Cairo University
Faculty of Science

Induction and development of heat shock proteins expressed as a cellular response of *Schistocerca gregaria* to heavy metal intoxication.

A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE (M.Sc.)

IN

ENTOMOLOGY

Presented by

Amira Afify Abdel-Hamed El-Menoufy

B.Sc., Fac. Sci., Cairo Univ., 2005

Entomology Department

Faculty of Science, Cairo University

2009

APPROVAL SHEET

Induction and development of heat shock proteins expressed as a cellular response of *Schistocerca gregaria* to heavy metal intoxication.

A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE (M.Sc.)

IN

ENTOMOLOGY AT THE CAIRO UNIVERSITY, EGYPT

Presented by

Amira Afify Abdel-Hamed El-Menoufy

B.Sc., Fac. Sci., Cairo Univ., 2005

Supervision Committee:

Prof. Dr. Afaf Abdel-Meguid

Professor of Entomology

Department of Entomology

Faculty of Science, Cairo University.

Prof. Dr. Hesham Ahmad Yousef

Professor of Insect Biochemistry

Department of Entomology

Faculty of Science, Cairo University.

Prof. Dr. Hany Mohamed Hassan

Professor of Immunology

Animal Reproduction Institute, Giza, Egypt.

Prof. Dr. Naeem M. Ali Eesa

Head of Entomology Department

Dedication

This work is dedicated to my charming parents

and my dearest husband

Acknowledgements

First of all, praise is to **Allah**, the Mighty, and the Merciful for all of **His** graces and for the completion of this work. I would have never ever done anything without **His** support and guidance.

I appreciate Prof. Dr. Afaf Abdel-Meguid, (Entomology department, Faculty of Science, Cairo University) for her supervision, encouragement and their valuable advices.

I wish to express my sincere gratitude to my supervisor, Prof. Dr. Hesham Ahmed Yousef, (Entomology department, Faculty of Science, Cairo University) for his Supervision, invaluable comments, patience, encouragement, sharing his inspiring ideas and guidance during the whole work.

I have no words to describe my deep and sincere appreciation to Prof. Dr. Hany Hassan, (Immunology department, Animal reproduction Institute, Giza, Egypt) for his Supervision, and helping me in the technical and practical study. I want to express my deepest thanks to the members of laboratory of Immunology, Animal reproduction Institute, Giza, Egypt, Dr. Abeer Mohamed Anwar for her kind help in comet assay and electrophoresis techniques and Dr. Salama Abdel-Hafez, for his help in ELISA technique and his support during this study. I had the privilege to work with them and their support is greatly acknowledged.

My grateful acknowledgement and thanks to the members of Entomology department, Faculty of Science, Cairo University.

Last, but not least**, my wonderful parents:** there are no words that I can use to pay you back all what you did for us, your understanding, patience and emotional support during my work. A well-deserved thank you also goes out to my sisters and my brother for all the support and love I got from them.

Contents

Introduction

Introduction

Heavy metals are among the most problematic causes of water pollution and consequently the soil and plants use this water. Since household waste also contains heavy metals, the added danger exists that heavy metals will enter the ground water and surface water through sewage from household waste, and a number of industries emit them. The heavy metals that reach the water are relatively quickly diluted either they decompose into carbonates, sulphates and sulphides, or mineral and organic sediment adsorbs them (Fellenberg, 1999).

Many studies have been conducted recently to investigate the genetic and biochemical effects of pollution on organisms and to establish species that may be considered as bioindicators for environmental hazards (Bolognesi and Roggieri,1996; Michailova *et al*., 2000a,b). In water pollution, the Chironomidae (Diptera) have been proposed as particularly suitable biological indicators of pollution (Warwick, 1990). Heavy metals have been found to induce genotoxic effects in chironomids (Michailova *et al*., 2001).

In the case of soil pollution, terrestrial insects, including orthopteran species, may provide good systems to evaluate the mutagenic effects of some environmental contaminants (Warchałowska-Śliwa and Maryańska-Nadachowska, 1991; Barsiene, 1994; Barsyte, 1997;1999). Some of these species may provide suitable bioindicators for heavy metal contamination (Schmidt, 1986; Hunter *et al*., 1987; Devkota and Schmidt, 2000; Warchałowska-Śliwa *et al*., 2005)

Cadmium is a widespread heavy metal, released into the environment by power stations, heating systems, waste incinerators, and metal working industries and from many other sources. Accumulation of cadmium in soils constitutes a remarkable danger to all kinds of organisms, including plants. Inconsistent results have been obtained with

respect to the genotoxic properties of cadmium (Koppen and Verschaeve, 1996; Steinkellner *et al*, 1998; Panda and Panda, 2002).

Cadmium is one of the most dangerous heavy metals on the human life. Its biological half-life is 10 years or more and its accumulation capacity is great. Cadmium ions are taken up through voltage-gated calcium channels in the plasma membrane of various cell types and accumulate intracellularly by binding to cytosolic and nuclear material (Beyersmann and Hechtenberg, 1997). The accumulative properties of cadmium induce varieties of effects and responses. The main toxicological symptoms of cadmium are painful shrinkage of the skeleton, anemia and renal failure. Cadmium can take an entirely different route to enter the food chains. It can be a substitute for the zinc in enzymes with a zinc content (hydroxylases) leading to loss of the enzyme effects. The carriers of the enzymes can become food for other living organisms, so that the cadmium slips into the food chain (Fellenberg, 1999).

Lead is highly toxic and is also considered a propable carcinogen. Lead poisoning has been recognized for many years. It can enter drinking water from solder used for connecting copper pipes. Most of the tetraethyl lead contained in gasoline is expelled to the atmosphere as lead oxide (Sawyer *et al*., 1994). Clinical, epidemiological, and toxicological studies have demonstrated that lead exposure can adversely affect human health. The most sensitive systems in the human body to lead are the hemobiotic and renal systems. The high blood levels of lead can inhibit enzymatic actions, alter physical and mental development, interfere with growing, decrease attention span and hearing, and interfere with children growth. In older men and women, lead can increase blood pressure (Arcadio and Gregoria, 2002). Pb^{2+}can enter the bones suppresses the synthesis of blood, inhibiting the enzyme 5-aminolevulin-acid dehydrate. This causes the well-known lead anemia, with very painful bone deformations, which is the reason for calling it the “Itai-Itai sickness”, since Itai-Itai is the approximate equivalent of “ouch-ouch” as acry of pain (Fellenberg, 1999).

Changes in the cell genome, caused by genotoxic agents leading to mutations are some of the lethal or sub-lethal effects induced by a complex mixture of pollutants. Agents that damage DNA are difficult to detect in epidemiological studies because their effects take a long time to appear. Therefore, they must be effectively and continuously monitored (Arnaiz, 1997). The mechanisms of DNA damage have not yet been recognized. Among recently used methods, the comet assay (SCGE -single cell gel electrophoresis) is often used in measurements of the level of DNA strand breaks in individual eukaryotic cells as a rapid, sensitive and inexpensive method (McKelvey-Martin *et al.,* 1993; Rojas *et al.,* 1999). Despite some difficulties in obtaining cell/nuclei suspension, this method has been frequently used to evaluate DNA damage in vertebrates, especially in human cells (Woźniak and Blasiak, 2003; Banu *et al.,* 2004) It has been successfully applied to bivalves, polychaetes, crustaceans, but seldom to insects. Only few studies were reported on DNA damage in *Drosophila melanogaster* (Mukhopadhyay *et al.,* 2004; Siddique *et al*., 2005), and one on studies in the weevil *Curculio sikkimensis* (Todoriki *et al*., 2006).

Bearing in mind usefulness of insects in biomonitoring studies the adaptation of comet assay to assess DNA damage in this group is important for comparative studies. The assessment of genotoxicological effects of heavy metals is more complete if we associate them with the information of the elements' contents and distribution in tissues, organs or the whole body. Previous experiments using comet assay and measuring DNA strand breaks indicated a slight genotoxic potential both *in vitro* and *in vivo* in rat and human cells (Delincée and Pool-Zobel, 1998; Delincée *et al.*, 1999).The Comet Assay can be used to detect DNA damage caused by double strand breaks, single strand breaks, alkali labile sites, oxidative base damage, and DNA cross-linking with DNA or protein, also the comet assay is used to monitor DNA repair by living cells (Collins, 2004).

Insects, which develop partly in the soil, are also exposed directly to metal ions present in the soil water. Among them are many grasshopper species which lay egg-pods directly in the soil.

Cells from bacteria and yeast to human respond to a variety of stresses by the rapid synthesis of a highly conserved set of polypeptides termed heat shock proteins (Hsps). They are present in the cytosol, mitochondria, endoplasmic reticulum, and nucleus, although these locations vary depending on the particular protein (Kregel, 2002).

Heat shock proteins were first discovered in *Drosophila* as a set of proteins induced by heat shock. The genes coding for these proteins were discovered as chromosome puffs in *Drosophila* after exposure to high temperatures, hence the name heat shock (Ritossa, 1962). Heat shock genes are a subset of a larger group of genes coding for molecular chaperones, i.e. proteins that are involved in 'house-keeping' functions in the cell (Sørensen *et al*., 2003). The molecular weights of Hsps range from ~15 to 110 KDa, and are categorized into families on the basis of their molecular weights (Tissieres *et al*., 1974; Welch, 1993).The most well-known are those which belong to the families of Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and the small Hsps (below 30KDa), and smaller co-factors (Parsell and Lindquist, 1993; Feder and Hofmann, 1999).

Exposure of organisms to different environmental pollutants has been found to induce a variety of stress response including the heat shock protein 70 (Hassanein, 1999; Schroder *et al*., 1999). Involvement of stress inducible Hsps in stress resistance has been documented and is reviewed in a number of papers (Lindquist, 1986; Feder and Hofmann, 1999). A number of investigations have confirmed the importance of Hsp in resistance towards heat and cold and a range of other stresses including insecticides, heavy metals, desiccation, diseases, parasites and inbreeding (Steinert and Pickwell 1993; Matz *et al*., 1996; Wong *et al*., 1996; Su and Gordon, 1997).

The present work aimed to detect the drastic effect of the environmental pollutant heavy metals cadmium and lead in *S. gregaria*. This includes estimation of expression profile of the heat shock proteins, with special reference to the Hsp 70. A preceding step of the effect of these heavy metals, DNA damage was also traced. The putative data are possibly used for assessment (as biomarker) of environmental pollution level.

Materials and Methodes

Materials and Methods

1. Colonization of *Schistocerca gregaria*:

Locusts were reared above oviposition tubes (sand containers). Heat and humidity were adjusted in the cages according to season so as to provide a constant day-time temperature of 34°C and a night time temperature of 28°C with a relative humidity of 50-60% at night and 30-50% at the day-time. Humidity was cut to a minimum or enough of it was supplied by the fresh vegetation used as food.

A daily supply of fresh clover is required. Suitable glass containers of about 9 cm in diameter and 12 cm deep are essential for egg-laying. They are full of tightly packed and moist and sterilized sand. Moisture is achieved by adding 15 parts of water to 100 parts by volume of dry sand. The containers should be changed from one to three days and replaced by fresh ones whether eggs have been laid or not. Cages must be regularly cleaned to maintain a healthy culture.

2. Heavy metals treatment:

Stock solutions of different concentrations of heavy metals (Cd^{+2} and Pb^{+2}) were prepared by dissolving 25 and 50 mg of $CdCl_2$ and $PbCl_2$ in one liter of double distilled water. Use 200 ml of each of stock solutions for only one treatment in different containers and immerse the roots of 250 gm of clover in each container for 24 h before feeding the different cages of locusts. The long term exposed insects were fed on treated clover at the newly hatched 1^{st} nymphal instar. The short term exposure; experimental stages were fed on treated clover 4 days before collecting them for sample preparation.

3. Sample preparation for alkaline single cell gel (SCG) assay:

Hemolymph samples were withdrawn from incision made near the hind coxae of the insects, using micropipette. 10 µl was taken from a pool of hemolymph from at least 4 individuals for each slide.

4. Detection of DNA damage using alkaline single cell gel (SCG) assay:

Biochemical techniques for detecting DNA single strand breaks, alkali-labile sites, and cross-linking with the single cell were done according to the alkaline (pH>13) SCG assay by Klaude *et al.* (1996) and Singh *et al.* (1998).

Reagents:

PBS (Ca^{2+}, Mg^{2+} free): 100 ml 10X stock:-

NaCl	8 gm
KCl	0.2 gm
Na_2HPO4	1.44 gm
KH_2PO4	0.24 gm

Dissolve and complete to 100 ml by dist. water. Adjust the pH of the used 1X PBS to 7.4.

Lysing Solution: Ingredients per 1000 ml:

2.5 M NaCl	146 gm
100 mM EDTA-	37 gm
10 mM Trizma base	1 gm