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EFFECT OF LEAD ON EXTERNAL MORPHOLOGY AND POLYTENE CHROMOSOMES OF *TABANUS BOVINUS*

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ABSTRACT

The influence of lead (lead acetate) on the cytogenetic and external morphology of *Tabanus bovinus* (Tabanidae) was investigated. The effect of lead acetate (9.65 mg/l) and (0.009 mg/l) treatment on five and one generations respectively of *T. bovinus* was studied experimentally. From F2 till F5 reduction in hatching and malformation were found. Malformations concerned the teeth of mandible, sub-mentum, colour of pupa, adults, shape of genitalia and wings. In the F4 and F5 generation individuals with elongated legs and larvae with two gular sclerites were observed. Structure and functional abnormalities of the polytene chromosomes were found; pulverization of the chromosomes, pericentric heterozygous inversions. In the I chromosomes a synapsis of the homologues is almost complete, probably owing to differences at a molecular level which are not reflected in the banding pattern. The presence of different degrees of polytene and different amounts of centromere heterochromatin of the homologues of this chromosome could be result of an inhabitory effect of lead on the replication activity of both homologues.

Key Words: Environmental studies, lead, chromosomes, *Tabanus bovinus.*

INTRODUCTION

Lead, a widely used industrial heavy metal, is a significant environmental pollutant that contaminates food, water, urban soil and air. The detection of possible hazardous effects of this metal on human health is therefore a matter of urgent concern. Although many studies have investigated the biological effects of lead, its genotoxic potential remains to be established. Lead has a definite cytogenetic effect on the bone marrow cells of mammals (Tachi and Nishime 1975; Michailova, 1987b; Short, 1990; Wilson, 1995; Watson, 1999; Walter, 2000; Porter, 2002; Ramel, 2003; Talbot, 2004; Margim. 2005).

Genetic investigation on *D. melanogaster* indicated a lead effect on meiotic nondisjunction (Ramel, 2003). Data on the action of lead on a group of insects such as the widely distributed species of the family Tabanidae (important from a practical and theoretical point of view) are missing in the literature. From studies carried out on natural populations of above mentioned species it has been established that contamination with heavy metals (Zinc, Lead etc.) can induce structural and functional modifications in the genome and a series of malformations.

Michailova, (1987a) and Timmermans (1988) reported on the effect heavy metals on the feeding behavior Chironomidae. The purpose of the present work was to trace under experimental conditions the cytogenetic effects of lead ions in various concentrations on the polytene chromosomes of 4th instar stage larvae of *T. bovines,* the Phenotypic modifications in the separate stages of metamorphosis, and to record the mortality rate of the 4th instar stage larvae in five generations.

MATERIALS AND METHODS

The stock of this species was reared and maintained by the method modified by Michailova (1985). Experiments were carried out at two concentrations of lead acetate on the larval stage of *T. bovinus.* One of the cultures where the larvae of this species were reared had a concentration of lead ions (lead acetate) in conformity with that of environmental (0.009 mg/l) while the other had high lead concentration (9.65 mg/l). The culture was carried out by the method described by Michailova (1985). One culture was made from one puddle mass. To ensure the normal development of the larvae in this culture, the water of the required lead acetate concentration was changed three time a week. Thus a culture of *T. bovinus* was maintained in the laboratory under the effect of 9.65 mg/l and 0.009 mg/l for five and one generations respectively. The culture was maintained by artificial crossing of image of this species under a binocular lens. The development of one generation (larva, pupa, and imago) at room temperature took 30 – 32 days. Mortality percentage of the 4th instar was recorded. The morphology of hatched adults and 4th instar larvae from each generation were subjected to detailed analysis.

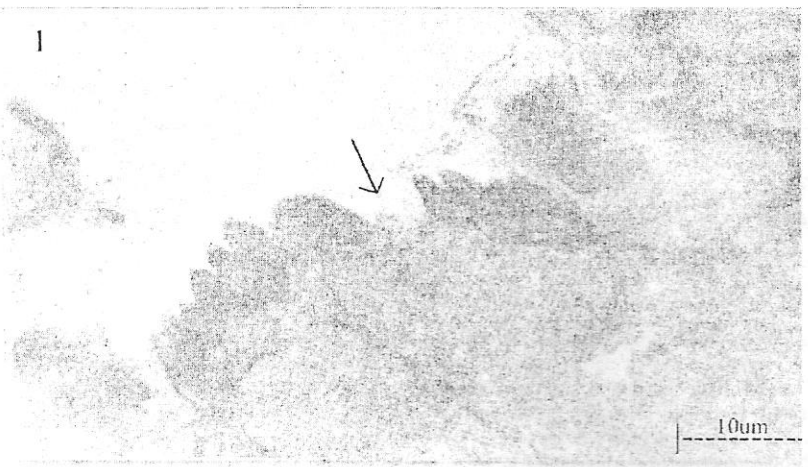

Fig. 1. Submentum with malformation.

Fig.2. I Chromosome with pericentric heterozygous inversion (after exposure to lead concetration).

The salivary gland chromosome map of *T. bovinus* constructed by Michailova (1987b) was used as standard. The chromosomes and their arms are designated as follows: I chromosome – AB, II – CD III-EF, IV-G. The inversions observed were marked as follows: paracentric heterozygous inversion as "Para. Het. Inv." Pericentric heterozygous inversion as "peri. Het. Inv."

From each larva salivary gland chromosome preparation and preparation from external morphology of the larva were made. External morphological and cytological analysis was also carried out on a maintained control larva culture. Twenty eight specimens (larvae) from Fi were analyzed after treatment with 0.009 mg/l lead acetate. Ten specimens (larvae) of each F1, F2, F3 and F4 were examined after treatment with 9.65 mg/l lead acetate. Sixty eight specimens were analyzed and twenty five cells of each specimen were studied.

RESULTS AND DISCUSSION

The larval survival rate in F1 was 89% when treated with 0.009 mg/l lead acetate as compared to control 95% (in both cases 500 larvae were treated). Females prevailed among hatched adults. Elongation of the body and legs was observed in adult males. Survival of larvae from F1 after treatment with 9.65 mg/l was 95% being the same for the control (500 larvae were treated). Among F2 and F3 generations mortality increased and reached 60% (250 larvae were treated) comparing with 90% survival control (500 larvae were treated). In F4 mortality was almost

50% and in F5 only 4 out of 54 fertile eggs survived to the last larval stage. Two of them developed into adults (♂♂). One of the larvae did not develop while the other showed abnormalities in its morphology: with double gular sclerites.

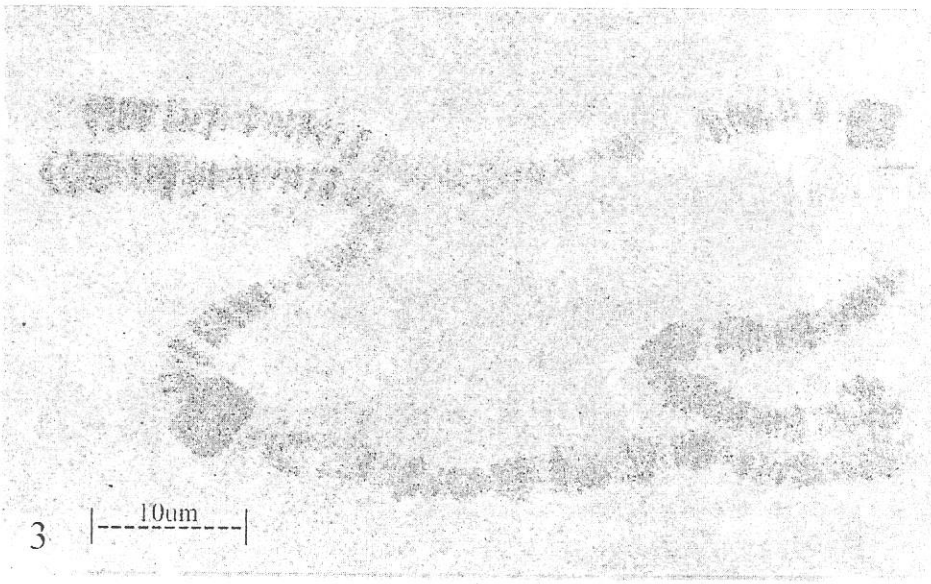

Fig.3. I Chromosome with a synapsis of both homologoues (after exposure to high lead concetration).

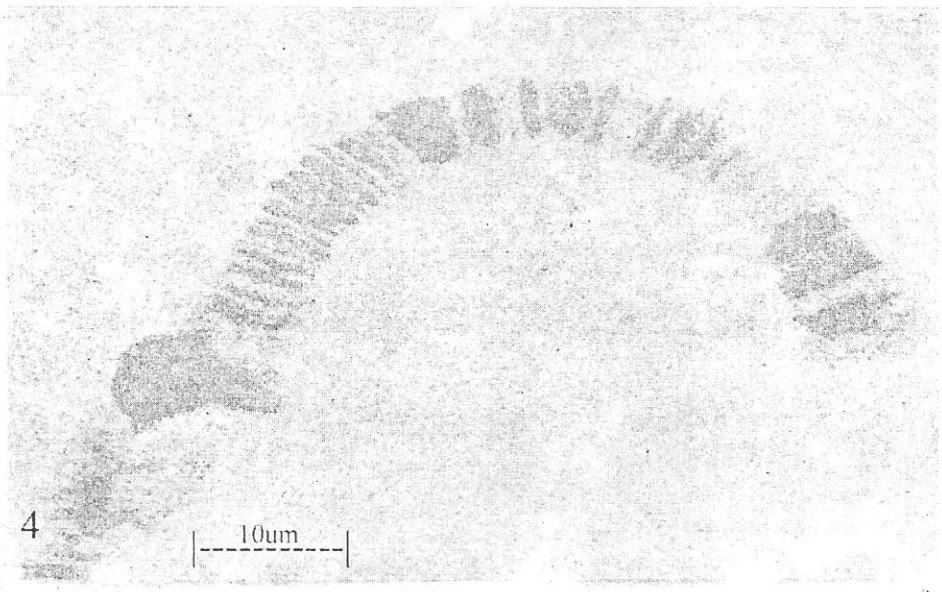

Fig.4. II Chromosome underrepliction in centromere region (after exposure to high lead concetration).

Adults with abnormalities in external morphology (elongated legs, hirsute bodies, yellow wings, yellow-spotted wings) were found as follows: F2 – 20 adults were analysed: 3 ♂♂ and 3 ♀♀ with abnormalities; F3 – 20 adults were analysed: 3 ♂♂ and 4 ♀♀ with abnormalities, F4 – 40 adults were analysed: 2 ♂♂ and 7 ♀♀ with abnormalities. Further more, while pupae were observed in F3 and F4, the larvae treated with low and high lead acetate concentrations had a number of abnormalities in external morphology: asymmetry in the submentum tooth number instead of lateral tooth of submentum a well formed exerscence (Fig I). *T. bovinus* has 2n = 8. The I,II, III and IV chromosomes have a well marked centromere region in the polytene chromosomes. Each chromosome has a

nucleolus. The IV chromosome carries two Balbiani rings. All chromosomes are well paired. This species was studied from a karyological point of view by many researchers (Dahl, 1988; Falck and Barke, 1999; Dryl, 2000; Haga, 2001; and Gortz, 2004).

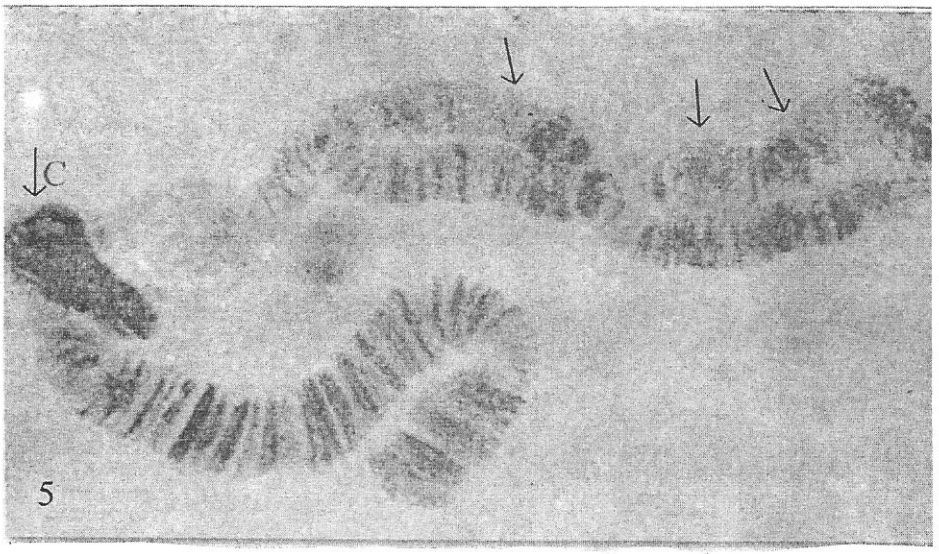

Fig.5. II Chromosome underrepliction in centromere region, a synapsis in arm 'C' and expression of puffs.

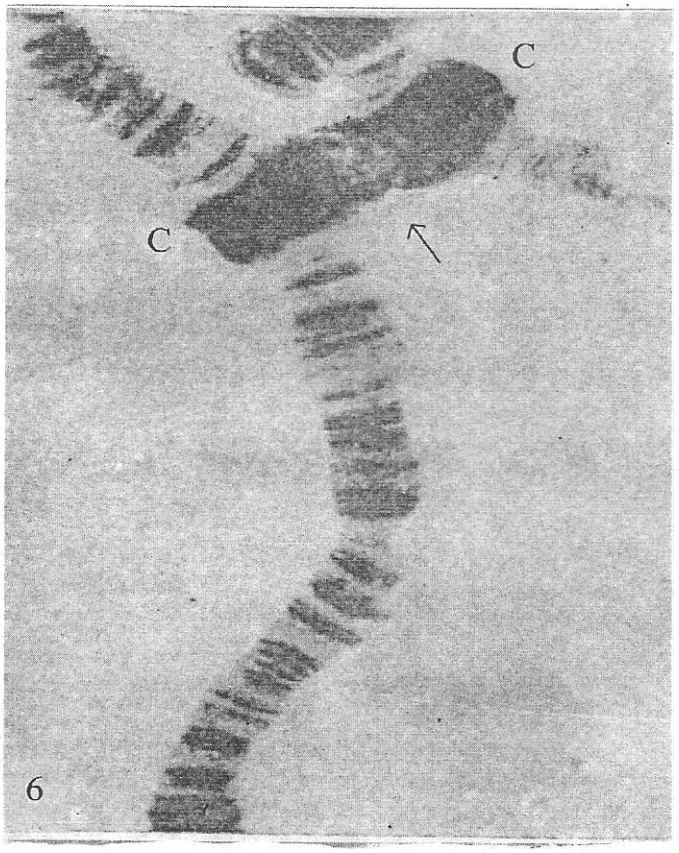

Fig.6. Centromere – centromere association.

It is a polymorphic species, with paracentric heterozygous inversion in all arms (Lach, 2000, Gordon, 2003; Srebro, 2005). No pericentric inversions, translocations, or deletions were found in the study of natural populations. The selective disadvantage of these aberrations must be considered in nature.

The results of the analysis of polytene chromosomes of *T. bovinus* treated with different lead concentrations are shown in Table 1 it is seen that a large proportion of specimens studied have chromosome aberrations and abnormalities in replication affected the I and II chromosomes the III chromosomes remaining unaffected. Unpairing was found in the IV chromosomes (Table 1)

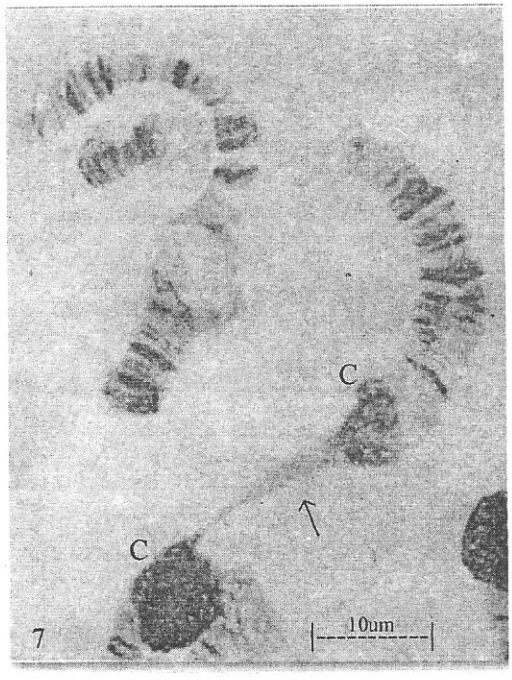

Fig.7. Ectopic pairing between centromere region, C- centromere region.

1. Chromosome (AB)

Under the action of low and high concentrations of lead acetate chromosome rearrangements were observed which were not present in the control. Pericentric heterozygous inversion (Fig. **2**) with a high frequency in both sexes was observed only after exposure to the low concentration of lead acetate (Table 1)

After treatment with lead acetate at high concentration, a synapsis in this chromosome was found in all the generations studied (Fig. **3**). The two homologues were oriented to each other but they were almost completely a synapses. Asynapsis was also distinct in the areas where there was a complete coincidence of the banding patterns.

Under the effect of the lead replication in both homologues was affected visibly in the centromere region (Fig. **4**). Most probably the replication activity in the centromere region of one of the homologues was inhibited: heterochromatin in the centromere region of one of the homologues was considerably less.

Such a change was observed with a high frequency in both sexes of F1, F2 and F4, While in F3 it was found only in ♂♂ (Table 1).

2. Chromosomes (CD)

Not with standing the lead concentration in this chromosome there was found underreplication in the centromere region of one of the homologues and incomplete pairing in the "C" arm (Fig. **5**). These abnormalities were observed in both sexes of specimens treated with of low concentration of lead and in F1, F2, F3 treated with a high concentration in ♂♂ only. In F4 they were observed in both sexes. An expression of a puff was noted in the telomere region in the II (Fig. **5**) and IV chromosomes. This was probably brought about by the lead ions.

At both concentrations, centromere, telomere, and centromere – centromere associations were noted quite often in both sexes (Figs. **5,6 and 7**) Probably the asynapsis in I chromosome was caused by difference at a molecular level which did not affact the banding pattern. The underreplication present in the chromosomes (narrowing, difference in the amount of centromere hetro chromatin in the I and II chromosomes) is presumably related to the inhibitory

effect of Pb^{+2} on the replication activity. Probably, as in some mammalian cells (Paton, 1973), in the present case lead ions may have inhibited enzymes required for DNA repair. Muro and Goyer (1969) suggested indirect chromosome damage associated with the raising of cellular DNase activity by exposure to lead. However, the lead damage to chromosomes remains obscure because of the scarcity of information on nucleic acid or the protein damaging effects of lead. The presented data indicate the same level of cytogenetic effect in spite of the lead concentration.

Table 1. Abnormalities in polytene chromosomes of *Tabanus bovines* after treatment with different concentrations of lead acetate.

Concentrations mg/L	Generation	Number of indiv. Male	female	Chromosomes	Chromosomes abnormalities	% of individual with abnormalities Male	Female
0.0009	F1	10	18	I	Per.Het.Indiv.	50.00	38.88
				II	underreplication + Asynapsis	40.00	38.88
				IV	Un paired	10.00	27.77
Control		10	20	II	Para.Het.Indiv.	15.00	50.00
9.65	F1	4	6	I	Underreplication + Asynapsis	25.00	50.00
				II	Underreplication + Asynapsis	--	60.00
9.65	F2	3	7	I	Underreplication + Asynapsis	66.66	57.14
				II	Underreplication + Asynapsis	-	14.28
				IV	Unpaired	--	42.85
9.65	F3	1	9	I	Underreplication + Asynapsis	--	33.33
				II	Underreplication	--	100.00
9.65	F4	3	7	I	Underreplication + Asynapsis	33.33	37.55
				II	Underreplication	66.66	25.00
Control		20	18	I	Para.Het.Indiv.	25.00	16.66
				II	Par.Het.Indiv.	10.00	22.22

Abnormalities in the I Chromosome were of different nature but their frequency was approximately the same. In the II chromosome different had an equivalent genetic effect.

The *T. bovinus* test presented is especially useful for the rapid screening of lead involved in environmental hazards. Irrespective of its concentration, lead (Lead acetate) has a definite mutagenic effect on Tabinidae, a group of insects important from a hydrobiological and freshwater marshes-ecological point of view, therefore the species *Tabanus bovinus* could be used as test system for heavy metals and to follow up the latter's mutagenic effects.

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