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Toxicological effect of lead nitrate on haemogram of eri silk worm (*Philosamia ricini*)

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Abstract
Lead is a widespread heavy metal and environmental pollutant which is released into the environment as automobile exhaust. It is toxic and affects different organisms including plants and animals. The present study was conducted to investigate the effect of lead on the haemogram of eri silkworms as haemogram serves as an indicator of the physiological status of an organism. Lead in the form of inorganic salt, lead nitrate was added to castor leaves at three different concentrations and fed to the 4th and 5th instar larvae of eri silkworm for 3 days to evaluate their effect. The data revealed that Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC) of larvae treated with lead nitrate were affected. Significant reduction in THC was recorded in both 4th and 5th instar larvae as compared to normal larvae. DHC of all the different haemocytes (PR, PL, GR, SP, OE) decreased more or less as compared to normal. Blood volume of the treated larvae also decreased slightly but the reduction was not significant.

Keywords: Lead, Haemogram, THC, DHC, Blood volume, eri silkworm

1. Introduction
Environmental pollution with toxic and dangerous heavy metals is a major problem of recent time. Air born pollution is directly implicated in the toxicological decline of many organisms in nature. The major factors or contribution of environmental pollution is rapid industrialization, agricultural practices and most importantly automobile exhaust. In India, petrol and diesel motor vehicle emit a wide variety of pollutants, mainly Benzene, Carbon monoxide, Lead, Sulphur dioxide and other heavy metal such as Cadmium, Cobalt, Copper etc.

Toxicity of heavy metals emitted from automobile exhaust have been shown to accumulate and cause damage and in extreme cases leads to death in living organisms including plants, animals and insects [1]. Heavy metals in insects have a clear effect on growth [2], mortality [3] and physiology [4, 5]. Lindquist [6] proved that an accumulation of heavy metal is of common occurrence in insects that feed on contaminated leaves. Heavy metal accumulated in the insects can exert toxic effects at all levels of the biological organisation [7].

Environmental pollution by heavy metal is a serious but largely unrecognised threat to sericulture. Sericulture, especially ericulture is a unique field of agriculture and environmental pollution is presently causing serious problems to the eri silk industry as the quality and quantity of the silk are being affected. Feeding of lead along with artificial diet is reported to affect the growth and development of the silk worm *Bombyx mori* [8]. The effect of lead on other insects is also reported to be profound [5].

The effect of lead on blood is more pronounced as it affects the circulating haemocytes. It is reported that Arsenic and Lead induction altered reactivity of haemocytes which may affect the propagation and survival of insect population by increasing its vulnerability to disease and parasitic effect [9].

Therefore the aim of the present study is to look into the possible effect of lead on the performance of the Eri silk worm (*Philosamia ricini*) as lead is reported to be one of the most widespread pollutants and is ranked second of all the hazardous substances by the Agency for Toxic Substance and Disease Resistant (ATSDR) [10]. The effect will be measured as changes in the haemogram of the insects. Haemogram represents the total number of haemocytes (THC) in relation to the blood volume as well as the different types of existing haemocytes (DHC).Haemocytes are responsible for various physiological processes, so the quantitative (THC) and qualitative (DHC) estimation of haemocytes is considered as an important measure of overall health of the insects [11].

2. Materials and Methods

The present study was carried out it the Department of Zoology, B. Borooah College as well as in the Bio Tech Hub of the same institution.

2.1 Insect: The 4th and 5th instar larvae of Eri silkworm were used for the study as the highest compliment of haemocytes are recorded during their late instars [12]. The larvae of Eri silkworm were collected from the eri seed grainage located in Oujari, Morigaon district and were reared on castor leaves.

2.2 Chemical and Dosage: Lead nitrate Pb $(NO_3)^2$ was used for the treatment in this current study. Here the leaf dipping method was used [13]. Lead nitrate solution was prepared with distilled water at three different concentrations. The applied concentrations were 25mg, 50mg and 75mg per 1000ml of distilled water, which was fed to the 4th and 5th instars larvae for 3 days. For each dosage group, 6 larvae were used. A control group of 6 larvae were maintained for comparison, which were fed with untreated leaves of the same host plant.

2.3 Total haemocytes count (THC): First the larvae of each instar were fixed in hot water at 56-60ºC for 2min. After heat fixation, the larvae were dried on a filter paper. A proleg present on the 7th abdominal segment of the larva was cut at the tip and first few drops of pale greenish yellow blood were collected on a clean glass slide. The blood drop was drawn into a Thoma white blood cell pipette up to 0.5 marks and diluted up to the 11 mark with the physiological saline solution. The pipette was then shaken for several minutes and the first drop was discarded and the haemocytometer was filled with it. Haemocytes were then counted in the four Corner Squares of the Neubaeur ruling haemocytometer and the total numbers of circulating haemocytes per cubic millimetre were calculated using the following formula suggested by Jones [14]:

$$\frac{\text{Haemocytes in x mm square} \times \text{dilution} \times \text{depth of chamber}}{\text{No. of 1mm square counted}}$$

Where, Dilution = 20 times
Depth factor of the chamber = 10 (constant) and
No. Of square counted= 4

2.4 Differential Haemocyte Count (DHC): One drop of haemolymph was placed on a sterilized glass slide and then drawn into a thin film by the edge of another slide and the film air dried. After that, few drops of methanol were applied on the slide as a fixative. After drying the slide, few drops of Giemsa stains were applied for staining the slide. After 3-5 min, the slide was washed with distilled water and observed under microscope.

2.5 Blood volume (BV): Blood volumes of Eri silkworm were recorded following Yeager and Munson method[15].

2.6 Data Analysis: Statistical analysis for cell data were performed using MS office excel 2017, and SPSS 21. The results were expressed as mean ± standard deviation (X ± SD).

3. Result

3.1. Effect of lead on Total haemocyte count (THC) in the 4th and 5th instar larvae of *Philosamia ricini*: It is evident from the data shown in Table 1 that the number of THC of both 4th and 5th instar larvae of eri silk worm decreases with the increase in lead concentration. Significant reduction of THC was recorded with all the three concentrations of lead used in the experiment. However, lowest THC value was recorded in both 4th instar (6233.33±225.46) and 5th instar (15166.66±725.14) larvae when treated with the highest concentration of 75mg/1000ml which was highly significant compared to control. Thus, from the above result, it is clear that increased concentration of lead nitrate affect the cell numbers.

Table 1: Total Haemocyte Count (THC) of normal and treated 4th and 5th instar larvae of *Philosamia ricini*

Developmental stage	Control Group (Mean ±S.D)	Treated group(Mean±S.D)		
		25mg/1000ml	50mg/1000ml	75mg/1000ml
4th instar	22166.66±2578.80	12300±1732.30*	7566±1147.10**	6233.33±225.46***
5th instar	72150±3130.10	26366.66±1275.18***	18733.33±971.25***	15166.66±725.14***

Mean ±S.D, (P>0.05), *=P<0.05, **=P<0.01, ***=P<0.001

3.2. Effect of lead on blood volume in the 4th and 5th instar larvae of *Philosamia ricini*: Table 2 depicts the blood volume of both 4th and 5th instar larvae of eri silk worm under normal and treated condition. Although change in blood volume was recorded among the control and treated groups, it was observed that the difference was not significant.

Table 2: Blood volume of normal and treated 4th and 5th instar larvae of *Philosamia ricini*

Developmental stage	Control Group (Mean±S.D)	Treated group(Mean±S.D)		
		25mg/1000ml	50mg/1000ml	75mg/1000ml
4th instar	0.187±0.021	0.183±0.015ns	0.155±0.008 ns	0.145±0.012 ns
5th instar	1.689±0.20	1.33±0.18 ns	1.02±0.21 ns	0.99±0.14 ns

Mean±S.D, ns= not significant (P>0.05), *=P<0.05, **=P<0.01, ***=P<0.001

3.3. Effect of lead on Differential Haemocyte count (DHC) in the 4th and 5th instar larvae of *Philosamia ricini*: The present study revealed the presence of different haemocytes namely, Prohaemocytes (PR), Plasmatocytes (PL), Granulocytes (GR), Spherulocytes (SP) and Oenocytes (OE) in variable numbers in the haemolymph of 4th and 5th instar larvae of *P. ricini*. Granulocytes were the most abundant (37.36±2.30 and 36.49±3.00) whereas the oenocytes (8.72±2.42 and 7.41±0.99) were the least abundant haemocytes in both 4th and 5th instar larvae. Table 3 shows the change in DHC after treatment with different concentration of lead extract.

In the 4th instar larvae, significant reduction in GR (21.80±1.23), SP (5.65±1.99) and OE (2.50±1.59) was recorded when treated with 50 mg/1000ml and 75 mg/1000ml concentration of lead. However, at lower concentration, decrease in the number of different haemocytes was not significant compared to control.

Table 3: Differential Haemocyte count (DHC) of normal and treated 4th and 5th instar larvae of *Philosamia ricini*

Types of haemocytes	Developmental stage	Control	Treated group (Mean±S.D)		
			25mg/1000ml	50mg/1000ml	75mg/1000ml
PR	4th instar	17.75±1.57	23.05±2.90^{ns}	19.02±1.70^{ns}	18.11±1.98^{ns}
	5th instar	25.65±3.21	23.02±2.35^{ns}	21.62±2.87^{ns}	14.52±1.87*
PL	4th instar	21.30±3.00	20.05±2.93^{ns}	19.87±1.22^{ns}	18.28±1.51^{ns}
	5th instar	18.92±2.66	18.86±0.83^{ns}	17.34±1.82^{ns}	15.55±1.23^{ns}
GR	4th instar	37.36±2.30	33.17±2.50^{ns}	30.07±2.13*	21.80±1.23*
	5th instar	36.49±3.00	34.67±2.18^{ns}	33.42±0.80^{ns}	31.41±2.14^{ns}
SP	4th instar	12.35±2.64	8.11±2.33^{ns}	6.73±1.25*	5.65±1.99*
	5th instar	13.33±3.33	10.28±2.23^{ns}	6.00±2.15*	5.25±1.28*
OE	4th instar	8.72±2.42	6.16±1.98^{ns}	3.86±1.95*	2.50±1.59*
	5th instar	7.41±0.99	5.19±1.40^{ns}	4.21±0.96*	2.30±1.11**

Mean±S.D, ns= not significant (P>0.05), *=P<0.05, **=P<0.01, ***=P<0.001

Similar results were also obtained in 5th instar larvae for SP and OE. But in case of GR no significant difference with the control group was observed, neither at low concentration nor at higher concentration. Highly significant difference was recorded between OE and control when treated with the highest concentration where the number of OE was found to be as low as 2.30±1.11.

4. Discussion

In our study we could identify five types of haemocytes, viz- Prohaemocyte, Plasmatocyte, Granulocyte, Spherulocyte and Oenocytoids based on their morphology, following Jones [14]. This was in accordance with the reports of Akai and Sato [16] working on *Bombyx mori*. Differences with other reported works on other species [17, 18] might be attributed to the differences in insect species or even developmental stages.

The observed variation in haemocytes is common in insect species as they are sensitive entities in the haemolymph. Their numbers are reported to fluctuate under insecticide stress and response to immune system against external exposure. Pesticides are known to intervene in the intermediate metabolism of the exposed targets which probably lead to the degeneration of the haemocytes resulting in their decreased numbers [19] as observed in our study.

The observed decline in THC in the treated worms may be correlated with the decrease of some of the haemocyte types (viz-PR, PL, GR, SP, OE etc) in the haemolymph as a counter measure against non-self materials. Reduction in THC may also be due to toxicity of the pollutant and their inhibitory effects on hematopoietic functions [20]. Decline may also be attributed to the death of cells by degeneration when exposed to the challenges which in this case is lead nitrate [12]. Barkat *et al*. [21] suggested that decline in THC may also occur due to the phagocytic activities of the haemocytes which constitute immunocytes in insects and their subsequent lysis. Agglutination induced by the external challenges may also be a cause of their reduced numbers on exposure to harmful chemicals [22]. However the observed reduction in PRs which serves as stem cells to other cells could be correlated to the transformation of PRs into other cell types [23] or destruction of haemopoietic organs responsible for the production of PRs [24]. Conversion of PRs to other cell types viz- GR, SP etc under stress condition is necessary as the other cell types get degenerated due to phagocytic activities, lysis, degeneration etc on being exposed. SP decline in treated insects may be due to effective utilization of fat reserves during the period of decreased respiratory metabolism and also to produce extra energy under stress condition [25]. The involvement of OEs in the synthesis of Phenoloxidase enzyme cascade may be responsible for the decrease in their number in the treated larvae [26].

So from the above discussion it is evident that exposure to lead pollutants present in automobile exhaust is detrimental to the health and performance of Eri silkworms. The observed effect on the haemogram of the larvae suggests the effect of lead on the physiology and immunity of the insects. Such effect on *Philosamia ricini* which is a commercially important species impact on the economy of the people involved in Eri silk culture. That is why lead pollution of the atmosphere is proving to be deleterious to such cultures as a whole and needs to be given serious attention by all concerned.

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6. Reference

1. Ahanger FA, Illahi I, Ramegowda GK, Rao RJ. Impact of road dust polluted mulberry leaves on the food ingestion, assimilation and conversion efficiency of silkworm, *Bombyx mori* L. in Kashmir valley. International Journal of Plant, Animals and Environmental Sciences. 2014; 5(1):2231-4490.
2. Warrington S. Relation between SO_2 dose and growth of the pea aphids, Acyrthosiphum pisum, on peas. Environmental Pollution. 1987; 43:155-162.
3. Mitterbock VF, Fuhrer E. Effects of Fluoride-polluted spruce leaves on nun moth caterpillars, *Lymantria monacha*. Journal of Applied Entomology. 1988; 105:19-27.
4. Fountain MT, Hopkin SP. Continuous monitoring of *Folsomia candida* (Insecta:Collembola) in a metal exposure test. Ecotoxicology and Environmental Safety. 2001; 48(3):275-286.
5. Ilijin L, Periac MV, Nenadovic V, Mrdakovic M. Effect of cadmium on protocerebral neurosecretory neurons and fitness components in *Lymantria dispar* L. Folia Biologica. 2009; 58(1-2):91-99.
6. Linquist L. Accumulation of cadmium, copper and zinc in five species of phytophagous insects. Environmental Entomology. 1992; 21(1):160-163.
7. Hare L. Aquatic insects and trace metals:bioavailability, bioaccumulation and toxicity. CRC Critical Reviews in Toxicology. 1992; 22(5-6):1-15.
8. Miyoshi T, Miyazawa F, Shimizu O, Machida J. Effects of heavy metals onn mulberry leaves and silk worm.L. The Journal of Sericultural Science of Japan. 1978; 47:70-76.
9. Guria S, Perveen S, Goswami DS. Alteration of morphology, phagocytic behaviour and aggregation of

insect Haemocytes exposed to contaminated food with arsenic and Lead. International journal of Pharmtech Research. 2016; 9(5):177-186.
10. Agency for Toxic Sustances and Disease Registry (ATSDR). Toxicological profile for lead. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2007.
11. Bardoloi S, Hazarika LK. Variation in haemocyte population during different larval instars of *Antheraea assama* (Lepidoptera: Saturniidae) Westwood and their roles in the defence mechanism of the insect. Journal of Assam Science Society. 1995; 37(2):96-102.
12. Sendi JJ, Salehi R. The effect of methoprene on total haemocyte counts and histopathology of haemocytes in *Papilio demoleus* L. (Lepidoptera). Munis Entomology and Zoology. 2010; 5(1):240-246.
13. Inamori Y, Kato Y, Kubo M, Sakai M. Mechanism of insecticidal action of Deoxypodophyllotoxin (Anthricin).I. in the tissue of 5th instar larvae of silkworm, *Bombyx mori* Linne. 1986; 31(12):4464-4468.
14. Jones JC. Current concepts concerning insect haemocytes. Amer. Zoo. 1962; 2:209-246.
15. Yeager JF, Munson SC. Arthropoda. 1950; 1:255.
16. Akai H, Sato S. Ultrastructure of the larval haemocytes of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). International Journal of Insect Morphology and Embryology. 1973; 2:207-231.
17. Ribeiro C, Brehelin M. Insect haemocytes: What type of cell is what? Journal of Insect Physiology. 2006; 52:417-429.
18. George PJE, Ambrose DP. Impact of insecticides on the haemogram of Rhynocoris kumarii Ambrose and Livingstone. Journal of Applied Entomolgy. 2004; 128(9-10):600-604.
19. Qamar A, Jamal K. Differential haemocyte counts of 5th instar nymphs and adults of *Dysdercus cingulatus* Fabr (Hemiptera: Pyrrhocoridae) treated with acephate, an organophosphorus insecticide. Biology and Medicine. 2009; 1(2):116-121.
20. Rajitha K, Savithri G, Sujathamma P. Haemocyte population dynamics in 5th instar silkworm *Bombyx mori* L. Inoculated with *Beauveria bassiana* (Bals.)Vuill. International Journal of Agricultural Science. 2013; 3(2):265-276.
21. Barakat EMS, Meshrif WS, Shehata MG. Changes in the haemolymph of the desert locust, *Schistocerca gregaria* after injection with *Bacillus thuringiensis*. Journal of Egyptian Acdemic Society for Environmental Development. 2002; 2(1):95-115.
22. Chavan JA, Chougale AK, Bhawane GP. Toxicity of Dimethoate and Chloropyriphos on Haemocyte count in male *Platynotus belli* Fairmaire (Coleoptera: Tenebrionidae). Journal of Entomology and Zoology studies. 2016; 5(1):126-133.
23. Bhatti ZS. Effect of some pyrethroids on the haemocytes of *Spodoptera litura* F. Canadian Journal of Zoology. 2002; 62:1461-1463
24. Tiwari RK, Pandey JP, Salehi R. Haemopoietic organs and effect of their ablation on total haemocyte count in lemon butterfly, *Papilio demoleus* L. Indian Journal of Experimental Biology. 2002; 40:1202-1205.
25. Begum R, Gohain R, Hazarika LK. Detoxifications of Deltamethrin by the haemocytes of the 5th instar *Philosamia ricini* Boisd. Journal of Environmental Biology. 1998; 17:149-155.
26. Suderhall K, Smith VJ. The prophenol oxidase activity system. The biochemistry of its activation and role in arthropoda cellular immunity with special reference to crustaceans. In: immunity in Invertebrates (M. Brechelin ed.) Springer Verlag, Berlin-Heidelberg, 1986.