TOXICITY OF SOME HEAVY METALS TO *DROSOPHILA MELANOGASTER.*

By

Al Ayoubi Dujana Y.
Girls Collage of Education-Genetics, Riyadh, K.S.A.

ABSTRACT

The present work was carried out to investigate the toxicity effect of some heavy metal compounds on some fitness components in *Drosophila melanogaster*. Seven heavy metals were tested for their effects on the percentage of survivors and larval period using LC_{50} for each metal. These metals were arsenic, mercury, cadmium, copper, lead, chromium and cobalt. A natural population of *Drosophila melanogaster* was used in theses investigation. The results indicated that rearing larvae on medium containing one of these metals had different effects on the percentage of survivors and larval period. Arsenic, mercury and lead reduced larval period, while copper, cadmium chromium and cobalt caused an increase in larval period. With respect to the toxicity effect, the seven metals were classified into three groups. The first group which had very toxic effect contained arsenic and mercury. The second group which had moderate toxicity contained copper and cadmium. The third group which had low toxic effect contained lead, chromium and cobalt. The results also showed that adding copper to either arsenic or mercury had different effects on the two fitness components.

INTRODUCTION

Heavy metals are present in different concentration levels in industrial wastes emitted into the environment. It is important that their toxic effect on bioassay media be recognized, in order to evaluate the combined effects created by the different wastes and to assess the toxicity of other constituents.

Metal ions are required for many biological processes in which bimacromolecules are engaged. These molecules frequently contain metal ions on the sites e.g., in carboxypeptidase, alkaline phosphates, carbonic anhydrase cytochrome C, etc. The metal ions in such substances are directly involved in the mechanism of the biological process, which the macromolecules are designed to mediate. Biologically active metal ions can sometimes be displaced from the active site by other metal ions. Thus the active site of a macromolecule is a potential locus for the interaction of toxic metals.

Toxicity is a relative term. Metal ions which are essential for biological activity at one concentration become toxic at a higher concentration. Metal toxicity however, probably involves other types of interaction in addition to those on the active site. Extensive studies on the metabolism of insecticides performed during the last 50 years have revealed that *Drosophila* micrososms are capable of facilitating similar enzymatic reactions to those from the mammalian liver (Wilkinson and Brattsten, 1972).

Biochemical characterization of microsomal enzymes in insects had demonstrated that a large number of foreign are oxdatively metabolized by isolated microsomes or other subcellular fractions. *Drosophila* had yielded positive response for the induction of lethal genes. It has versatile non-specific substrate in its enzyme system. *Drosophila*, unlike the other test organisms, is composed of specialized tissues and organs and reproduces by sexual means only. It is, therefore, very much more akin to man.

MATERIALS AND METHODS

The natural population of *Drosophila melanogaster* used in the present experiments was collected from the Matrouh area, about 350 km west of Alexandria, where industrial pollution is practically absent. The population was maintained in the laboratory by mass mating under optimal feeding conditions at 25 C°. The meal used consisted of ordinary maize-meal (35.5 gm), molasses (32.5 ml), agar (5.0 gm), propionic acid (0.5 ml) and live yeast (3 gm). For the control, 375 ml of double-distilled water was used. The same volume of different

solutions of heavy metals was used to test the effect of each metal on the percentage emergence and developmental time for larvae. Different concentrations of each metal were used to determine the concentration required to kill a certain proportion of the population.

The value of LC_{50} was estimated using the dosage mortality regression line. Statistical fitting of the regression lines and slope values and the LC_{50} confidence limits were calculated according to Litchfield and Wilcoxon (1949). Table 1 summarizes the range of concentrations applied for each metal. Five vials were prepared for each concentration, each containing 70 eggs. The cultures were kept at 25C°. After emergence, exhausted and no further larval period was expressed in terms of natural logarithms of days to pupation measured as the total developmental time minus the average pupal period of 4.3 days.

Based on the results of each metal, the combined effects of copper and arsenic, as well as copper and mercury, were studied using the concentrations shown in Table 2.

RESULTS AND DISCUSSION

Estimation of the LC_{50} for the seven elements under the investigation was carried out to help in the study of mutagenicity created by these toxic elements in later stages in the present writer's research.

Table 3 shows the LC_{50} values and their confidence limites, as well as the slope values for each tested metal. Slope values are measured to give an idea concerning the genetic differences in population reaction towards the metals.

The high slope value indicates a homogeneous reaction while the low slope value indicates heterogeneity. The effect of each metal on larval development time and on survival of larvae to produce, finally, fly, will discussed with respect to each metal.

Table I: Concentration of metals added to the culture media

Metal salt	Concentration tested µg/gm of diet												
Na As O2	0.96	4.8	9.6	24.0	48	96	192	as As					
Hg Cl2	0.96	4.8	9.6	24.0	48	96	192	as Hg					
Cu SO4SH2O	0.96	4.8	9.6	24.0	48	96	115	134	154	173	192	as Cu	
CO Cl2	0.96	4.8	9.6	24.0	48	96	192	384	as Co				
Pb (CH2 COO)2 – 3HO2	0.96	4.8	9.6	24	48	96	192	384	576	as Pb			
K2Cr2O7	0.096	0.84	0.96	4.8	9.6	84	96	192	384	576	768	960	1440 as Cr
Cd SO4	4.8	9.6	24	48	96	192	384	as Cd					

Table 2: Concentration of metals added to the culture medium to study their combined effects.

Metal	LC Applied	Concentration in µg/gm of Medium
Cu SO4 5H2O	LC_{10}	1.92
	LC_{30}	5.76
	LC_{50}	9.60
Na ASO2	LC_{10}	2.4
	LC_{30}	14.4
	LC_{50}	28.8
Hg Cl2	LC_{10}	2.4
	LC_{30}	14.4
	LC_{50}	28.8

Table 3: Estimates of LC_{50}, confidence limits and slope values of the various metals.

Metal	LC50 µg/mg	Confidence limits	Slope µg/mg
Arsenic	25	34.6 – 25.6	7.660
Mercury	26	33.6 – 21.5	4.050
Cadmium	96	129.7 – 71.2	6.100
Copper	105	126.9 – 88.5	6.473
Cobalt	269	278.8 – 260.5	1.190
Chromium	423	528.9 – 338.5	3.705
Lead	471	574.4 – 376.9	3.330

1-Arsenic:

Arsenic and arsenicals have many diversified industrial uses. They are used in the alloy industry, in pigmentation and paints, in cloth and electrical semiconductors as well as in the formulation of herbicides used in agriculture.

According to Frost (1967) the most toxic arsenicals are well tolerated at concentrations of 10 to 20-ppm arsenic in the human diet. The least toxic arsenicals can be fed with out injury at levels, which contribute up to at least 1000-ppm arsenic in the diet.

Trivalent arsenicals (organ arsenicals as well as in the organic arsenite) are regarded as being primarily sulfhydryl (SH) reagents, with the result that they inhibit a number of thiol-dependent enzyme systems in various tissues, as tested by Wagner and Weswig (1974).

Arsenicals also comprise one of the few compounds which block metabolism at a lower level than that which affects cell division. This explains the data in Table 4 which shows progressive decrease in the percentage obtained by emergence increasing the concentration of arsenic, while the larval developmental time did not vary significantly from that of untreated population at the 5% level. The calculated LC_{50} for sodium arsenite was 25 µg/gm of diet for larval development in *Drosphila melanogaster*.

2-Cadmium:

Biologically, cadmium is a nonessential, beneficial element recognized to be high toxic potential. Within the past decades industrial production and use of this metal have increased, leading to accumulation of more of it in the environment. (U.S.E.P.A. 1998).

The data obtained for the effects are shown in Table 5. The results indicated that cadmium was less toxic than arsenic, (its LC_{50} being represented by 96 µg/gm of diet). Its presence increased the larval time slightly more than arsenic. The increase in the development time was progressive with the increase of the applied dose of cadmium.

Table 4: The effect of adding arsenic to the larval diet on survival and larval developmental time.

Concentration µg/gm	Survival %	Larval Period
4.8	- 20	- 0.9
9.6	- 27	- 1.5
240	- 29	- 2.5
48.0	- 55	+ 2.3
96.0	- 61	+ 2.0

-ve sign: indicates a decrease in the survival percentage.

Table 5: The effect of adding lead to diet deviation from control

Concentration µg/gm	Survival %	Larval Period
9.6	- 10	+ 0.4
48	- 16	+.3.1
96	- 33	+ 3.4
192	- 40	+ 4.3

This is in arguments with the findings of Inoue and Watanabe (1978) who stated that cadmium was highly toxic at every developmental stage of *Drosophila Melanogaster,* resulting in prolongation of the developmental time, lowering of viability and decrease egg production.

3-Chromium:

The effects of chromium are shown in Table 6. Heaxavalent chromium is irritating and corrosive to the mucous membrane. It is absorbed via ingestion, through the skin, and by inhalation and is toxic when introduced into laboratory animals systemically. The NAS (1996) study designed by Mackenzie *et al.* (1958) to show the toxicity of hexavalent and trivalent chromium ions to rats for one year at levels of 0.45 to 25 mg/L showed no evidence of toxic response in body weigh, food consumption, blood changes or mortality. With *Drosophila* this was also supported by the high LC_{50} value of 423 µg/gm as Cr in the diet.

4-Copper:

It is important in invertebrate blood chemistry for the synthesis of hemoglobin. An overdose of ingested copper in mammals acts as an emetic.

The activity of carbonxypepide enzyme and carbonic anhydrase which is responsible for the interaction of carbon dioxide with water is practically diminished when copper bon dioxide with water is practically diminished when copper replaces the zinc ion present in their chemical structure. Copper ions also bind the certain amino groups as well as the histidine group and two peptides nitrogen, leading to nullification of their metabolic activities (Eickhorn, 1985).

The effects of copper on the percentage of survival and larval period are shown in Table 7. The LC_{50} in *Drosophila* was found to be 105 µg/gm. This dose significantly increased the developmental period.

Table 6: The effect of chromium addition to the larval diet deviation from untreated control.

Concentration µg/gm	Survival %	Larval Period x 100
192	- 12	+ 0.3
348	- 23	+ 8.0*
576	- 39	+ 10.8*
769	- 53	+ 23.2*

* indicates significance at the 0.05 level of probability.

Table 7: The effect of adding copper to larval diet, as deviation from untreated control.

Concentration µg/gm	Survival %	Larval Period x 100
96	- 25	- 0.7
115	- 26	+ 11.3*
134	- 31	+ 21.0**
153	- 37	+ 22.0**
173	- 50	+ 25.9**

* & ** indicate significance at the 0.05 and 0.01 levels of probability respectively.

5-Lead:

Lead has no beneficial or desirable nutritional effects. It is a toxic metal which tends to accumulate in the tissues of men and other animals.

Lead was the least toxic metal to *Drosopila melanogaster*, its LC50 was 471 µg/gm. This can be attributed to the inhibiting effect of pb on enzymatic reaction which is less severe than pb toxic reaction in hemobiosynthesis. Table 8 shows the effects of lead on survival and larval period. There was a general tendency towards reducing the developmental time at the highest concentration tested which was 769 µg/gm. The decrease was proportional to the increase in the applied lead dose.

6-Mercury:

Mercury is widely distributed in the environment and biologically is a nonessential or non-beneficial element.

Historically, it was recognized to posses a high toxic potential and was used as a germicidal or fungicidal agent for medical and agricultural purposes. Mercurials exhibit a wide variety of effects of mitochondria; this depends to a large extent on whether or not the mercurial can penetrate the inner membrane.

The thiol groups of the phosphate transporter are extremely reactive to mercurials. Blocking the phosphate transporter results in inhibitions of oxidative phosphorylation and strongly inhibits matrix enzyme activities (Brierley *et al.*, 1971).

In *Drosophila melanogaster* the response to the mercuric chloride was very close as regards the dose required to induce 50% of larval mortality. The estimated LC50 was 26 µg/gm of diet as Hg++. A significant reduction in the larval developmental time was observed at 4.8 µg/gm Hg++, as shown in Table 9. The decrease in developmental time was reduced by increasing the concentration of mercury added to the diet. Mercury induced a slight decrease at lower doses and an insignificant increase at the higher doses.

Table 8: The effect of adding lead to larval diet, as deviation from untreated control.

Concentration µg/gm	Survival %	Larval Period x 100
129	- 5	- 0.7
484	- 14	- 4.2
576	- 28	- 3.6
769	- 50	- 5.8

Table 9: The effect of adding mercuric chloride to larval diet, as deviation from untreated control.

Concentration µg/gm	Survival %	Larval Period x 100
4.8	- 15	- 10.8*
9.6	- 30	- 10.5*
24.0	- 32	- 10.7*
27.0	- 47	- 10.8*
96.0	- 62	- 9.6

* indicates significance at the 0.05 level of probability.

7-Cobalt:

The effects of cobalt on the percentage of survival and larval period are shown in Table 10. The concentrations used of this metal ranged from 192 to 769 µg/gm. The results clearly indicated that percentage of survival considerably decreased below the untreated control level. The reduction of survival reached to about 50% of that in the control level (769 µg/gm). On the other hand, rearing larvae on media containing cobalt caused significant increase in the larval period especially with the increase with cobalt concentration in media.

The Combined effects of Arsenic, Mercury and Copper:

Estimates of the LC_{50} values for different metals presented in Table 3 clearly indicate that arsenic and mercury have almost the same level of toxic effect on viability measured as percentage emergence. The toxic effect of copper was one-fourth of arsenic and mercury toxicity when applied separately. Table 11 presents the combined effects of copper with either arsenic or mercury, using concentrations of LC_{10}, LC_{30}, and LC_{50} for each metal.

The results indicated that the combined effects of copper and mercury were synergistic and both significantly decreased the larval viability. The combined effects of copper and arsenic were significantly less than the combined effects of copper and mercury, except at LC_{30} which showed the least lethal effect in this case.

DISCUSSION AND CONCLUSION

The nutritional requirements of a species or strain can be specified in terms of the minimal concentration of essential nutrients, including metal ions, which are required for most rapid growth to adulthood. The metal ions content is likely to vary quantitatively and qualitatively in the natural diets of insects. Changing the balance of the diet constitution due to environmental pollution or other means provides unfavorable feeding conditions. Rearing *Drosophila* larvae on unbalanced media for one or more of these essential nutrients may result in reducing the percentage of survival and an increase or

Table 10: The effects of cobalt on percentage emergence and larval development time as deviation from untreated control.

Concentration µg/gm	Survival %	Larval Period x 100
269	- 22	+ 9.6*
307	- 48	+ 24.8*
346	- 50	+ 26.0*
384	- 60	+ 25.9*

* Indicates significance at the 0.05 level of probability.

Table 11: Combined effects of arsenic, mercury and copper at different concentrations.

LC_{10}	Arsenic and Copper Survival %	Mercury & Copper Survival %
LC_{10}	19.5	33.8
LC_{30}	18.6	28.6
LC_{50}	10.5	3.8
Control	66.7	

decrease in larval duration period (Schultz *et al.*, 1946; Begg and Robertson, 1950; Hinton *et al.*, 1951 and Sang, 1956). A very interesting result was encountered when the diet was altered by creating an artificial deficiency of metal ions by adding a chelating agent. Steffenson (1957) reported that addition of EDTA (ethylenediaminetetraacetic acid) to the media led to lower survival and longer development time and that these unfavorable effects were removed by adding zinc ions or ions of metals of higher stability constant at the appropriate concentration. The results of the present investigation indicated clearly that rearing *Drosophila* larvae on diet containing one of the viability and the duration of the growth period according to the concentration added.

These elements could be classified with respect to their toxicity into three groups, namely highly, moderately and slightly toxic. This classification is mainly based on the LC_{50} estimates for *Drosophila* population (Table3) and this does not necessarily apply for other organisms. Arsenic and mercury could be considered as highly toxic; their LC_{50} ranged from 22 to 25 µg/gm. The moderately toxic group included copper and cadmium; their LC_{50} values ranged from 72 to 130 µg/gm. The other three elements, cobalt, chromium and lead, have LC_{50} values ranging from 262 to 574; therefore they could be considered as the least toxic. However, the presence of two elements in the media may affect their level of toxicity.

Adding copper to either arsenic or mercury had different effects on larval viability, although the latter two metals had almost the same level of toxicity.

The population reacted differently with respect to the different metals, as the slope values indicate Table 3). With arsenic, cadmium and copper, the population showed a homogeneous reaction towards these elements, since it had the highest slope values, ranging from 6 to 7.7. In contrast, the larvae reacted in a heterogeneous way towards cobalt, the lowest slope value of about 1.2 having been found in this case.

The reaction of the larvae towards the other three elements, i.e. mercury, chromium and lead, was in turn compared with the previous

two cases. This may indicate a difference in genetic system responsible for the reaction against the metals.The larval period was also affected by adding the metals to the diet. Arsenic, copper and lead had an affect on reducing the larval period below the normal level. The other four elements, i.e. copper, cadmium, cobalt and chromium, caused a considerable lengthening of the larval period.

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الملخص العربي

سمية بعض العناصر الثقيلة للدروسوفيلا ميلانوجاستر

اجرى هذا البحث لدراسة مدى سمية بعض العناصر الثقيلة وتأثيرها على بعض صفات الموائمة في حشره الدروسوفيلا ميلانوجاستر. اختبر هذا البحث تأثير سبعة عناصر ثقيلة هي الزرنيخ الزئبق- النحاس- الكوبلت- الكروميوم والرصاص وذلك بالنسبة للتركيز الذي يؤدي إلى موت ٥٠% من اليرقات (LC_{50}) الحيوية وطول مدة تطور اليرقات.

استخدمت عشيرة طبيعية من ذبابه الدروسوفيلا جمعت من منطقة مطروح على بعد حوالي ٣٥٠ كم غرب الإسكندرية. اوضحت النتائج المتحصل عليها ان تربية اليرقات على بيئة تحتوي احد هذه العناصر له تأثيرات مختلفة على كل من الحيوية وطول فترة التطور وذلك تبعاً للتركيز المستخدم. ولقد وجد ان كل من الزرنيخ والزئبق والرصاص لهم تأثير على تقصير مدة التطور بينهما النحاس والكادميوم والكوبلت لهم تأثير على تطويل مدة التطور وذلك بالمقارنة بالأفراد المرباه على بيئة غذائية خالية من أي من هذه العناصر. أما بالنسبه لدرجة السمية فقد قسمت هذه العناصر إلى ثلاث مجاميع حسب نتائج ال LC_{50} المتحصل عليها. المجموعة الأولى وهى شديدة السميه وتشمل الزرنيخ والزئبق ويتراوح ال LC_{50} لهما ما بين ٢٢- ٢٥ جزء في المليون. والمجموعة الثانية وهى متوسطة السميه وتشمل النحاس والكادميوم ويتراوح ال LC_{50} لهما ما بين ٣٠-٧٢ جزء في المليون والمجموعه الثالثة وهى الأقل سميه وتشمل الكوبلت والكروميوم والرصاص ويتراوح ال LC_{50} لهم مابين ٢٦١- ٥٧٤ جزء في المليون. كما لوحظ ان اضافة النحاس إلى الزرنيخ او الزئبق له تأثير مختلف في كل حالة بالنسبة للحيوية بالرغم من أن الزرنيخ والزئبق لهما نفس مستوى السميه.