sustainability

MDPI

Article

Dietary Lead Exposure in Mealworm Larvae (*Tenebrio molitor*) and Its Implications for the Safety of Edible Insects

Konrad Matyja [1], Justyna Rybak [2,*], Beata Hanus-Lorenz [2], Wojciech Dobicki [3] and Przemysław Pokorny [3]

[1] Department of Micro, Nano and Bioprocess Engineering, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland; konrad.matyja@pwr.edu.pl
[2] Faculty of Environmental Engineering, Wroclaw University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland; beata.hanus-lorenz@pwr.edu.pl
[3] Department of Limnology and Fishery, Institute of Animal Breeding, Wrocław University of Environmental and Life Sciences, Chelmonskiego 38C, 51-630 Wrocław, Poland
* Correspondence: justyna.rybak@pwr.edu.pl

Abstract

This study examined the toxicological, and bioaccumulative effects of dietary lead (Pb) on *Tenebrio molitor* larvae, increasingly considered for human and animal consumption within sustainable food and feed production systems. Larvae were exposed for 21 days to an oat-based diet contaminated with lead salts (125–2000 mg Pb/kg). Body mass, and Pb accumulation in the intestine, internal tissues, and cuticle were analyzed. Pb concentrations in larval compartments increased with increasing nominal Pb levels in feed, with concentrations in internal tissues and cuticle reaching 5–6 times higher than in the control. Estimated bioaccumulation factors (BAF) were below 1 in all cases, indicating no biomagnification. Despite high exposure levels, no significant effects on larval survival or biomass gain were observed. These findings indicate that growth-related parameters are not sensitive indicators of Pb exposure. At the same time, substantial Pb accumulation occurred, particularly in the gut and cuticle, highlighting a risk of "hidden" contamination in insect-based production systems. The results emphasize the need for substrate monitoring and the inclusion of sensitive chemical indicators in food safety risk assessment.

Keywords: accumulation; heavy metals; toxicity; mealworm; growth inhibition

1. Introduction

The growing global population increases the demand for food, placing pressure on agricultural systems and available resources [1]. This challenge has intensified the search for sustainable and resource-efficient food production systems. Trace elements cannot be metabolized [2], and when they enter the food chain, their presence in the environment poses a serious threat to food safety. In many regions of the world, the extensive use of metals, including lead (Pb), a highly hazardous metal, has resulted in environmental degradation and health issues. Pb was previously found in petrol, household paint, water supply pipes, batteries, children's toys, and metal fixtures such as faucets and valves, and it is still present in various industrial and consumer products [3]. Although the general population's exposure to Pb is primarily caused by food and drinking water, Pb contamination originates from multiple pathways. Pb enters the environment through industrial emissions, vehicular exhaust, and waste disposal, eventually accumulating in air, soil, and water. Plants absorb Pb from contaminated soil, introducing it into the food web through herbivorous organisms [2–4].

Academic Editors: Dragan R. Milicevic, Nataša Kilibarda and Zeki Bayramoğlu

Received: 17 March 2026
Revised: 10 April 2026
Accepted: 13 April 2026
Published: 15 April 2026

Unlike essential metals such as Zn, Cu, and Mn, Pb has no biological function. Elements such as Pb, As, and Cd are among the most toxic metals affecting animal and human health. Pb is harmful even at extremely low concentrations [2], and exposure to sub-lethal levels adversely affects multiple physiological and biochemical systems [2]. It can cause disorders of the cardiovascular, neurological, and reproductive systems, as well as damage to the liver, gastrointestinal tract, respiratory system, and endocrine system [5]. Poisoning via food remains a concern, especially for the developing central nervous system of children. Since no safe exposure threshold has been established, there is no recommended acceptable intake level.

Legislative actions have progressively reduced Pb exposure by eliminating it from petrol, paint, food containers, and water pipes. According to the European Food Safety Authority (EFSA), dietary Pb exposure in Europe ranges widely depending on food category, with a median of 21.4 µg/kg and a mean lifetime exposure of 0.68 µg/kg body weight per day [6]. The European Union sets a maximum limit of 0.10 mg Pb per kilogram of fresh meat intended for human consumption.

In recent years, interest in edible insects as a food source has grown [7]. Compared to traditional livestock, insects may provide a reliable source of protein and micronutrients. They are increasingly recognized as a promising component of sustainable food systems due to their low environmental footprint, high feed conversion efficiency, and potential integration into circular bioeconomy models. Larvae of *Tenebrio molitor* are among the most commonly consumed species and have nutritional values comparable to conventional meat [8,9]. The growing interest in mealworms as a sustainable food source has led to their regulatory approval in the European Union. In 2021, the European Commission authorized dried *T. molitor* larvae as a novel food under Commission Implementing Regulation (EU) 2021/882, in accordance with Regulation (EU) 2015/2283 [10]. Additionally, *T. molitor* larvae are a valuable source of nutrients such as Zn, P, and Mg and have relatively low salt content, which is beneficial for human health. They are also widely used as a protein source in animal feed, where their inclusion has been shown to improve growth performance in poultry and pigs [8,11]. However, eating insects carries various concerns, including those related to toxic chemicals, which are mostly related to the polluted environment in which they are raised, or the formation of toxins at a particular stage of development. Heavy metals have been detected in various edible insect species, including *T. molitor*.

Several studies have addressed the bioaccumulation and potential toxicity of these elements, raising concerns about food safety. Previous works reported variable bioaccumulation factors (BAF) for Pb in *T. molitor*, typically below 1, but used much lower Pb concentrations (5–100 mg/kg) and lacked regression analyses of dose-dependent accumulation [12,13]. There is increasing evidence in the literature that edible insects can accumulate heavy metals, which poses a significant problem for food safety. A review by Schrögel and Wätjen [14] indicates that the heavy metal content in edible insects—such as mealworms and the fruit fly *Hermetia illucens*—depends on the species, developmental stage, and substrate type. On the other hand, Lindqvist and Block [15] showed that *T. molitor* larvae can excrete cadmium during molting and metamorphosis, suggesting a natural detoxification mechanism, although not entirely effective. However, the latest research by Gori et al. [16], analyzing e-commerce insect products available on the EU market, found the presence of Pb, Cd, and Ni in some samples at levels that are potentially hazardous for typical consumption. In this context, the level of harmful elements should be monitored as a standard. Unfortunately, data on this issue are available only from a few scientific articles [8,11–13]. There is no broader analysis of how toxic metals affect the metabolism and development of *T. molitor* either. Moreover, the influence of metals on the growth-related parameters of the larvae of this species has not been studied so far at extremely high Pb concentrations up to

https://doi.org/10.3390/su18083931

2000 mg/kg. To our knowledge, the previous studies have focused on the bioaccumulation of metals and the risk of food contamination only [13]. While the Pb concentrations applied in this study exceed levels typically encountered in commercial insect production systems, they were intentionally selected to represent a worst-case exposure scenario. This approach allows for the identification of physiological limits of Pb accumulation and provides a safety margin relevant for risk assessment in circular production systems, where substrate contamination may occur unpredictably.

This issue is particularly relevant in sustainable insect farming systems, where organic side-streams and waste-derived substrates are increasingly used as feed, potentially introducing contaminants into food and feed chains.

Therefore, the objective of this study was to determine how different concentrations and chemical forms of Pb salts in the diet affect Pb accumulation, growth of *T. molitor* larvae, with a focus on exceptional tolerance to extreme Pb exposure (up to 2000 mg/kg), to better assess the toxicological risk associated with consuming contaminated insects.

2. Materials and Methods

2.1. Experimental Design

T. molitor larvae were purchased from the inbred strain "Cricket Farm" (Lublin, Poland). Larvae in the mass range of 0.03–0.04 g were selected for experiments. Sixteen samples, comprising 30 randomly chosen individuals, were placed in polypropylene boxes with smooth walls and perforated lids to ensure proper air circulation. The mealworm larvae were reared for 21 days in the incubator at a constant temperature of 25 °C and 80% humidity. Larvae were maintained under constant dark conditions (no photoperiod), which reflects standard industrial rearing practices for *T. molitor*. Raw larval body mass data are provided in Supplementary Table S2.

Larvae used in this study were reared from hatching under controlled experimental conditions, ensuring that Pb detected in their tissues originated exclusively from the experimental exposure. No fasting period was applied prior to dissection; therefore, Pb measured in the gut may partly reflect the presence of undigested feed in addition to assimilated metal. Consequently, gut Pb concentrations should be interpreted with caution, and future studies should include a defined fasting period to better distinguish between ingested and absorbed Pb fractions.

Each sample was subjected to a different treatment: fed oatmeal contaminated with three different lead salts (chloride, acetate, and nitrate) at concentrations of 125, 250, 500, 1000, and 2000 mg Pb/kg. The control sample was fed with non-contaminated oatmeal. Fresh food was supplied every 7 days. The actual Pb content in the feed used (oat flakes) after supplementation with lead salts was not analyzed; it was assumed that the Pb concentrations corresponded to the nominal values obtained during the feed preparation process.

Food preparation was conducted according to Bednarska and Świątek [11]. Oatmeals were soaked with distilled water for control and with aqueous solutions of metal salts ($PbCl_2$—POCh, Gliwice, Poland, $Pb(CH_3COO)_2 \times 3H_2O$—CHEMPUR, Piekary Śląskie, Poland, $Pb(NO_3)_2$—POCh, Gliwice, Poland), dried with hot air at 105 °C, and crushed into flour. Larvae were fed ad libitum during the whole experiment. The number of living individuals in each sample was counted, and their body mass was recorded (Radwag AS 220.3Y, Radwag, Radom, Poland) on the 1st, 7th, and 21st day of the experiment.

Three individuals from each sample were stored at −18 °C for further analyses. After being removed from the freezer, the larvae were thoroughly rinsed with a stream of distilled water using a spray bottle. Then, the larvae were dried on filter paper, and preparation began. The larvae were attached to a preparation tray and cut open to gain access to the viscera. First, the digestive system was dissected using tweezers, then the fat body and remaining tissues were scraped from the cuticle, and lastly the cuticle was cleaned of tissues. The Pb content was assessed in the larvae's gut, internal soft tissues, and cuticle. Additionally, Pb content was also assessed in the exuviae (shed cuticle) of larvae that had brief surface contact with food contaminated at 2000 mg Pb/kg, to evaluate possible metal adsorption on the outer cuticle. The collected material was dried at 100 °C until a constant weight was established. Then, the samples were weighed on an analytical balance with an accuracy of 0.0001 g and digested with nitric acid in a CEM MARS 5 microwave oven (CEM MARS 5, CEM Corporation, Matthews, NC, USA).

The amount of Pb in the larval parts was determined using flame atomic absorption spectroscopy (SpectrAA 220, Varian Inc., Mulgrave, VIC, Australia) after prior wet mineralization. The measurement process was validated using reference material DOLT-4 (Dogfish Liver Certified Reference Material for Trace Metals, National Research Council Canada). The mean recoveries in five replicates of certified material expressed the precision of the method, or the difference between the mean value obtained by analyzing a certified reference material and its certified value for Pb performed on the same sample, were within 5% of the concentrations announced for the reference material.

2.2. Statistical Analysis

To assess the impact of lead exposure on larval growth, larval mass was analyzed using a series of two-way analyses of variance (ANOVA) at a significance level of $\alpha = 0.05$. These analyses were conducted separately for three distinct time points: day 1, day 7, and day 21. For each analysis, the statistical model included two fixed factors: type of lead salt and concentration of lead salt (125, 250, 500, 1000, and 2000 mg Pb/kg).

Statistical analysis was also performed to evaluate the effect of Pb concentration on its accumulation in larval tissues (cuticle, internal tissues, and gut). Two-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test ($p < 0.05$) was applied to assess the effects of tissue type and metal dose on metal accumulation. For statistical purposes, measurements obtained for different Pb forms (acetate, nitrate, and chloride) were pooled within each concentration level.

The analysis was performed in Statistica 14.3 (TIBCO Software Inc., Palo Alto, CA, USA). Additionally, linear regression analysis was performed for each combination of tissue (gut, internal soft tissues, cuticle) and Pb salt type to evaluate dose-dependent Pb accumulation. The analysis included the determination of regression coefficients and an F-test for the overall significance of the model at a significance level of $\alpha = 0.05$. The regression model was described by the equation:

$$C_L = b_0 + C_F b_1 \tag{1}$$

where C_L is Pb content in larvae [mg Pb/kg dry mass], C_F is Pb content in food [mg Pb/kg dry mass], b_0 and b_1 are regression coefficients, and b_1 is an estimate of the bioaccumulation factor (BAF).

3. Results

3.1. Survival and Larval Growth

After 21 days of exposure, larval survival exceeded 89% across all experimental groups, including those fed the highest Pb concentration (2000 mg Pb/kg). Mortality was low and randomly distributed between control and contaminated groups, with no apparent relationship to Pb concentration. Two-way ANOVA revealed that neither Pb concentration nor lead salt type (acetate, nitrate, chloride), nor their interaction, had a statistically significant effect at the $\alpha = 0.05$ on larval body mass growth (Table S1a–c, all $p > 0.05$) indicating that growth performance was not affected by Pb exposure. Thus, despite exposure to Pb concentrations up to 2000 mg/kg, larval biomass gain remained comparable to the control group throughout the experiment.

Statistical comparison of Pb content between dietary concentrations (Table 1) revealed that significant differences were observed only between the control group and the highest Pb concentration (2000 mg Pb/kg), while no statistically significant differences were found among intermediate concentrations.

Table 1. Lead content in larval compartments after short-term and 21-day exposure. Lead (Pb) concentrations assessed in the gut, internal soft tissues, and cuticle of *T. molitor* larvae following either short-term surface contact or 21-day dietary exposure to Pb-contaminated feed. Values are expressed as mg Pb/kg dry mass. Nominal Pb concentrations in feed ranged from 125 to 2000 mg Pb/kg and were applied in the form of acetate, nitrate, or chloride salts. Control values represent larvae reared on uncontaminated feed. Different letters indicate statistically significant differences between Pb concentrations (Tukey test, $p < 0.05$). Abbreviations: Acet.—acetate; Nit.—nitrate; Chl.—chloride.

Pb Concentration in Food [mg Pb/kg]	Cuticle			Internal Tissues			Gut		
	Acet.	Nitr.	Chlor.	Acet.	Nitr.	Chlor.	Acet.	Nitr.	Chlor.
0	3.92 [a]	3.92 [a]	3.92 [a]	9.06 [a]	9.06 [a]	9.06 [a]	6.75 [a]	6.75 [a]	6.75 [a]
125	11.67 [a]	13.35 [a]	15.22 [a]	8.23 [a]	15.23 [a]	10.16 [a]	83.01 [a]	112.87 [a]	105.15 [a]
250	8.91 [a]	21.16 [a]	20.25 [a]	14.17 [a]	20.68 [a]	7.64 [a]	55.57 [a]	175.24 [a]	75.82 [a]
500	27.59 [a]	13.89 [a]	26.79 [a]	23.09 [a]	14.56 [a]	13.96 [a]	103.67 [a]	162.73 [a]	12.60 [a]
1000	20.51 [a]	24.24 [a]	16.59 [a]	76.49 [a]	25.55 [a]	15.09 [a]	502.32 [a]	333.34 [a]	119.23 [a]
2000	46.17 [b]	38.71 [b]	23.13 [b]	31.02 [b]	35.89 [b]	29.46 [b]	1095.29 [b]	483.19 [b]	1349.31 [b]

These results indicate that *T. molitor* larvae can tolerate high levels of Pb exposure without measurable effects on survival or biomass gain, even at concentrations as high as 2000 mg/kg. The absence of significant differences between treatments suggests that neither Pb concentration nor its chemical form had a detectable impact on overall growth performance under the experimental conditions.

This is particularly noteworthy given the wide range of Pb concentrations tested, which substantially exceeds levels typically reported in previous studies. The consistently high survival and stable biomass across all treatments indicate a strong physiological capacity of larvae to cope with elevated metal exposure.

At the same time, the lack of response in growth parameters, despite increasing Pb levels in the diet, suggests that biomass-based endpoints are relatively insensitive to Pb exposure in this species. This indicates that growth performance alone may not adequately reflect the presence of contaminants in insect rearing systems, even under conditions of substantial dietary exposure.

These findings highlight a clear decoupling between exposure and observable growth effects, emphasizing the need for more sensitive indicators when assessing the impact of contaminants in edible insect production.

3.2. Lead Accumulation in Larval Compartments

The content of Pb in larvae tissues and gut after 21 days of exposure to contaminated feed is presented in Figure 1 and Table 1. The general trend shows that higher Pb content in feed is associated with higher Pb accumulation in all three larval compartments (gut, internal soft tissues, and cuticle). However, statistical analysis indicated that significant differences were detectable only at the highest exposure level. The highest Pb levels were found in the gut, approaching (with only slight dilution) the nominal Pb concentrations in the feed. In internal soft tissues and cuticle, Pb concentrations were 5–6 times higher than in the corresponding control groups across most treatments (Table 1).

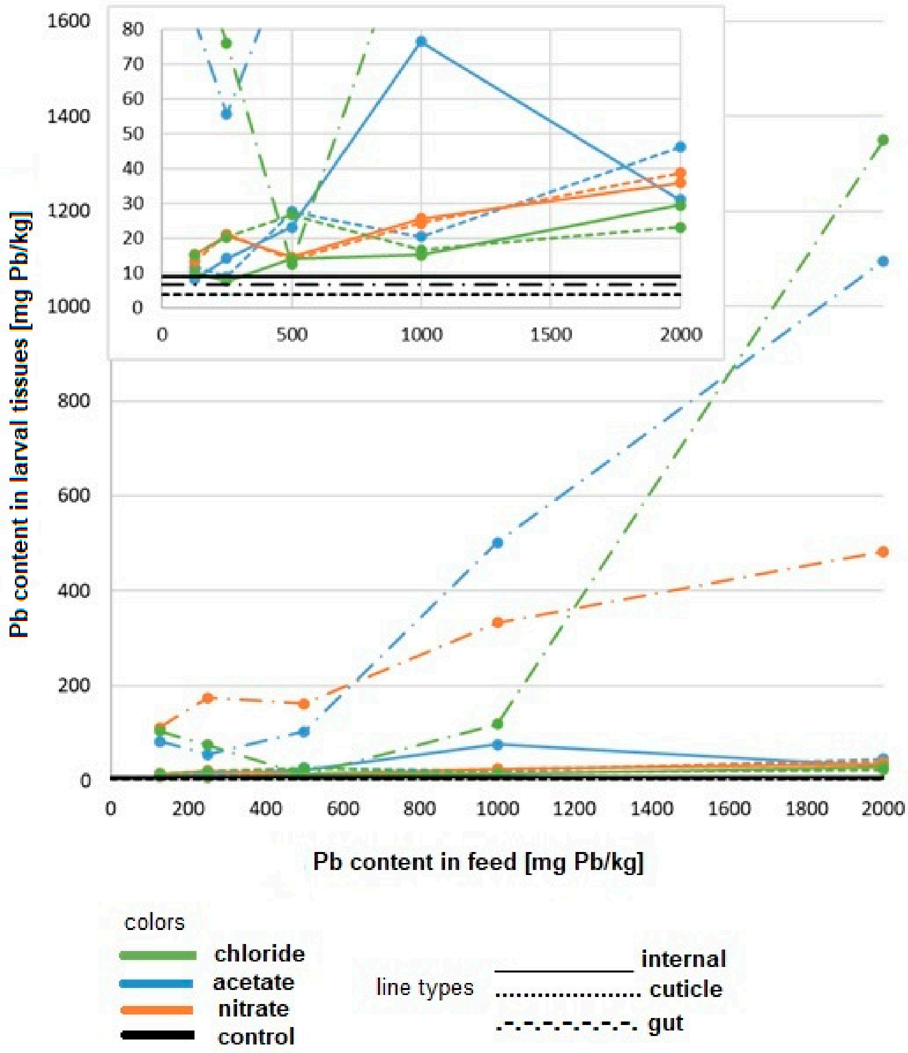

Figure 1. Pb content in larval compartments: gut (dash-dotted line), internal soft tissues (solid line), and cuticle (dotted line), plotted against nominal Pb concentrations in feed. Different colors indicate Pb salts: acetate (blue), nitrate (orange), chloride (green), and control (black). The inset (upper left) shows a magnified view of Pb concentrations in internal soft tissues and cuticle using a different y-axis scale. Each value represents a pooled sample of larvae from a given treatment group (n = 30); and therefore error bars are not shown.

Linear regression analyses were performed separately for each compartment (gut, internal soft tissues, cuticle) and lead salt type to assess the relationship between nominal Pb concentration in feed (x) and Pb concentration in larvae (y). Regression equations, coefficients, R^2 values, and significance of the models are summarized in Table 2. Most regressions were statistically significant (F-test, $p < 0.05$), indicating a general dose-related trend in Pb levels in larval tissues. However, the strength of these relationships varied, with some models showing relatively low R^2 values, indicating substantial variability in Pb accumulation, particularly at intermediate exposure levels. Two exceptions were observed: the regressions for the cuticle/chloride and internal tissues/acetate combinations were not statistically significant ($p > 0.05$). This lack of significance may reflect higher biological variability, weaker dose dependence in these specific compartment–salt combinations, or the influence of factors such as heterogeneous Pb distribution in feed and differences in metal bioavailability.

Table 2. Results of linear regression analysis describing the relationship between nominal Pb concentration in feed and Pb content in larval compartments (gut, internal soft tissues, cuticle) for each Pb salt. Reported parameters include the estimated bioaccumulation factor (BAF, regression slope), coefficient of determination (R^2), F-statistic, and corresponding *p*-value. Statistical significance was evaluated at $\alpha = 0.05$. Bold values indicate statistically significant results ($p < 0.05$).

Compartment	Salt	$b_1 = BAF$	b_0	R^2	F	p
Cuticle	acet.	0.02	7.54	0.85	23.17	**0.01**
	nitr.	0.01	9.81	0.85	22.28	**0.01**
	chlor.	0.01	14.26	0.25	1.30	0.32
Internal tissue	acet.	0.02	15.58	0.27	1.45	0.29
	nitr.	0.01	12.49	0.87	27.90	**0.01**
	chlor.	0.01	7.59	0.93	56.47	**<0.001**
Gut	acet.	0.56	−52.55	0.97	141.11	**0.0003**
	nitr.	0.22	71.53	0.93	55.72	**0.0017**
	chlor.	0.63	−128.36	0.81	16.69	**0.02**

Estimated BAF values (b) were consistently below 1 in all compartments and salt types, suggesting limited accumulation and no biomagnification of Pb in larvae. Although regression analysis revealed overall increasing trends, post hoc comparisons (Table 1) indicated that statistically significant differences between individual exposure levels were detectable only at the highest Pb concentration. This suggests that while a general dose–response pattern exists, variability within intermediate treatments limits the statistical resolution of pairwise comparisons.

4. Discussion

4.1. Influence of Pb Salt Type on Feed Contamination and Metal Bioavailability

Oatmeal used as a feed for larvae was contaminated with Pb in three different scenarios using three Pb salts: chloride, acetate, and nitrate. These salts are composed of Pb (II) cations and different anions, which results in different dissociation constants and solubilities in water. The method of feed contamination involved dissolving the salts in water and adding the resulting solution to oatmeal [11]. Oatmeal is composed of carbohydrates, fiber, proteins, fat, and other organic compounds. All of these compounds provide different chemical functional groups that may participate in Pb bounding [17]. The physical and chemical adsorption of metal ions can be highly influenced by metal speciation in the solution, which depends on the anions present [17,18]. Moreover, the process of precipitation during drying may vary in each scenario.

A methodological limitation of the present study is that the actual Pb concentrations in the prepared feed were not analytically verified and were assumed to correspond to nominal values based on the preparation protocol. Given the multi-step preparation procedure (solution application, drying at 105 °C, and grinding), it is possible that local heterogeneity in Pb distribution or changes in metal speciation occurred. Such effects could influence both the absolute exposure levels and Pb bioavailability. Therefore, the observed dose–response relationships should be interpreted with some caution, particularly in a quantitative context. However, since all treatments were prepared using the same standardized procedure, relative comparisons between experimental groups remain valid.

Therefore, the main aim of this setup was to assess how the type of Pb salts used for contamination affects metal bioavailability and toxicity. It is worth emphasizing that despite theoretical differences in bioavailability resulting from different Pb speciation, this effect did not translate into substantially different accumulation patterns or inhibition of larval growth. This indicates that in the studied range, it was not the chemical form of

https://doi.org/10.3390/su18083931

Pb, but the larvae's physiological ability to tolerate and compartmentalize the metal that determined the organism's response.

From a sustainability perspective, these findings suggest that the chemical form of contaminants present in insect rearing substrates may influence toxicity without necessarily affecting production efficiency.

4.2. Pb Accumulation Patterns and Compartment-Specific Differences

The Pb content in internal soft tissues and cuticle was similar for all used salts and nominal meta amount in feed, except for the group exposed to Pb acetate at 1000 mg Pb/kg, where accumulation was notably higher than in corresponding samples (Figure 1, Table 1). The elevated lead levels observed in the cuticle can be partially attributed to surface adsorption of metal ions from the feed, rather than internal accumulation (Table 1). However, this observation does not affect the general conclusion that Pb assimilation in internal soft tissues and cuticle is generally independent of Pb salt used for feed contamination. Moreover, the Pb concentration in the gut showed greater variability (Figure 1). This variation likely results from a combination of differences in feed intake, gut retention time, and the absence of a fasting period prior to dissection, which limits the ability to distinguish between ingested and absorbed Pb fractions. As a result, inter-individual variability in feeding behavior and gut clearance limits the ability to reliably assess the effect of different Pb salts on gut Pb concentrations. The most distinct and consistent pattern was Pb compartmentalization, with the metal predominating in the gut and cuticle, and relatively lower concentrations in internal tissues. However, this pattern should be interpreted with caution, as the experimental design did not include a fasting period prior to dissection. Consequently, Pb measured in the gut likely reflects not only assimilated metal but also the presence of undigested, Pb-contaminated feed. This may have led to an overestimation of gut-associated Pb and contributed to the observed variability. This should be considered a limitation of the present study when interpreting intestinal Pb data. Despite this limitation, the comparatively low Pb levels in internal soft tissues still support the conclusion that a substantial fraction of Pb remains externally bound or compartmentalized in non-metabolically active fractions.

This distribution indicates that a significant portion of Pb detected in larvae is not directly involved in metabolism but remains bound to the gut contents or protective structures. This mechanism likely constitutes a significant barrier limiting the toxic effects of Pb on growth processes. However, from a food safety perspective, this compartmentalization may mask contamination risk, as Pb remains present in the organism despite limited physiological impact.

An additional limitation of the present study is the lack of frass analysis, which prevents a complete assessment of Pb mass balance within the system. Without quantifying Pb excreted by larvae, it is not possible to distinguish between metal assimilation and elimination processes. Consequently, the relative contributions of retention and excretion to the observed Pb distribution among compartments remain uncertain. Future studies incorporating frass analysis would allow for a more comprehensive understanding of Pb toxicokinetics in *T. molitor*.

4.3. Comparison with Previous Studies on Pb Bioaccumulation in Mealworm Larvae

Our results align with several studies showing dose-dependent Pb accumulation in *T. molitor* but generally no biomagnification (BAF < 1; Table 2). In studies of van der Fels-Klerx et al. [12], a significant relationship between the metal concentration in the substrates and the accumulation of metals (Cd, Pb, Ni, As, and Hg) by *T. molitor* was observed. Additionally, mealworm larvae exhibited the ability to bioaccumulate As, but not Cd and Pb. Pb levels in mealworm larvae were lower than in their feed. It was also noted that Pb concentrations in residual material were lower than in the larval body, which indicates that Pb is not sequestered in the exoskeleton. However, the results of van der Fels-Klerx et al. [12] confirm our observations that with the increase in the concentration of Pb in feed, higher concentrations in the tissues of larvae were recorded. Moreover, their observation that Pb concentrations in larval exuviae were lower than in the larval body agrees with that which was observed in our study, where the highest level of Pb in the larvae' gut was recorded. In studies by Noyens et al. [19] the highest quantity of Pb was found in the fermented chicory roots. The larvae of mealworms fed fermented chicory roots had significantly higher Pb concentrations in their body. Grown on soil, chicory roots are well-known for their ability to absorb metals. The studies by Noyens et al. [19] are in contrast with our findings that Pb concentrations in *T. molitor* tissues were always lower than concentrations in feed. This discrepancy may be explained by differences in Pb bioavailability related to the composition of the exposure matrix. In the study by Noyens et al., Pb was present in plant-derived material, where metal uptake and binding may differ from that in artificially contaminated feed used in the present study. Additionally, differences in Pb chemical speciation, fermentation processes, and interactions with organic compounds may influence Pb absorption efficiency and distribution in larval tissues.

In the studies of Truzzi et al. [13], Cd, Pb, Ni, As, Se, and Hg have been assessed in feeding substrates, in *T. molitor* larvae, and their frass. The limit of chemicals in animal feed (2002/32/EC) was not exceeded by any of the metals in the substrates. Larvae had various concentrations of studied elements, but Hg only showed a statistically significant relationship between feeding substrates and larval concentration. Pb content varied significantly amongst larvae raised on various substrates, but its concentration in substrates and larvae did not show a statistically significant correlation, suggesting that the Pb content of the feeding substrate does not affect the content of the larvae. On the other hand, authors obtained high bioaccumulation factor (BAF) values, indicating this metal's bioaccumulation ability. Truzzi et al. [13] suggested that high Pb concentrations may have resulted from an extra source, like carrots offered to the insects as a water source. In the same studies, a significant correlation was observed between the Pb content of the feeding substrate and frass, suggesting that its concentration influences its content in the frass. It exhibited a two to three times higher Pb content than the feeding substrate. We acknowledge that the experimental design did not include a fasting period before dissection or analysis of frass. At the time of planning, our primary focus was on the tissue-level accumulation of Pb rather than digestive transit. However, we recognize that the absence of a defined fasting protocol likely contributed to variability in gut Pb concentrations, as undigested substrate may have influenced the results. Future studies should incorporate both fasting and frass analysis to better distinguish between ingested and absorbed metal fractions. In studies of Vijver et al. [20], *T. molitor* larvae were used to assess the effects of soil properties on Cd, Cu, Pb, and Zn intake. In Zn-spiked OECD soils, field soils, and soil-sediment combinations, the larvae' body content of Cu and Zn was relatively steady. Cd and Pb body concentrations were found to vary between field soils and soil-sediment mixes and were linked with the soil's overall metal pool [20]. Therefore, in this study authors suggested that the accumulation pattern of Cd and Pb varies depending on the type of substrate, which is in line with the conclusion of Truzzi et al. [13], although the content of studied metals was related to their level in the soil. As research shows, the results are very diverse, and various factors may have a significant impact on the bioaccumulation of Pb in the tissues of mealworm larvae. It should be emphasized that although the accumulation of Pb in *T. molitor* larvae has been previously described, previous studies included much lower Pb concentrations in feed (usually $\leq$100 mg/kg). In this context, the key novelty of this study is the demonstration that even at extremely high Pb concentrations (up to 2000 mg/kg), the larvae retain the ability to grow comparable to the control, which substantially exceed levels commonly reported in previous studies. Despite such extreme exposure, T. molitor larvae maintained normal growth and survival, indicating a high tolerance to Pb. Importantly, our results demonstrate that growth-related parameters, such as larval biomass, are not sensitive indicators of Pb exposure. In contrast, Pb concentrations in larval tissues and changes in larval morphology provide a more reliable reflection of contamination. This highlights a critical limitation of using biomass alone as a proxy for safety in insect production systems and emphasizes the need for more sensitive physiological or chemical indicators.

This variability highlights the need for standardized assessment protocols in insect-based production systems, particularly in the context of sustainable food supply chains.

From an application perspective, the ability of larvae to tolerate and accumulate Pb may be relevant for environmental remediation strategies. However, this characteristic is of limited benefit in the context of food and feed production, where insects must be reared on safe and controlled substrates. Therefore, Pb accumulation capacity should be considered primarily in relation to environmental applications rather than edible insect production.

In the context of sustainable food production, edible insects are increasingly reared on organic side-streams and waste-derived substrates [21,22]. While this approach improves resource efficiency, it may also introduce contaminants such as heavy metals into the production system. The present results indicate that high levels of Pb accumulation may occur without visible effects on larval growth, which creates a risk of "hidden contamination" in insect-based food and feed chains.

Although the exposure levels applied in this study exceed those typically encountered in practice, they provide insight into Pb accumulation under high-load conditions and support the development of conservative safety thresholds and monitoring strategies in sustainable insect production systems.

It should be emphasized that, according to current European Union legislation, edible insects and insects intended for feed production must be reared on substrates approved for farm animals and cannot be fed waste-derived materials. This is regulated under Commission Regulation (EU) 2017/893 and its subsequent amendments. Consequently, the use of contaminated substrates, including those containing elevated levels of heavy metals, is not permitted in commercial insect production systems within the EU.

4.4. Metal Accumulation in Phytophagous Insects

The analysis of the possibility of accumulation of selected metals in the tissues of different species of invertebrates confirms this. Phytophagous insects, including mealworm larvae as plant consumers and clue energy transmitters via food webs, have a crucial role in the terrestrial ecosystem [23]. As insectivorous invertebrates hunt on them, they are the most important link in the accumulation and movement of trace metals to higher trophic levels [24]. Invertebrate species differ widely in their capacity to regulate their intake of metals. Non-essential trace metals like Cd and Pb are less regulated and better biomagnified than essential metals, e.g., Cu and Zn [4]. However, scientists found differences in metal accumulation and biomagnification depending on how phytophagous invertebrates consume food. For instance, it appears that phytophagous insects that eat plants via chewing do not accumulate Pb [24], although biomagnification of the essential elements Cu and Zn was observed [25]. Referring to the insects that feed on plant juices, the picture is much more complicated, as sap may have an impact on bioaccumulation ability depending on the metal form. Metal bioavailability also depends on the solubility of sap molecules. The transfer of metals from plants to invertebrates may also depend on metal concentrations in

https://doi.org/10.3390/su18083931

sap. Therefore, the bioaccumulation ability depends on the element's biochemistry and physiology of invertebrate tissue [4].

In studies of Bednarska and Świątek [11], mealworm larvae were fed with flour contaminated with Zn or Cd to examine the internal compartmentalization of these elements. The larvae's cellular components were divided into fractions: G (metal-rich granule fraction), S2 (cellular fraction), and S1 (cytosolic fraction containing organelles and proteins). While Zn concentration in feed only affected its concentration in the S2 and G fractions, the Cd content in the flour affected its concentration in each subcellular fraction. The levels of Cd and Zn in mealworms in all three fractions did not change after exposure. The percentage of Cd in the S1 fraction was unaffected by the flour's Cd concentration, and Zn content in the flour did not affect its percentage in any of the three fractions. About 30% of Cd and Zn were sequestered by larvae in the S1 fraction [11]. The authors did not study the effect of Pb, although these studies show that every metal could bioaccumulate differently and in various cell fractions in the larvae of *T. molitor*. Compared to other phytophagous invertebrates, **T. molitor** appears to demonstrate particularly effective mechanisms for limiting internal Pb bioavailability, which may be due to adaptation to variables and potentially contaminated food substrates. This strategy is consistent with the observed metal compartmentalization and lack of biomagnification.

4.5. Toxicity and Growth Effects

The increased metal concentration in larval tissues can exhibit toxic effects. Jucker et al. [26] focused on the possibility of Cd bioaccumulation from feeding media in the Black soldier fly (*Hermetia illucens*) and mealworm larvae (*T. molitor* and *Zophobas morio*). For Black soldier fly, the concentration of Cd in larvae and pupae was high with a decline in the later phases of development. On the contrary, bioaccumulation of Cd was lower for mealworm larvae. Cd affected superworms' final larval weight, but there were no differences in the survival rates of mealworms fed on the contaminated and control diets. Authors suggested that *H. illucens* had a high capacity for Cd accumulation, whereas mealworm larvae's prolonged growth probably allowed for their removal. Therefore, there is much evidence that the amount of metal accumulated varies depending on its type, species, and developmental stage.

The concentration of metals in feed affects their distribution in the invertebrate body. From previous studies, we know that metals that are present in the cytosol and bound to proteins are much more bioavailable than metals associated with such structures as exoskeletons, cell walls, and granules [11].

The "available fraction" of Pb can probably be attributed to the toxic effects. In our experiment, the highest content of Pb was noted in the gut and the lower content in internal soft tissues and cuticle (Figure 1, Table 1). It is worth noting that despite the lack of observed influence of Pb salt type on Pb accumulation, the chemical form of Pb may still affect its bioavailability and toxicity. This is suggested by differences in larval responses observed between treatments, indicating that Pb speciation can influence biological effects even when accumulation patterns remain similar [17,27].

The most significant toxicological result of this study is the lack of inhibition of larval biomass growth despite very high exposure to Pb. Larvae maintained normal growth across all treatments, even at the highest Pb concentrations. This indicates a high tolerance of *T. molitor* to Pb exposure and suggests that biomass-based parameters alone may not reflect the presence of contamination. As a consequence, reliance solely on growth performance may lead to underestimation of potential risks in insect production systems. This has important implications for sustainability-oriented risk assessment, where production efficiency indicators are often prioritized [28,29].

https://doi.org/10.3390/su18083931

From a practical perspective, the predominant accumulation of Pb in the gut and cuticle suggests that simple technological measures, such as fasting, could significantly reduce the metal content in insect biomass prior to processing. At the same time, the ability of larvae to grow normally under high Pb exposure increases the risk of "silent" contamination entering the food and feed chain if appropriate chemical monitoring is not implemented.

Therefore, ensuring the safety of insect-based products requires not only systematic monitoring of metal concentrations but also strict control of rearing substrates within sustainable production systems.

5. Conclusions

In conclusion, this study demonstrates that *Tenebrio molitor* larvae accumulate Pb in a dose-related pattern, reflecting its concentration in the diet, while maintaining normal biomass growth and high survival even at very high exposure levels (up to 2000 mg/kg). A key novelty of this work is the application of exceptionally high Pb concentrations and the observation that growth-related parameters are insensitive indicators of metal exposure. Estimated bioaccumulation factors (BAF) were below 1, indicating no biomagnification. Although the exposure levels applied in this study exceed those typically encountered in practice, they provide a conservative framework for assessing contamination risks in circular production systems.

Despite the lack of growth effects, substantial Pb accumulation occurred, particularly in the gut and cuticle, indicating a risk of "hidden" contamination in insect-based food and feed systems.

From a food safety perspective, these findings highlight the need for strict substrate control and systematic chemical monitoring. Although current European Union regulations restrict the use of waste-derived substrates, contamination may still occur due to variability in raw materials or unintended exposure.

The preferential accumulation of Pb in the gut and cuticle suggests that processing steps, such as fasting or removal of external structures, may reduce contaminant levels; however, this was not directly tested and requires further investigation. The absence of a fasting period prior to analysis should be considered when interpreting gut Pb concentrations, as residual feed may contribute to the measured values. Future studies should focus on identifying more sensitive biomarkers of contamination and on mechanisms regulating metal uptake and compartmentalization.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su18083931/s1, Table S1a–c: Analysis of variance (ANOVA) results for larval mass and perimeter; Table S2: (excel-raw data of larvae mass during the experiment).

Author Contributions: Conceptualization, J.R. and K.M.; methodology, K.M.; formal analysis, K.M.; investigation, K.M., B.H.-L., W.D. and P.P.; data curation, K.M.; writing—original draft preparation, K.M. and J.R.; writing—review and editing, J.R.; visualization, K.M.; supervision, J.R.; project administration, J.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study described in our manuscript was conducted exclusively on insect larvae (*Tenebrio molitor*), which are invertebrates. According to European and national regulations, ethical approval is not required for research involving invertebrates. Specifically, Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes applies only to live vertebrate animals (Article 1). As insects are invertebrates, they are not covered by this di-rective, and therefore no approval from an Institutional Review Board or Ethics Committee is re-quired. This directive is implemented in Poland through national legislation concerning the protec-tion of animals used for scientific or educational purposes, which likewise applies only to vertebrates.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

Acknowledgments: The authors would like to thank the technical staff from the Wrocław University of Science and Technology for their support.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Pb	lead
BAF	bioaccumulation factor
ANOVA	analysis of variance

References

1. Malematja, E.; Manyelo, T.G.; Sebola, N.A.; Kolobe, S.D.; Mabelebele, M. The accumulation of heavy metals in feeder insects and their impact on animal production. *Sci. Total Environ.* **2023**, *885*, 163716. [CrossRef]
2. Abd Elnabi, M.K.; Elkaliny, N.E.; Elyazied, M.M.; Azab, S.H.; Elkhalifa, S.A.; Elmasry, S.; Mouhamed, M.S.; Shalamesh, E.M.; Alhorieny, N.A.; Abd Elaty, A.E.; et al. Toxicity of heavy metals and recent advances in their removal: A review. *Toxics* **2023**, *11*, 580. [CrossRef]
3. Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B.B.; Beeregowda, K.N. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.* **2014**, *7*, 60–72. [CrossRef]
4. Dar, M.I.; Green, I.D.; Khan, F.A. Trace metal contamination: Transfer and fate in food chains of terrestrial invertebrates. *Food Webs* **2019**, *20*, e00116. [CrossRef]
5. Carrington, C.D.; Bolger, P.M. An assessment of the hazards of lead in food. *Regul. Toxicol. Pharmacol.* **1992**, *16*, 265–272. [CrossRef]
6. EFSA Panel on Contaminants in the Food Chain (CONTAM). Lead dietary exposure in the European population. *EFSA J.* **2012**, *10*, 2831. [CrossRef]
7. van Huis, A. Insects as food and feed, a new emerging agricultural sector: A review. *J. Insects Food Feed* **2019**, *6*, 27–44. [CrossRef]
8. Costa, S.; Pedro, S.; Lourenço, H.; Batista, I.; Teixeira, B.; Bandarra, N.M.; Murta, D.; Nunes, R.; Pires, C. Evaluation of *Tenebrio molitor* larvae as an alternative food source. *NFS J.* **2020**, *21*, 57–64. [CrossRef]
9. Rumpold, B.A.; Schlüter, O.K. Nutritional composition and safety aspects of edible insects. *Mol. Nutr. Food Res.* **2013**, *57*, 802–823. [CrossRef] [PubMed]
10. European Commission. Commission Implementing Regulation (EU) 2021/882 of 1 June 2021 authorising the placing on the market of dried *Tenebrio molitor* larva as a novel food. *Off. J. Eur. Union* **2021**, *L194*, 16–18.
11. Bednarska, A.J.; Świątek, Z. Subcellular partitioning of cadmium and zinc in mealworm beetle (*Tenebrio molitor*) larvae exposed to metal-contaminated flour. *Ecotoxicol. Environ. Saf.* **2016**, *133*, 82–89. [CrossRef]
12. van der Fels-Klerx, H.J.; Camenzuli, L.; van der Lee, M.K.; Oonincx, D.G.A.B. Uptake of cadmium, lead and arsenic by *Tenebrio molitor* and *Hermetia illucens* from contaminated substrates. *PLoS ONE* **2016**, *11*, e0166186. [CrossRef]
13. Truzzi, C.; Illuminati, S.; Girolametti, F.; Antonucci, M.; Scarponi, G.; Ruschioni, S.; Riolo, P.; Annibaldi, A. Influence of feeding substrates on the presence of toxic metals in larvae of *Tenebrio molitor*: Risk assessment for human consumption. *Int. J. Environ. Res. Public Health* **2019**, *16*, 4815. [CrossRef]
14. Schrögel, P.; Wätjen, W. Insects for food and feed—Safety aspects related to mycotoxins and metals. *Foods* **2019**, *8*, 288. [CrossRef]
15. Lindqvist, L.; Block, M. Excretion of cadmium during moulting and metamorphosis in *Tenebrio molitor*. *Environ. Pollut.* **1995**, *89*, 189–195. [CrossRef]
16. Gori, A.; Armani, A.; Pedonese, F.; Benini, O.; Mancini, S.; Nuvoloni, R. Heavy metals (Pb, Cd, Ni) in insect-based products for human consumption sold by e-commerce in the EU market. *Food Control* **2025**, *167*, 110781. [CrossRef]
17. Wang, J.; Chen, C. Biosorbents for heavy metals removal and their future. *Biotechnol. Adv.* **2009**, *27*, 195–226. [CrossRef]
18. Di Toro, D.M.; Allen, H.E.; Bergman, H.L.; Meyer, J.S.; Paquin, P.R.; Santore, R.C. Biotic ligand model of the acute toxicity of metals. *Environ. Toxicol. Chem.* **2001**, *20*, 2383–2396. [CrossRef] [PubMed]
19. Noyens, I.; Schoeters, F.; Van Peer, M.; Berrens, S.; Goossens, S.; Van Miert, S. Nutritional profile and heavy metal uptake of mealworms reared on sidestreams. *Sci. Rep.* **2023**, *13*, 11604. [CrossRef] [PubMed]

20. Vijver, M.; Jager, T.; Posthuma, L.; Peijnenburg, W. Metal uptake from soils by *Tenebrio molitor* larvae. *Ecotoxicol. Environ. Saf.* **2003**, *54*, 277–289. [CrossRef] [PubMed]
21. Kłobukowski, F.; Śmiechowska, M.; Skotnicka, M. Edible Insects from the Perspective of Sustainability—A Review of the Hazards and Benefits. *Foods* **2025**, *14*, 1382. [CrossRef] [PubMed]
22. Lange, K.W.; Nakamura, Y. Edible Insects as Future Food: Chances and Challenges. *J. Future Foods* **2021**, *1*, 38–46. [CrossRef]
23. Andersen, A.N.; Hoffmann, B.D.; Müller, W.J.; Griffiths, A.D. Using ants as bioindicators in land management. *J. Appl. Ecol.* **2002**, *39*, 8–17. [CrossRef]
24. Devkota, B.; Schmidt, G.H. Accumulation of heavy metals in food plants and grasshoppers. *Agric. Ecosyst. Environ.* **2000**, *78*, 85–91. [CrossRef]
25. Zhang, C.; Song, N.; Zeng, G.-M.; Jiang, M.; Zhang, J.-C.; Hu, X.-J.; Chen, A.-W.; Zhen, J.-M. Bioaccumulation of metals from sediments. *Environ. Monit. Assess.* **2014**, *186*, 1735–1745. [CrossRef]
26. Jucker, C.; Defilippo, F.; Lupi, D.; Gigante, P.; Savoldelli, S.; Ferretti, E.; Curatolo, M.; Bassi, A.; Bonilauri, P.; Dottori, M. Bioaccumulation of cadmium in *Tenebrio molitor*, *Zophobas morio* and *Hermetia illucens*. In Proceedings of the Insects to Feed the World 2020, Virtual, 23–26 November 2020.
27. Lemire, J.A.; Harrison, J.J.; Turner, R.J. Antimicrobial activity of metals. *Nat. Rev. Microbiol.* **2013**, *11*, 371–384. [CrossRef]
28. Amorello, D.; Barreca, S.; Orecchio, S. Critical Evaluation of Hazardous Pollutants in Edible Insects: A Simple Review. *J. Food Compos. Anal.* **2024**, *135*, 106675. [CrossRef]
29. Jankowski, W.M.; Przychodniak, D.; Gromek, W.; Majsiak, E.; Kurowski, M. Edible Insects as an Alternative Source of Nutrients: Benefits, Risks, and the Future of Entomophagy in Europe—A Narrative Review. *Foods* **2025**, *14*, 270. [CrossRef]