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TOXICOLOGICAL STUDIES ON THE LONG TERM EFFECTS OF HEAVY METALS (PB AND CD) TREATED SOIL ON THE DEVELOPMENT AND REPRODUCTIVE CAPACITY OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA* (FORSKAL.).

Ibrahim, N. M.; Othman K. S.; Rodina A. Hassan and Yasmin E. Abdelmobdy.

Department of Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

Influence of heavy metals (Pb and Cd) treated soil on the development and reproductive capacity of S. *gregaria* was studied. Oviposition rate was not affected at any soil concentrations of the metals 50-500 Pb(NO2)3 and 10-100ppm CdCl2 used, laying females were evidently unable to distinguish between treated and untreated soil. The hatchability rate of the egg laid in heavy metals treated soil was reduced compared to eggs laid in untreated soil.

High concentrations of heavy metals caused an increase in the duration of immature stages resulting from eggs laid in treated soil. Emerged adults were lighter and their life span were also shorter as compared with those in control.

Adult emerged from egg laid in treated soil revealed a higher accumulation of the Pb and Cd compared to the untreated control. It could be stated that the accumulation of metals content in the different body parts and most of the found in the testes, was affected by the level and the type of metals in the soil.

INTRODUCTION

The heavy metals in the environment cause a real problem, because in addition to their acute toxicity which may be lethal, it may cause an insidious chronic disease of serious character developing over a number of generations or years (Schmidt et al. 1991). Heavy metals can affect the organisms as toxic substances in soil, water and sediment or as a toxicant in the food chain (Zyadah and Abdel- Baky, 2000).

A correlation between the chronic ingestion of heavy metals and the reduction of reproduction has been suspected since antiquity. It was even proposed as a factor in the decline of the Roman Empire (Gilfillan, 1965).

Less attention has been paid to the biological effects of such metals on vital organs in insects. A variety of approaches may be taken into consideration to examine the acute or chronic effects of these metals. These range from reproductive success to lifespan and include such factors as growth adaptation to environmental stress and interaction with cellular components causing functional changes in some vital organs (Ibrahim and El-Sayed, 1997). The biological impact of heavy metals on insects has been extensively studied both in nature and in the laboratory. Insects are sensitive bioreporters of heavy metal contamination because exposure also occurs during critical stages of insect development such as embryogenesis, larval

development, and pupation. In addition to mortality, exposure of insects to heavy metals can result in changes in locomotion, behavior, oviposition, and mating (Fargasova, 2001).

The objective of this study was to determine the influence of heavy metals in soil on the development, reproduction and viability of the desert locust *Schistocerca gregaria* (Froskel.) (Orthopetra, Acrididae), which can be used as an indicator to environmental pollution. Nymphal duration, adult body weight, reproduction ability, mortality percentage and other studies were taken into consideration. Moreover, the distribution of heavy metal was determined in the insect eggs and various organs. Some of these tests can be used to assess the magnitude of environmental pollution by chemicals, particularly in soil.

MATERIALS AND METHODS:

The desert locust *S. greigaria* were reared under normal condition in wooden cages measured (60cm long x 40cm wide x 50cm high) with a wire in the front for observation purpose and ventilation, in a room temperature of 32 $\pm$ 2 °C and humidity ranged between 50% -70%. The insects have been reared and handled to satisfy the crowded breeding conditions described by Hunter-Jones (1966). The laying media for ovipostion was six plastic pots for each experiment. Each pot was filled up with 450gm dried sand. One of them was used as a control without any treatment, the others were treated by different concentrations of heavy metal salts (lead nitrate and cadmium chloride) which was dissolved in added water 50ml to give 500 gm wet test substrate.

Twenty adult females four weeks old were kept with males in the laying cage. Each experimental series lasted 14 days for oviposition.

Water content of the substrate was controlled daily, any loss being compensated by adding pure water. The position of the pots was changed in a planned sequence, thus, no box was favored by a higher temperature and mass laying.

After two weeks, the deposited eggs were incubated in incubator at 30$\pm$2 °C. Hatching larva was checked daily for 20-25 days and recorded (Schmidt *et al.*, 1991). After hatching the larval number under each concentration was counted and set up till the next ovipostion in a separate cage in which the nymphal survival and other toxicological observations were registered.

After the incubation period the egg pods of non hatched eggs were investigated by dipping the pots containing the egg-pods in water, sand and the foams of the egg pods were removed, then the remaining egg-mass become visible.

Analysis and determination of heavy metals accumulated in insect tissues and egg samples were carried out after ashing using atomic absorption spectrophotometer model (929), according to A.O.A.C. report (1990).

Data obtained were statistically analyzed according to procedures outlined by Gomez and Gomez (1984) using MSTAT-C (1989) computer program. The differences among treatment means were compared by Least Significant Differences test (L.S.D) at 0.05 level of probability.

RESULTS AND DISCUSSION

Data in Tables (1 and 2) show the effect of lead nitrate and cadmium chloride treated soil on the egg pods numbers, hatchability and mortality rates in nymphs of *S. gregaria.*

Table (1): Effect of lead nitrate and cadmium chloride treated soil on egg-pod numbers and hatchability rate of desert locust, *S . gregaria.*

Treatment (ppm)	Pb(NO2)3		CdCl2	
	No.of egg pods (M)	% of Hatchability	No.of egg pods (M)	% of Hatchability
Control	4.750	91.165	6.000	92.795
10	----	----	5.750	65.378
30	----	----	5.500	55.960
50	5.000	77.810	5.500	44.802
100	4.750	69.878	5.250	36.947
250	5.250	60.740	----	----
500	4.250	55.978	----	----
L.S.D	N.S	2.049	N.S	4.606

The results in table (1) show the effect of heavy metal treated soil on the mean pod numbers laid by twenty females through two weeks and their hatchability percentage. At heavy metals treated soil concentration, number of egg pods was approximately equal and no pronounced differences in the mean pod numbers between control and treated could be observed. It could be seen that, the laying females were evidently unable to distinguish between treated and untreated soil.

This finding is similar to that described by Devkota and Schmidt (1999 b). They reported that treating the egg-laying substrate of *Eyprepocnemis plorans* with mercury (Hg2+) dose not cause any significant effect on the number of egg pods laid by females at all the soil concentrations.

The hatchability percentages of *S . gregaria* eggs in the different soil concentration of heavy metal given in table (1) show that hatchability rates at the five concentrations of both (Pb and Cd) used, decreased with increasing concentrations in the soil. The reduction of hatchability at the two higher concentrations of (250 and 500ppm) Pb and (50 and100ppm) Cd was found highly significant. It is apparent that exposure during oviposition reduces and prevents the complete development of the embryos.

Similar results were found by Devkota and Schmidt (1999 a). They reported that the hatchability rates of the two grasshoppers, *Aiolopus thalassinus* and *E. plorans* were affected by mixing each of (Hg2+, Cd2+, Pb2+) with the

ovipositional substrate. This effect varied from a little reduction in nymphal hatching with some concentrations until almost no hatching occurred in the case of *A. thalassinus* eggs.

Emerged nymphs from eggs layed in heavy metal treated soil were reared in separate cages, so that the toxicological effects could be observed. The morta'ity percentages of nymphs developing from eggs deposited in treated soii are given in table (2), it can be seen that the heavy metals had a slight influence on the nymphal stage, the mortality increased gradually from the control (38.730 Pb and 37.605 Cd) till the highest concentrations (49.075 Pb and 47.500 Cd).

Table (2): Mortality percentages of *S . gregaria* nymphal stages resulted from egg pods laid in lead and cadmium treated soil.

Treatment (ppm)	Pb(NO2)3	CdCl2
	% Mortality in of nymphs	% Mortality in of nymphs
Control	38.730	37.605
10	----	42.290
30	----	43.542
50	42.290	45.105
100	44.662	47.500
250	47.383	----
500	49.075	----
L.S.D	2.684	2.164

In a similar experiment, Nascarella *et al.* (2003) stated that higher doses of cadmium inhibited pupation success of blowfly larvae *Calliphora erythrocephala* and died in the rearing box, also cadmium at all exposure levels adversely affected the emergence of the adult fly from the pupal case.

Data in Table (3) show that the nymphal duration was found to be prolonged in the hoppers resulting from the treated soil, especially in the highest concentrations, the duration increased by 25 days and 19 days in Pb and Cd treatments over the control. It is evident that metals in soil, affect the nymphal duration and it is suggested that such metals can affect not only the embryonic development but also the post-embryonic stages (nymphs and adults). Similarly Cervera *et al.* (2004) found that the duration of the nymphal stages of *Oncopeltus fasciatus* increased when exposed to various concentrations of CdCl2. At the lowest Cd concentration the median duration was significantly prolonged by one day, while at the highest concentration it was increased by 10 days over the control group. They showed that development and reproduction of *O. fasciatus* are seriously impaired at sub lethal Cd concentrations.

The data in table (4) indicate the influence of heavy metals on the adult body weight. After emergence, adults were removed, sexed and individually weighed. The mean adult fresh weight had been taken on the first day after emergence.

Table (3):Effect of lead nitrate and cadmium chloride treated soil on the total nymphal duration of *S. gregaria*.

Treatment (ppm)	Pb(NO2)3	CdCl2
	Total nymphal duration (days)	Total nymphal duration (days)
Control	37.000	39.500
10	----	45.000
30	----	49.250
50	39.750	54.500
100	43.500	58.500
250	60.250	----
500	62.250	----
L.S.D	2.156	2.179

Table (4): Effect of lead nitrate and cadmium chloride treated soil on the adult body weight (female and male) of *S. gregaria* (only the embryogenesis took place in treated soil).

Treatment (ppm)	Pb(NO2)3		CdCl2	
	Female weight (mg)	Male weight(mg)	Female weight (mg)	Male weight(mg)
Control	1463.750	991.000	1443.250	996.000
10	----	----	1124.500	893.750
30	----	----	1027.750	826.750
50	1108.250	895.500	938.250	751.500
100	1013.500	795.500	899.000	660.500
250	989.250	720.000	----	----
500	977.750	698.750	----	----
L.S.D	38.59	13.85	37.14	16.62

It could be seen from table (4) that the females and males emerging from higher concentrations are exclusively weighed much less than the control, and the adult emerged weight from the low concentrations did not show high differences from the control.

These results are in agreement with Schmidt *et al* (1991) who found that the adult fresh body weight of *A. thalassinus* emerging from soil treated with heavy metals (Hg, Cd, Pb) was significantly low during two successive generations.

Table (5) shows the mortality percentages of the adults. The results indicated that the total period of the adults emerged from eggs laid in both heavy metals contaminated soil was generally shorter in the highest concentrations, on the other hand, no remarkable variations were found between the other concentrations and control. The treated adults lifespan of both female and male ranged from 7 to 9 weeks. Control adults were able to live longer than 10 weeks.

Table (5): Effect of lead nitrate and cadmium chloride treated soil on the adult life span of *S. gregaria*.

Treatment (ppm)	Pb(NO2)3		CdCl2	
	Total adult period (days)		Total adult period (days)	
	Female	Male	Female	Male
Control	73.750	69.000	74.500	69.500
10	----	----	71.000	66.500
30	----	----	68.000	62.750
50	72.500	67.750	63.750	57.500
100	71.000	64.750	59.500	53.500
250	63.500	61.250	----	----
500	61.250	57.750	----	----
L.S.D	1.151	1.801	2.096	1.334

Also Devkota and Schmidt (2000) reported that adult of *A. thalassinus* emerged from egg-laying substrate contaminated with (Hg, Pb) had significantly shorter life spans in comparison to the control adult.

The results in Tables (6 and 7) show the determination of the heavy metal content of various tissues of *S. greigaria*. The adults resulting from eggs laid in treated soil, three weeks old after emergence were taken for chemical analysis. The results indicate that there was a remarkable accumulation of heavy metals (Pb and Cd) in the adults body resulting from eggs laid in treated soil.

Table (6): Lead contents (ppm dry weight) found in the different organs of three. weeks old adults resulting from eggs laid in lead chloride treated soil.

	Metal contents in different organs					
Treatment (ppm) (Pb)	Malpighian tubules	Eggs	head	testes	ovary	Eggs of F1 generation
Control	0.082	0.067	0.057	0.042	0.068	0.055
50	0.180	2.647	0.197	0.938	0.238	1.113
100	0.303	3.353	0.290	1.522	0.295	1.365
250	0.438	11.453	0.330	1.930	0.355	1.522
500	1.022	18.965	0.410	2.397	0.520	2.080
L.S.D	0.004872	1.855	0.006890	0.7484	0.01089	0.04872

Table (7): Cadmium contents (ppm dry weight) found in the different organs of three weeks old adults resulting from eggs laid in cadmium chloride treated soil.

	Metal contents in different organs					
Treatment (ppm) (Cd)	Malpighian tubules	Eggs	head	tests	ovary	Eggs of F1 generation
Control	0.063	0.040	0.047	0.040	0.052	0.047
10	0.137	1.657	0.103	0.572	0.193	0.878
30	0.297	2.887	0.158	0.865	0.217	1.730
50	0.505	5.250	0.210	1.243	0.242	2.075
100	0.900	8.990	0.358	2.120	0.320	2.750
L.S.D	0.04872	0.3479	0.04872	0.06890	0.04872	0.06890

The heavy metals used exhibited the highest accumulation in eggs layed in treated soil followed by adult testes and Malpighian tubules. There was a big increase in lead content in all tissues of the contaminated adults in comparison to control ones. The testes showed high accumulation.
The amounts of metals found in F1 eggs are presented in the same tables. It is evident that the metals had the ability to be transferred to eggs laid in untreated soil through the parent females.
These findings are in agreement with consistent with Schmidt and Ibrahim (1994); they found that the highest concentration of Pb2+ in testes, gut and ovaries of *A. thalassinus* exposed to PbCl2 treated soil or diet. The accumulation of heavy metals in head of *A. thalassinus* was reported by Ibrahim (1988). The metal contents of eggs laid in heavy metal treated soil of *A. thalassinus* and laid by adult emerging of heavy metals treated were stated by Schmidt *et al* (1991).

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دراسات سمية على تاثيرات المعادن الثقيلة(الرصاص والكاديوم) على نمو وتكاثرالجراد الصحراوى

نبيل محفوظ إبراهيم – كمال سيد احمد عثمان - ردينه احمد عبد الجواد و ياسمين امام عبد المبدىء

قسم المبيدات – كلية الزراعه - - جامعة القاهره - الجيزه

تتعرض البيئة التي نعيش فيها إلى العديد من مصادر التلوث و يعتبر التلوث بالمعادن الثقيلة و المبيدات من أهم مشكلات التلوث البيئي و خاصة في العقود الستة الأخيرة نظرا لإزدياد الصناعات الكيميائية بالإضافة إلى استخدام مركبات الرصاص العضوية في الخلط مع البنزين المستخدم كوقود للسيارات.

و لقد تعرض الكثير من الباحثين لمشكلة التلوث بالمعادن الثقيلة، و تعتبر التربة هي المخزن الرئيسي لمعظم هذه الملوثات، فهي تحتوى تحت الظروف الطبيعية على تلك المعادن كجزء من تركيبها الجيولوجي و لكن مع توسع الإنسان في المشروعات الزراعية و الصناعية أدى ذلك إلى زيادة تركيزات هذه المعادن في التربة أو تلوث الأجزاء الخضرية المعرضة للهواء أو امتصاصها من التربة الملوثة ثم وصول هذا التلوث إلى الحيوان و الإنسان.

و جدير بالذكر أنه في دراسات تراكم الملوثات في الكائنات الحية بصفة عامة و المعادن الثقيلة بصفة خاصة لم تنل الحشرات القسط الوافر من البحث مما قد يسبب نقص كبير في المعلومات حيث أن الحشرات تمثل جزء هام من السلسة الغذائية التي بها الطيور و الحيوانات بما فيها الإنسان على قمة هذه السلسلة و بالتالي يعتبر دراسة تراكم الملوثات فى الحشرات ذات أهمية كبيرة عند تقدير تلك الملوثات فى البيئة و التى منها المعادن الثقيلة.

تهدف الدراسة إلى تقييم التأثير الحاد و المزمن لبعض المعادن الثقيلة مثل نترات الرصاص Pb(NO2)3 و كلوريد الكادميوم CdCl2 على حشرة الجراد الصحراوي (*Schistocerca gregaria*) و ذلك من خلال معاملة التربة بتلك المعادن و تأثيرها على الكفاءة الحيوية و التناسلية للحشرة، حيث يمكن أن تعطينا مؤشر لما يحدث في الكائنات الأرقى كالإنسان و الحيوان.

■ **يمكن تلخيص النتائج المتحصل عليها فيما يلى:**

□ **التأثير على كفاءة و صلاحية البيض:**

١- لم يتأثر معدل وضع البيض فى التربة المعاملة بكل من الرصاص و الكادميوم على مستوى جميع التركيزات، حيث فشلت الإناث فى التفريق بين التربة المعاملة و غير المعاملة كبيئة لوضع البيض و كان عدد كتل البيض تقريباً متشابه.

٢- انخفض معدل الفقس بصورة معنوية فى حالة الكتل الموضوعة فى التربة المعاملة عن التربة الغير معاملة.

□ **التأثير الطويل للمعادن الثقيلة على العمر الحورى الناتج من التربة المعاملة:**

١- لم تلاحظ أى زيادة معنوية فى نسب الموت للحوريات الناتجة من تربة معاملة بالرصاص أو الكادميوم.

٢- لوحظ طول مدة عمر الحورية فى جميع المعاملات الناتجة من تربة معاملة، حيث كانت الزيادة فى طول عمر الحورية تدريجيه بزيادة التركيز.

□ **التأثير طويل المدى للمعادن الثقيلة على طور الحشرة الكاملة:**

١- انخفض وزن كل من الذكور و الإناث فى جميع المعاملات لجميع التركيزات، وكان الإنخفاض تدريجى بزيادة التركيز.

٢- قصر طول عمر الحشرة الكاملة فى جميع المعاملات (رصاص – كادميوم)، فعلى سبيل المثال طول عمر الحشرة الكاملة الناتجة من تربة غير معاملة تراوح ما بين ١٠ – ١١ أسبوع فى الإناث و من ٨ – ٩ أسابيع فى الذكور اما الحشرات الناتجة من تربة معاملة فطول العمر تراوح ما بين ٨ – ٩ أسابيع فى الإناث و من ٦ – ٧ أسابيع فى الذكور.

□ **تقدير تركيز الرصاص و الكادميوم فى الإنسجة المختلفة للطور الكامل:**

تم التقدير فى انسجة و أعضاء الحشرات الكاملة الناتجة من البيض الموضوع فى تربة معاملة و كانت النتائج متقاربة الى حدا كبير، فقد لوحظ تراكم معنوى فى جميع الأنسجة و الأعضاء و خاصتا الخصية، حيث احتوت على ٢,٣٩ ppm و ٢,١٢ لكل من الرصاص و الكادميوم على الترتيب ، مقارنة ٠,٠٤٠ppm – ٠,٠٤٢ للخصية الناتجة من الحشرات غير المعاملة.

كما تم التقدير تركيز الرصاص و الكادميوم فى كل من البيض الموضوع فى تربة معاملة و غير معاملة، و قد أظهرت النتائج ان مكونات المعادن الثقيلة فى البيض الذى وضع فى تربة معاملة تزايد تدريجيا مع زيادة تركيز المعادن فى التربة، و كذالك لوحظ وجود تلك المعادن بكمية اقل فى البيض الذى وضع بواسطة الجيل الأول فى تربة غير معاملة و بكميات ايضا تتناسب مع الكميات الأولية مما يؤكد قدرة المعادن على الإنتقال الى البيض خلال الاباء المحملة بالمعادن الثقيلة.