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Deleterious Effect of Cement Dust Pollution on Chromosomes and Free Amino Acids of Two Beetles, *Blaps sulcata* (Laporte) and *Akis reflexa* (Fabricius) in the Western-Coastal Desert of Egypt

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Abstract

This study was initiated to evaluate the effect of exposure to hazardous cement dust on the coleopterous insect, *Blaps sulcata* and *Akis reflexa* inhabiting the Maryout region of Egypt at two different sites using physicochemical and organic criteria. The expected anoxicity of cement dust site was evidenced by the elevated metal content and physicochemical parameters. Emerging evidence suggests that insects respond differently according to environmental stress index in each site. Cement dust caused structural and numerical chromosomal aberrations and induced significant increase in abnormal metaphases. It changed free amino acids (FAAs) concentrations in the whole body homogenate of both sexes of the two insects. Almost all the FAAs were sensitive to cement dust pollution. Most recorded changes were increase in FAAs concentrations rather than a decrease.

Keywords: cement dust pollution; chromosome aberration; free amino acids; biomarkers; beetles; insects.

Introduction

Developing countries such as Egypt are facing the challenge of managing the increased environmental pollution that is accompanying its economic development. The risk of atmospheric pollution has increased, especially in the industrial areas and air pollution has become far more serious and more difficult to control than previously. Cement dust is considered a major pollution problem at various steps of cement manufacture (Eckert *et al.*,1999). The cement kilns are the major source of toxic air emissions. Moreover, the cement dust contains many toxic substances such as calcium carbonate ($CaCO_3$), and sodium sulphate (Na_2SO_4) as well as heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), zinc (Zn), nickel (Ni) and copper (Cu). These pollutants have adverse health effects (Legator *et al.*, 1998). Many chemical pollutants that are released in the environment through industrial processes, beside their toxicity, may induce mutations and chromosomal aberrations (Schneider *et al.*, 1984).

Free amino acids (FAAs) in insects have an important role as a major blood buffer, make a major contribution to osmotic pressure, make up the contractile elements of protein and the enzymes that catalyze the release of energy, have a transport function when they are present in the blood. Moreover, some amino acids such as Tyrosine and its derivatives have an important role in the formation of exoskeleton, hardening of the cuticle and body pigmentation (Karison, 1963).

Free amino acids (FAAs) in insects are in a state of dynamic equilibrium. The amount fluctuates only within regulated limits in spite of the rapid turnover due to the influx

from feeding or catabolism of cellular proteins and the efflux due to cellular growth or protein secretion (Woodring and Blakeney, 1980). There are many studies which demonstrate the influence of metal-contaminated diet on free amino acid composition in insects (Ortel, 1995; Jamil *et al*., 1995) but very little is known about the effects of industrial wastes. The present study is aimed at studying the effects on chromosomal structure and free amino acid concentrations in the coleopterous insects, *Blaps sulcata* (Laporte) and *Akis reflexa* (Fabricius) exposed to cement dust from a cement factory in the Western Coastal Desert of Egypt.

Materials and methods

Study sites

The study was carried out in the Maryout region which is a strip of land about 100 km long and 30 km wide West of Alexandria on the Mediterranean coast, with an annual precipitation of about 150 mm which decreases sharply inland. Two uncultivated sites were chosen for sampling of the coleopterous insects. The uncultivated areas are occupied by many halophytic plant species shrubs like *Thymelea hirsute* and *Atriplex halmus* and are used for herding sheep and goats. Also, the soil in the two studied sites is mixed with different sizes of rocks, stones, pebbles and gravels. These sites were: (A) at Burg El-Arab city, which is 53 km West of Alexandria and 5 km South of the Mediterranean sea-shore and (B) at El-Hammam city which is 62 km West of Alexandria and 10 km South of the sea-shore (Fig. 1). The area surveyed at El-Hammam city is 1 km down-wind from a recently built cement factory. The area showed signs of cement deposition on the vegetation as well as the soil surface.

Sampling procedure

In the studied sites the coleopterous insects, *B. sulcata* and *A. reflexa* were confined to the spaces beneath rocks and canopy of the shrubs and were available during the early mornings. Sampling was accordingly restricted to such places and was taken during the breeding (October-November) and non-breeding (December-October) periods (Shalaby *et al.*, 1987). Twelve sampling areas (each 1m x1m) were randomly chosen in each site during each season. All the insects collected were sexed and were maintained alive on native soil and plants in suitable jars until processing. Simultaneously with insect collection, soil samples at a depth of 30 cm below the surface were collected from the specific sampling sites, air dried and passed through 0.2 mm sieve to eliminate gravel and debris.

Soil analysis

The determination of heavy metals copper (Cu), lead (Pb), cobalt (Co), zinc (Zn), cadmium (Cd), chromium (Cr) and nickel (Ni) concentrations, in sieved soil samples was carried out according to Loring and Rantala (1992) using atomic absorption spectrophotometer (Perkin-Elmer model 2380) under the recommended conditions and detection limits (DL) in the manual for each metal. Physicochemical characteristics: electric conductivity (EC), hydrogen ion concentration (pH), calcium carbonate ($CaCO_3$), total phosphorous (P), total organic matter (O.M), hygroscopic moisture (H.M) and fractions of slit and clay were carried out according to Allen *et al.* (1974).

Chromosome preparation

Chromosomal aberrations were obtained from the analysis of metaphases in testes cells as they were difficult to get from ovarian cells. Adult males of each species collected from sampling sites were injected in the abdominal region with a dose of 0.05 ml of 0.05% colcemid for 2 hours. Specimens were then dissected in insect Ringer solution

where testes were removed by fine forceps and instantly immersed in 1.0% of sodium citrate solution for 10 mins. at room temperature. The testes were then fixed in ethanol-acetic acid (3:1) for at least 1 hour. Chromosomal analysis was carried by squash technique used by Osman (1994). The dissected testes were stained with 20% aceto-orcein solution then fixed for about 10 minutes in 45% acetic acid. The best spread metaphase cells were selected. Chromosome number and structure were examined in 100 intact metaphase cells. The types frequencies of numerical as well as structural aberrations of chromosomes in all groups were then recorded.

Free amino acids assessment

Samples of males and females of insects which were collected from the studied sites during the breeding and non-breeding periods were oven dried to constant weight at 60°C and pulverized by hammer mill. 200 mg grind insects were homogenized in 3 ml sulfosalicylic acid (3.5%). The mixture was centrifuged at 3500 rpm for 5 minutes then filtered. The filtrate (0.1 ml) was injected into a Beckman amino acid analyzer, Model 119 CL.

Statistical analysis

Chromosomal aberration data were subjected to χ^2-test. Soil analysis and free amino acid assessments were tested with analysis of variance (SAS Institute, 1988).

Results

Soil chemistry

Table 1 shows the metal concentrations in soils of the selected sites. The soil at cement dust site has significantly higher values of all metals as compared to the reference site, which is the unpolluted site and thus served as control. Table 2 shows other physicochemical and organic matter of soils at the selected sites. The pH values were generally on the alkaline side in the studied sites with increased alkalinity at cement dust site. Soil at cement dust was significantly highest in all parameters except hygroscopic moisture which exhibited indifferent amounts. In general, Burg El-Arab site had the least amounts of pollutants hence its selection as reference or control.

Chromosomal aberration

The diploid chromosome number of *Blaps sulcata* is 2n = 34 while that of *Akis reflexa* is 2n = 16 (Fig. 2. A-B). Cytological examinations in testes cells of the studied species from the selected sites revealed several types of structural and numerical chromosomal aberrations. The structural aberrations are fragments, stickiness and chromatid deletion. The numerical aberrations are polyploidy (Fig. 2. C-D).

Data for structural and numerical chromosomal aberrations are summarized in Table 3. All types of aberrations as well as total aberrant metaphase were significantly increased (χ^2 -test, $P < 0.01$) in testes of both species collected from cement dust site.

Free amino acid assessment

Seventeen amino acids comprising nine essential ones: Thrionine (Thr), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Phenyl.Alanine (Phe), Histidine (His), Lysine (Lys), Arginine (Arg) and eight non-essential ones: Aspartic (Asp), Serine (Ser), Glutamic (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Cysteine (Cys), Tyrosine (Tyr) were identified and quantified from the protein hydrolysate run data of adults of both species during the study period (Fig. 2). In all groups the most prominent

amino acids were Asp, Glu, Pro, Ala and Arg amounting to about more than half of the amino acid pool. The amino acid Proline was found to be the major component.

Conspicuous differences in the relative amounts of individual amino acids occurred between cement dust and the reference groups. The number of individual free amino acids (FAAs) which showed significant increase in concentration due to cement dust stress was higher than those that showed significant decrease (Fig. 2).

Remarkable changes in TFAAs concentrations were observed among sexes of each species during the sampling periods at the selected sites (Table 4). Females of *B. sulcata* showed significant lower concentration than males during non-breeding period (χ^2-test, $P < 0.05$). The reverse was true for *A. reflexa*. During the breeding period, the profile of TFAAs concentrations of the sexes of *B. sulcata* remained rather constant throughout the reference and cement dust group whereas that of *A. reflexa* remained constant throughout the reference groups and decreased significantly (χ^2-test, $P < 0.05$) in cement dust females.

Table 4 also shows the effect of cement dust on TFAAs concentrations as well as the ratio of essential to non-essential amino acids (EAAs/NEAAs) in sexes of both species sampled during different periods. ANOVA indicated that cement dust significantly increased TFAAs in whole body homogenate. The ratio EAAs/NEAAs decreased markedly in cement dust insects. From the eight groups tested, four proved to be statistically significant that is, males and females *Akis reflexa* during the non-breeding and breeding periods.

Discussion

The data from the present study indicate that cement dust increased the concentrations of metals significantly. Earlier reports indicated that cement dust contains several metals (Carrasco *et al.,* 1998; Eckert *et al.,* 1999). Cement dust was also found to cause changes in soil characteristics which is in agreement with Migahid and El-Darier (1995).

Soil analysis assessment would be of limited value without the development of a comprehensive risk approach. Such an approach would, concurrently, include both chemical and biological monitoring advances. With the latter approach, it is reported here that cement dust was capable of inducing significant increase in abnormal metaphases in populations of the studied insects. Testes were found to contain high frequency of fragments, stickiness, chromatid deletion and polyploidy. Such results provide evidence that exposure to high level of air pollution by cement dust increases the risk of chromosomal aberration. Various studies have shown that exposure to high levels of air pollution increases the risk of chromosomal aberrations (Husby *et al.,* (1999); Perreault *et al.* (2000); Rubes *et al.* (2000); Seleven *et al.* (2000). Fatima *et al.* (2001) also showed that workers occupationally exposed to cement dust increased their chromosomal aberrations. Data from current study supports the assertion by Michailova, Petrova, Bovero, Cavicchioli, Ramella and Sella (2000) that chromosomal aberration is an appropriate parameter for assessing metal pollution.

Chromosomal aberrations in the present study could have arisen from the interaction of cement dust toxicants with DNA and other cellular macromolecules as previously documented in white-footed mice *Peromyscus leucopus* collected from abandoned coal

strip mines (Husby and McBee, 1999) and in the chub *Leuciscus cephalus* exposed to chemical pollutions of the river Rhone (Norway *et al.*, 1998).

With regards to free amino acids (FAAs), the results indicated variations in TFAAs concentrations among the sexes of each species. This sex specific difference may be attributed to the differences in the metabolic requirements of male and female reproductive system as reported earlier by Pant and Cupta (1980) in *Philosamid ricini*. Also, it was clear that cement dust enhanced TFAAs concentrations of both sexes of the two species during the study period. The profile of the relative abundance of individual amino acids also changed. It has been demonstrated in many insect species that exposure to sublethal concentrations of pollutants causes an increase in the levels of FAAs concentrations (Islam and Roy 1983; Carr *et al.*, 1985; Jamil *et al.*, 1995; Ortel, 1995).

The marked decrease in the ratio of essential/non-essential amino acids (EAAs/NEAAs) detected in cement dust insects may be indicative of the fact that protein synthesis, but not synthesis of amino acids themselves, is impaired. It might also have resulted from the imbalance between the anabolic and catabolic rates of these endogenous amino acids, as Mansingh (1965) concluded from his results of insecticide treated cockroaches. Such imbalances are possible due to pollutants-induced changes in enzyme activities. Such reduced enzyme activities were reported by Bream (2003). In conclusion, the biochemical and genetic parameters that responded, in a quantitative manner, to the deleterious effect of cement dust could be ranked as indicators of pollution. Moreover, when no serious tissue damage occurs, the enhanced FAAs concentration is considered as a promising "biomarker" for appraising sublethal contamination of insects.

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Table 1. Metal concentrations (µg/g) in soil samples (means based on two replicates from the reference and three replicates from cement dust). Data were subjected to analysis of variance.

Site	Cu	Pb	Co	Zn	Cd	Cr	Ni
Reference	25.5	35.0	11.0	55.0	0.50	26.0	27.5
Cement dust	22.3	146.0	13.0	63.3	0.90	34.7	32.3
F-ratio	30.94	1848.1	11.0	93.75	14.40	101.4	72.09
P	<0.05	<0.001	<0.05	<0.002	<0.05	<0.01	<0.01

F at 5% level = 10.13, F at 1% level = 34.10.

Table 2. Levels of some physiochemical characteristics of soil at the selected sites (means based on 5 replicates from each site). Data were subjected to analysis of variance.

Site	Electrical conductivity (mm hos/cm)	pH	$CaCO_3$ (%)	Total phosphorous (µg/g)	Organic matter (%)	Hygroscopic moisture (%)	Clay Frac. (clay & silt) (%)
Reference	10.13	7.10	50.0	134.3	2.19	1.04	16.0
Cement dust	25.62	7.8	59.1	241.5	0.83	1.01	23.0
F-ratio	59.91	0.73	124.2	5398	21.34	0.001	73.5
P	<0.01	>0.05	<0.01	<0.01	<0.05	>0.05	<0.01

F at 5% level = 5.32, F at 1% level = 11.26.

Table 3. Chromosomal aberrations in testes of *Blaps sulcata* and *Akis reflexa* collected from the selected sites.

Insect species	Site	Types of aberrant chromosomes				Total aberrant metaphase (%)	χ^2 for total aberrant metaphase
		Fragments (%)	Stickiness (%)	Chromatid deletion (%)	Polyploidy (%)		
B. sulcata	Reference	5	4	1	1	11	
	Cement dust	9	16	10	6	*41	17.31
A. reflexa	Reference	2	3	1	1	7	
	Cement dust	8	11	6	8	*33	16.90

Note: 100 metaphase cells were examined for each test.
*P < 0.01, $\chi^2_{0.01} = 6.6$

Table 4. Total free amino acids concentrations (TFAAs) and the ratio of essential to non-essential amino acids (EFAAs/NEFAAs) in whole body homogenate of sexes of both insects sampled during different periods at the selected sites. Values represent mean of three replicates.

Period		Total free amino acid concentrations (mg/100g sample)											
		Male *B. sulcata*			Female *B. sulcata*			Male *A. reflexa*			Female *A. reflexa*		
		Reference site	Cement dust	F-ratio P	Reference site	Cement dust	F-ratio P	Reference site	Cement dust	F-ratio P	Reference site	Cement dust	F-ratio P
Non-breeding	TFAAs	1400.53	1977.35	373.64 0.000	1184.53	1409.00	333.3 0.000	1092.38	1672.31	223.38 0.000	1482.26	2012.77	315.55 0.000
	EFAAs	445.37	612.48		395.80	449.86		413.77	565.13		564.18	720.94	
	NEFAAs	995.16	1364.87		788.73	959.14		678.61	1107.18		918.08	1291.83	
	EFAAs/NEFAAs	0.466	0.448	1.65 0.268	0.501	0.469	0.01 0.99	0.609	0.510	12.42 0.024	0.614	0.558	6.26 0.05
Breeding	TFAAs	1317.25	1529.71	375.93 0.000	1297.44	1509.13	720.0 0.000	1036.60	1405.83	1712.28 0.000	1057.37	1163.07	129.31 0.000
	EFAAs	340.32	357.28		313.22	348.05		343.83	425.79		350.1	310.79	
	NEFAAs	976.93	1172.43		984.22	1161.08		713.54	1246.52		707.27	852.28	
	EFAAs/NEFAAs	0.348	0.304	6.0 0.07	0.318	0.299	3.13 0.152	0.481	0.341	26.87 0.007	0.495	0.364	45.56 0.003

F at 5% level = 7.71, F at 1% level = 21.20.

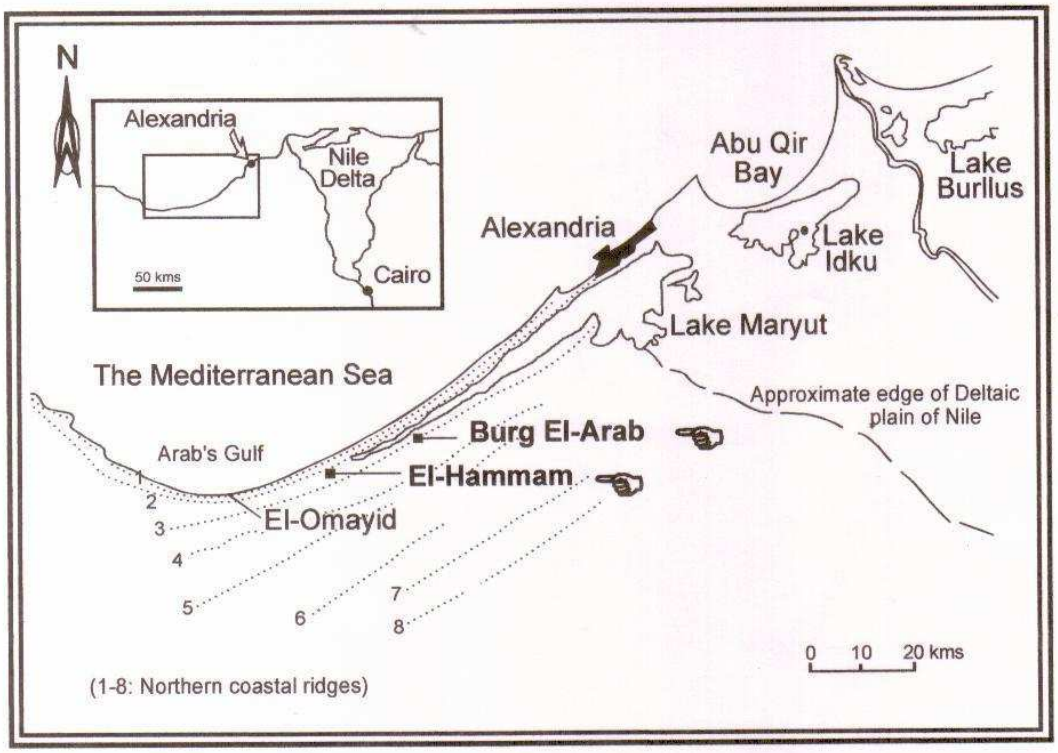

Fig. 1 . Location map of the study area.

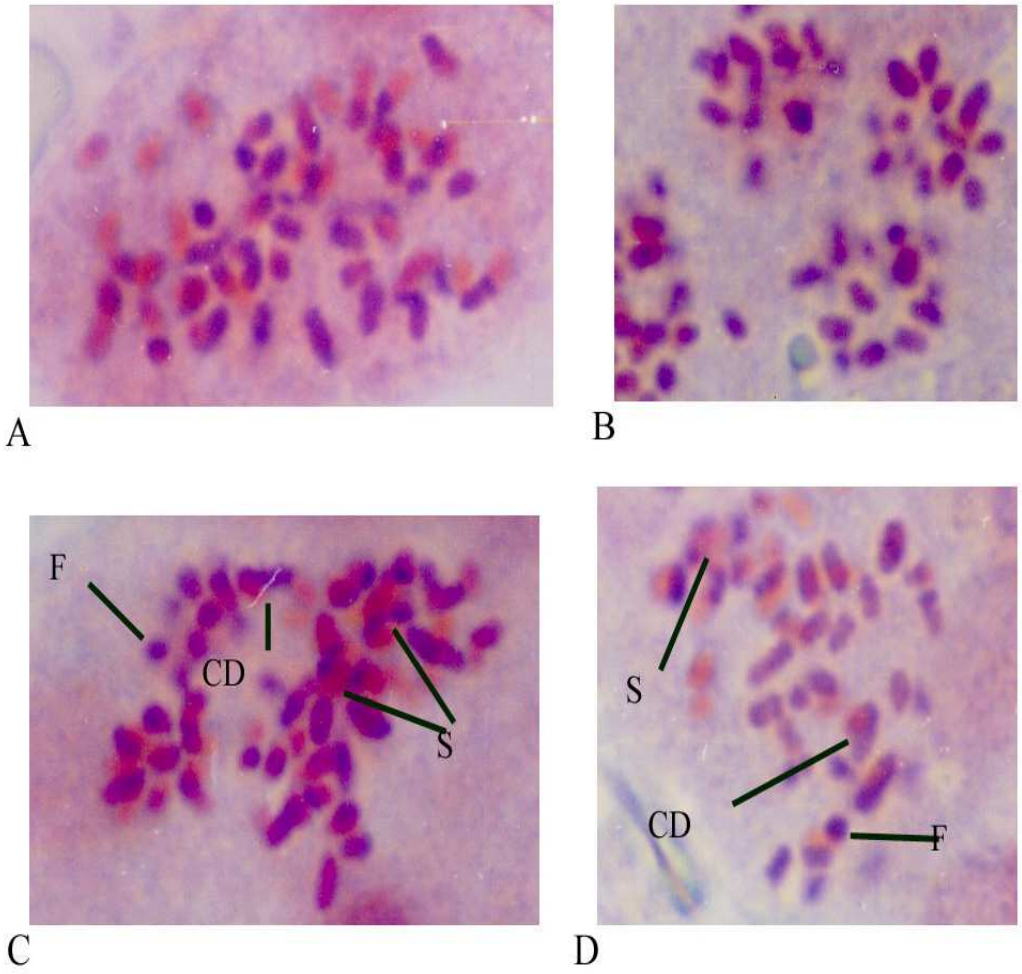

Fig. 2. A-B. Photomicrographs showing normal metaphase stages in testicular cells of (A) *Blaps sulcata* with 2n = 34 chromosomes and (B) *Akis reflexa* with 2n = 16 chromosomes in each cell (X4500). C-D. Photomicrographs of abnormal metaphase stages in testicular cells of (C) *Blaps sulcata* and (D) *Akis reflexa* showing stickiness(S), fragment (F) and chromatid deletion (CD) (X4500).