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Abstract: *Drosophila melanogaster* serves as a important research model both for genetic investigations and for the study of metal toxicity, facilitating the elucidation of physiological mechanisms comparable to those of human organisms. In our research, we evaluated the toxicity effect resulting from exposure to various concentrations of lead and copper on the prolificity rates throughout the life cycle (egg-adult) of four genotypes of *Drosophila melanogaster*: *wild-type* (control), *white*, *brown*, and *white-vestigial*. During our study, the prolificity rates were examined across three repetitions under the impact of exposure to concentrations of 0.50, 1.00, 2.00, and 4.00 mM of copper ($CuSO_4$) and lead ($Pb(C_2H_3O_2)_2$). Prolificity rates throughout the life cycle exhibited variations as a direct consequence of genetic factors, the concentration of exposure, and the specific type of metal, either copper or lead. The mutant *white-vestigial* genotype revealed an IC_{50} concentration for prolificity inhibition at lower doses of 2.00 mM for copper and 4.00 mM for lead, in contrast to the control genotype (*wild-type*), which exhibited an inhibition concentration rate >IC_{50} of 4.00 mM only in the case of copper. Our results concluded that the (i) dose influences the prolificity rate in a directly proportional manner, (ii) comparative analyses between copper and lead revealed that copper displayed toxicity across all genotypes within the concentration range of 0.50 mM to 2.00 mM. In contrast, lead exhibited toxicity within the concentration range of 1.00 mM to 4.00 mM, highlighting a (iii) more acute toxicity characteristic in the case of copper. Thus, the results of this research reflect the importance of using *Drosophila melanogaster* as a genetic model in the comparative study of the interaction between genetic factors and the toxicity of metals, offering significant insights into monitoring their impact and defining the maximum permissible doses on organisms.

Keywords: *Drosophila melanogaster*; genotype; prolificity; heavy metals; toxicology; lead; copper

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1. Introduction

Drosophila melanogaster is a standard model for the study of various diseases, being an important bioindicator for testing the influence of chemical factors [1,2], for the development of treatments [3,4], diagnosis and understanding of toxicity phenomena in the body [5–9], as well as in the study of some neurodegenerative diseases [10].

Drosophila melanogaster (2n=8), exhibits a wide array of mutant genotypes alongside with a short life cycle, providing a significant advantage in the comparative study of toxicology [5,11]. Studies under the influence of heavy metals in *D. melanogaster* have stimulated increased numbers of reactive oxygen species (ROS) [6,12–14], leading to vacuolation of cells, a phenomenon associated with programmed cell death [15–18], damage to genetic material [19] and eventually apoptosis [18,20]. Numerous studies have highlighted the possibility of using antioxidants to reduce the toxic effects of heavy metals in *Drosophila melanogaster* [16,21,22]. Being thus an excellent bioindicator, the assay provides valuable information for the development of therapeutic strategies and personalized treatments for various diseases such as Parkinson's [10,23–26], Alzheimer [27–29], cancer [30–32], kidney disease [33] and diabetes [34–36], being a model for investigating insulin action [37]. In terms of the action of metals, *D. melanogaster* may be an essential indicator for determining and understanding how they act on organisms beyond normal limits [6,38–40]. *D. melanogaster* is not only used for understanding the physiological aspects of essential metals [41–43], but also for assessing the toxic impact of heavy metals [44,45] having numerous genes and detoxification mechanisms in its composition similar to those found in humans [46–49].

Metals with metabolic implications, such as copper, although indispensable for the growth and development of organisms by participating in enzymatic reactions and maintaining homeostasis in the body [50,51], become toxic if they accumulate in high concentrations [52,53].

Copper intake in the body is provided by copper-rich foods such as vegetables, fruit [54–56], in meat products [57] or in cereals [1,2]. Drinking water also contributes about 0.12-0.26 mg (≈20%) [1] of copper to the average daily intake. The maximum concentration of copper contamination in drinking water is about 1.3 mg. The natural concentration of copper in soil is about 50 mg/kg. The atmospheric copper content also varies between 5 and 20 ng Cu/m^3 [61].

Copper is found in nature in combinations, mainly as sulphides [62], participating in numerous biochemical processes based on oxidation-reduction reactions [63], via the various enzymes in whose composition it enters. In normal concentrations it has a positive impact on the functioning of the nervous and immune systems. In the nervous system, it facilitates the transmission of signals and the maintenance of neuronal integrity and in the immune system it contributes to the activation of defence cells and the protection of the body against pathogens.

The link between copper and these systems highlights its importance in maintaining health, with recommended doses of 1.5-3.0 mg in adults and 1 mg in children [52]. High concentrations of copper [64] act as an enzyme inhibitor and limit the activity of alkaline phosphatase [65], catalase [22], xanthine oxidase [66] and ribonuclease [67,68].

Heavy metals, such as lead, are toxic to the body [69–71], affecting a number of physiological and biochemical processes, such as metabolism, functioning of internal organs, and can contribute to diseases [5] and in plants affect [6,19,72], nutrition, photosynthesis [73], respiration [74], growth and development their [77,78].

Accumulation of heavy metals [78] leads to altered nutrient absorption capacity, deregulation of metabolic functions and eventually even death of organisms in case of intoxication [72,79,80]. In the context of heavy metal, pollution and climate change have a negative impact on the quality of life [81].

The maximum concentration limits for lead according to the EU Food Regulation are 0.1 mg/kg in meat products, 0.2 mg/kg in cereals and vegetables, 0.1 mg/kg and 0.02 mg/kg in milk. The normal limits for total lead in the human body in a person weighing 70 kg is on average 120 mg lead, with 0.2 mg/L in blood and between 0.2 and 3 in tissues. The U.S. Centers for Disease Control and Prevention has established standard elevated blood lead levels for adults and children (10 μg/dL and 5 μg/dL) [82].

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Lead poisoning in concentrations between 0.2 mM-5 mM can manifest acute or chronic forms, and clinical symptoms usually become evident after 2-3 days [83].

Looking at the impact of lead on organisms, we see that exposure to lead can have significant health consequences. Studies show that lead can affect various organs and systems, including the nervous, cardiovascular and reproductive systems [16,84].

In the present study we aimed to monitor the effect of heavy metals on the life cycle of several mutant genotypes and the standard genotype, with a view to using them as bioindicators of comparative toxicity between copper and lead, as well as identifying toxicity limits. *Drosophila melanogaster* offers the possibility of establishing toxicity limits between heavy metals, their combinations, and interactions between them, which recommends it as a toxicological bioindicator for assessing impacts on ecosystems and organisms.

2. Materials and Methods

2.1. Description of biological Material

In the toxic effects research, we opted to use four genotypes of *Drosophila melanogaster*, including both wild and mutant variants.

The *wild* type genotype was used as a control variant, having a light grey body, scarlet (dull red) eyes and wings longer than the body (Figure 1a) [85].

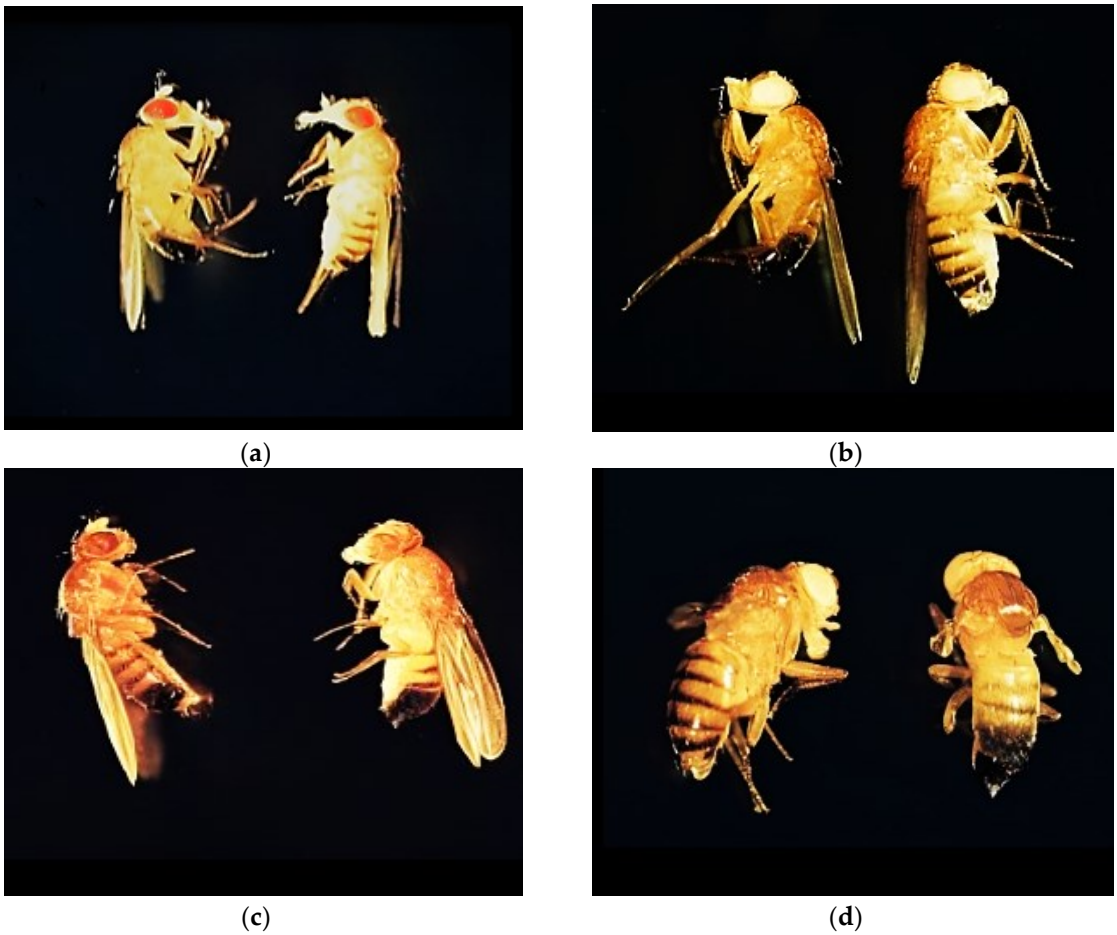

Figure 1. Genotypes of *Drosophila melanogaster* used, (a)-*wild* genotype, (b)- *white* mutant genotype (c)- *brown* mutant genotype, (d)- *white-vestigial* mutant genotype.

The *white* mutant genotype is different from the wild type due to the presence of the recessive *white* gene which determines the white colour of the eyes. This gene is located on chromosome I (heterozygous X) at a distance of 1.5 centimorgans from the 0 end and is denoted by „w" (Figure 1b) [86].

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The *brown* mutant genotype has as a defining feature the presence of the recessive bw gene, located on chromosome II at 104 cMo from the 0 end. This gene blocks the formation of the normal red pigment, resulting in a brown eye colour (Figure 1c) [87].

The *white-vestigial* mutant genotype is characterized by the white colour of the eyes, generated by the presence of the recessive gene w, located on chromosome I and the presence of reduced and rudimentary wings determined by the presence of the recessive gene ,,vg'', located on chromosome II (Figure 1d).

2.2. Preparation of culture medium and inoculation of D. melanogaster.

The preparation of the culture medium consists of 200 ml of water to which 2 g of gelatine, 3.6 g of brewer's yeast, 16.4 g of sugar and 20 g of grey are added.

The mixture obtained is boiled for 20 minutes and then 1 ml of propionic acid is added to the hot medium. The resulting composition is poured (30 ml) into culture dishes and covered with sterile stoppers until the culture medium solidifies. Subsequently, the medium is treated with a solution of yeast and distilled water, using a brush over which fine particles of dried yeast are sprinkled. Varying concentrations of lead acetate and copper sulphate were added to the prepared medium where adults of *Drosophila melanogaster* were inoculated. After etherization and examination, selected individuals were inoculated onto the culture media.

2.3. Growing environment and experimental metal varinates Cu^{2+} ($CuSO_4$) and Pb^{2+} ($Pb(C_2H_3O_2)_2$)

Four concentrations of Cu^{2+} and Pb^{2+} were used in this study to determine the effect on lifecycle proliferation. Due to the varying toxicity limits between Cu^{2+} and Pb^{2+} according to the literature we used intermediate epidermal variants to determine the proliferation inhibition concentration (>IC_{50}) in the four genotypes of *D. melanogaster* studied (*wild*, *white, brown* and *white-vestigial*) and to compare the toxicity levels between the two metals.

The experimental variants used were:

- normal environment with grey (control) [81];
- medium with grey, supplemented with copper sulphate solution $CuSO_4$ -$5H_2O$ (0.50 mM, 1.00 mM, 2.00 mM and 4.00 mM);
- medium with grey, supplemented with $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ lead acetate solution (0.50 mM, 1.00 mM, 2.00 mM and 4.00 mM);

Determination of the inhibition index (CI_{50}) of proliferation following exposure to Cu^{2+} ($CuSO_4$) and Pb^{2+} ($Pb(C_2H_3O_2)_2$)

After pregrafting the experimental variants, *Drosophila melanogaster* adults were inoculated in a 1:1 ratio (♀ : ♂) for the *wild-type* and mutant genotypes: *white, brown* and *white-vestigial*.

The experiment was carried out in three replicates during the life cycle (egg-adult, Figure 2) ,during which the proliferation of different genotypes of *Drosophila melanogaster* was monitored under normal conditions and exposed to *Cu^{2+} and Pb^{2+}* concentrations of 0.50 mM, 1.00 mM, 2.00 mM and 4.00 mM.

In the research, the impact of Cu^{2+} and Pb^{2+} was monitored at all developmental stages of *Drosophila melanogaster* (egg, larvae I, II, III, pupa and adult) (Figure 1) in order to determine the prolificacy as well as their inhibition concentrations.

After the establishment of the proliferation indices under normal conditions, they were compared with the experimental variants with different concentrations of Cu^{2+} and Pb^{2+} to determine the proliferation inhibition index (IC) according to formula (1):

$$IC = 100 - \frac{Number\ of\ individuals\ in\ experimental\ group}{Number\ of\ individuals\ in\ control\ group} * 100 \quad (1)$$

The proliferation inhibition index >IC_{50} was considered the maximum toxicity level of the metal taken in the study [89,90].

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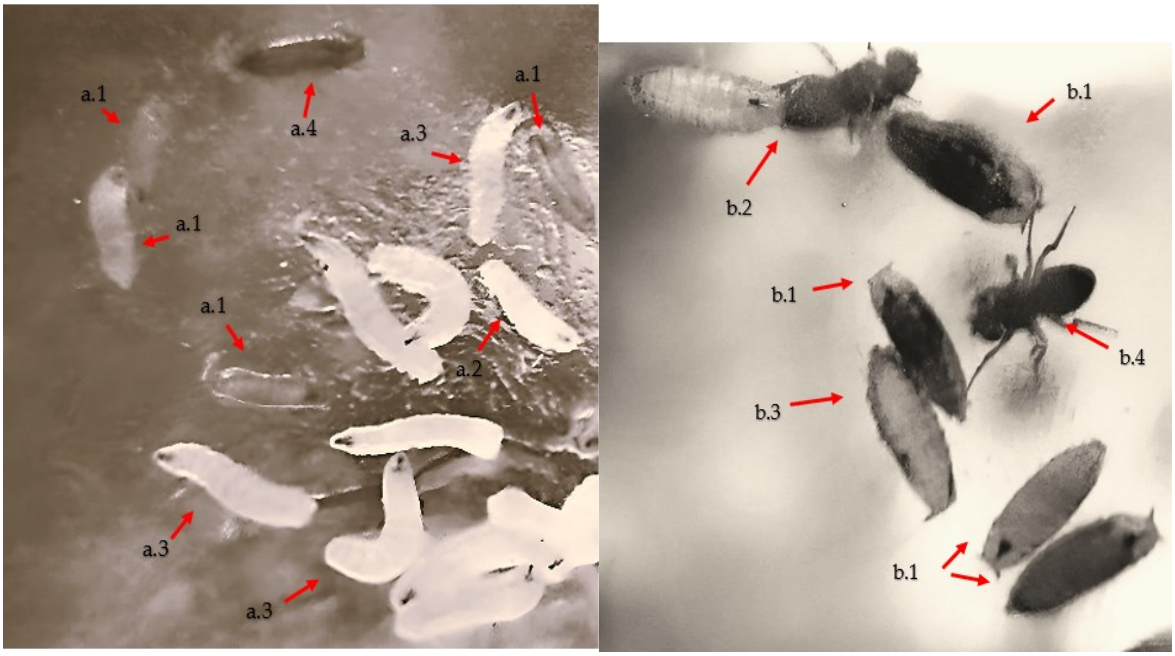

Figure 2. Developmental stages of *Drosophila melanogaster* larvae, (a.1)- larva stage I, (a.2)- larva stage II, (a.3)- larva stage III, (a.4)- larva pupation; Evolution of pupal to adult formation: (b.1)- pupal, (b.2)- hatching, (b.3)- hatched pupal, (b.4)- adult;**.**

2.4. Statistical calculation methods used to determine proliferation and toxicity levels between Cu^{2+} and Pb^{2+} .

In this study, we investigated the behavior of the genotypes concerning adaptability and prolificacy under exposure to various concentrations of heavy metals. We conducted statistical analysis using a multivariate ANOVA test, which uncovered the effects of genotype, metal type (Cu^{2+} or Pb^{2+}), toxicity levels, and concentration on the developmental period from egg to adult. Significance was determined at a confidence level of p <0.05. The obtained results underwent comparative analysis using the Tukey test. The statistical interpretation was carried out using Rstudio software.

3. Results

This study determined the prolificacy of *D. melanogaster* genotypes following the application of different concentrations of copper and lead at different life cycle stages.

The results showed differences in the evolution of the number of larvae (I, II and III), as well as in the formation of pupae and adults, depending on the genotype, the type of metal used (Cu^{2+} or Pb^{2+}) and the concentration used. Using the *D. melanogaster* genetic model, research has identified the influence of genotype and metal concentrations used in determining toxicity levels.

3.1. Influence of genotype, concentration and type of metal Cu^{2+} and Pb^{2+} on proliferation in Drosophila melanogaster.

In the study of the influence of different *wild-type* genotypes (control) and variations between *brown*, *white* and *white-vestigial* mutant genotypes, a significant difference in the number of larvae formed in stages I, II and III under different experimental conditions was observed.

The results show a directly proportional correlation between the concentrations of heavy metals used and their type (Cu^{2+} and Pb^{2+}), as well as the nature of the genotype, in terms of the number of larvae formed ($p < 0.001$).

There was also a significant influence of genotype on the number of individuals resulting from exposure to these metals.

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Thus, from the results obtained, it can be deduced that the type of genotype studied can exert a significantly different influence on the number of individuals resulting from exposure to Cu^{2+} and Pb^{2+}, thus confirming the significant role of genetic predisposition to toxicity (Table 1).

Table 1. Analysis of factors involved in the process of inhibition of proliferation at different concentrations of copper ($CuSO_4$) and lead $Pb(C_2H_3O_2)_2$ in larval stages.

	Analysis factor	df	SS	S^2	F	Pr(>F)
Larval stage I	Concentration	4	55264	13816	240.429	< 2e-16 ***
	Genotype	3	24097	8032	139.782	< 2e-16 ***
	Metal	1	3936	3936	68.499	2.74e-16 ***
	Concentration: Genotyp	12	1240	103	1.798	0.043 *
	Concentration:Metal	4	1200	300	5.219	0.000356 ***
	Genotype:Metal	3	290	97	1.680	0.169 Ns
	Concentration: Genotip: Metal	12	232	19	0.337	0.982 Ns
	Residuals	1520	87344	57		
Larvar stage II	Concentration	4	37452	9363	200.948	< 2e-16 ***
	Genotype	3	9527	3176	68.159	< 2e-16 ***
	Metal	1	2763	2763	59.292	2.56e-14 ***
	Concentration: Genotyp	12	1617	135	2.892	0.000583 ***
	Concentration:Metal	4	1037	259	5.563	0.000192 ***
	Genotype:Metal	3	130	43	0.933	0.423 Ns
	Concentration: Genotip: Metal	12	284	24	0.508	0.910 Ns
	Residuals	1392	64859	47		
Larvar stage III	Concentration	4	31284	7821	248.496	< 2e-16 ***
	Genotype	3	8277	2759	87.665	< 2e-16 ***
	Metal	1	2266	2266	71.992	< 2e-16 ***
	Concentration: Genotyp	12	1084	90	2.870	0.000646 ***
	Concentration:Metal	4	748	187	5.943	9.72e-05 ***
	Genotype:Metal	3	114	38	1.209	0.304 Ns
	Concentration: Genotip: Metal	12	199	17	0.526	0.899 Ns
	Residuals	1278	40223	31		

SS- sum of square, dF-Degrees of freedom; S^2 -Mean square. "Ns"-not significant, "*"- $p < 0.05$, "**"- $p < 0.01$, *** Significant at $p < 0.001$.

As in the larval stage, the influence of genotype and metal concentrations used was found to show significant variation in prolificacy levels throughout the life cycle, including in pupation and adult formation ($p < 0.001$) (Table 2).

Analysis of the interaction between the genetic factor and metal concentration levels (Cu^{2+} and Pb^{2+}) significantly influenced proliferation in *D. melanogaster* throughout the life cycle, both in the larval stages (Table 1) and in the pupal and adult formation process (Table 2) ($p < 0.001$). Thus, as

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concentration increases, proliferation capacity specifically affects certain genotypes, with some being more tolerant (showing a lower inhibition concentration) and others more sensitive.

Looking at the interaction between concentration and metal type (Cu^{2+} or Pb^{2+}), it had a significant impact on the number of individuals during the developmental cycle ($p < 0.001$). This reflects differences in the response of proliferation rates depending on the type of metal to which *D. melanogaster* genotypes are exposed.

The results obtained show the same impact of decreased prolificacy for both Cu^{2+}, and Pb^{2+}, obtaining the same effect of decreased number of inidivids in larval stages of pupal formation (Genotype*Metal, Concentration*Genotype: Metal, $p > 0.05$). Toxicity levels (degree of inhibition of proliferation, $>IC_{50}$) are influenced by genotype, concentrations and type of metal (Cu^{2+} or Pb^{2+}) used in the medium (Concentration, Genotype, Metal, Concentration*Genotype, Concentration*Metal, $p < 0.001$) (Tables 1 and 2).

Table 2. Analysis of factors involved in the process of inhibition of proliferation at different concentrations of copper ($CuSO_4$) and lead ($Pb(C_2H_3O_2)_2$ in pupal and adult formation.

	Analysis factor	df	SS	S^2	F	Pr(>F)
Pupal stage	Concentration	4	24437	6109	203.090	< 2e-16 ***
	Genotype	3	5526	1842	61.234	< 2e-16 ***
	Metal	1	1687	1687	56.094	1.45e-13 ***
	Concentration: Genotype	12	1132	94	3.136	0.000211 ***
	Concentration:Metal	4	585	146	4.861	0.000691 ***
	Genotype:Metal	3	80	27	0.884	0.448785 Ns
	Concentration: Genotype: Metal	12	179	15	0.496	0.918151 Ns
	Residuals	1060	31886	30		
Adult stage	Concentration	4	12209	3052.3	135.964	< 2e-16 ***
	Genotype	3	4569	1522.9	67.837	< 2e-16 ***
	Metal	1	893	893.5	39.799	5.55e-10 ***
	Concentration: Genotype	12	551	45.9	2.044	0.0189 *
	Concentration:Metal	4	274	68.5	3.051	0.0166 *
	Genotype:Metal	3	65	21.7	0.968	0.4075 Ns
	Concentration: Genotype: Metal	12	89	7.4	0.331	0.9836 Ns
	Residuals	586	13155	22.4		

Note: SS- sum of square, dF-degrees of freedom; S^2 -mean square. "Ns"-not significant, "*"- $p < 0.05$, "**"- $p < 0.01$, ***significant at $p < 0.001$.

3.2. Influence of copper ($CuSO_4$) on proliferation in Drosophila melanogaster during the life cycle (egg-adult).

Investigations of the prolificacy rate of *Drosophila melanogaster* genotypes at various copper concentrations show a decrease directly proportional to the increase in copper concentration, both in the three larval developmental stages (stage I, II and III) and in the pupal and adult formation stages (Table 3).

Significant differences were observed in the response to copper concentrations between the genotypes used ($p < 0.05$). *Brown* and *white-vestigial* genotypes showed higher sensitivity to the highest Cu^{+2} concentrations compared to *wild* and *white* genotypes.

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The *wild* genotype showed prolificacy rate values below 50% (>IC_{50}) at copper concentrations of 4.00 mM in larval stages I, II and III. Close values of proliferation inhibition >IC_{50} were observed at 2.00 mM concentration of Cu^{+2}. The same toxicity effect was observed in pupal and adult formation with >IC_{50} values being present at 4.00 mM concentration (Table 3).

Table 3. Results on the proliferation of *D. melanogaster* genotypes following exposure to various concentrations of copper.

Genotype	Concentration (mM)	*Copper ($CuSO_4$)*									
		Stage I	IC	Stage II	IC	Stage III	IC	Pupal	IC	Adult	IC
		Mean		Mean		Mean		Mean		Mean	
wild	Control	35.97^{a}	0.00	28.33^{a}	0.00	26.39^{a}	0.00	25.41^{a}	0.00	24.87^{a}	0.00
	0.50	29.85^{b}	17.03	24.58^{abc}	13.24	22.24^{ab}	15.73	20.67^{ab}	18.66	20.27^{abc}	18.50
	1.00	25.41^{bcd}	29.37	19.58^{cdef}	30.88	17.85^{bcde}	32.38	15.67^{cde}	38.34	16.40^{cdef}	34.05
	2.00	21.56^{de}	40.06	16.58^{defgh}	41.47	15.03^{defg}	43.05	13.33^{def}	47.52	14.20^{def}	42.90
	4.00	13.23^{fgh}	*63.22*	10.14^{ijk}	64.22	9.24^{hijk}	64.98	7.90^{ghi}	68.92	8.39^{gh}	66.26
brown	Control	27.08^{bc}	0.00	20.83^{cde}	0.00	19.52^{bcd}	0.00	18.74^{bc}	0.00	17.73^{bcd}	0.00
	0.50	22.28^{cde}	17.71	17.83^{defgh}	14.40	16.39^{cdef}	15.99	14.96^{cdef}	20.16	14.47^{def}	18.42
	1.00	19.97^{de}	26.23	16.03^{efgh}	23.07	14.88^{efg}	23.76	13.04^{def}	30.43	12.73^{defg}	28.20
	2.00	16.85^{efg}	37.78	12.56^{hij}	39.73	11.67^{ghij}	40.22	10.22^{fgh}	45.45	10.80^{fgh}	39.10
	4.00	10.82^{hi}	60.04	7.03^{kl}	66.27	6.48^{kl}	66.77	6.00^{hi}	67.98	6.94^{hi}	60.84
white	Control	30.54^{b}	0.00	26.64^{ab}	0.00	26.18^{a}	0.00	24.48^{a}	0.00	22.31^{ab}	0.00
	0.50	28.00^{bc}	8.31	21.47^{bcd}	19.40	19.82^{bc}	24.31	17.26^{bcd}	29.50	18.33^{bcd}	17.83
	1.00	22.33^{cde}	26.87	17.14^{defgh}	35.66	16.30^{cdef}	37.73	14.07^{cdef}	42.51	14.27^{def}	36.06
	2.00	17.85^{ef}	41.56	13.53^{fghi}	49.22	12.30^{fghi}	53.01	10.85^{efg}	55.67	11.67^{efgh}	47.71
	4.00	10.46^{hi}	65.74	8.33^{ghij}	68.72	7.18^{jkl}	72.57	7.30^{ghi}	70.18	6.56^{hi}	70.62
white-vestigial	Control	23.10^{cd}	0.00	21.85^{bcd}	0.00	19.70^{bc}	0.00	18.56^{bc}	0.00	16.93^{bcde}	0.00
	0.50	19.79^{de}	14.32	18.42^{defg}	15.72	15.94^{cdefg}	19.08	14.63^{cdef}	21.16	13.87^{defg}	18.11
	1.00	17.05^{efg}	26.19	15.19^{fghi}	30.47	13.27^{efgh}	32.62	12.74^{def}	31.34	11.33^{efgh}	33.07
	2.00	11.51^{ghi}	50.17	9.03^{jkl}	58.69	8.45^{ijkl}	57.08	7.22^{ghi}	61.08	6.27^{hi}	62.99
	4.00	5.97^{i}	74.14	4.53^{l}	79.27	4.58^{l}	76.77	3.64^{i}	80.37	2.94^{i}	82.63

*Superscript letters are assigned based on the results of the Tukey pairwise comparison test, indicating the significance levels of each column value obtained relative to the copper concentration and exposure time. The highest-ranked values are denoted, beginning with the first letter "a". Similar superscript letters indicate statistically non-significant differences between the obtained values ($p > 0.05$).**IC denotes the prolificity inhibitaion concentration of copper.

For the *brown* genotype, at the concentration of 4.00 mM Cu^{+2}, the results indicate a decrease in proliferation for larval stages I, II and III (>IC_{50}), highlighting a manifestation of increased toxicity in this genotype ($p < 0.05$). A significant decrease in the number of pupae and adults compared to the control variant is also noted, indicating the negative impact of copper on their formation ($p < 0.05$).

In the case of the *white* genotype, the concentration of 4.00 mM (>IC_{50}) of copper shows the same toxic effect, causing a reduction in the proliferation of this genotype during the life cycle.

The highest sensitivity was recorded in the *white-vestigial* genotype in which the 2.00 mM Cu^{+2} concentration (>IC_{50}) showed a decrease in proliferation of more than 50% compared to the control group for all developmental stages ($p < 0.05$).

According to the results obtained (Table 3 and Figure 3), the proliferation rate of the genotypes was inhibited by the presence of copper in all larval stages, showing the significant reduction of the proliferation rate starting with the dose of 0.50 mM Cu^{+2}($p < 0.05$).

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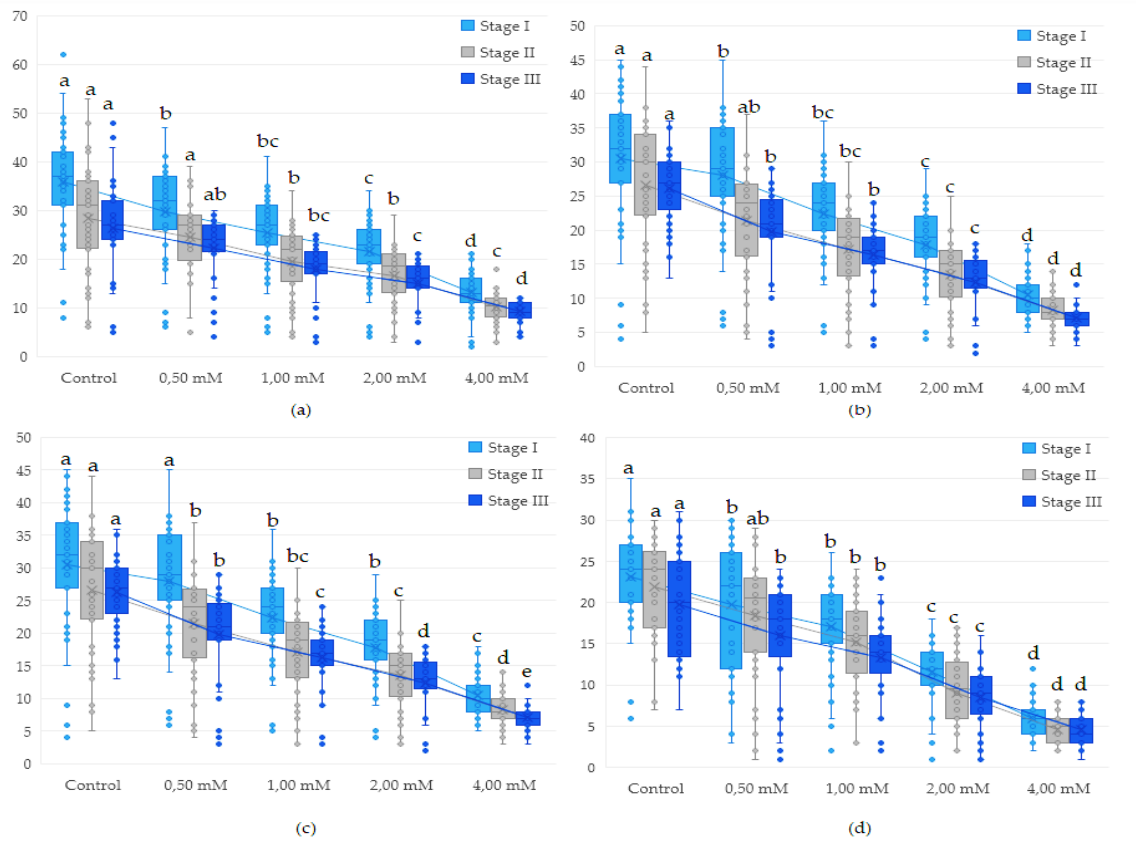

Notes: Different superscript letters (a, b, c, d, e, f) indicate significant differences ($p < 0.05$), with "a" representing the best.

Figure 3. Effect of copper on different genotypes of *Drosophila melanogaster* in adult formation: (a)- wild *wild* genotype, (b)- *brown* genotype (c)- *white* mutant genotype, (d)- *white-vestigial* mutant genotype.

In the early stages of the developmental cycle, concentrations of 0.50-1.00 mM Cu^{+2} resulted in decreases in the proliferation rate, which increased as the concentration of Cu^{+2} increased.

Pup and adult formation of the genotypes studied was influenced directly proportional to increasing concentrations for all stages followed (Table 3 and Figure 4).

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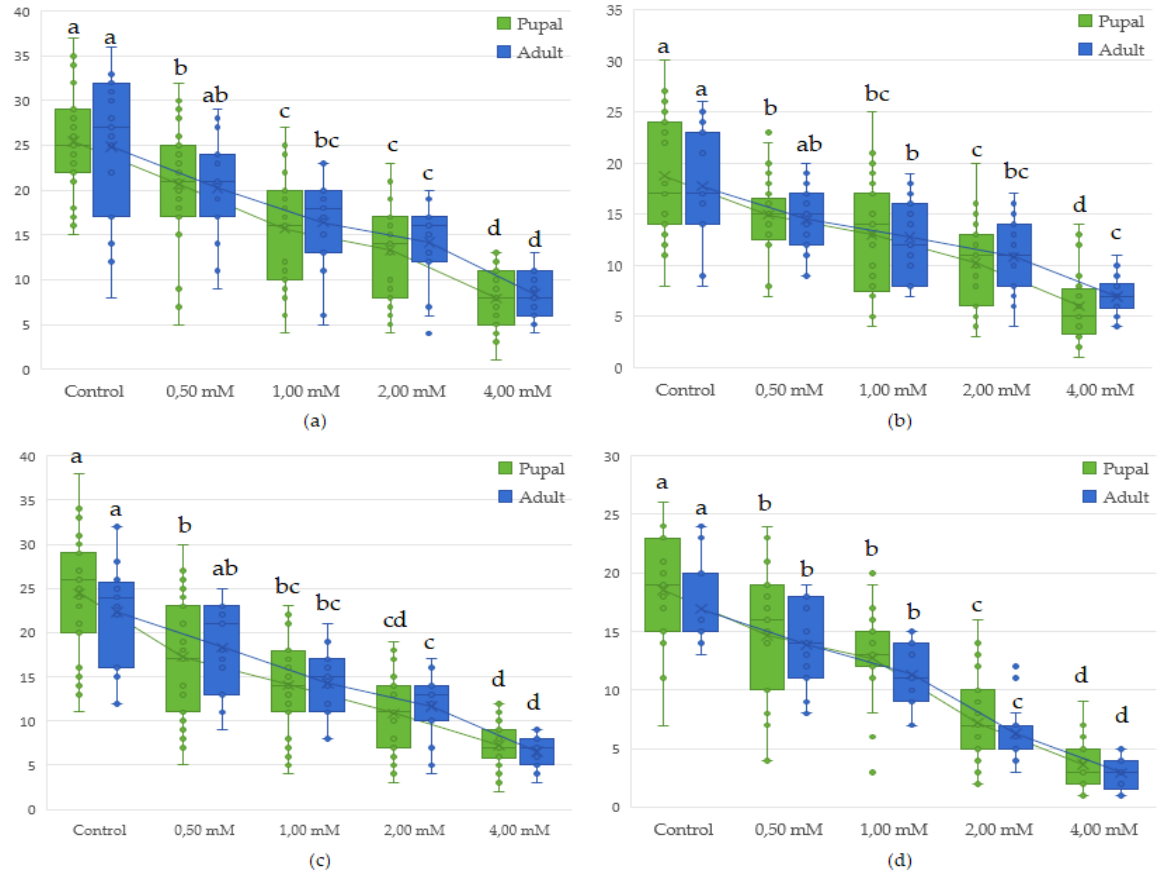

Notes: Different superscript letters (a, b, c, d, e, f) indicate significant differences ($p < 0.05$), with "a" representing the best.

Figure 4. Effect of copper on different genotypes of *Drosophila melanogaster* in adult formation: (a)- *wild* genotype, (b)- *brown* genotype (c)- *white* mutant genotype, (d)- *white-vestigial* mutant genotype.

Thus, from the presented results it was evident that the value of $>IC_{50}$ at the concentration of 4.00 mM is present in all studied genotypes (4/4). At 2.00 mM concentration of Cu^{+2} the *white-vestigial* genotype showed the $>IC_{50}$ value throughout the development cycle (1/4).

The results obtained in this study highlight the impact of copper concentrations ($CuSO_4$) on the proliferation of *Drosophila melanogaster* during their life cycle, showing an inhibition with increasing copper concentration and genotype under study.

3.3. Influence of lead ($Pb(C_2H_3O_2)_2$ on proliferation in Drosophila melanogaster during the life cycle (egg-adult).

Analysis of the number of stage I, II and III larvae of *Drosophila melanogaster* genotypes exposed to varying concentrations of Pb^{2+} revealed significant differences in the rate of proliferation as an effect of toxicity. The results showed significant variation in the number of individuals developed under different experimental conditions.

Investigations found that genotype had a significant influence on development in *D. melanogaster* following exposure, confirming a higher genetic predisposition to toxicity for certain genotypes. The highest values of prolificacy were recorded for the *wild* and *white* genotypes, *the* average values for the *brown* genotype and the lowest values for the *white-western* genotype (Table 4). The $>IC_{50}$ value at all developmental stages is present only in the *white-vestigial* genotype at a concentration of 4.00 mM (1/4, of genotypes).

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Table 4. Results on *D. melanogaster* genotype proliferation and viability following exposure to different lead concentrations $Pb(C_2H_3O_2)_2$.

Genotype	Concentration (mM)	*Lead* $(C_2H_3O_2)_2$									
		Stage I	IC	Stage II	IC	Stage III	IC	Pupal	IC	Adult	IC
		Mean		Mean		Mean		Mean		Mean	
wild	Control	35.9^{a}	0.00	28.33^{a}	0.00	26.39^{a}	0.00	25.41^{a}	0.00	24.87^{a}	0.00
	0.50	34.3^{ab}	4.63	27.47^{ab}	3.04	25.76^{a}	2.41	23.48^{abc}	7.58	22.93^{ab}	7.77
	1.00	31.3^{abc}	12.97	24.19^{abcd}	14.61	19.92^{b}	24.54	19.11^{bcd}	24.78	20.20^{abc}	18.77
	2.00	26.4^{cdefg}	26.37	20.31^{defg}	28.33	16.72^{bcde}	36.64	16.37^{defg}	35.57	17.13^{bcde}	31.10
	4.00	19.4^{hijk}	46.04	15.89^{fghi}	43.92	15.70^{bcdef}	40.53	13.66^{defgh}	46.26	13.28^{def}	46.60
brown	Control	27.08^{cdefg}	0.00	20.83^{cdefg}	0.00	19.52^{b}	0.00	18.74^{cd}	0.00	17.73^{bcd}	0.00
	0.50	25.97^{cdefg}	4.07	19.83^{defgh}	4.80	18.70^{bc}	4.19	17.30^{def}	7.71	16.60^{bcde}	6.39
	1.00	24.6^{defgh}	8.90	19.44^{defgh}	6.67	16.81^{bcde}	13.60	16.07^{defg}	14.23	15.53^{cde}	12.41
	2.00	20.7^{ghij}	23.30	15.47^{ghi}	25.73	13.17^{cdef}	32.53	12.59^{fgh}	32.81	13.33^{def}	24.81
	4.00	16.18^{ijk}	40.25	14.06^{hi}	32.53	11.24^{efg}	42.39	10.97^{gh}	41.48	11.06^{ef}	37.66
white	Control	30.54^{abcd}	0.00	26.64^{abc}	0.00	26.18^{a}	0.00	24.48^{ab}	0.00	22.31^{ab}	0.00
	.50	28.7^{bcde}	5.71	22.61^{abcde}	15.12	21.12^{ab}	19.33	18.42^{cde}	24.75	20.53^{abc}	7.97
	1.00	27.72^{cdef}	9.24	21.28^{cdefg}	20.13	18.42^{bc}	29.66	17.33^{def}	29.20	17.40^{bcd}	22.02
	2.00	22.26^{fghi}	27.12	16.75^{efghi}	37.12	13.83^{cdef}	47.16	13.59^{defgh}	44.48	14.53^{cde}	34.86
	4.00	14.62^{jk}	52.14	14.03^{hi}	47.34	12.67^{def}	51.62	13.13^{efgh}	46.35	12.39^{def}	44.48
white-vestigial	Control	23.10^{efgh}	0.00	21.85^{bcdef}	0.00	19.70^{b}	0.00	18.56^{cde}	0.00	16.93^{bcde}	0.00
	0.50	21.54^{fghi}	6.77	20.22^{defg}	7.46	18.29^{bcd}	7.14	16.97^{def}	8.57	15.88^{cde}	6.21
	1.00	20.92^{ghij}	9.43	18.89^{defgh}	13.56	16.42^{bcde}	16.62	15.56^{defg}	16.17	14.13^{cde}	16.54
	2.00	14.13^{k}	38.85	11.19^{ij}	48.77	10.48^{fg}	46.77	8.93^{hi}	51.90	7.47^{fg}	55.91
	4.00	7.41^{l}	67.92	5.65^{j}	74.16	5.82^{g}	70.46	4.69^{i}	74.73	3.59^{g}	78.81

*Superscript letters are assigned based on the results of the Tukey pairwise comparison test. indicating the significance levels of each column value obtained relative to the copper concentration and exposure time. The highest-ranked values are denoted. beginning with the first letter "a". Similar superscript letters indicate statistically non-significant differences between the obtained values ($p > 0.05$). **IC denotes prolificity inhibition concentration of lead.

In the early stages of the developmental cycle, larval stages exposed to concentrations ranging from 0.50 to 1.00 mM of lead exhibited a decrease in prolificacy. However, this decrease was not statistically significant concerning genotype prolificacy ($p > 0.05$).

The *wild* genotype showed a significant reduction in the number of larvae in stages I. II and III at higher concentrations, especially at 4.00 mM. The same trend was observed for the *white* and *brown* genotypes, suggesting a common sensitivity to lead.

As for the *white-vestigial* genotype, a more pronounced sensitivity to lead was observed. with a significant reduction in larval numbers in stages I, II and III starting at 1.00 mM concentration. in

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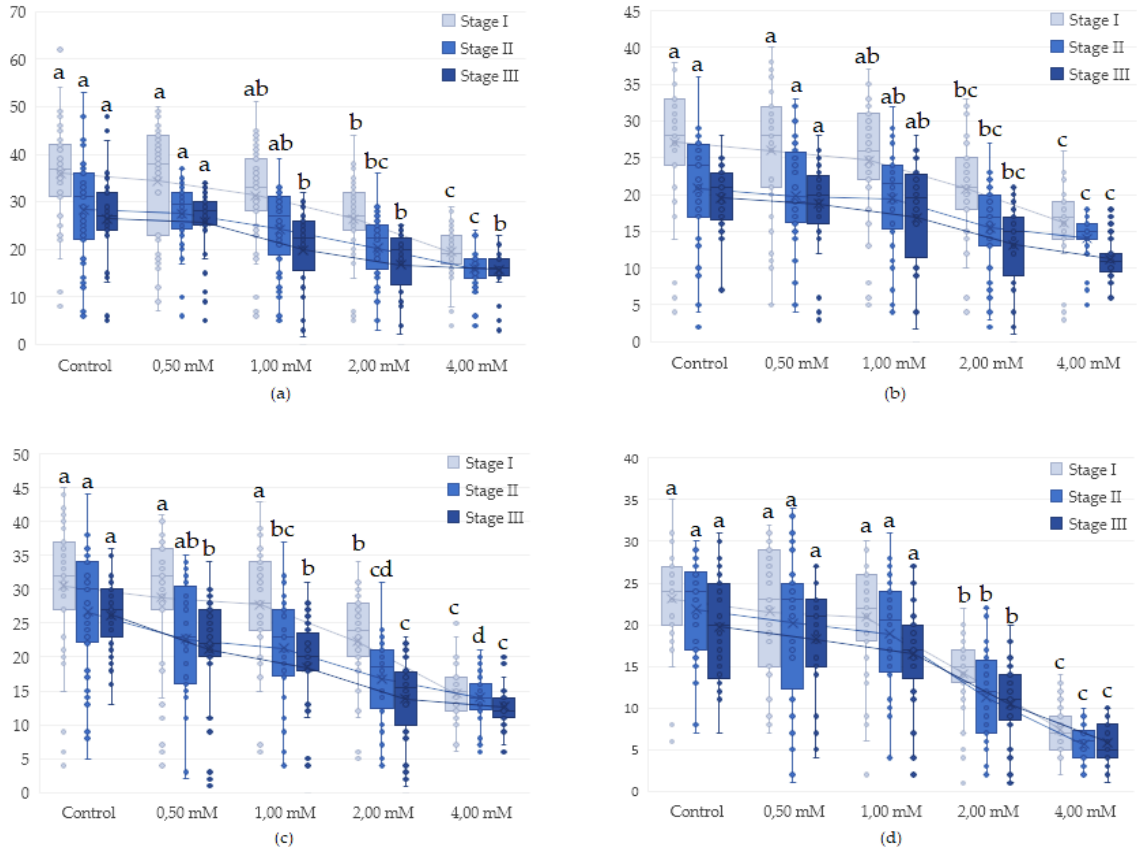
contrast to the other genotypes which showed a higher tolerance at this concentration. The results suggest that genotypes show different responses to lead concentrations. influencing development in *D. melanogaster* to varying degrees.



Notes: Different superscript letters (a. b. c. d. e. f) indicate significant differences ($p < 0.05$). with "a" representing the best.

Figure 5. Effect of lead on different genotypes of *Drosophila melanogaster* in adult formation: (a)- *wild* genotype. (b)- *brown* genotype, (c)- *white* mutant genotype, (d)- *white-vestigial* mutant genotype.

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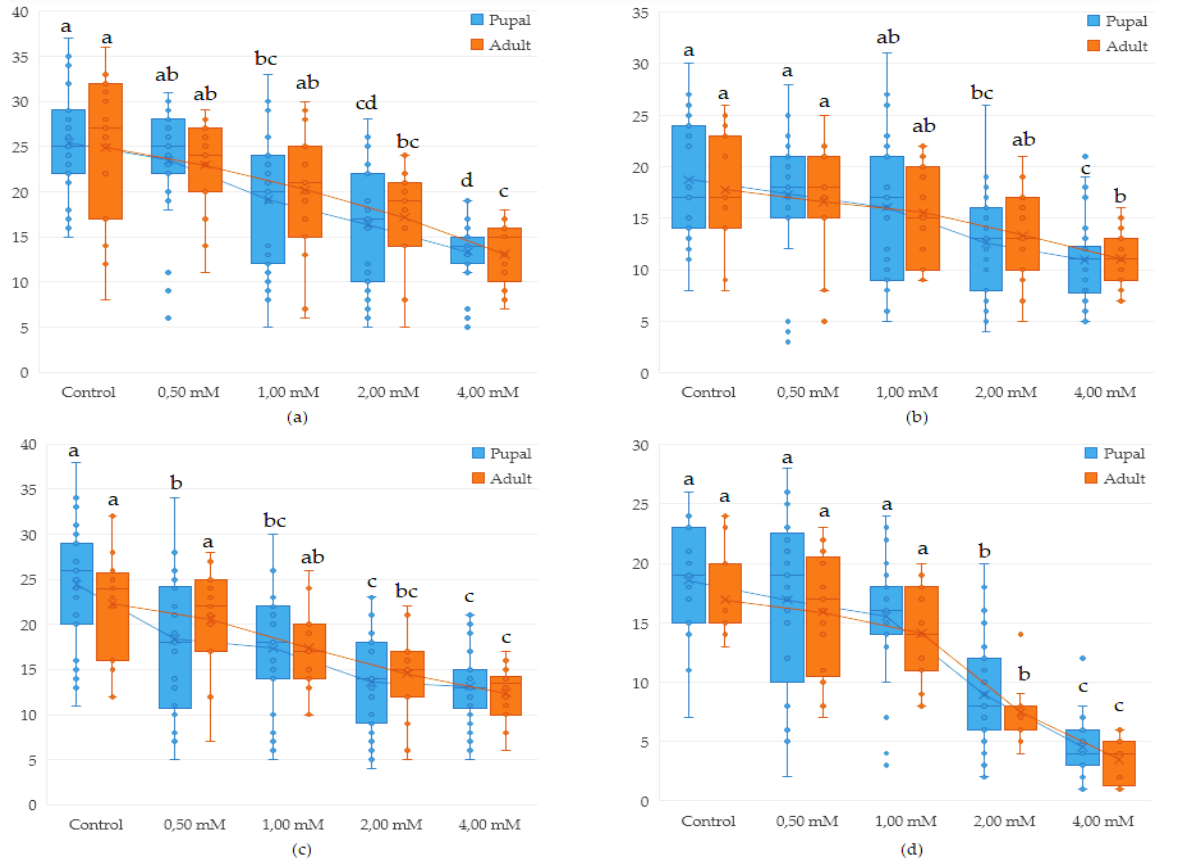

Notes: Different superscript letters (a. b. c. d. e. f) indicate significant differences ($p < 0.05$). with "a" representing the best.

Figure 6. Effect of lead on different genotypes of *Drosophila melanogaster* in adult formation: (a)- wild *wild* genotype (b)- *brown* genotype (c)- *white* mutant genotype (d)- *white-vestigial* mutant genotype.

Analysing the influence of *Drosophila melanogaster* genotypes on the number of pupae and adults obtained we found that the diversity of genotypes had a significant impact on the results. while also influencing the number of pupae in the control samples (p <0.001). In the case of lead exposure, the results obtained indicate a significant link between lead concentration and the effects generated.

Studies on mutant genotypes revealed that the *white-vestigial* genotype showed significantly fewer pupae formed in most of the variants studied, compared to the wild *wild* genotype which showed the highest tolerance at the pupal and adult stages.

Analysis of the effect of lead in *Drosophila melanogaster* reveals the significant impact of this metal on the development and health of organisms highlighting the importance of further research in this field and the development of effective strategies for environmental and public health protection.

Comparative study of toxicity effects on drosophila melanogaster at various concentrations of lead and copper.

The comparative study of copper and lead toxicity revealed significant variations in the formation of pupae and the emergence of adult flies. The analysis of toxicity differences demonstrated that copper exhibited higher toxicity at both low and high concentrations compared to lead. In the context of adult development, it was observed that at a concentration of 0.50 mM, no significant differences between the two metals were identified ($p < 0.05$).

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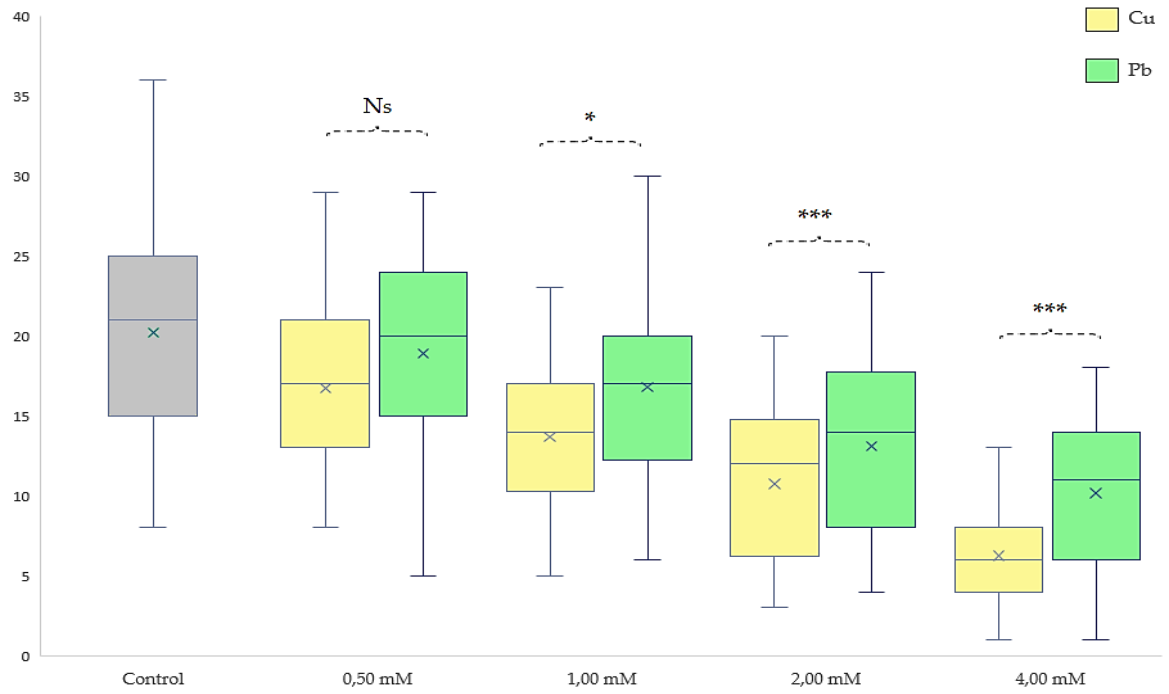

***"Ns"- not significant, "*"- $p < 0.05$, "**"- $p < 0.01$, *** Significant at $p < 0.001$*

Figure 7. Effect of copper on various genotypes of *Drosophila melanogaster* in adult development.

These differences persisted across other concentrations, indicating a continuous dominant effect of copper toxicity over that of lead. Consequently, the level of significance increased with rising concentrations. Therefore, at a concentration of 1.00 mM, the toxicity degree was significantly more pronounced for copper with $p < 0.05$ compared to lead. At concentrations of 2.00 mM and 4.00 mM, the degree of significance reached $p < 0.001$. The intervals were significantly different from each other (Figure 8) and also compared to the control group. These findings demonstrate a more acute toxic effect of copper compared to lead. This outcome might be inherently understandable considering that copper ($CuSO_4$) is often used as an antimicrobial and antibacterial agent, playing a crucial regulatory role in homeostasis, whereas the accumulation of lead exhibits a significantly different toxicological profile.

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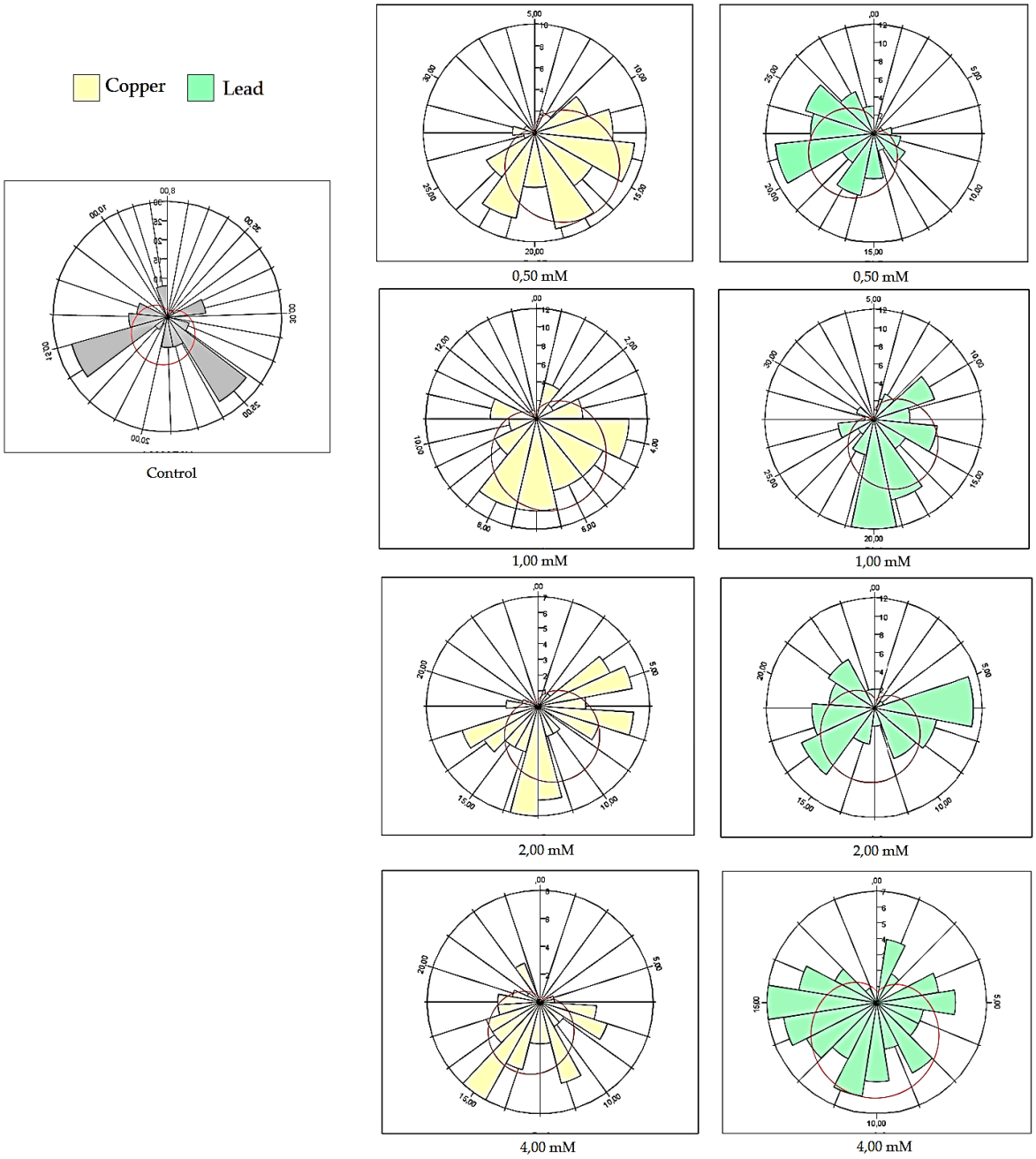

Figure 8. Polar histrogram of the effect of copper on various genotypes of *Drosophila melanogaster* in adult development.

The study on *D. melanogaster* can provide valuable insights into both types of toxicity, considering its rapid life cycle and the ease with which multiple generations can be generated and observed.

4. Discussion

Following literature reviews the results obtained complete the explanation of the Cu^{2+} toxicity phenomenon. (i) The same toxic effect has been observed by other authors using *wild* type genotype at concentrations of 0.50 mM [61–63], the most damaging concentration of 3.00 mM of Cu^{2+}, values that justify the toxicity values obtained by us (2 mM-4 mM, Table 3). (ii) In addition to the results we can say that there is a significant influence of the genotype used, as the authors also indicate an inhibition of proliferation on Cu^{2+} depending on the sex [93,94] or the developmental stage [2], the

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number of individuals starting to decrease significantly depending on the dose applied 0.40 mM for females and 0.75 mM for males [93]. (iii) Research shows the possibility of development of genotypes with increased tolerance to Cu^{2+} concentrations up to 50 mM of more than 75% as opposed to other genotypes with survival rates below 18%. As a result. genotype influence plays a determined role in the assessment of metalor toxicity above normal doses.

Copper is an essential element for the proper functioning of all organisms having an essential role in the composition of enzymes [95,96], antimicrobial and antiviral against *Escherichia coli* [97,98], *Staphylococcus aureus* (MRSA) [99–101], *SARS-coronavirus* [102–104], influencing virus A [104–106] and fungi [107]. However, even though the effects of copper are beneficial at high concentrations the results obtained for *D. melanogaster* indicate a negative effect of Cu^{2+}, manifested by reduced numbers of larvae. pupae and adults at concentrations starting at 0.50 mM Cu^{2+}.

Research has shown that the toxicity causes a reduction in the number of individuals and the impact is significant in all genotypes at the highest concentrations used in the experiment of 2.00-4.00 mM. Our results indicate different degrees of toxicity depending on the genotype of *D. melanogaster* used, which was observed for both Cu^{2+}, and Pb^{2+} as well as results obtained by other authors [20].

As regards lead accumulation research has shown the appearance of oxidative stress [109] at concentrations of 0.20 mM Pb $^{2+}$ [16] from the larval stage and even the appearance of spots on the wings. showing a mutagenic effect of Pb^{2+} from concentrations of 0.40 mM [110].

Cumulative cytotoxic effects of Pb^{2+} and Cu^{2+}, have also been shown in the organisms *Ceriodaphnia dubia* and *Daphnia carinata* [111].

Studies by other researchers on the impact of heavy metals on *Emiliania huxleyi* indicate a stimulation of copper uptake in the presence of lead at low concentrations [111]. However, at higher lead levelsm a reverse effect was observed. characterized by a significant reduction in copper uptake [112].

Influence on the reduction of absorption has also been identified for iron (Fe) indicating a decrease in Fe absorption under the influence of Pb^{2+}, at a concentration of 342 µg Pb/mL [109].

These results were also obtained in mouse experiments, which indicated that DMT1, responsible for iron (Fe) uptake, facilitates lead transport across the blood-brain barrier to the brain [113] (2.00 mM Pb^{2+}). This explains the toxic effect of lead on the nervous system with long-term exposure, with an even more pronounced impact on children [84].

Due to the considerable number of generations undergone by *Drosophila melanogaster*, this species offers a precise opportunity for localizing Quantitative Trait Loci (QTL), not only concerning genetic effects and biological impacts in heavy metal resistance, but also for analyzing the toxic effects of various chemicals and treatments. Consequently, by furnishing substantial and efficient biological support, *Drosophila melanogaster* emerges as a pivotal model and valuable indicator in health analysis and maintenance. This contributes significantly to the identification, monitoring, and development of solutions for various diseases, including cases of heavy metal poisoning [26].

5. Conclusions

This study unveiled the influence of genetic factors on the proliferation capacity of *Drosophila melanogaster*. The most sensitive genotype, *white-vestigial*, exhibited lower prolificacy compared to *wild* genotypes or other mutant variations. Prolificity varied across both control and experimental copper and lead variants throughout the life cycle.

Metals toxicity was elucidated through the proliferation inhibition index, which fluctuated according to genotype and concentration, with proliferation inhibition directly correlating with increasing concentration.

Copper displayed toxicity across all genotypes within the 0.50 mM to 2.00 mM concentration range (>IC_{50} valid for all genotypes), emphasizing the necessity of regulating and monitoring copper levels in the environment. Lead exhibited toxicity within the concentration range of 1.00 mM to 4.00 mM, with an inhibitory concentration exceeding IC_{50} solely for the white-vestigial genotype.

The comparative analysis between copper and lead highlighted that the adverse effects of copper are more enduring and acute than those of lead, suggesting the requisite for appropriate management

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of heavy metal exposure in natural environments. Differences in proliferation toxicity in *D. melanogaster* between copper and lead are substantial, escalating with increasing concentrations.

These findings underscore the significance of further research in ecotoxicology and the formulation of effective strategies for environmental protection and public health concerning heavy metal pollution, utilizing the *D. melanogaster* genetic model.

In conclusion, we aim to replicate the experiments with other metals and develop genotypes resistant to the most influential health-affecting factors. These research directions hold the potential to offer significant insights and practical solutions in addressing complex health issues, drawing upon the contributions of *Drosophila melanogaster* in the experiments.

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