Impact of Lead Exposure on Wing Condition in *Drosophila melanogaster*

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Abstract. Lead is a heavy metal that often contaminates the environment and human food. The purpose of this study was to analyze the effect of lead exposure on changes in the morphology of living things. *Drosophila melanogaster* was selected as a model organism while wing length and amount of wing damage were selected as objects of observation. By applying the pretest-posttest control group design, the flies were divided into four groups with different exposure conditions: 0, 100, 150 and 200 ppm. Lead exposure was given for two generations on fly culture medium. The analysis of covariance test results show that lead exposure has a significant effect on wing length. Furthermore, flies that were not exposed to lead were not damaged at all, while in the group exposed to lead there were always flies whose wings were not perfect. The Kruskal-Wallis H test showed that lead exposure had significant effect on the amount of wing imperfection. Based on the findings of this study, lead contamination for more than one generation needs to be considered more seriously because it has the potential to have a negative impact on the morphology of living things.

1 Introduction

Environmental pollution caused by various pollutants has become a serious issue in environmental health [1–2] and sustainability [3]. Various pollutants such as heavy metals have the potential to damage ecosystems and affect the living creatures that live there [4–5]. One heavy metal of concern is lead (Pb), which is known to have toxic effects on living organisms, including animals and humans [6–7]. Lead exposure comes from various sources, such as industrial activities [8], motor vehicle traffic [9], and domestic waste [10]. The adverse effects of lead exposure on organisms can include growth disruption, damage to the nervous [11] and reproductive system [12–13], and impacts on ecosystem balance [14–15]. This exposure will have an impact on the biological conditions of living creatures, such as developmental disorders and damage to organ systems [16–17]. Therefore, ongoing studies

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regarding the impact of lead on organisms still need to be explored to explore knowledge in assessing risks and taking protective measures for the environment and human health.

Several previous studies also reported that lead can affect body morphology [18–19]. These changes can take many forms, such as changes in body size, deformation of body structure, or even changes in certain anatomical shapes. This impact on body morphology can disrupt the function of the body structure, affect reproduction, and can even threaten the survival of populations. These studies have often involved lead exposure in certain periods of the lives of the research subjects involved.

In nature, lead exposure often exposes organisms continuously and across generations [20]. Exposure over several generations can have a cumulative effect on organisms [21–22]. This condition will have a deeper effect on biological systems and produce more profound impacts on the development and growth of those organisms [21–22]. Correspondingly, the possibility of more pronounced or significant morphology changes in subsequent generations is a key consideration in relation to cross-generational lead impacts. To study this condition, research that examines the long-term impact of lead exposure on morphological changes needs to be carried out.

In efforts to understand the cross-generational effects of lead exposure on body morphology, the existence of model organisms that can be utilized efficiently for research is needed. Long life cycles, high costs, and a number of ethical factors pose obstacles in studying the cross-generational impacts of environmental exposure on mammalian organisms [23–24]. Therefore, studies using model organisms with short life cycles become a rational solution. *Drosophila melanogaster* is one such model organism that excels in this regard. This organism is proven to be a wise choice for this research [25].

D. melanogaster has short life cycle allowing for the observation of cross-generational impacts in a relatively short time [26]. In addition, its ease of maintenance and reproduction, as well as its ability to produce many offspring in a short time, make it highly suitable for cross-generational studies [27]. Specifically, in body morphology studies, Drosophila wings offer several advantages [19]. Drosophila wings are easily measured with high accuracy, and morphological changes in the wings can clearly indicate environmental impacts [19–28]. The potential for rapid adaptation and change in Drosophila wings in response to environmental exposure provides a great opportunity to understand the effects of environmental changes on body morphology, including cross-generational lead exposure impacts.

Given the high levels of heavy metal contamination, research on the effects of heavy metals on morphology or other variables has received attention in the scientific literature. Several previous studies have revealed a relationship between heavy metal exposure and changes in body morphology in some organisms, such as rice [29], rapeseed [30], and crustacea [31]. However, despite initial understanding of the impacts of heavy metals on morphology, there is still room for further research, particularly in the context of cross-generational impacts and a focus on organisms with short life cycles such as *D. melanogaster*. In addition, previous research has tended to focus more on the acute impacts of heavy metal exposure, while cross-generational research in this context is still limited. Thus, this study seeks to fill this gap and provide new insights into how morphological changes can develop from one generation to the next due to heavy metal exposure.

In this study, methodological aspects have been developed to ensure valid and credible results. The adopted pretest-posttest group design approach provides flexibility in observing morphological changes before and after exposure, while the use of lead concentration variations provides in-depth insight into the potential dose-effect on body morphology.

2 Method

2.1 Model organism and experimental design

The model organism used in this study is the wildtype strain of *D. melanogaster*. This organism was chosen because it has a short life cycle, allowing for the observation of morphological changes in a relatively short time frame. The selection of the wildtype strain from the Genetics Laboratory of Malang State University was based on availability and genetic diversity relevant to this study.

This study adopts a pretest-posttest group design research design. Each treatment group was given lead exposure at different concentrations, namely 0 ppm, 100 ppm, 150 ppm, and 200 ppm. Each treatment group had six replicates, resulting in a total sample size of twenty-four replicates for this study. Lead exposure was carried out for two generations, with data collection taking place before treatment (generation 0) and after treatment (generation 2). Morphological data on Drosophila wings, including wing perfection and wing size, will be measured, and analyzed to identify the impact of lead exposure on this organism.

2.2 Wing imperfection observation

Each observation involves ten flies, with five male and female individuals. These flies are placed in a bottle and then put in a freezer to facilitate more accurate observation of wing morphology. Next, the wings of individual *D. melanogaster* are carefully examined to identify signs of damage. Damaged wings tend to have a non-oval shape, may have tears on one or both wings, and appear to not develop normally. Meanwhile, normal *D. melanogaster* wings have recognizable characteristics, such as having an oval shape with a length exceeding the fly's abdomen, non-serrated wing edges, and being free from tears. This observation of wing imperfection allows for the identification of changes in wing shape and condition because of exposure to various lead concentrations in this organism.

2.3 Wing length measurement

The procedure for measuring the morphology of Drosophila wings is carried out with careful steps. In each data collection, ten flies, consisting of five males and five females, are placed in a bottle. The bottle containing the flies is then placed in a freezer to facilitate more accurate observation of wing morphology. In the next step, a millimeter block paper with a 1 mm grid is placed on the preparation table. Using a digital microscope, the flies are placed on the millimeter block paper for observation. During observation, the number of grid lines printed on the Drosophila wings is carefully counted to determine their length. The data obtained during this measurement is then carefully documented. It is important to note that the normal wing length of *D. melanogaster* ranges from 2.6 to 3 mm. This measurement procedure ensures accuracy in identifying changes in wing size because of various lead concentrations given to this organism.

2.4 Data analysis techniques

The analysis performed on the wing perfection data consists of two stages. The first stage is a descriptive analysis, where the data is described in the form of descriptive statistics. After that, further analysis is carried out using a non-parametric statistical test, namely Kruskal-Wallis H test. The post hoc test used in this analysis is the Games-Howell Test, which allows for the identification of significant differences between treatment groups. Meanwhile, for wing length data, analysis is carried out using a one-way analysis of covariance (ANCOVA) approach. Previously, a Kolmogorov-Smirnov test was performed to test the normality of data distribution and a Levene test to ensure homogeneity of variance between groups. The results of these normality and homogeneity tests form a strong basis for continuing the analysis with ANCOVA. The post hoc test used in this analysis is the Dunn test.

3 Results and Discussion

After the data was collected, several data analyses were performed. The wing condition observed in the group that was not given lead exposure showed that all Drosophila wings had a perfect shape, with characteristics including an oval shape, length, straightness, and being free from tears or abnormalities. However, in groups exposed to various lead concentrations, there were changes indicating that not all wings maintained their perfect state.

Further analysis also included counting and evaluating the number of wings that experienced imperfections in various groups. The results of the Kruskal-Wallis statistical test, presented in Table 1, revealed that lead exposure had a significant impact on the number of wings experiencing imperfections ($H = 17.943$, $p < 0.001$). This finding illustrates that the higher the concentration of lead given, the greater the number of wings experiencing damage or abnormalities. The implication is that there is a significant negative impact of lead exposure on the morphological integrity of Drosophila wings. These results provide further understanding of organism vulnerability to heavy metal impacts in the environment and encourage the urgency of this research in examining the effects of environmental exposure on organism morphological characteristics.

Table 1. Summary of the Kruskal-Wallis H Test analysis of wing imperfection data.

Factor	Statistic	df	p
Treatment	17.943	3	< .001

Furthermore, the post hoc test results using the Games-Howell test presented in Table 2 shows that the group without lead exposure has a significantly lower mean than the other three groups exposed to lead. Furthermore, the average data in the 100-ppm treatment group was not significantly different from 150 ppm but significantly lower than the 200-ppm group.

Table 2. Summary of Games-Howell Post Hoc Comparisons for wing imperfection data.

Comparison	Mean Difference	SE	t	df	p_{tukey}
0 ppm - 100 ppm	-15	9.574	-1.567	5	0.47
0 ppm - 150 ppm	-50	6.831	-7.319	5	0.003
0 ppm - 200 ppm	-66.667	4.216	-15.811	5	< .001
100 ppm - 150 ppm	-35	11.762	-2.976	9.043	0.062
100 ppm - 200 ppm	-51.667	10.462	-4.939	6.869	0.007
150 ppm - 200 ppm	-16.667	8.028	-2.076	8.327	0.236

Based on the results of the ANCOVA test for wing length data (Table 3), it was found that lead had a significant effect on the wing length of *D. melanogaster* ($F = 7.344$, $p = 0.002$). This result indicates that lead exposure has the potential to affect wing length size in this organism. Further, post hoc analysis using the Dunn test was performed to identify differences between treatment groups.

The post hoc analysis results in Table 4 present interesting findings regarding wing length in different treatment groups. The condition without lead exposure had a significantly longer wing length compared to flies exposed to lead at concentrations of 150 and 200 ppm. This indicates that lead, at those exposure levels, can contribute to a reduction in Drosophila wing length. In addition, the finding that the shortest wing length occurred in flies exposed to 200 ppm lead and this size was not significantly different from flies exposed to 150 ppm, indicates a dose-response in the effect of lead on wing length.

Table 3. One-Way ANOVA test results for wing length data.

Cases	Sum of Squares	df	Mean Square	F	p
Treatment	1.039	3	0.346	7.344	0.002
G0 Wing Length	0.176	1	0.176	3.73	0.069
Residuals	0.896	19	0.047		

Table 4. Dunn's Post Hoc Comparisons for wing length data.

Comparison	z	W_i	W_j	p
0 ppm - 100 ppm	0.287	18.417	17.25	0.774
0 ppm - 150 ppm	2.847	18.417	6.833	0.004
0 ppm - 200 ppm	2.683	18.417	7.5	0.007
100 ppm - 150 ppm	2.56	17.25	6.833	0.01
100 ppm - 200 ppm	2.396	17.25	7.5	0.017
150 ppm - 200 ppm	-0.164	6.833	7.5	0.87

The findings of this study are in line with other studies that report another parameter. A study inform that lead accumulation in fruit flies can reduce their lifespan [32]. Other studies also highlight the effects of lead exposure on fly larvae, especially in their neuromuscular junction [33]. Articles from other researchers also reveal that lead exposure can block Ca^{2+} channels [34]. In fact, chronic exposure to heavy lead can also reduce Drosophila's cognitive skills and affect its sensory function.

A previous study have linked heavy metal exposure to developmental and body morphology disorders in other organism [35]. Lead is also known to damage the internal organs of organisms [6]. In the context of Drosophila, lead exposure can interfere with several developmental processes, including the formation of morphological structures such as wings. One mechanism that may be involved is its impact on cell and tissue growth. Lead toxicity may disrupt mitosis and cell proliferation processes, which in turn can result in disruption in tissue and organ formation. In the case of Drosophila wings, lead exposure may affect the formation and growth of cells that contribute to wing structure and shape, causing morphological changes that appear as imperfections.

Lead can affect biological signaling pathways [36] and physiological processes in an organism's body [15–37]. In addition, the endocrine-disrupting effects of lead may also play a role in body morphology changes. Lead may disrupt hormonal function through various mechanisms, including inhibition of hormone synthesis and interaction with hormone receptors. In some cases, hormonal changes can affect organism growth and development, including body morphology formation such as wings. Disruption of this pathway can inhibit timely exoskeleton shedding, resulting in abnormal wing development. In addition, lead can inhibit cell growth and differentiation processes, which are necessary for forming proper morphological structures, including wing length.

In addition to biological mechanisms, chemical interactions between lead and cellular components may also play a role in its influence on wing length. This toxin can disrupt mitochondrial function [38–39], the organelle that produces energy within cells. Disruption of mitochondria affecting energy availability for growth and development [40–41]. At the cellular level, this lead can trigger oxidative stress [42–43] that damages cell structures and disrupts normal processes [44–45], including wing growth.

In this sequence, lead's potential to affect Drosophila wing length illustrates the serious impact of heavy metal exposure on organisms. These findings also reflect the complexity of interactions between the environment and organism development. Given the importance of Drosophila as a model for understanding environmental effects on morphology and development, this study provides insight into the potential toxic hazards of lead to other organisms and reinforces the urgency of protecting the environment and human health from lead contamination.

4 Conclusion

In conclusion, this study provides a deeper understanding of the impact of lead exposure on the wing morphology of *D. melanogaster*. The analysis results reveal that lead exposure contributes significantly to wing imperfections, with exposed groups showing a higher number of imperfect wings compared to the unexposed group. In addition, lead exposure also affects wing length, with flies exposed to lead having shorter wing lengths compared to the unexposed group, and this difference was found to be significant. These findings imply the potential toxic effects of lead on the growth and development of Drosophila wings.

The interpretation of these findings reinforces our understanding of the impact of heavy metals on organisms, with a focus on observed morphological changes. The underlying mechanisms for these changes involve the complexity of biological and biochemical interactions affected by lead exposure. Although the influence of lead on wing morphology appears significant, this study also provides insight that the impact may be more significant if lead exposure occurs over a longer period or involves subsequent generations. This underscores the importance of understanding the long-term effects of heavy metal exposure on organisms and encourages protection of the environment and human health from the risk of lead contamination. In a broader context, this study highlights the role of Drosophila as a powerful model organism for studying environmental impacts on morphology and development and reinforces the need for monitoring and prevention measures against heavy metal contamination in our environment.

References

1. I. Manisalidis, E. Stavropoulou, A. Stavropoulos, & E. Bezirtzoglou, Environmental and health impacts of air pollution: A review. *Frontiers in Public Health*, **8** (2020). https://doi.org/10.3389/fpubh.2020.00014.
2. D. A. Kusumastuty, N. Mahmudati, E. Purwanti, I. Hindun, & A. Fauzi, The quality of air at petroleum refining area in Bojonegoro, Indonesia: Morphological condition and chlorophyll level changes of Muntingia calabura L. *IOP Conference Series: Earth and Environmental Science* (2019), p. 012029. https://doi.org/10.1088/1755-1315/276/1/012029.
3. B. Elleuch, F. Bouhamed, M. Elloussaief, & M. Jaghbir, Environmental sustainability and pollution prevention. *Environmental Science and Pollution Research*, **25** (2018) 18223–18225. https://doi.org/10.1007/s11356-017-0619-5.

4. A. F. Tumanyan, A. P. Seliverstova, & N. A. Zaitseva, Effect of heavy metals on ecosystems. *Chemistry and Technology of Fuels and Oils*, **56** (2020) 390–394. https://doi.org/10.1007/s10553-020-01149-z.
5. H. Ali, E. Khan, & I. Ilahi, Environmental chemistry and ecotoxicology of eazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, **2019** (2019) 1–14. https://doi.org/10.1155/2019/6730305.
6. A. Kumar, A. Kumar, C.-P. M.M.S., A. K. Chaturvedi, A. A. Shabnam, G. Subrahmanyam, R. Mondal, D. K. Gupta, S. K. Malyan, S. S. Kumar, S. A. Khan, & K. K. Yadav, Lead toxicity: Health hazards, Influence on food chain, and sustainable remediation approaches. *International Journal of Environmental Research and Public Health*, **17** (2020) 2179. https://doi.org/10.3390/ijerph17072179.
7. M. A. Assi, M. N. M. Hezmee, A. W. Haron, M. Y. M. Sabri, & M. A. Rajion, The detrimental effects of lead on human and animal health. *Veterinary world*, **9** (2016) 660–71. https://doi.org/10.14202/vetworld.2016.660-671.
8. D. J. Roberts, S. M. Bradberry, F. Butcher, & A. Busby, Lead exposure in children. *BMJ*, (2022) e063950. https://doi.org/10.1136/bmj-2020-063950.
9. M. Megasari, P. Wahyono, R. Latifa, L. Waluyo, A. Fauzi, & D. Setyawan, Lead (Pb) level of fresh and smoked mackerel tuna (Euthynnus affinis) in Tuban, Indonesia. *IOP Conference Series: Earth and Environmental Science* (2019), p. 012032. https://doi.org/10.1088/1755-1315/276/1/012032.
10. N. Vongdala, H.-D. Tran, T. Xuan, R. Teschke, & T. Khanh, Heavy metal accumulation in water, soil, and plants of municipal solid waste landfill in Vientiane, Laos. *International Journal of Environmental Research and Public Health*, **16** (2018) 22. https://doi.org/10.3390/ijerph16010022.
11. L. H. Mason, J. P. Harp, & D. Y. Han, Pb neurotoxicity: Neuropsychological effects of lead toxicity. *BioMed Research International*, (2014) 1–8. https://doi.org/10.1155/2014/840547.
12. O. L. L. León & J. M. S. Pacheco, Effects of lead on reproductive health. In P. Chooto,ed., *Lead Chemistry* (IntechOpen, 2020). https://doi.org/10.5772/intechopen.91992.
13. P. Massányi, M. Massányi, R. Madeddu, R. Stawarz, & N. Lukáč, Effects of cadmium, lead, and mercury on the structure and function of reproductive organs. *Toxics*, **8** (2020) 94. https://doi.org/10.3390/toxics8040094.
14. S. Kumar, R. Islam, P. B. Akash, M. H. R. Khan, R. Proshad, J. Karmoker, & G. R. MacFarlane, Lead (Pb) contamination in agricultural products and human health risk assessment in Bangladesh. *Water, Air, & Soil Pollution*, **233** (2022) 257. https://doi.org/10.1007/s11270-022-05711-9.
15. M. Balali-Mood, K. Naseri, Z. Tahergorabi, M. R. Khazdair, & M. Sadeghi, Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology*, **12** (2021). https://doi.org/10.3389/fphar.2021.643972.
16. S. Dai, Z. Yin, G. Yuan, H. Lu, R. Jia, J. Xu, X. Song, L. Li, Y. Shu, X. Liang, C. He, C. Lv, & W. Zhang, Quantification of metallothionein on the liver and kidney of rats by subchronic lead and cadmium in combination. *Environmental Toxicology and Pharmacology*, **36** (2013) 1207–1216. https://doi.org/10.1016/j.etap.2013.10.003.
17. J. Vizuete, M. Pérez-López, M. P. Míguez-Santiyán, & D. Hernández-Moreno, Mercury (Hg), lead (Pb), cadmium (Cd), selenium (Se), and arsenic (As) in liver, kidney, and feathers of gulls: A review. In P. de Voogt,ed., *Reviews of Environmental*

Contamination and Toxicology (Cham: Springer International Publishing, 2019), pp. 85–146. https://doi.org/10.1007/398_2018_16.

18. M. Aslam, A. Aslam, M. Sheraz, B. Ali, Z. Ulhassan, U. Najeeb, W. Zhou, & R. A. Gill, Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action, and management. *Frontiers in Plant Science*, **11** (2021). https://doi.org/10.3389/fpls.2020.587785.
19. D. Fatmawati, D. Khoiroh, S. Zubaidah, H. Susanto, M. Agustin, & A. Fauzi, Wing morphological changes of Drosophila melanogaster exposed with Lead in nine generations. *AIP Conference Proceedings* (AIP Publishing, 2022).
20. Hokkaido University, Cross-generational consequences of lead poisoning. *ScienceDaily*, (2021).
21. D. Spurgeon, E. Lahive, A. Robinson, S. Short, & P. Kille, Species sensitivity to toxic substances: Evolution, ecology and applications. *Frontiers in Environmental Science*, **8** (2020). https://doi.org/10.3389/fenvs.2020.588380.
22. University of Rochester Medical Center, Environmental toxins impair immune system over multiple generations. *ScienceDaily*, (2019).
23. R. A. Powell & G. Proulx, Trapping and marking terrestrial mammals for research: Integrating ethics, performance criteria, techniques, and common sense. *ILAR Journal*, **44** (2003) 259–276. https://doi.org/10.1093/ilar.44.4.259.
24. O. Van Cauwenbergh, A. Di Serafino, J. Tytgat, & A. Soubry, Transgenerational epigenetic effects from male exposure to endocrine-disrupting compounds: a systematic review on research in mammals. *Clinical Epigenetics*, **12** (2020) 65. https://doi.org/10.1186/s13148-020-00845-1.
25. F. P. Fischer, R. A. Karge, Y. G. Weber, H. Koch, S. Wolking, & A. Voigt, Drosophila melanogaster as a versatile model organism to study genetic epilepsies: An overview. *Frontiers in Molecular Neuroscience*, **16** (2023). https://doi.org/10.3389/fnmol.2023.1116000.
26. D. P. Snustad & M. J. Simmons, *Principles of genetics*, 7th ed (Wiley, 2015).
27. M. Yamaguchi & H. Yoshida, Drosophila as a model organism. *Drosophila Models for Human Diseases, Advances in Experimental Medicine and Biology* (Singapore: Springer Nature Singapore Pte Ltd, 2018), pp. 1–10. https://doi.org/10.1007/978-981-13-0529-0_1.
28. D. Houle, J. Mezey, P. Galpern, & A. Carter, Automated measurement of Drosophila wings. *BMC Evolutionary Biology*, **3** (2003) 25. https://doi.org/10.1186/1471-2148-3-25.
29. M. Khan, T. N. I. A. L. Azzawi, M. Imran, A. Hussain, B. G. Mun, A. Pande, & B. W. Yun, Effects of lead (Pb)-induced oxidative stress on morphological and physio-biochemical properties of rice. *Biocell*, **45** (2021) 1413–1423. https://doi.org/10.32604/BIOCELL.2021.015954.
30. M. Bilal Shakoor, S. Ali, A. Hameed, M. Farid, S. Hussain, T. Yasmeen, U. Najeeb, S. Aslam Bharwana, & G. Hasan Abbasi, Citric acid improves lead (pb) phytoextraction in brassica napus L. by mitigating pb-induced morphological and biochemical damages. *Ecotoxicology and Environmental Safety*, **109** (2014) 38–47. https://doi.org/10.1016/j.ecoenv.2014.07.033.
31. V. Mazzei, G. Longo, M. V. Brundo, F. Sinatra, C. Copat, G. Oliveri Conti, & M. Ferrante, Bioaccumulation of cadmium and lead and its effects on hepatopancreas morphology in three terrestrial isopod crustacean species. *Ecotoxicology and*

Environmental Safety, **110** (2014) 269–279. https://doi.org/10.1016/j.ecoenv.2014.09.015.

32. H. R. Massie, V. R. Aiello, & S. J. P. Whitney, Lead accumulation during aging of Drosophila and effect of dietary lead on life span. *AGE*, **15** (1992) 47–49. https://doi.org/10.1007/BF02435023.
33. E. J. Morley, H. V. B. Hirsch, K. Hollocher, & G. A. Lnenicka, Effects of chronic leadexposure on the neuromuscular junction in Drosophila Larvae. *NeuroToxicology*, **24** (2003) 35–41. https://doi.org/10.1016/S0161-813X(02)00095-5.
34. H. V. B. Hirsch, G. Lnenicka, D. Possidente, B. Possidente, M. D. Garfinkel, L. Wang, X. Lu, & D. M. Ruden, Drosophila melanogaster as a model for lead neurotoxicology and toxicogenomics research. *Frontiers in genetics*, **3** (2012) 68. https://doi.org/10.3389/fgene.2012.00068.
35. D. G. Sfakianakis, E. Renieri, M. Kentouri, & A. M. Tsatsakis, Effect of heavy metals on fish larvae deformities: A review. *Environmental Research*, **137** (2015) 246–255. https://doi.org/10.1016/j.envres.2014.12.014.
36. J. G. Paithankar, S. Saini, S. Dwivedi, A. Sharma, & D. K. Chowdhuri, Heavy metal associated health hazards: An interplay of oxidative stress and signal transduction. *Chemosphere*, **262** (2021) 128350. https://doi.org/10.1016/j.chemosphere.2020.128350.
37. Z. Chen, X. Huo, G. Chen, X. Luo, & X. Xu, Lead (Pb) exposure and heart failure risk. *Environmental Science and Pollution Research*, **28** (2021) 28833–28847. https://doi.org/10.1007/s11356-021-13725-9.
38. L. J. M. Bandaru, N. Ayyalasomayajula, L. Murumulla, & S. Challa, Mechanisms associated with the dysregulation of mitochondrial function due to lead exposure and possible implications on the development of Alzheimer's disease. *BioMetals*, **35** (2022) 1–25. https://doi.org/10.1007/s10534-021-00360-7.
39. L. J. M. Bandaru, L. Murumulla, B. L. C., K. P. D., & S. Challa, Exposure of combination of environmental pollutant, lead (Pb) and β-amyloid peptides causes mitochondrial dysfunction and oxidative stress in human neuronal cells. *Journal of Bioenergetics and Biomembranes*, **55** (2023) 79–89. https://doi.org/10.1007/s10863-023-09956-9.
40. Y. Qin, X. Jiang, Q. Yang, J. Zhao, Q. Zhou, & Y. Zhou, The functions, methods, and mobility of mitochondrial transfer between cells. *Frontiers in Oncology*, **11** (2021). https://doi.org/10.3389/fonc.2021.672781.
41. S. Madan, B. Uttekar, S. Chowdhary, & R. Rikhy, Mitochondria lead the way: Mitochondrial dynamics and function in cellular movements in development and disease. *Frontiers in Cell and Developmental Biology*, **9** (2022). https://doi.org/10.3389/fcell.2021.781933.
42. G. Flora, D. Gupta, & A. Tiwari, Toxicity of lead: A review with recent updates. *Interdisciplinary toxicology*, **5** (2012) 47–58. https://doi.org/10.2478/v10102-012-0009-2.
43. J.-W. Lee, H. Choi, U.-K. Hwang, J.-C. Kang, Y. J. Kang, K. Il Kim, & J.-H. Kim, Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental Toxicology and Pharmacology*, **68** (2019) 101–108. https://doi.org/10.1016/j.etap.2019.03.010.
44. O. Sytar, A. Kumar, D. Latowski, P. Kuczynska, K. Strzałka, & M. N. V. Prasad, Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. *Acta Physiologiae Plantarum*, **35** (2013) 985–999. https://doi.org/10.1007/s11738-012-1169-6.

45. U. Ashraf, S. Hussain, S. A. Anjum, F. Abbas, M. Tanveer, M. A. Noor, & X. Tang, Alterations in growth, oxidative damage, and metal uptake of five aromatic rice cultivars under lead toxicity. *Plant Physiology and Biochemistry*, **115** (2017) 461–471. https://doi.org/10.1016/j.plaphy.2017.04.019.