environment differs from other environments by dark brown color, and on its background it is easy to identify the laid eggs, 3^{rd} instar larvae, and pupae. Besides, molasses and malt provide it with rich aroma and taste that increases the attractive properties. Finally, due to the soya flour the environment is enriched with protein and mineral substances. We have used Rye Malt Extract Dark "Harboes Bryggeri" company (Denmark), soya food flour of Agrosnab Ltd (Kyiv, Ukraine) and fast-acting baking yeast of “Lvivski drizhdzi” TM (Lviv, Ukraine) in this research.

The traps (4 pieces each) were placed on the ground in the shade of the trees on the territories of sanitary- protective zones adjacent directly to the working zone and exhibited with opened hole for 2 days. In two days the flies sat down being attracted by the smell of the inoculated fruit. The neck of the bottle was closed by cotton swab and the side hole with scotch tape and transported to the laboratory.

In the laboratory the flies were etherized with sulfuric ether. The females were separated from the males and placed in separate cultivators with standard environment (5 specimens in each) and returned them to the places where they had been caught for three days. Both openings of the cultivators were closed with pieces of two-layer gauze not to prevent from air passing of technotypes into the cultivators. When this period is over the specimen of both sexes (5 females and 5 males each) under the field conditions were placed in four new cultivators with fresh standard environment for crossbreeding for 1 day. In a day the cultivators were transported to the laboratory, the males were removed and each female of a certain variant was placed in a separate cultivator with fresh standard environment to drop the eggs. In 8 hours, having released the females from the cultivators, the eggs were counted. The cultivators with the eggs were returned to the places of natural localization. On the 5^{th}

and the 6th day from the day of egg laying (including this day) the number of 3rd instar larvae on the walls and on the surface of the very cultivator was counted, and on the 9th and 10th day the number of pupae was counted. On the 12th day the cultivators were transported to the laboratory and the number of imago was counted.

The results of cohort analysis were presented as the curves of survival in a semi-logarithmic scale: the logarithm of the number of living specimen was placed on the axis of ordinates ($lg\ a_x$), while the stage of ontogenesis was placed on the abscissa axis.

To calculate the squares under the curves of survival (S) the program ImageJ for analysis and processing images was used as well as the application to it – magic tool (Plugin) (Koniuhov, 2012). The calculation of the squares and, respectively, $OCV_{D.m.}$ was performed according to the following algorithm (Figure 1):

1. encircled the square under the curve of survival in the program "Paint" using the instrument "polygon";

2. installed Plugin in the ImageJ program which was later shown in the toolbar in a way of an arrow;

3,4. set the scale using the tab "Set Scale". The segment on the abscissa axis from the beginning of cohort formation (egg stage) to its death was taken for 10 cm was chosen as a unit of scale;

5,6. set the upper and lower values of the threshold to segment the square under the survival curve using the tool Threshold (Ctrl + Shift + T). Red color was used to show the threshold on the image;

7. received the square of the survival curve encircled with red outline;

8. determined the size of the square under the survival curve (S) using the function "Area". As the size of the square expressed in cm^2 does not convey any biological sense load, it was considered to be conventional units (c.u.)

The criterion of reliable significant difference, Tukey HSD test, within one-factor dispersion analysis ANOVA (one-way Analysis of Variation) in Statistica 6 program was used to analyze the difference between $OCV_{D.m.}$ of control and technogenic biotypes. Tukey test was used only after the confirmation of statistically significant difference for the entire ANOVA model using F-test.

The method involves the binding of the enterprises to the landscape areas of the city and determining control (background) $OCV_{D.m.}$ Values for each of them separately. The $OCV_{D.m.}$ values in park areas of appropriate landscape areas are considered to be control (background).

The indicator of oppression of overall *D. melanogaster* cohort viability (IO $OCV_{D.m.}$) was calculated for sanitary-protective zones only of those enterprises for which the Tukey criterion showed the reliable $OCV_{D.m.}$ difference as compared with the control. Meanwhile IO $OCV_{D.m.}$ was determined as percentage of reliable decrease of $OCV_{D.m.}$ in relevance to the appropriate control. For the sanitary-protective zones of enterprises where Tukey test did not reveal the reliable $OCV_{D.m}$. difference as compared to the control, IO OCV $_{D.m}$ was taken for zero.

The level of atmospheric air pollution of urban landscapes was estimated with the four-level scale built based on IO $OCV_{D.m.}$ values (Table 1).

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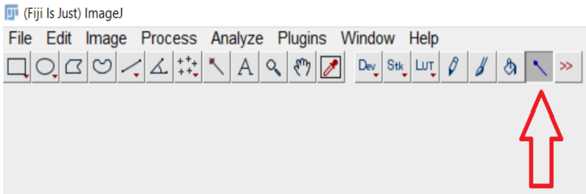

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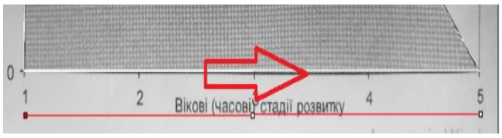

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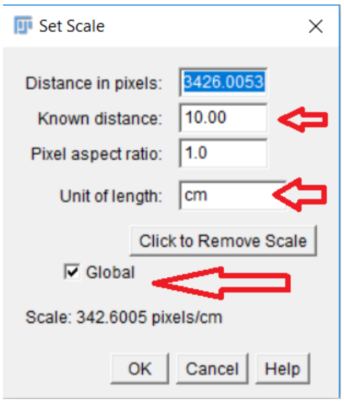

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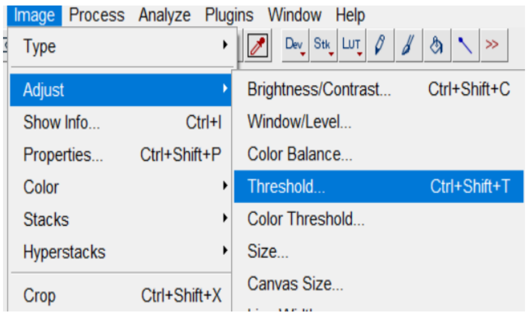

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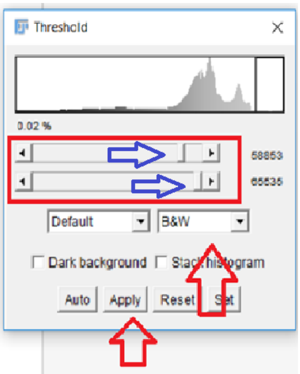

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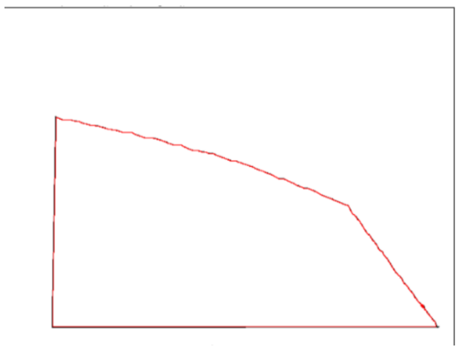

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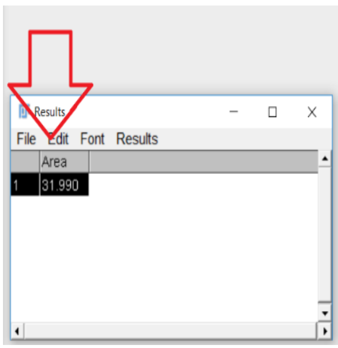

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Fig. 1. Algorithm of determining overall *D. melanogaster* cohort viability (OCV$_{D.m.}$) as square figures under the survival curves with ImageJ program (stage description is presented in the text)

Table 1. The scale of the levels of atmospheric air pollution depending on inhibition index of general *D. melanogaster* cohort vitality (IO $OCV_{D.m}$)

IO $OCV_{D.m.}$, %	Level of atmospheric air pollution
≤25	Low
From 26 to 50	Medium
From 51 to 75	High
≥ 76	Very high

RESULTS AND DISCUSSION

The method was approbated based on biomonitoring of technogenic landscapes of the city of Chernivtsi, the smallest of 24 regional centers of Ukraine with the population of 265 682 people (Timonina, 2018) and relatively low level of environment pollution (Prokopenko, 2018). This allowed evaluating the sensitivity of the elaborated methodology to even relatively not high level of technogenic transformation of the environment. The physical geographical zoning by Hutsuliak (2006) was used to relate the enterprises to the landscape areas of the city of Chernivtsi. The main enterprises in Chernivtsi are located within three landscape areas: Sadhora, Central City and Southern ones. Generally, the level of atmospheric air pollution in sanitary-protective zones of 23 enterprises was estimated using the suggested method. The territories of tuberculosis dispensary park, Shevchenko Park, Zhovtnevy Park within the appropriate landscape areas served as the control (background) territories.

The results of bioindication of the level of atmospheric air pollution by Chernivtsi enterprises based on $OCV_{D.m.}$ are presented below. The detailed analysis of the results is presented for the enterprises showing medium or higher than medium level of pollution.

The one-factor dispersion ANOVA analysis showed relatively high level of statistical significance of general impact on $OCV_{D.m}$ of the enterprises located in **Sadhora landscape area** ($F_{10,\ 209}$=2.99; p=0.0015) (Table 2).

On the basis of the interval chart inferred by ANOVA program we may assume that average $OCV_{D.m.}$ values in sanitary-protective zones of the investigated enterprises of Sadhora landscape area are lower than the mean background one. The vertical bars denoting 0,95 confidence intervals, control (No1) and none of the studied options (No2-11) do not overlap (Figure 2).

Tukey test has proved the validity of graphically fixed $OCV_{D.m.}$ decrease in sanitary-protective zones of all enterprises under study of Sadhora landscape area in relevance to the control (Table 3). In all sanitary-protective zones compared with an appropriate control, the value of *p* turned out to be lower than 0,005, an admissible limit of error level.

In sanitary-protective zone of 4 enterprises in Sadhora landscape area IO $OCV_{D.m.}$ exceeds 25% that corresponds to the medium level of atmospheric air pollution (Figure 3).

Table 2. The results of one-factor dispersion analysis for the variable "Overall *D. melanogaster* cohort viability" on the investigated territories of Sadhora landscape area in Chernivtsi

	Multiple R	Multiple R^2	R-adjusted	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
Overall viability of cohorts	0.8964	0.8035	0.7142	551	10	55.10	3838	209	18.37	2.99	0.0015

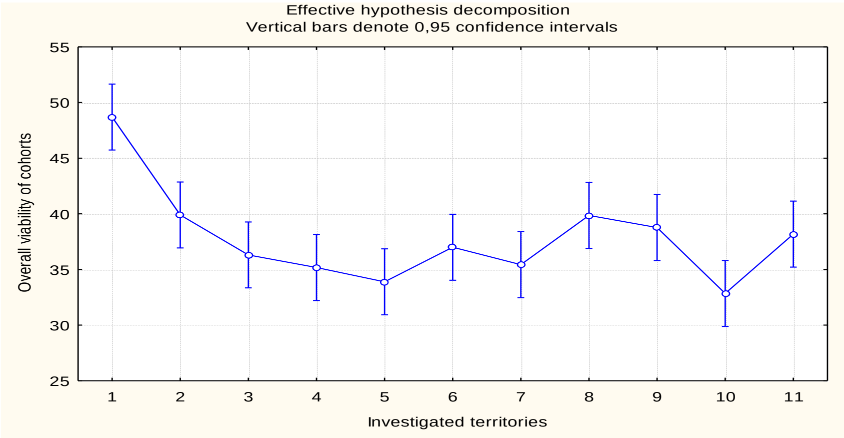

Fig. 2. The chart of average values of the analyzed variable "Overall *D. melanogaster* cohort viability " for various values of the group variable " The studied territories of Sadhora landscape area of Chernivtsi": *1 – tuberculosis dispensary park (background territory);sanitay--protective zones: 2 – thermoisolation materials plant, 3 – oil and fat company, 4 – chemical plant, 5 – furniture factory, 6 – "Kalynivsky market" parking place, 7 – reinforced concrete and concrete structures plant, 8 – "Ukrnafta" gas station No24, 9 – "Denysivka-1" limited liability company , 10 – bus station No 3, 11 – city garbage dump*

Table 3. The paired comparisons of mean values of overall *D. melanogaster* cohort viability (OCV*D.m.*) in sanitary-protective zones of the enterprises and the control (background) territory of Sadhora landscape area in Chernivtsi by a posteriori Tukey test (n=20)

Number of variants	Places of formation and development of cohorts	OCV*D.m.*, c.u.	Probabilities for Tukey HSD
1	Tuberculosis dispensary park (background territory)	48.70 ± 3.40	-
	Sanitary-protective zones:		
2	thermoisolation materials plant	39.90 ± 2.12	0.0091
3	oil and fat company	36.31 ± 2.89	0.0003
4	chemical plant	35.18 ± 2.44	0.0002
5	furniture factory	33.89 ± 1.77	0.0002
6	"Kalynivsky market" parking place	37.00 ± 3.39	0.0005
7	reinforced concrete and structures	35.43 ± 2.24	0.0002
8	"Ukrnafta" gas station No 24	39.86 ± 2.35	0.0087
9	"Denysivka -1" limited liability company	38.77 ± 2.25	0.0026
10	bus station No3	32.85 ± 1.76	0.0002
11	city garbage dump	38.18 ± 1.92	0.0014

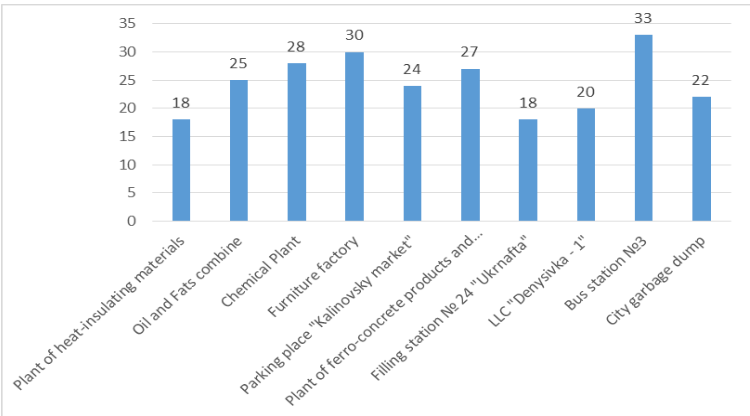

Fig. 3. The indicator of oppression of overall cohort viability *D. melanogaster* (IO OCV*D.m.*) in sanitary-protective zones of the enterprises in Sadhora landscape area of Chernivtsi, %

Among them there is bus station No 3 with buses in 80 directions and 431 routes. Bus station No 3 is an important transport hub connecting Chernivtsi with other settlements of Chernivtsi region and other regions of Ukraine and even other countries. But a very small area of the station built still in the soviet times does not match its high modern capacity which results in increasing of pollutants concentration in the air. The evidence of this is also IO OCV $_{D.m.}$, which is 33 % here.

The proof of the negative impact of automobile emissions on *D.melanogaster* is fixed in the investigations of many authors. At the end of the 70s and the beginning of the 80s the impact of petrol fuel (Nylander et al., 1978) and diesel emissions (Schuler & Niemeier, 1981) on a fruit fly organism was studied. Later the attention of the researchers was focused on certain ingredients of waste gases of internal combustion engines. Thus, it was on the basis of a fruit fly that detoxication transmembrane P-gp (glycoprotein penetrating) pump was discovered which perform a transporting function from cells of such products of incomplete combustion of fuel as benzo[a]pyren and other polycyclic aroma hydrocarbons. In this case the P-gp expression demonstrates clear dependence on the dose of the pollutants of this class. (Vache et al., 2006). The assessment of the impact of such constant component of automobile exhaust gas as nitrogen oxide on *D. melanogaster* was also unequivocal. On the one hand, the biological significance of NO as an important negative regulator of proliferation of cell-precursors during the morphogenesis of tissues and organs was proved and its decisive role in organizing neural connections (Enikolopov et al., 1999; Jaszczak et al., 2015; Rabinovich et al., 2016), as well as the positive role in induction of innate immune reaction on infecting by gram-negative bacteria (Foley & O'Farrell, 2003) was established. On the other hand, it was established that excessive NO concentrations inhibit DNA synthesis and cell proliferation and also inhibit NO- synthase (NOS) in imaginal discs of larvae of Drosophila which may result in hypertrophy of tissues and organs of an imago (Enikolopov et al., 1999). Apart from this, it was established that decrease in nitrogen oxide concentration in Drosophila's organism continues its life expectancy (Mit' et al., 2014). But the widest range of research focuses on the impact of tetraethyl lead as an anti-knock petrol additive on *D. melanogaster*. At early stages the mutagenic and teratogenic tetraethyl lead effects were established (Kennedy et al., 1971) and genetics of *D. melanogaster* resistance to this pollutant was studied. (Nassar, 1979). A fruit fly was used as a model for the investigation of toxicity of other lead compounds emitted in the air by a vehicle engine while using leaded gasoline, lead nitrate and lead acetate in particular (Castañeda et al., 2001). It was proved later that led inhibits the D. *melanogaster* development (Cohn et al., 1992). It has been established that D. melanogaster easily accumulates lead during its life and the level of accumulation increases in proportion to the presence of Pb in the environment. Besides, wandering larvae of the third age accumulate more Pb than adult species (Peterson et al., 2017). It has been shown that lead inhibits the quickness of egg hatching and the quickness of transforming of larvae in pupae, and pupae into imago and also inhibits the growth of larvae, pupae, and imago in length and width. The authors have come to the conclusion that these effects are due to the inhibiting of mitochondrial ATP synthesis (Safaee et al., 2014). With the increase of lead concentration a noticeable change is observed in number of offspring and D. melanogaster behavior, besides, a long lasting action of lead may dramatically effect the population survival, even if the concentration is low (Mathew & Krishnamurthy, 2018). The study of lead impact on the development of nervous system of *Drosophila* has allowed to understand better the mechanism of neurotoxicity of Pb in a human nervous system (Morley, 2003; Hirsch et al., 2012).

The second position by IO $OCV_{D.m.}$ is taken by the furniture factory. This fact is of special consideration as recently formaldehyde has been acknowledged as one of the most stable pollutant of the atmospheric air of Chernivtsi and Chernivtsi region (Ecological passport, 2018). The main source of this pollutant emission in the environment is the furniture factory and timber industry, major economies in Chernivtsi region. It is common knowledge that formaldehyde is used and emitted in the environment while manufacturing plywood, pressed wood, chipboard, MDF (Salthammer et al., 2010; Salthammer, 2015).

It has been established that formaldehyde while inhaling causes serious toxicity in Drosophila resulting in significant changes in genes expression and behavior (Eom et al., 2017). On the basis of the results obtained the authors recommend to use Drosophila as a potential alternative model for inhalation toxicity screening.

The effect of formaldehyde on life expectancy and stress resistance of Drosophila depends on the concentration. Low concentrations of formaldehyde prolong *D. melanogaster* life expectancy and increase its tolerance to starvation and heat shock (Li & He, 2016). The confirmation of positive impact of low concentrations of formaldehyde on *D. melanogaster* development can be found in other papers (Liang et al., 2018). The authors have put forward a hypothesis that some increase of the percentage of survival of a fruit fly at the stage of its transformation from an egg into a larva which takes place with low concentrations of the pollutant may be conditioned by antimicrobial properties of formaldehyde. They believe that the decrease of the number of microorganisms on the egg shell makes easier larvae hatching.

Rather, higher formaldehyde concentrations decrease life expectancy and *D. melanogaster* tolerance to starvation and heat shock (Li & He, 2017).

IO $OCV_{D.m.}$ in the sanitary- protective zone of the furniture factory is 30% which is the evidence that emissions of this enterprise exceed the concentrations which may be considered as favorable for *D. melanogaster* development.

The oppression of 28% of overall *D. melanogaster* cohort viability is fixed in the sanitary-protective zone of the chemical plant. On the whole, this enterprise is among the leaders in technology modernization and diversification of products. Chernivtsi chemical plant manufactures and offers the consumers more than 40 types of products. Penthaphtalic, polyurethane, acrylic, acrylate paints and enamels of 2024 colors and shades, parquet lacquer, drying oil, old paint and varnish remover and wood bioprotector. Products of auto-chemistry are also in demand. They are lutes, putties, primers, different solvents, coolants and quick-drying enamels for automobile, agricultural and railway transport. Besides, the chemical plant produces fireproof pastes for metal constructions and high voltage cables, pastes and lutes for special machinery and paste polish for furniture. Still, recognizing a tangible contribution of the chemical plant to the city economy, the results of the research performed show that more attention should be paid to the search for new possibilities of production greening.

The variety of products of the chemical plant makes us consider it as a potential source of emission of volatile organic compounds (VOC) in the air. It has been proved that VOC have a detrimental effect on larvae survival and *D. melanogaster* life expectancy (Trivino et al., 2017), adversely affect female fertility and progeny survival (MacGregor et al., 2018). The neurotoxic effect of VOC on *D.melanogaster* manifesting itself in the form of locomotor defects has been established where one of the reasons of VOC-mediated neurotoxicity is the formation of active forms of oxygen (Inamdar, 2010). It has been shown that Drosophila is a perfect model to explain the mechanistic properties of various toxic VOC (Inamdar, 2014).

The medium level of pollution of atmospheric air was also fixed in the sanitary-protective zone of the reinforced concrete and structures plant where IO $OCV_{D.m.}$ value was 27%. Concrete is the second popular substance in the world economy after water and, at the same time, the most destructive material on the Earth. Its advantages conceal serious danger for the planet, for the health of the people and the civilization in general. The concrete and cement production is first of all associated with large emissions of CO_2 (Meyer, 2013a). Though concrete requires less CO_2 per unit of volume than other materials but its production volume creates more CO_2 than any other material. Some of the biggest concrete producing companies reduced the carbon intensity of their production investing into more economical fuel stoves but most of the gained improvements were eclipsed by considerable increase of the world production of concrete. The population increases, active urbanization takes place, there is a great demand in construction of dams, roads, and houses, and, besides, the increase of all-world level of personal wealth has increased the demand in concrete.

Meanwhile, it is well known that *D. melanogaster* is very sensitive to CO_2. It is not by accident that carbon dioxide is used as analgesic for *D.melanogaster* in many laboratories of the world. The authors (Colinet & Renault, 2012) have shown that CO_2 blocks synaptic transmission in skeletal neuromuscular junction. The inhibiting effect of CO_2 on the heart (including its cardiac arrest) and skeletal neuromuscular junction in *D. melanogaster* takes place at the expense of sensitivity reduction to glutamate, neuromediator released by the ends of motor nerves.

It has been proved that carbon dioxide influences on the physiology and behavior of *D. melanogaster* larvae (Badre et al., 2005).

Thus, the medium level of the atmospheric air pollution in the sanitary-protective zone of the reinforced concrete and structures plant demonstrates the general tendency of air pollution of urban landscapes by carbon dioxide as a result of concrete production. Meanwhile, modern construction concrete may use 75% energy less in its life cycle (Meyer, 2013b). And to reduce CO_2 emissions as suggested by the European Commission new breakthrough technologies are required. Among such, the author refers to the new technology of concrete production which was elaborated and implemented by the Canadian company CarbonCure. The technology lies in CO_2 transformation into solid mineral within concrete, i.e. its binding to the latter. The liquefied carbon dioxide gets caught from the industrial processes and is introduced into concrete during its production that facilitate the process of mixing cement and other ingredients.

F-test has proved the statistical importance of the regressive ANOVA model which describes the general effect on $OCV_{D.m.}$ of the enterprises located in **the Central city landscape area** of Chernivtsi (Table 4). And though the value of *p* was considerably lower than that for the appropriate model of Sadhora landscape area, it was still lower than 0.05 ($F_{5, 114}$=2.5; p=0.035).

The integral chart built by ANOVA program for the Central city landscape area demonstrates the approximation of mean values of $OCV_{D.m.}$ in the sanitary-protective zone of "Rosma" limited liability company (variant No2) and the control (variant No1) and some remoteness of these variants from others (Figure 4). The vertical bars denoting 0,95 confident intervals of these two variants overlap while they do not overlap with appropriate bars of other variants. This allows to assume that in variants No 3,4,5,6 the values of $OCV_{D.m.}$ are lower than in variants No1 and 2. To verify this hypothesis a posteriori Tukey test was used.

Table 4.The results of one-factor dispersion analysis for the variable "Overall *D. melanogaster* cohort viability" on the investigated territories of the Central city landscape area of Chernivtsi

	Multiple R	Multiple R^2	R-adjusted	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
Overall viability of cohorts	0.8972	0.805	0.7237	365	5	73.04	3330	114	29.22	2.5	0.035

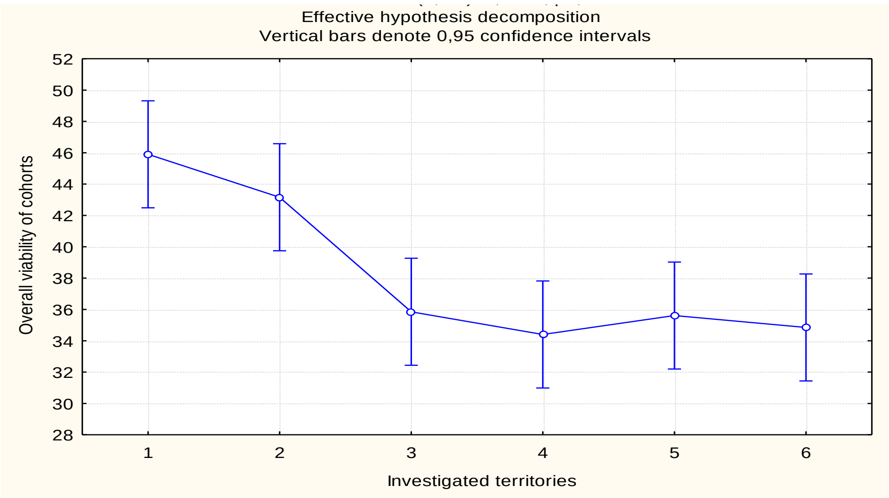

Fig. 4. The chart of mean values of the analyzed variable "Overall *D. melanogaster* cohort viability" for various values of the grouping variable "The investigated territories of the Central city landscape area of Chernivtsi": *1 – Shevchenko park (background territory); sanitary-protective zones: 2 – "Rosma"Ltd, 3 – brick factory No3, 4 –machine engineering plant, 5 – bus station No 2, 6 – "Tourist" motor transport depot*

The Tukey test confirmed the existence of significant difference in $OCV_{D.m}$ value in the control (variant No1) and variants No 3,4,5,6 and the absence of the significant difference from the control of variant No2 –"Rosma" Ltd (Table 5). The latter enterprise is known in Ukraine as a leader in production modernization. Due to the constant renewal of material and technical base and purchase of import high technological equipment "Rosma" Ltd has set up the export of its products (polypropylene pipes and fittings for cold and hot water supply and heating, rubber and polymeric footwear, rubber and technical products) to the countries of Europe and Middle East. And though the enterprise started its activity in 1935 the constant investment of costs from profit into new equipment enabled it to withstand the economic crisis in the country and in the world on the market (Kobevko, 2015). The results of the research prove that reconstruction and technical refit of "Rosma" Ltd facilitated the production greening.

As the mean $OCV_{D.m.}$ value in the

sanitary-protective zone of "Rosma" Ltd did not differ considerably from the control, IO $OCV_{D.m.}$ for this enterprise equaled zero. IO $OCV_{D.m.}$ in the variants where the mean $OCV_{D.m}$ values differed significantly from the control and equaled or was less than 25%, i.e. corresponded to the low level of air pollution (Figure 5).

It is noteworthy to mention that, likewise in Sadhora landscape area, among the variants different from the control are the enterprises associated with construction industry (brick factory No 30 and automobile transport (bus station No 2 and "Tourist" motor depot). One of the ecological problems of Ukraine is out of date buses. The bus park of the Ukrainian transport operators consists of 80% of the vehicles over 10 years old due to the absence of bus production for international routes in Ukraine and high rates of import duty (20% of customs value + 20% of VAT). This causes negative consequences in both the issues of their technical maintaining and ecology (Statistical Data, 2018).

As performed with F-test the diagnosis of SS general model ANOVA versus the SS residues proved the statistical significance of the established regressive dependence of $OCV_{D.m.}$ on the type of an enterprise in **the Southern landscape area** of Chernivtsi ($F_{8,\ 171}$ =2.46; p=0.015) (Table 6).

Table 5. The paired comparison of mean values of overall *D. melanogaster* cohort viability ($OCV_{D.m.}$) in the sanitary-protective zones of the enterprises and the control (background) territory of the Central city area of Chernivtsi with the a posteriori Tukey test (n=20)

No of variants	The places of formation and development of cohorts	OCVD.m., c.u.	Probabilities for Tukey HSD
1	Shevchenko park (background territory)	45.90 ± 3.62	-
	Sanitary-protective zones:		
2	"Rosma" Ltd	43.16 ± 2.33	0.7825
3	brick factory No 3	35.85 ± 2.26	0.0049
4	machine engineering plant	34.40 ± 2.30	0.0017
5	Bus station No 2	35.61 ± 2.69	0.0041
6	"Tourist" motor depot	34.85 ± 2.84	0.0024

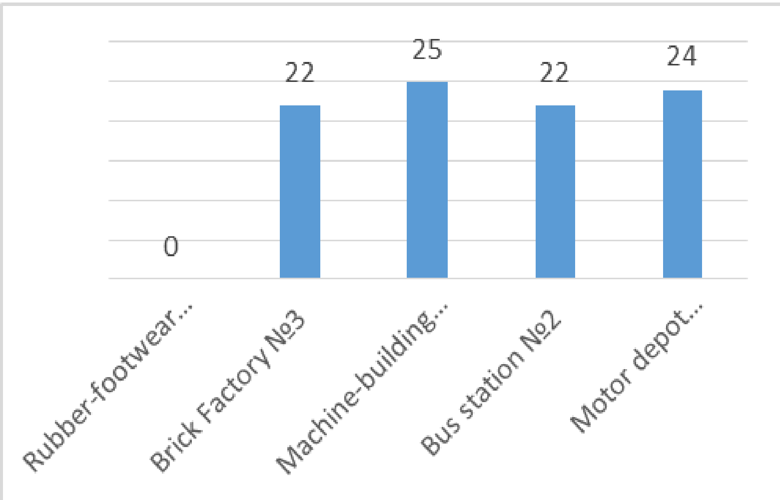

Fig. 5. The indicator of oppression of D. melanogaster overall viability of cohorts (IO OCVD.m.) in the sanitary-protective zones of the enterprises of the Central city landscape area of Chernivtsi, %

Table 6. The results of one-factor dispersion analysis for the variable "Overall *D. melanogaster* cohort viability" on the investigated territories of the Southern landscape area of Chernivtsi

	Multiple R	Multiple R^2	R-adjusted	SS model	df model	MS model	SS Residual	df Residual	MS Residual	F	p
Overall iability of cohorts	0.820	0.6724	0.5267	258	8	32.25	2238	171	13.09	2.46	0.015

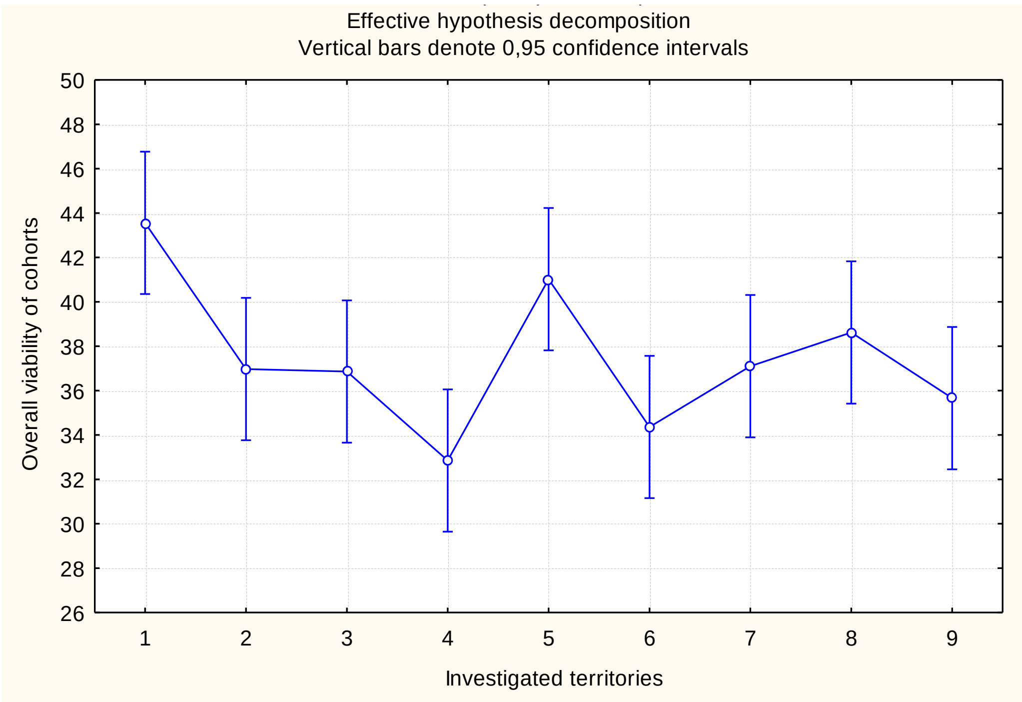

Fig. 6. The chart of the mean values of the analyzed variable "Overall viability of *D. melanogaster* cohorts " for various values of the group variable " The investigated territories of the Southern landscape area of Chernivtsi": *1 – Zhovtnevy park (background territory);sanitary-protective zones: 2* – bread factory*; 3* – "MAVEKS-PETROL" gas station; 4 – "Ukrnafta" gas station No 1; 5 – "ALPHA-NAFTA" gas station, affiliated branch No 16*; 6* – building structures plant; *7* – boiler houses of "Chernivtsioblteplomerezha" company*; 8* – Central city bus station*; 9* – *"*KORFOS*"* gas station

The integral chart built with the help of ANOVA program for the Southern landscape area demonstrates rather diverse picture as for the overlapping of 0,95 confident intervals of the investigated and the control variants: high and low level of overlapping and its absence (Figure 6).

The a posteriori analysis with the help of Tukey test proved the existence of reliable difference in $OCV_{D.m.}$ values as compared to the control in the sanitary-protective zones only three of eight enterprises of the Southern landscape area of Chernivtsi: "Ukrnafta" gas station No 1, "KORFOS" gas station and the building structures plant (Table 7). Thus, automobile transport and construction industry brought about the transformation of the atmospheric air sensitive for the bioindicator in this landscape area too.

However, in significantly different from the control variants of the Southern landscape area, likewise in the Central city landscape area, the mean $OCV_{D.m}$ value equaled zero or was less than 25% (Figure 7).

Table 7. The paired comparison of the mean values of overall viability of *D. melanogaster* cohorts ($OCV_{D.m.}$) in the sanitary-protective zones of the enterprises and the control (background) territory of the Southern landscape area of Chernivtsi with a posteriori Tukey test (n=20)

No of variants	The places of formation and development of cohorts	$OCV_{D.m.}$, c.u	Probabilities for Tukey HSD
1	Zhovtnevy park (background territory)	43.56 ± 3.62	-
	Sanitary-protective zones:		
2	bread factory	36.97 ± 2.48	0.1171
3	“MAVEKS-PETROL” gas station	36.86 ± 2.47	0.1068
4	“Ukrnafta: gas station No 1	32.85 ± 1.76	0.0027
5	“ALPHA-NAFTA” gas station, affiliated branch No 16	41.02 ± 3.40	0.9517
6	building structures plant	34.36 ± 2.12	0.0109
7	“Chernivtsioblteplomerezha” boiler houses	37.10 ± 2.77	0.1305
8	central city bus station	38.62 ± 2.57	0.3967
9	“KORFOS” gas station	35.66 ± 2.03	0.0368

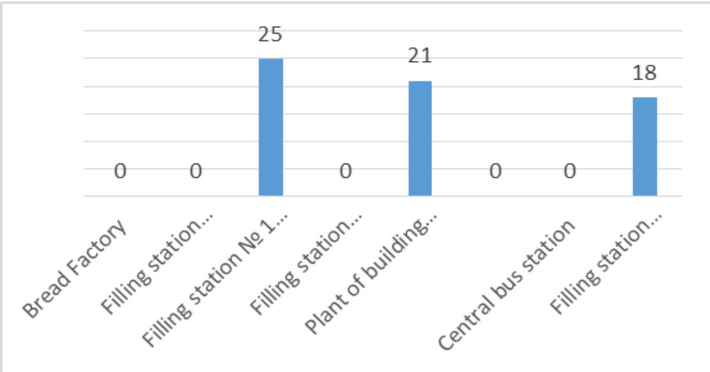

Fig. 7. The indicator of oppression of overall viability of *D. melanogaster* cohorts (IO $OCV_{D.m.}$) in the sanitary-protective zones of the enterprises of the Southern landscape are of Chernivtsi, %

The effectiveness of IO $OCV_{D.m.}$ as an indicator of air pollution of urban landscapes is also proved by the fact that its values turned out to be different in the sanitary-protective zones of one type of enterprises. It may be demonstrated by gas stations in Chernivtsi. Thus, IO $OCV_{D.m.}$ in the sanitary-protective zone of “Ukrnafta” gas station No 1 (Southern area) was 25%, “KORFOS” gas station (Southern area) and “Ukrnafta” gas station No 24 (Sadhora area) – 18%, and “MAVEKS-PETROL” (Southern area) and “ALPHA-NAFTA” (Southern area) was at the level of the control. The results obtained prove the interdependence established by other authors (Cherniak & Radomska, 2012) between fuel losses at gas stations, on the one hand, and its quality and available vapor recovery systems of petroleum products, on the other hand. So, IO $OCV_{D.m}$ demonstrates a sensitive response to various levels of one type enterprises greening.

CONCLUSION

The effectiveness of the application of the suggested method of assessment of air pollution of urban landscapes on the basis of indicator of oppression of overall viability of *D. melanogaster* cohorts (IO $OCV_{D.m.}$) has been proved. On the basis of the city of Chernivtsi (Ukraine) it has been established that IO $OCV_{D.m.}$ is responsive to various pollutants of air. The city of Chernivtsi is not a large industrial centerbut $OCV_{D.m}$ in sanitary-protective zones of various enterprises demonstrates different change character in relevance to the control: it is at the level of the control, undergoes medium or low level of inhibition. All the enterprises causing the highest level of oppression of $OCV_{D.m}$ are located in Sadhora landscape area of Chernivtsi. The highest percentage of $OCV_{D.m}$ oppression is caused by the overloaded bus station and enterprises manufacturing furniture, chemical products and concrete. In all landscape areas of the city the transformation of the atmospheric air on the part of automobile and construction enterprises has been revealed.

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CONFLICT OF INTEREST
The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING
No life science threat was practiced in this research.

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