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PHYSIOLOGICAL ALTERATIONS IN HEMOLYMPH OF BLAPS SULCATA BEETLE AS AN INDICATOR OF ENVIRONMENTAL POLLUTION EFFECTS

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ABSTRACT

Environmental pollution of air, water, soil and food became a threat for the sustainability of several plant and animal communities and may ultimately threaten the human survival. The present study was carried out to evaluate the effect(s) of some pollutants, namely, Cadmium, Cd; copper; Cu; Iron, Fe, and Lead, Pb, in soil of two different localities on some physiological parameters (Glucose, total protein, total lipid, phospholipid, uric acid (u.a.), cholinesterase, acid phosphatase (ACP), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) in the hemolymph of the beetle *Blaps sulcata*.

The results obtained in the present study showed that location "M" (Moharam Bey botanival garden at the Faculty of Science, Alexandria University, Egypt) could be considered as polluted as compared to the location "R" (reference site at Burg El-Arab, 50Km west of Alexandria). In soil, levels of Fe, Pb, Cu and Cd were significantly elevated in location "M" as compared to the location "R". In addition, the concentrations of all measured parameters in hemolymph of *B. sulcata* collected from "M" site were found to be lower than reference site this could be due to an increased the previously mentioned pollutants.

KEY WORDS: pollutants; Blaps sulctata; insect physiology.

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INTRODUCTION AND REVIEW

Pollution is essentially wrong substance, in the wrong place, in the wrong concentration, at the wrong time. More formally, pollution can be defined as the introduction of human-made substances (or natural substances released by humans) and forms of energy into the environment that are likely to damage ecosystems or their constituents, amenities, or structures, (Paijo 2010).

Heavy metal ions are natural components of Earth's crust. Their content in soil varies from very low (Femtograms) to high (milligrams). However due to anthropogenic activities their content can be elevated at the site of the action. High concentrations of heavy metal ions can injure human health and pollute the environment. It is a common knowledge that toxic heavy metal ions as Lead and cadmium are able to enter organisms and interfere with several important metabolic processes, (Krystofova et al. 2010).

Insects, like other living organisms, are affected by pollution. However, insects are also used to assess the effects of pollution as surrogates or representatives of the larger assemblages of organisms in communities and ecosystems. We refer to insects in this latter role as bio-monitoring agents since they present a number of advantages: 1- They are ubiquitous, so they are exposed to pollution in many different habitats. 2- The large number of species offers a range of responses. 3- The sedentary nature of many insects allows spatial analysis of pollutant effects. 4- Their long life cycles allow temporal analysis of pollutant effects. Unlike relying on instantaneous measurement of physical and chemical variables, the use of living organisms, like insects, provides a temporal integration of pollutant effects over their life span, (Paijo 2010).

The effects of pollution on insects occur at a variety of spatial and temporal scales. For example, effects can occur at the molecular level in fractions of seconds (i.e., biochemical effects) and at the ecosystem level over several decades, and at various scales in between these extremes, (Paijo 2010).

Food-borne heavy metal intoxication is mostly limited to long-term consumption of water and food products from environments that contain high levels of metals such as fluoride or that are contaminated by mining, smelting, and industrial discharge, e.g. mercury, lead, cadmium, copper and zinc (Schmidt et al. 1991). Eisler (1988) reported that toxicity of lead is influenced by biotic species, age of the organisms, and length of exposure. Philip et al. (2007) reported that organic lead compounds are fat-soluble and are more toxic than other forms.

Metals bound to membranes are likely to modify membranes' function, for instance inhibiting trans-membrane transport of electrolytes, sugars, amino acids and other solutes (Shainskaya *et al.,* 2000). The toxic outcomes cover virtually every adverse effect from the cellular to the whole animal level, where multi-organ toxicity was made by metals (Timbrell 2000; Archer *et al.,* 2001). Several human inheritable diseases are related to imbalance in metal metabolisms (Walker *et al.,* 2001). Proteins are major targets of damage caused by metals, (Chao and Yang 2001). Metals may act as mutagens and carcinogens, modifying the structure of DNA or interfering with the transcription processes. Moreover, toxic metals can displace essential metals acting as cofactors of enzymes or supporting key structures in the cytoskeleton (Chen and Shi 2002). Metals can block functional sites through binding to sulfhydryl groups. Heavy metals can also increase the acidity of the blood (Ostrovskii *et al.,* 2000; Ejnik *et al.* 2002).

Hemolymph analysis is valuable as a means of evaluating physiological conditions of insects and diagnosing a disease, so this study was made for the purpose of tracing the effects of pollutants (Cadmium, Cd; copper; Cu; Iron, Fe and Lead, Pb) in soil and plant leaves in a certain period and specified locations, on the concentrations of some biochemical constituents (Glucose, total protein, total lipid, phospholipids, uric acid, cholinesterase, acid phosphatase (ACP), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), in hemolymph of *B. sulcata*, as a bio-monitoring insect agent.

MATERIALS AND METHODS

2.1 Insect used:

In the present study *B. sulctata* (Coleoptera: Tenebrionidae) was selected as an experimental model, because it is an abundant beetle species, which inhabits all terrestrial environments, and is available in the different seasons of the year (Tolerant to different environmental stressors). It is herbivorous and variously adapted to feed on the decayed roots, stems or leaves of their host plants. The interrelationship between the insect and its host plant is affected by environmental conditions. The insects selected during winter 2014 (February 22) from the two selected locations.

2.2 Selected locations:

Soil and *B. sulcata* were sampled at two locations: Moharram Bey "M", near the fence of the botanical garden at the Faculty of Science' garden, Alexandria, as polluted area and Burg El Arab "R", which is located in the western Mediterranean desert of Egypt 50 km. west of Alexandria as a reference area (Uncontaminated).

2.3 Determination of metals in soil:

Soil granules were added in a flask or beaker, heating and adding concentrated HNO_3 as necessary was continued until digestion was completed as shown by a light-color clear solution. Sample should not be left drying during digestion. Flask or beaker walls should be washed down with water (Distilled) and then filtered if necessary. Filtrate was transferred to a 100ml volumetric flask with two 5 ml portions of water (Used for washing). These rinsing were added to the volumetric flask, cooled, diluted to mark with distilled water, and mixed thoroughly. Portions of this solution were taken for required metal determinations.

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In flame atomic absorption spectrometry, samples were aspirated into a flame and atomized. A light beam was directed through the flame, into a monochromator, and onto a detector that measured the amount of light absorbed by the atomized element in the flame. For some metals, atomic absorption exhibits superior sensitivity over flame emission. Since each metal has its own characteristic absorption wavelength, a source lamp composed of that element was used. This makes the method relatively free from spectral or radiation interferences. The amount of energy at the characteristic wavelength absorbed in the flame was proportional to the concentration of the element in the sample over a limited concentration range (Henry *et al*., 1974).

2.4 Hemolymph collection:

Hemolymph was collected from the insect according to method reported by Willott *et al.* (1994**)**. By removing one of the insect legs or cutting between 2 legs, the insect was chilled on ice prior to collection. Hemolymph should be stored in the freezer till analysis.

2.5 Biochemical analysis:

Various physiological parameters including: glucose (According to Singh and Singh 1977), total protein (According to Gornall *et al.*, 1949), total lipid (According to Kinght *et al.,* 1972), phospholipids (According to Takeyana, *et al.,* 1977), cholinesterase activity (According to Weber 1966), acid phosphatase activity (According to Abbott and Kaplan 1984), alkaline phosphatase catalytic activity (According to Kind and King 1954), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (According to Reitman and Frankel 1957) were determined in the total hemolymph of 35 females and 35 males of *B. sulctata* from the two selected locations.

Since the hemolymph samples have slight colour so preparation of a blank by adding 2.50 ml of physiological solution to 0.05ml of sample was a must. The absorbance of this blank is red against distilled water and subtracted from the absorbance of the sample.

All data are represented as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by LSD for multiple comparison.

RESULTS AND DISCUSSION

Results deduced from this study shows that location M could be considered as polluted location as manifested by significantly elevated levels of Fe, Cd and Pb Compared to R site (Figure 1).

The significant elevation in levels of Fe at site M could be attributed to the neighbourhood of this location, just few meters away; a local plant (Badawi plant, Moharram Bey) that used to work on manufacturing several metal and metal alloys including iron was located. This plant collapsed leaving a contamination problem with heavy metals. As for the value of Fe found in the R location it is mostly related to the metal trace concentrations naturally occurring in the soil, since it is found in almost all rocks, minerals, soils, plants, and animals (Mckee and Wolf 1963).

Also, Pb in the soil of M location was found to be higher than in the reference site; this could be attributed to being located in big city that is highly exposed to extensive car fumes and other industrial emissions This suggestion agreed with Khakbaz et al. (2012).

The cadmium present in soil of location M could be arise principally from point industrial sources including combustion of fossil fuels, since this location near Badawi plant witch used to work on manufacturing several metal and metal alloys, and from phosphate fertilizers coming from agricultural drainage discharges, since this location is also near botanical garden of Faculty of Science where fertilizers which may contain Cd in their structure are customarily used, Lenntech, (1993). The most important factor for release Cd will be (acidic) exclusion from and/or dissolution of the cation exchange complexes (WHO 2003).

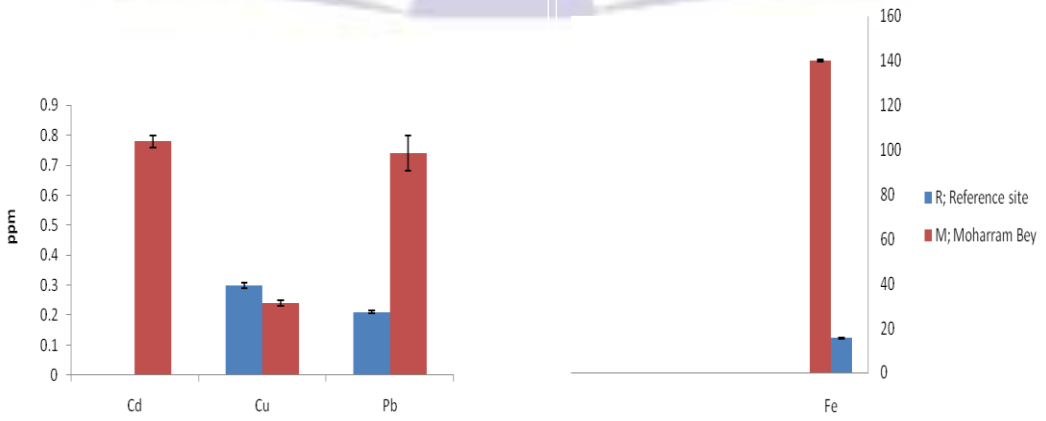

Figure 1: Concentrations of Cd, Cu, Pb and Fe in soil from the selected locations

The results also showed that all the biochemical parameters (Including; Glucose, total protein, total lipid, phospholipids, uric acid, cholinesterase, acid and alkaline phosphatase and ALT and AST) concentrations were lower in hemolymph of insects from M location than from R location, (Figures. 2a, 2b, 3a and 3b).

Since insect's trehalose must be converted into glucose before it can enter the cell so measuring glucose concentration could be an indicator for carbohydrate metabolism in insects (Friedman 1985).

Glucose concentrations in hemolymph of *B. sulcata* from location M were insignificant lower than from R location. The high glucose level from location R could be due to the fact that it is desert so insects may convert fats into carbohydrates; what follows is carbohydrate metabolism to get the energy needed to mediate the effects of stress and to serve as energy buffers during periods of harsh environmental conditions and food shortages. As for the lower concentration from location M that could be because pesticides and some plant extracts act as insecticides which reduced the haemolymph carbohydrates, and location M is a garden. This suggestion agreed with Abo El- Ghar et al. (1995).

The concentration of hemolymph total protein in *B. sulcata* from location M was significantly lower than those in the reference site in response to exposure to pollutants. The present results come in agreement with Assem et al. (1992); Dabrowska – Bouta et al. (1996) and Li et al. (2012) who stated that in response to pesticides, insects might show a significant decrease in hemolymph protein and also reported that the changes in protein are important to indicate the susceptibility of organ systems to pollutants by altering function.

The present results are also confirm the report of Ostrovskii et al. (2000) who reported a decrease in blood serum total protein *in Cyprinus Carpio* after exposure to two non- essential (mercury and lead) and two essential (copper and nickel) heavy metal salts at lethal and subleathel concentrations. Another confirmation for the present data came from Chao and Yang (2001) who found that serum protein can bind normal physiological constituents in the body as well as some foreign compounds (such as heavy metals) leading to total protein reduction.

The hemolymph of *B. sulcata* from location M was found to have lower concentration values of lipid. A possible reason for that could be due to the fact that the absorption of metals in excess disturbs the metabolism of lipids, as suggested by Claudia (1995) and Mayer and Candy (1969).

Generally, there was a direct relationship between phospholipids and total lipid concentrations (as phospholipids are one of the components of total lipid) an it was found that both were less in serum of insects collected from location M than from the reference site and as phospholipids are considered as important elements for the stability of lipid complexes in insect haemolymph so variations in phospholipids concentration could be expected to be of great biological importance. That comes in agreement with Hopf (1940) and Mayer and Candy, (1969).

The concentration of uric acid was found to be lower in serum of *B. sulcata* from location M. This could be related to the accelerated catabolism of total protein and total lipid in serum of *B. sulcata* from location R since both constituents were higher in reference site and lower in location M. This is quite expected since uric acid is one of the degradation products and indicators of carbohydrates, lipid and protein metabolism as reported by Florkin (1958). That could also be due to the fact that insects from location M have enough quantity of water while location R is desert so the insects convert urea to uric acid as mentioned by Wigglesworth (1972).

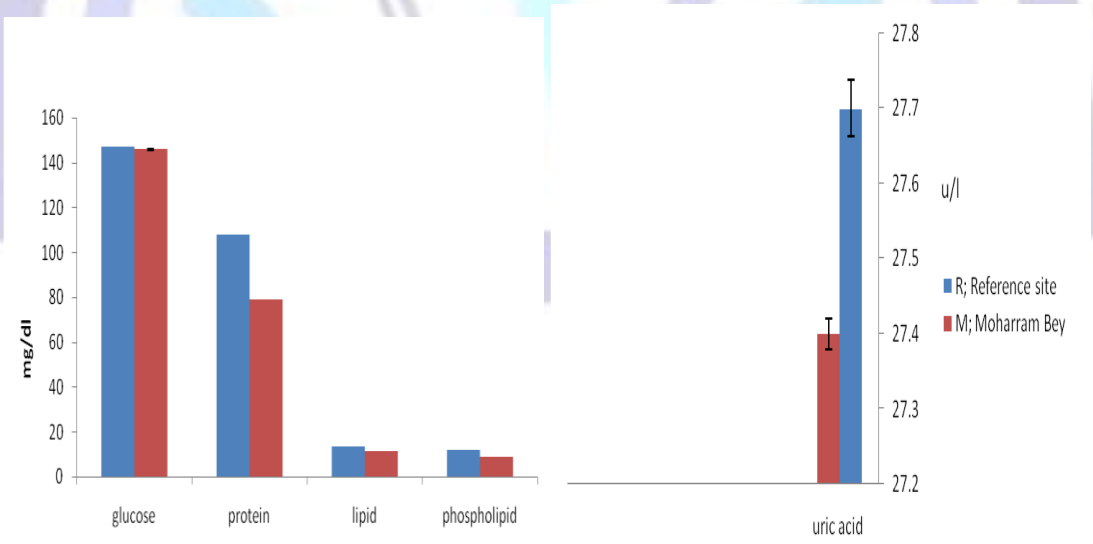

Figure 2a: Biochemical parameters in hemolymph of male *B. sulcata*

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Figure 3a: Biochemical parameters in hemolymph of female *B. sulcata*

Hemolymph cholinesterase activities were generally inhibited in *B. sulcata* from location M, a result that could be attributed to the use of insecticide (for controlling mosquitoes) and It is well documented that insecticides are cholinesterase' inhibitors as denoted by Harlin and Ross (1990); Van Vuren et al. (1994); Elena et al. (2003) and Paijo (2010).

From the above mentioned results it was obvious that the mean content of acid and alkaline phosphatase in hemolymph of insects from M location were found to be less than that from R location, and that agreed with Abdel- Hafez *et al.* (1993) who found significant reduction in acid and alkaline phosphatase when using two OP (Organophosphorus) insecticides on laboratory strain of *Spodoptera littoralis*.

The results showed that ALT and AST in *B. sulcata* hemolymph from location M were less than that from location R, and it was obvious that the inhibition of AST were more than in ALT. that could be due to damage of specific organs and tissues resulting from chemical toxicity, and these two enzymes showing the greatest diagnostic potential, since ALT and AST have important roles in the transfer of amino groups and in the Kreb's cycle. In other words; the transaminases form a link between the metabolism of amino acids, lipids and carbohydrates, as denoted by Sharma and Singh (1977); Azmi *et al.* (1998) and Hassan (2002).

This results was also supported by the results of Hunt and Hooper (1993); William (1997) and Céron et al. (1999) who found that blood enzymes have a role in monitoring and detecting stress or disease and that has led to growing concern in using them as biochemical indicators to trace environmental pollutants.

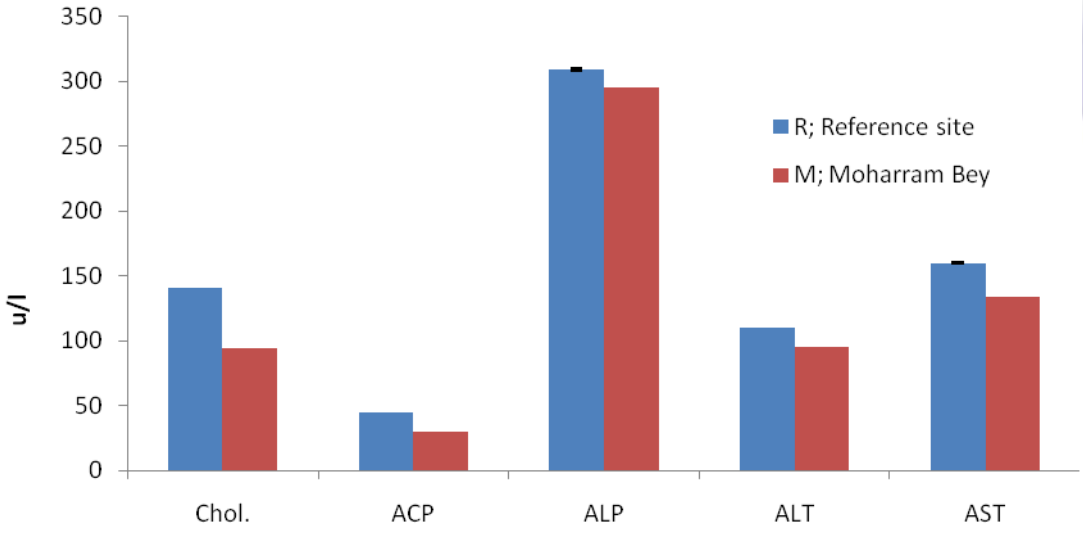

Figure 2b: Estimating enzymatic levels in hemolymph of male *B. sulcata*

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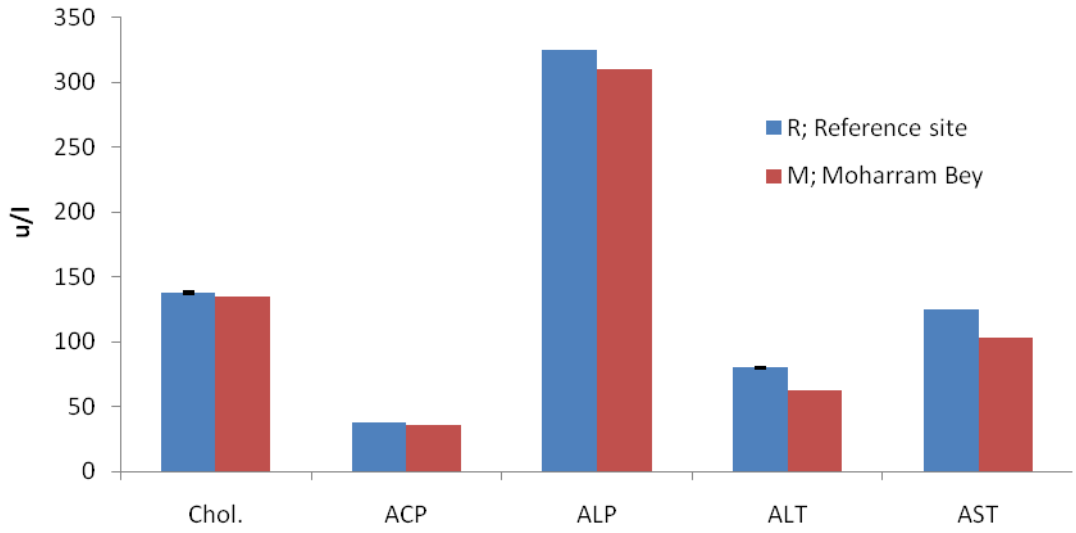

Figure 3b: Estimating enzymatic levels in hemolymph of female *B. sulcata*

CONCLUSIONS

From all the previous results, it was concluded that location M is a polluted area because of Badawi plant, that used to work on manufacturing several metal and metal alloys including iron also the use of insecticides and the Pb contaminated air from cars fumes in their vicinity. This in turn created a dramatic effect on the physiological status of *B. sulcata*.

As an output of this study, it is recommended that banning or at least minimizing the use of insecticides and pesticides is a major priority, or replacing them with natural controllers or less polluted ones. Another recommendation concerning Pb emissions from car fumes is to periodically check up car exhausts and to try to find an alternative less toxic fuel. And as for the contamination from factories they must be belt outside cities.

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