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MYCOLOGICAL EVALUATION OF POULTRY FEEDS, CONCENTRATES AND FEED INGREDIENTS

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ABSTRACT :

A mycological evaluation of total eighty two samples of apparently normal poultry rations (22),concentrated mixtures(18) and feed ingredients (42) were performed. The samples were collected from some poultry processing plants in different localities at Assiut Governorate, Egypt. The moisture content of the examined samples were moderately low with mean values ranged from 4.58±0.72 to 7.06±0.17. The mean values of the total mould and yeast colony count/g were varied from $1.9x10^4$ to $5.2x10^4$. The broiler concentrate mixtures showed the minimum count ($4.4x10^3$/g) while the maximum count was observed in broiler rations ($1.3x10^5$/g). Twenty fungal genera represented by 42 species and 1 species variety were identified in the present investigation . The most prevalent fungal species were found to belong to Aspergillus followed by Penicillium. Moreover, many fungal species of less frequency distributions were also detected including *Cladosporium, Gibberella, Paeciliomyces, Emericella, Hypomyces, Mucor species* and others. The hygienic significance of the fungal isolates and the restrictive measures that must be considered were discussed.

INTRODUCTION :

Most significant researches on fungi have been conducted in the last decade, although the older literature revealed many instances where mouldy feedstuffs caused toxic results, altered nutrient values, or reduced the palatability. In 1959, at least 100,000 turkey poults and ducklings died in England as a result of feeding contaminated peanut meal

imported from Brazil (Purchase, 1971). The U.S. broiler industry showed sporadic occurrence over the years of haemorrhagic syndrome suspected to be due to *Alternaria, Aspergillus clavatus, A. flavus* and *Penicillium purpurogenum* (Schaible, 1970). All these events changed the attitude towards moulds in foods and feeds.

Recently special attention was paid to provide information on the incidence and activities of fungi in the mixed feeds. The poultry feeds have not received special mycological attention, in spite of the fact that they are prepared basically from the raw plant materials (Corn, Soybean, Wheat bran, etc.) and concentrate mixtures, where fungi are the most important contaminant microorganisms (Lagrandeur & Poisson, 1968; Lovett et al., 1971; Hesseltine et al., 1976; Muntanola-Cvetkovic & Borisavljevic, 1979; Ogundero, 1980; Clarke & Hill, 1981; McGimpsey & Malone, 1981; Tabib et al., 1981; El-Kady et al., 1982. Moreno-Rome & Fernandez, 1986; Ranjan & Sinha, 1991; Chang- Yen et al., 1992 and Abarca et al., 1994). These microorganisms may be present in the final product or originate directly or indirectly from cross contamination. The unfavourable conditions of storage as high temperature and humidity were found to stimulate the fungal growth in the poultry feedstuffs (Abdel-Fattah et al.,1979 ; Ogundero, 1980; Bartov et al., 1982 and Ogundero 1987).

The mycological study of poultry feeds and feed ingredients are of significant importance for determining the distribution of the mycotoxin producing fungi as their toxic metabolites have been detected in many feed stuffs. In this sense, poultry feeds are the first stage of the chain that may carry mycotoxins to man. The present work was planned to throw light on the mycological status of commercial poultry mixed feeds and their ingredients, as well as their fungal contamination and to arise the public health awareness of such contamination on human health through poultry, as well as rescuing the birds from such contamination. Moreover, special attention was paid to the mycotoxin producing fungi, which will be reported elsewhere.

MATERIALS & METHODS :

A- Collection of the samples:

Eighty two samples of poultry feeds were collected from some poultry processing plants in different localities at Assiut Governorate (Beni-Mor, Arab El-Awamer, Dronka, Rifa and Manfalout). Out of 82 samples collected, 22 were poultry rations(final product), 18 concentrate mixtures and 42 were feed ingredients . The samples were collected in sterile polyethylene bags and transported to the laboratory for their mycological analysis.

B- Determination of the moisture content :

The moisture content of the poultry feeds was determined according to Tabib et al. , (1981) and Swelim et al. , (1994) by the oven method. Replicates of the samples of 10 gm for each were dried at 105 °C in an electric oven

to a constant weight. The moisture content was calculated as percentage on oven dry basis (Tabib et al., 1981 and Swelim et al., 1994).

C-Enumeration and identification of fungi:

The dilution-plate method described by Johnson & Curl (1972) was used for the enumeration of fungi. Dilutions were prepared by shaking 10 gm of each sample in 90 ml sterile saline solution (0.88 w/v NaCl). Serial tenfold dilutions were prepared and 1 ml aliquots of the appropriate dilution were transferred into sterile petri dishes. Dicloran rose bengal medium of King et al., (1979) was used for the isolation of fungi. Five replicates for each sample were incubated at 25 °C for 7-15 days.

The growing fungal colonies were enumerated and identified based on their macro- and microscopic characteristics using the methods of Raper & Fennel (1965), Ellis (1971)& (1976); Pitt (1979); Domsch et al., (1980); Sivanesan (1984)& (1987), Kozakiewicz (1989) and Moubasher (1993).

RESULTS & DISCUSSION :

Poultry mixed feeds and their ingredients are naturally contaminated by different species of fungi, that is mainly due to their exposure to the surrounding environment and storage under humid conditions (Beer, 1969).

In general the moisture content in such feeds is considerd as the most important factor contributing to their moulding. From table (1), a moderate moisture content was observed in the examined samples. The highest moisture content was detected in the white corn (7.06±0.17) while the lowest was observed in the yellow corn (4.58±0.72).

Data in table (2) revealed that the highest mean of total fungal count /g was noted in the yellow corn ($5.2x10^4$) followed by laying rations ($4.4x10^4$), broiler rations and white corn ($4.1x10^4$), wheat bran ($3.7x10^4$), concentrate mixture of broiler ($3.3x10^4$), soybean meal ($2.2x10^4$). The lowest mean count was recorded in the concentrate mixture for layers ($1.9x10^4$). Concerning the total mould count/g, it easily demonstrated that the highest count was observed in the yellow corn and laying rations (table 2). This could be attributed to the high starch content (Shotwell et al., 1966) and/or high moisture content, beside the improper handling and storage specially in case of yellow corn (Bartov et al., 1982 and Ogundero, 1987). The high humidity enhances the fungal growth which is typical for the tropical and subtropical regions.

The obtained results revealed that the mean fungal count/g were ranged from $1.9x10^4$ to $5.2x10^4$. The maximum count was found in both broiler rations and yellow corn ($1.3x10^5$) and a minimum count in the broiler concentrate mixture ($4.4x10^3$). The obtained results are more or less coincident with fungal densities ($7x10^2$ - $3.2x10^5$/g) obtained by Lovett et al. (1971) and 5 to $1.2x10^5$/g obtained by Tabib et al. (1981). On the other hand,

obtained results are lower than that recorded by Abarca et al.,1994 (up to 10^8 CFU/g).

Concerning the differential mould & yeast count in poultry feeds, concentrate mixtures and feed ingredients that recorded in tables (3, 4, 5). The present data showed that the most dominant species are *Aspergillus fumigatus, A. flavus, Penicillium chrysogenum, Gibberella fujikuroi, Aspergillus niger and A. terreus.* It was observed that *A. fumigatus* mean count/g was $8.5x10^3$; $4.8x10^3$, $9.7x10^3$; $7.0x10^3$; $1.0x10^4$; $1.1x10^4$ and $8.4x10^3$ in laying rations, laying concentrate mixture, broiler concentrate mixture, soybean meal, wheat bran, yellow corn and white corn, respectively. Moreover, the *Aspergillus flavus* mean count was $8.3x10^3$; $3.7x10^3$; $8.8x10^3$ in broiler rations, wheat bran and yellow corn, respectively. However, the mean count of *Aspergillus niger* was $1.4x10^3$; $1.7x10^3$ in the laying concentrate mixture and soybean meal respectively while *A. terreus* was $7.3x10^3$ in the broiler concentrate mixture. On the other hand, tables 3,4 and 5 revealed that *Penicillium chrysogenum* mean count/g was $9.6x10^3$; $7.1x10^3$; $7.1x10^3$; $5.4x10^3$; $8.2x10^3$; $1.2x10^4$ and $8.5x10^3$ in laying rations, laying concentrate mixture, broiler concentrate mixture, soybean meal, wheat bran, yellow corn and white corn respectively. *Gibberella fujikuroi* was isolated from laying rations, broiler rations, white corn by $5.2x10^3$; $1.2x10^4$ and $1.3x10^4$, respectively.

Table (1): Statistical analysis of moisture content in the different poultry feed, concentrates and feed ingredients.

Poultry feeds, concentrates and feed ingredients	No.of ex. samples	Moisture content %		
		Min.	Max.	Mean
Poultry rations :-				
Laying rations	9	5.99	6.62	6.33±0.08
Broiler rations(starters)	13	6.12	6.58	6.33±0.06
Concentrates:				
Conc.(Layers)	7	5.64	6.12	5.87±0.06
Conc.(Broilers)	11	4.00	5.88	5.04±0.28
Feed Ingredients :-				
Soyabean meal	13	3.92	8.16	5.61±0.59
yellow corn	14	2.00	7.14	4.58±0.72
White corn	3	6.48	7.84	7.06±0.17
Wheat bran	12	6.12	8.51	7.0±0.29

Table (2): Statistical mould & yeast count of poultry feeds, concentrates and feed ingredients.

Feeds & feed ingredients	No. of samples	Total mould & yeast count/g		
		Min	Max	Mean
Laying rations	9	$1.9x10^4$	$9.8x10^4$	$4.4x10^4 \pm 9.7x10^3$
Broiler rations	13	$4.6x10^3$	$1.3x10^5$	$4.1x10^4 \pm 9.9x10^3$
Conc. layer	7	$5.2x10^3$	$5.3x10^4$	$1.9x10^4 \pm 7.0x10^3$
Conc. broiler	11	$4.4x10^3$	$8.7x10^4$	$3.3x10^4 \pm 8.7x10^3$
Soybean meal	13	$6.4x10^3$	$4.7x10^4$	$2.2x10^4 \pm 3.8x10^3$
Yellow corn	14	$1.6x10^4$	$1.3x10^5$	$5.2x10^4 \pm 9.7x10^3$
White corn	3	$2.6x10^4$	$5.3x10^4$	$4.1x10^4 \pm 9.6x10^3$
Wheat bran	12	$1.2x10^4$	$8.3x10^4$	$3.7x10^4 \pm 5.4x10^3$

Table (3): Differential mould & yeast count/g in poultry rations.

Fungal species	Laying rations (9)			Broiler rations (13)		
	Min	Max	Mean	Min.	Max.	Mean
Alternaria alternata	0	0	0	0	$2x10^2$	1.5x10
A. chlamydospora	0	0	0	0	$2x10^2$	1.5x10
Aspergillus alutaceus	0	$4.0x10^2$	4.4x10	0	$1.4x10^3$	$1.2x10^2$
A. flavus	$8x10^2$	$1.9x10^4$	$4.8x10^3$	$4.0x10^2$	$3.2x10^4$	$8.3x10^3$
A. fumigatus	$1.2x10^3$	$2.6x10^4$	$8.5x10^3$	$8.0x10^2$	$1.6x10^4$	$6.2x10^3$
A. niger	$2.0x10^2$	$2.4x10^4$	$4.7x10^3$	$2.0x10^2$	$5.2x10^3$	$2.5x10^3$
A. oryzae	0	0	0	0	$6.0x10^2$	4.6x10
A. sydowii	0	$1.2x10^3$	$1.3x10^2$	0	0	0
A. tamarii	0	$2.0x10^2$	2.2x10	0	0	0
A. terreus	0	$3.8x10^3$	$9.3x10^2$	0	$3.4x10^3$	$1.2x10^3$
Cladosporium cladosporioides	0	$4.0x10^2$	6.7x10	0	$4.0x10^2$	3.0x10
Emericella nidulans	0	$4.0x10^2$	6.7x10	0	$1.2x10^3$	$2.0x10^2$
E. quadrilineata	0	0	0	0	$2.4x10^3$	$1.8x10^2$
Fennellia flavipes	0	$6.0x10^2$	$1.3x10^2$	0	$2.0x10^2$	1.5x10
Gibberella fujikuroi	0	$2.1x10^4$	$5.2x10^3$	0	$7.6x10^4$	$1.2x10^4$
Paecilomyces voriotii	0	$1.2x10^4$	$1.8x10^3$	0	$6.0x10^2$	6.1x10
Penicillium aurantiogriseum	0	$9.0x10^3$	$2.9x10^3$	0	$7.6x10^3$	$2.2x10^3$
P. chrysogenum	0	$3.1x10^4$	$9.6x10^3$	$2.0x10^2$	$1.6x10^4$	$7.3x10^3$
P. citrinum	0	$8.0x10^3$	$1.2x10^3$	0	$3.8x10^3$	$2.9x10^2$
P. duclauxii	0	$2.6x10^3$	$2.9x10^2$	0	$1.0x10^3$	$1.4x10^2$
P. funiculosum	0	$2.2x10^4$	$3.9x10^3$	0	$1.2x10^3$	$1.4x10^2$
P. variabile	0	0	0	0	$2.0x10^2$	1.5x10
Trichoderma harzianum	0	$2.0x10^2$	2.2x10	0	0	0

* N.C.I. = Number of cases of isolations .

Table (4): Differential mould & yeast count/g in poultry concentrate mixtures.

Fungal species	Conc. layers (7)			Conc. broilers (11)		
	Min.	Max.	Mean	Min.	Max.	Mean
Acremonium strictum	0	$2.0x10^2$	2.9x10	0	2.0x102	1.8x10
Alternaria alternata	0	0	0	0	$2.4x10^3$	$2.2x10^2$
A. chlamydospora	0	0	0	0	$1.2x10^3$	$2.0x10^2$
Aspergillus alutaceus	0	0	0	0	$2.0x10^2$	3.6x10
A. carbonarious	0	$2.0x10^2$	2.9x10	0	0	0
A. flavus	0	$2.4x10^3$	$1.0x10^3$	$2.0x10^2$	$4.0x10^3$	$1.1x10^3$
A. fumigatus	$8.0x10^2$	$1.1x10^4$	$4.8.x10^3$	$2.0x10^3$	$2.6x10^4$	$9.7x10^3$
A. niger	0	$5.2x10^3$	$1.4x10^3$	0	$1.4x10^4$	$2.3x10^3$
A. oryzae	0	$6.0x10^2$	$1.1x10^2$	0	$1.0x10^3$	9.1x10
A. sydowii	0	$2.4x10^3$	$4.3x10^2$	0	$2.0x10^2$	1.8x10
A. terreus	0	$8.4x10^3$	$1.6x10^3$	0	$6.0x10^4$	$7.3x10^3$
A. ustus	0	$2.0x10^2$	2.9x10	0	$2.0x10^2$	1.8x10
A. versicolor	0	$2.0x10^2$	2.9x10	0	0	0
Cladosporium cladosporioides	0	$2.0x10^2$	8.6x10	0	0	0
Emericella nidulans	0	$6.0x10^2$	$2.0x10^2$	0	$2.4x10^3$	$4.2x10^2$
E. quadrilineata	0	$6.0x10^2$	$1.1x10^2$	0	0	0
Eupenicillium spp.	0	$2.0x10^2$	2.9x10	0	0	0
Eurotium chevalieri	0	$2.0x10^2$	2.9x10	0	0	0
Fennellia flavipes	0	0	0	0	$6.2x10^3$	$5.6x10^2$
Gibberella fujikuroi	0	$2.0x10^2$	2.9x10	0	$4.0x10^2$	3.6x10
Humicola minima	0	0	0	0	$2.0x10^2$	1.8x10
Paecilomyces voriotii	0	$1.2x10^3$	$1.7x10^2$	0	$1.4x10^3$	$1.3x10^2$
Penicillium aurantiogriseum	$2.0x10^2$	$4.2x10^3$	$1.4x10^3$	0	$6.8x10^3$	$1.9x10^3$
P. chrysogenum	$6.0x10^2$	$2.4x10^4$	$7.1x10^3$	$8.0x10^2$	$2.2x10^4$	$7.1x10^3$
P. citrinum	0	0	0	0	$1.8x10^3$	$1.6x10^2$
P. duclauxii	0	0	0	0	$3.6x10^3$	$3.5x10^2$
P. funiculosum	0	0	0	0	$7.0x10^3$	$8.0x10^2$
Pleospora tarda	0	$6.0x10^2$	8.5x10	0	0	0
Scopulariopsis brevicaulis	0	$2.0x10^2$	2.9x10	0	0	0
S. candida	0	$4.0x10^2$	8.6x10	0	0	0

Table (5): Differential mould & yeast count/g in poultry feed ingredients.

Fungal species	Soybean meal (13)			Wheat bran (12)			Yellow corn (14)			White corn (3)		
	Min	Max	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min	Max	Mean
Acremonium strictum	0	$2.0x10^2$	1.5x10	0	$2.4x10^3$	$2.0x10^2$	0	$4.0x10^2$	2.9x10	0	$4.0x10^2$	1.3x10
Alternaria alternata	0	$6.0x10^2$	9.2x10	0	0	0	0	$6.0x10^2$	7.1x10	0	0	0
A. chlamydospora	0	$2.6x10^3$	$2.2x10^2$	0	$2.0x10^2$	1.7x10	0	0	0	0	$1.6x10^3$	$5.3x10^2$
Aspergillus alutaceus	0	0	0	0	$2.0x10^2$	1.7x10	0	$1.6x10^3$	$2.0x10^2$	0	0	0
A. clavatus	0	$6.0x10^2$	6.4x10	0	0	0	0	0	0	0	0	0
A. flavus	0	$3.4x10^3$	$1.0x10^3$	0	$1.2x10^4$	$3.7x10^3$	0	$3.6x10^4$	$8.8x10^3$	$8.0x10^2$	$3.2x10^3$	$2.1x10^3$
A. flavus var columnaris	0	0	0	0	$6.0x10^2$	5.0x10	0	0	0	0	0	0
A. fumigatus	$2.2x10^3$	$1.6x10^4$	$7.0x10^3$	$3.2x10^3$	$2.3x10^4$	$1.0x10^4$	$3.0x10^3$	$2.6x10^4$	$1.1x10^4$	$1.4x10^3$	$1.4x10^4$	$8.4x10^3$
A. melleus	0	$6.0x10^2$	4.6x10	0	0	0	0	$2.0x10^2$	2.9x10	0	0	0
A. niger	0	$1.1x10^4$	$1.7x10^3$	$2.0x10^2$	$1.0x10^4$	$3.1x10^3$	0	$3.2x10^4$	$4.8x10^3$	$2.6x10^3$	$7.4x10^3$	$4.5x10^3$
A. oryzae	0	$2.0x10^2$	1.5x10	0	0	0	0	0	0	0	0	0
A. sydowii	0	$2.4x10^3$	$2.0x10^2$	0	2.0x102	1.7x10	0	$1.2x10^3$	8.6x10	0	0	0
A. tamarii	0	$2.0x10^2$	1.5x10	0	0	0	0	0	0	0	0	0
A. terreus	0	$3.6x10^3$	$6.6x10^2$	0	$5.6x10^3$	$2.3x10^3$	0	$2.2x10^4$	$2.9x10^3$	0	$1.0x10^3$	$3.3x10^2$
A. versicolor	0	$4.0x10^2$	3.1x10	0	$1.8x10^3$	$2.0x10^2$	0	$8.0x10^2$	$1.0x10^2$	0	0	0
Chrysosporium tropicum	0	$6.0x10^2$	4.6x10	0	$2.0x10^2$	1.7x10	0	0	0	0	0	0
Cladosporium cladosporioides	0	$2.2x10^3$	$3.8x10^2$	0	0	0	0	0	0	0	0	0
Emericella nidulans	0	$1.2x10^3$	$2.5x10^2$	0	$3.2x10^3$	$4.8x10^2$	0	$1.2x10^3$	$1.4x10^2$	0	$2.6x10^3$	$1.0x10^3$
E. quadrilineata	0	0	0	0	$2.0x10^2$	1.7x10	0	0	0	0	0	0
Fennellia flavipes	0	0	0	0	$2.4x10^4$	$3.2x10^3$	0	0	0	0	0	0
Gibberella fujikuroi	0	$7.6x10^3$	$1.8x10^3$	0	$4.2x10^3$	$6.0x10^2$	0	$3.0x10^4$	$3.4x10^3$	0	$4.1x10^4$	$1.3x10^4$
Humicola grisea	0	$2.0x10^2$	1.5x10	0	0	0	0	0	0	0	0	0
Hypomyces chrysospermus	0	$6.0x10^2$	6.2x10	0	$2.0x10^2$	1.7x10	0	$9.6x10^3$	$6.9x10^2$	0	$2.0x10^2$	$1.3x10^2$
Mucor racemosus	0	0	0	0	0	0	0	$4.0x10^2$	2.9x10	0	0	0
Paecilomyces voriotii	0	$1.4x10^3$	$1.7x10^2$	0	6.0x10	6.7x10	0	$5.4x10^3$	$9.4x10^2$	0	$2.0x10^2$	6.7x10
Penicillium aurantiogriseum	0	$3.8x10^3$	$9.4x10^2$	$8.0x10^2$	$7.6x10^3$	$3.4x10^3$	0	$1.7x10^4$	$3.6x10^3$	$2.0x10^2$	$3.6x10^3$	$1.7x10^3$
P. chrysogenum	$6.0x10^2$	$2.1x10^4$	$5.4x10^3$	$1.4x10^3$	$3.6x10^4$	$8.2x10^3$	0	$5.8x10^4$	$1.2x10^4$	$3.0x10^3$	$1.8x10^4$	$8.5x10^3$
P. citrinum	0	$6.0x10^2$	6.2x10	0	$6.0x10^2$	5.0x10	0	$1.0x10^4$	$7.6x10^2$	0	0	0
P. duclauxii	0	$2.2x10^3$	$2.0x10^2$	0	0	0	0	$2.8x10^3$	$4.1x10^2$	0	0	0
P. funiculosum	0	$7.2x10^3$	$1.1x10^3$	0	$4.8x10^3$	$6.7x10^2$	0	$3.6x10^3$	$7.0x10^2$	0	0	0
P. oxalicum	0	$4.0x10^2$	3.1x10	0	0	0	0	0	0	0	0	0
Pleospora tarda	0	$2.0x10^2$	1.5x10	0	$2.0x10^2$	1.7x10	0	0	0	0	0	0
Scopulariopsis brevicaulis	0	$6.0x10^2$	4.6x10	0	0	0	0	0	0	0	0	0
Scytalidium lignicola	0	0	0	0	0	0	0	$7.2x10^3$	$5.1x10^2$	0	0	0
Torula spp.	0	0	0	0	$2.0x10^3$	$1.7x10^2$	0	0	0	0	0	0
Yeasts	0	$4.0x10^2$	3.1x10	0	0	0	0	0	0	0	0	0

Table (6): Frequency distribution and incidence percentages of moulds & yeasts in poultry rations.

Fungal isolates	Laying rations (9 samples)			Broiler rations (13 samples)			Overall incid. %	Overall freq. %
	N.C.I.*	Incid. %	Frq. %	No. of isolates	Incid. %	Frq. %		
Alternaria alternata	0	0	0	1	1	7.69	0.58	4.54
A. chlamydospora	0	0	0	1	1	7.69	0.58	4.54
Aspergillus alutaceus	1	1.39	11.11	2.	2	15.38	1.74	13.64
A. flavus	9	12.5	100	13	13	100	12.79	100
A. fumigatus	9	12.5	100	13	13	100	12.79	100
A. niger	9	12.5	100	13	13	100	12.79	100
A. oryzae	0	0	0	1	1	7.69	0.58	4.54
A. sydowii	1	1.39	11.11	0	0	0	0.58	4.54
A. tamarii	1	1.39	11.11	0	0	0	0.58	4.54
A. terreus	5	6.94	55.55	10	10	76.92	8.72	68.18
Cladosporium cladosporioides	2	2.78	22.22	1	1	7.69	1.74	13.64
Emericella nidulans	2	2.78	22.22	3	3	23.08	2.91	22.73
E. quadrilineata	0	0	0	1	1	7.69	0.58	4.54
Fennellia flavipes	2	2.78	22.22	1	1	7.69	1.74	13.64
Gibberella fujikuroi	6	8.33	66.66	9	9	69.23	8.72	68.18
Paecilomyces voriotii	3	4.17	33.33	2	2	15.38	2.91	22.73
Penicillium aurantiogriseum	7	9.72	77.78	10	10	76.92	9.88	77.27
P. chrysogenum	8	11.11	88.88	13	13	100	12.21	95.45
P. citrinum	2	2.78	22.22	1	1	7.69	1.74	13.64
P. duclauxii	1	1.39	11.11	2	2	15.38	1.74	13.64
P. funiculosum	3	4.17	33.33	2	2	15.38	2.91	22.73
P. variable	0	0	0	1	1	7.69	0.58	4.54
Trichoderma harzianum	1	1.39	11.11	0	0	0	0.58	4.54
Total	72			100				

* N.C.I. = Number of Cases of Isolations .

Table(7): Frequency distribution and incidence percentages of moulds & yeasts in poultry concentrate mixtures.

Fungal isolates	Laying concentrates (7 samples)			Broiler concentrates (11 samples)			Overall incid. %	Overall freq. %
	N.C.I.*	Incid. %	Frq. %	No. of isolates	Incid. %	Frq. %		
Acremonium strictum	1	1.67	14.28	1	1.19	9.09	1.39	11.11
Alternaria alternata	0	0	0	1	1.19	9.09	0.69	5.55
A. chlamydospora	0	0	0	3	3.57	27.27	2.08	16.67
Aspergillus alutaceus	0	0	0	2	2.38	18.18	1.39	11.11
A. carbonarious	1	1.67	14.28	0	0	0	0.69	5.55
A. flavus	4	6.67	57.14	11	13.09	100	10.42	83.33
A. fumigatus	7	11.67	100	11	13.09	100	12.50	100
A. niger	6	10	85.71	9	10.71	81.81	10.42	83.33
A. oryzae	2	3.33	28.57	1	1.19	9.09	2.08	16.67
A. sydowii	2	3.33	28.57	1	1.19	9.09	2.08	16.67
A. terreus	5	8.33	71.43	9	10.71	81.81	9.72	50.0
A. ustus	1	1.67	14.28	1	1.19	9.09	1.39	11.11
A. versicolor	1	1.67	14.28	0	0	0	0.69	5.55
Cladosporium cladosporioides	3	5.0	42.86	0	0	0	2.08	16.67
Emericella nidulans	3	5.0	42.86	0	0	0	2.08	16.67
E. quadrilineata	2	3.33	28.57	4	4.76	36.63	4.17	33.33
Eupenicillium spp.	1	1.67	14.28	0	0	0	0.69	5.55
Eurotium chevalieri	1	1.67	14.28	0	0	0	0.69	5.55
Fennellia flavipes	0	0	0	1	1.19	9.09	0.69	5.55
Gibberella fujikuroi	1	1.67	14.28	1	1.19	9.09	1.39	11.11
Humicola minima	0	0	0	1	1.19	9.09	0.69	5.55
Paecilomyces voriotii	1	1.67	14.28	1	1.19	9.09	1.39	11.11
Penicillium aurantiogriseum	7	11.67	100	9	10.71	81.81	11.11	88.89
P. chrysogenum	7	11.67	100	11	13.09	100	12.50	100
P. citrinum	0	0	0	1	1.19	9.09	0.69	5.55
P. duclauxii	0	0	0	2	2.38		1.39	11.11
P. funiculosum	0	0	0	3	3.57	18.18	2.08	16.67
Pleospora tarda	1	1.67	14.28	0	0	27.27	0.69	5.55
Scopulariopsis brevicaulis	2	3.33	28.57	0	0	0	1.39	11.11
S. candida	1	1.67	14.28	0	0	0	0.69	5.55
Total	60			84		0		

* N.C.I. = Number of Cases of Isolations .

Table (8): Frequency distribution and incidence percentages of isolated mould & yeast from poultry feed ingredients.

Fungal Isolates	Soybean meal (13)			Wheat bran (12)			Yellow corn (14)			White corn (3)			Overall	Overall
	N.C.I	Inc.%	Frq. %	NCI	Inc%	Frq.%	N.CI	Inc%	Frq.%	N.CI	Inc %	Frq.%	Incid. %	Frq. %
Acremonium strictum	1	0.88	7.69	1	1	8.33	1	0.9	7.14	1	4.17	33.33	1.15	9.52
Alternaria alternata	3	2.65	23.1	0	0	0	2	1.8	14.28	0	0	0	1.44	11.9
A. chlamydospora	2	1.77	15.38	1	1	8.33	0	0	0	1	4.17	33.33	1.15	9.52
Aspergillus alutaceus	0	0	0	1	1	8.33	2	1.8	14.28	0	0	0	0.86	7.14
A. clavatus	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
A. flavus	10	8.8	76.9	11	11	91.67	12	10.81	85.71	3	12.5	100	10.34	85.71
A. flavus var columnaris	0	0	0	1	1	8.33	0	0	0	0	0	0	0.29	2.38
A. fumigatus	13	11.5	100	12	12	100	14	12.61	100	3	12.5	100	12.1	100
A. melleus	1	0.88	7.69	0	0	0	2	1.8	14.28	0	0	0	0.86	7.14
A. niger	8	7.1	61.54	12	12	100	12	10.81	85.71	3	12.5	100	10.1	83.33
A. oryzae	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
A. sydowii	2	1.77	15.38	1	1	8.33	1	0.9	7.14	0	0	0	1.15	9.52
A. tamarii	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
A. terreus	7	6.19	53.8	11	11	91.67	12	10.81	85.71	1	4.17	33.33	8.91	73.81
A. versicolor	1	0.88	7.69	2	2	16.67	2	1.8	14.28	0	0	0	1.44	11.9
Chrysosporium tropicum	1	0.88	7.69	1	1	8.33	0	0	0	0	0	0	0.57	4.76
Cladosporium cladosporioides	7	6.19	53.8	0	0	0	0	0	0	0	0	0	2.01	16.67
Emericella nidulans	5	4.42	38.5	4	4	33.33	3	2.7	21.43	2	8.33	66.7	4.02	33.33
E. quadrilineata	0	0	0	1	1	8.33	0	0	0	0	0	0	0.29	2.38
Fennellia flavipes	0	0	0	4	4	33.33	0	0	0	0	0	0	1.15	9.52
Gibberella fujikuroi	3	2.85	23.1	4	4	33.33	6	5.4	42.86	1	4.17	33.33	4.02	33.33
Humicola grisea	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Hypomyces chrysospermus	2	1.77	15.38	1	1	8.33	1	0.9	7.14	2	8.33	66.7	1.72	14.28
Mucor racemosus	0	0	0	0	0	0	1	0.9	7.14	0	0	0	0.29	2.38
Paecilomyces voriotii	4	3.54	30.77	2	2	16.67	4	3.6	28.57	1	4.17	33.33	3.16	29.19
Penicillium aurantiogriseum	11	9.73	84.6	12	12	100	12	10.81	85.71	3	12.5	100	10.92	90.48
P. chrysogenum	13	11.5	100	12	12	100	13	11.71	92.86	3	12.5	100	11.78	97.62
P. citrinum	2	1.77	15.38	1	1	8.33	2	1.8	14.28	0	0	0	1.44	11.9
P. duclauxii	2	1.77	15.38	0	0	0	3	2.7	21.43	0	0	0	1.44	11.9
P. funiculosum	7	6.19	53.8	3	3	25.0	5	4.5	35.71	0	0	0	4.31	35.71
P. oxilacum	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Pleospora tarda	1	0.88	7.69	1	1	8.33	0	0	0	0	0	0	0.57	4.76
Scopulariopsis brevic aulis	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Scytalidium lignicola	0	0	0	0	0	0	1	0.9	7.14	0	0	0	0.29	2.38
Torula sp.	0	0	0	1	1	8.33	0	0	0	0	0	0	0.29	2.38
Yeasts	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Total	113			100			111			24				

The isolated mycoflora from mixed poultry feeds pose problems due to the ability of some of them to invade the animal tissues (*A. fumigatus*) as well as their ability to grow on feed, causing their spoilage beside production of mycotoxins (Moreno-Rome & Fernandez, 1986). So it is necessary to note the high prevalence of these mycoflora in general and with special emphasis on which comprise the most important and well known mycotoxin producing fungi affecting the mixed poultry feeds and their ingredients.

The frequency distribution and incidence percentages of the isolated fungi were shown in tables (6-8). A total of 20 genera; 42 species and one species variety were isolated from all examined samples. The most prevelant genera were *Aspergillus* that represented by overall frequency percentages of 100% in both of laying and broiler rations, followed by *Penicillium* spp., *Cladosporium* spp., *Gibberella* spp., *Paciliomyces* spp., *Emericella* spp., *Hypomyces* spp., *Fennellia* spp., *Alternaria* spp., *Trichoderma* spp. and *Mucor* species. The remaining identified genera had lower frequencies of isolation and they were designated as mycoflora of rare occurrence in poultry mixed feeds and their ingredients (Ogundero, 1980).

A. fumigatus was the dominant species isolated from all examined samples with an overall frequency of 100 %. *The* fungus causes respiratory disease in man and poultry (Lacey, 1975). The ingestion of spoiled poultry feeds with the toxic fungal metabolites is responsible for the poultry toxicosis (Lovett, 1972). Furthermore, *A. fumigatus* is very dangerous for patients with reduced immunological competence as a consequence of chronic diseases including diseases of lung, liver and kidney, leukemia, tuberculosis, diabetes and kidney and AIDS (Reiss, 1995).

A. flavus is considered as a dominant species in the poultry feeds, concentrate mixtures and feed ingredients (tables 3-5). The mean values/g of *A. flavus* were $8.8x10^3$, $8.3x10^3$,$4.8x10^3$, $3.7x10^3$, $2.1x10^3$, $1.1x10^3$, $1.0x10^3$ and $1.0x10^3$ in yellow corn; broiler rations; laying rations; wheat bran; white corn; broiler concentrate mixtures; layer concentrate mixture and soybean meal respectively. It was recovered with an over all frequency of 100% from laying and broiler rations; 85.71% from poultry feed ingredients and 83.33% from laying and broiler concentrate mixtures. *A. flavus* is considered the main fungus that produces aflatoxins causing mycotoxicosis which has been recognized throughout the world as a major problem in poultry and animal industry (Mirocha & Christensen, 1974 and Abarca et al., 1994).

Generally *Aspergillus* species are incriminaed in poultry mycotoxicosis as many of them are toxic producer including *A. flavus, A. fumigatus, A. terreus, A. clavatus* and *A. glaucus* (Forgacs, 1966 and Lovett, 1972). Furthermore, avian aspergillosis which is caused by several species of *Aspergilli* as *A. fumigatus, A. nidulans* and *A. niger* (Singh & Singh, 1970; Hubbert et al., 1975 and Muller, 1984).

Penicillium species were recovered with high frequency distributions as *P. chrysogenum* which showed an overall frequency of 100; 97.62 and 95.45% in poultry concentrate mixtures, feed ingredients and poultry rations respectively. *Penicillum* spp. are implicated in pulmonary mycosis, gastrointestinal disturbances and poultry mycotoxicosis (Christensen et al., 1968 and Lovett, 1972).

The two species of *Alternaria* that could be isolated were *Alternaria alternata* and *Alternaria chlamydospora*. *A. alternata* was recovered with overall frequency of 11.9; 5.55 and 4.54% from feed ingredients, concentrate mixtures and poultry rations respectively. On the other hand, *A. chlamydospora* was recorded with overall frequencies of 16.67; 9.52 and 4.54% in concentrate mixtures, feed ingredients and poultry rations respectively. *Alternaria* spp. are reported as saprophytes however, they have been recorded as opportunistic causative agent of poultry mycotoxicosis (Abrams, 1965 and Forgacs et al., 1962).

The highest overall frequency of *Gibberella fujikuroi* was obsereved in poultry rations(68.18%), followed by feed ingredients (33.33%). The fungus produces estrogenic substance which inhibits ovulations and significantly reduce egg production and feed consumption in laying hens (Schaible, 1970 and Adams & Tuite, 1976).

The remaining identified genera including *Cladosporium cladosporioides; Mucor racemosus; Paeciliomyces voriotii; Scopulariopsis brevicaulis* and *Trichoderma harzianum* occurred with variable overall frequencies in the examined samples. From the hygienic point of view *Cladosporium* spp. are implicated in some cases of dermatitis in chickens and poultry mycotoxicosis (Christensen et al., 1968 and Lovett, 1972). The *Mucor* spp. representing health hazard of the birds as they incriminated in mucormycosis of birds and man (Jand & Dhillon, 1973 and Al-Doory, 1980). *Paeciliomyces* spp. are incriminated in some pulmonary affections of chickens and usually isolated from the lungs and air sacs of diseased birds (Chute et al., 1956 and Eckman & Morgan, 1979). Furthermore, *Paeciliomyces* were implicated in poultry mycotoxicosis (Abrams, 1965 and Forgacs, 1966). *Scopulariopsis* spp. are incriminated in pulmonary affection of chickens and in some cases of mycotoxicosis (Chute, et al., 1956; Christensen et al., 1968 and Lovett, 1972). Moreover, *Scopulariopsis* have been reported as a causative agent for nail affections and in deep seated granulomatus lesions (Al-Doory, 1980). *Trichoderma* spp. were recorded as a pathogen to young broiler chicks and usually isolated from the lungs and air sacs beside they are incriminated in avian mycotoxicosis (Chute, et al., 1956; Christensen et al., 1968 and Lovett, 1972). Finally the fungal contamination of poultry feeds and their ingredients leads to losses of their nutrient constituents beside the determintal effects on poultry health and production, so, it is of urgent need to evaluate and control the mycological quality (Buhatel et al., 1982).

CONCLUSION :

The results of this study showed a high incidence of a wide variety of fungi from the poultry feeds and their ingredients. Presence of *Aspergillus flavus* and others in some of these feeds may include pathogenic strains producing aflatoxins which may constitutes a potential health hazards to poultry and man. Additional comprehensive monitoring programmes with regular intervals must be considered to evaluate the hygienic fitness of such poultry feeds and their ingredients to overcome and minimize the economic losses in the Egyptian poultry farms and to safeguard human health. Moreover, the authors recommended the prevention of initial mould growth by using good hygienic stores for the poultry feeds and the use of proper antifungal agents. In this respect the use of mould inhibitors in the mixed feeds may be of economical importance during periods when feeds may have to be stored under conditions where heating is the major significant factor (Hafez et al.,1995).

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التقييـــــم الفطـــرى لعلائق الدواجـــن وبعض المركزات والمكونات خاصتها

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أجريت هذه الدراسة للوقوف على الحالة الصحية لبعض علائق الدواجن ومكوناتها وذلك بالتقييم الفطرى لها . وإستيفاءاً لهذا الغرض فإنه قد تم تجميع وإجراء الفحوص الميكولوجية لإجمـالى عدد ٨٢ عينة شملت ٢٢ عينة عليقة منتج نهائى ، ١٨ عينـة مركـزات أعـلاف هذا بالإضافـة إلـى ٤٢ عينة من المكونات الأولية لهذه العلائق والتـى تـم تجميعهـا مـن وحـدات متخصصـة لإنتـاج علائـق الدواجن المختلفة .

وقد أظهرت النتائج وجود محتوى مائى تمثل فى متوسطات قيم الرطوبـة النسبية لبعض هذة العلائق ومكوناتها والتى تراوحت بين ٤,٥٨ ± ٠,٧٢ إلى ٧,٠٦ ± ٠,١٧ هذا بالإضافة إلـى وجـود أعداد من الفطريات والخمائر تمثلت بمتوسطات قيم تقع بين ($١,٩ \times ١٠^{٤}$ إلى $٥,٢ \times ١٠^{٤}$) وذلك للعد الطبقى القياسى لكل ١ جرام عند درجة حرارة الغرفة والتـى قـدرت بقيمـة عـد أ دنـى $٤,٤ \times ١٠^{٣}$ لكل ١ جم من مركزات العلائق وكذلك عد أقصى قدره $١,٣ \times ١٠^{٥}$ لكل ١ جم من علائق الدواجن . وقد أسفرت الدراسة عن عزل العديد من عترات الفطريات والتى لها أهمية بالغة لصحة وإنتاجيـة الدواجن وصناعتها والتى بلغ إجمالها ٦٦٤ عترة لعدد ٢٠ جنس من الفطريات المختلفـة وقـد تمـت مناقشة الأهمية الصحية لأهم عترات الفطريـات المعزولـة هـذا بالاضافـة للتوصيـات والاشـتراطات الصحية الواجب توافرها للحصول على منتج ذى مواصفات جيدة يخلو من مسببات الأمراض والتى قد تنجم عن وجود بعض الفطريات المفرزة للسموم حفاظا على الثروة الداجنة .

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ORGANOCHLORINE RESIDUES IN BUFFALOE AND CATTLE TISSUES IN ASSIUT GOVERNORATE

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ABSTRACT :

Pesticide residues were studied in 164 tissue samples (120 fat, 20 liver and 24 muscle samples) collected from Assiut Governorate during March-April 1992 by GC-ECD.

Liver and muscle samples were found to contain negligible amounts of organochlorine pesticides that never exceeded Extraneous Residue Limit (ERL) ERL's, while fat samples contained relatively higher amounts that rarely exceeded the permissible limits.

DDT derivatives (p,p'-DDT, o,p'-DDT, p,p'-DDD and p,p'-DDE), total HCH isomers (α-, β-, γ- and δ-isomers) and hexachlorobenzene (HCB) were the most frequent pesticides, followed by aldrin and dieldrin, endrin and heptachlor and heptachlor epoxide. Only one buffaloe fat sample (1.3%) exceeded endrin released by the Codex Committee on Pesticide Residue (CCPR) of FAO/WHO in 1990.

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INTRODUCTION :

There is a growing awareness and alarm regarding the possible hazards and economic losses to ever increasing number of toxic substances that enter the food chain. Many of the contaminants enter food directly or indirectly as a result of human activities.

Pesticides occupy a rather unique position among the many chemicals that man encounters daily, in that they are deliberately added to the environment for many purposes. Over the years, vast quantities of chlorinated pesticides have been used in Egypt for crop protection and control of disease transmitting insects.

Public concern about the hazards of health and the environment from the use of agricultural chemicals had been increased dramatically in recent years. Much of the public concern is focused on the residues of the organochlorine compounds. Some of the most prominent pesticides of this group includes DDT, aldrin, dieldrin, lindane (gamma-HCH) and heptachlor. Such chemicals are characterized by their great tendency to accumulate and persist in animal tissues as they are all fat soluble pesticides. They reach man through the food chain and accumulate in various organs, but mainly in fatty tissues. However, pesticides from other groups, such as organophosphates and carbamates that are not free of problems associated with their use, do not pose serious long term residue problems (Fries, 1970).

In Egypt, although the use of chlorinated hydrocarbon pesticides had been curtailed since the early 1970s, high amounts of these chemicals were found in meat, milk and fish samples collected from some Egyptian Governorates (Saleh, 1986; El-Shafei, 1988; Dogheim et al., 1988 and 1990 and salem et al., 1995).

The present work reports the levels of organochlorine pesticide residues in buffaloe and cattle tissues from Assiut Governorate, Egypt.

MATERIALS AND METHODS :

A total number of 120 perinephric fat samples (80 buffaloes and 40 cattle), 20 liver samples (10 of each) and 24 muscle samples (14 of buffaloes and 10 of cattle) from buffaloe and cattle carcasses generally consumed by man were collected randomly during March-April 1992 from the slaughter houses in Assiut Governorate. The samples were transferred into the laboratory, minced and frozen until analysis.

Extraction of tissue samples:

Liver and muscle samples were extracted according to Food and Drug Administration, PAM1 (1988).

Adipose tissue samples were extracted according to the method established by the Federal Institute for the Control of Infectious Diseases in Livestock in Austria (Anonymous,

1988) and related to Stijve and Cardinale, (1974).

Clean-up was carried out according to the method that followed in fat samples (Anonymous, 1988).

Pesticide reference standards:

Alpha-HCH (Supelco Nr. 4-8493), Beta-HCH (Supelco Nr. 4-8494), Gamma-HCH (Supelco Nr. 4-9049) and delta-HCH (Supelco Nr. 4-8495), p,p'-DDE (Supelco Nr. 4-9017), p,p'-DDD (Supelco Nr. 4-9009), o,p'-DDT (Ehrenstorfer P1111), p,p'-DDT (Supelco Nr. 4-9019), Heptachlor and heptachlor epoxide (Supelco Nr. 4-9041 and 4-9042), Aldrin and dieldrin (Supelco Nr. 4-9000 and 4-9024), Hexachlorobenzene (Supelco Nr. 4-8508) and endrin (Supelco Nr. 4-9032). Standard solution of reference materials were prepared in petroleum ether.

Gas chromatograph- Carlo Erba MEGA HRGC 5330 with ^{63}Ni ECD and split-splitless injector and column, HP ULTRA1 50m X 0.2mmX 0.33um was used, according to the method of Suzuki, et al (1979).

RESULTS AND DISCUSSION :

The results of organochlorine pesticide residues are presented in table 1 which show that liver, muscle and fat samples of buffaloes and cattle were found to contain DDT and its metabolites, HCH isomers, heptachlor , heptachlor epoxide, aldrin and dieldrin, HCB and endrin residues with different frequencies and levels (table 1)

According to the previous studies carried out in many countries, i.e., Egypt (Saleh, 1986; El-Shafei, 1988 and Dogheim et al., 1988, 1990 and 1991), Denmark (Bro-Rasmussen, 1968); France (De Lavaur and Hascoet, 1974); Italy (Pastore and Vecchia, 1974), the pesticides most commonly found in the various animal foodstuffs are lindane, the isomers of BHC (HCH), DDT and its metabolites (DDE and DDD), dieldrin and heptachlor epoxide. HCB and HCH gamma and alpha isomers were the most frequently detected pesticides in Germany (Knoeppler, 1976) and in Austria (Jarc, 1980).

DDT derivatives o,p´ and p,p´, DDE and DDD contributing the DDT complex were detected in all tissue samples of liver, muscle and adipose tissues with 100% frequency. Highest amounts of the DDT complex were determined in adipose tissue samples. This could be attributed to the high solubility and tendency of DDT and its metabolites to accumulate and store in fatty tissues (WHO, 1979 and 1989).

Residual amounts detected in buffaloe tissue samples were almost higher than that in cattle.

DDE constituted more than 80% of the DDT complex residues in both animal tissues, but p,p´-DDT was represented by 8% (table 2) . DDE was the most frequently presented

TABLE (1) : Mean, range values (ppm)[a] and percentage's frequency (%F) of organochlorine pesticide residues detected in buffaloe and cattle tissue samples collected from Assiut Governorate in 1992.

		Buffaloes			Cattle		
Pesticides	Values	Liver	Muscle	Fat	Liver	Muscle	Fat
DDT complex	Mean	0.041	0.023	0.213	0.023	0.018	0.108
	Range	0.011-0.080	0.010-0.053	0.025-0.869	0.015-0.029	0.004-0.051	0.009-0.758
	Frequency	100	100	100	100	100	100
Total HCH isomers	Mean	0.032	0.010	0.030	0.024	0.012	0.017
	Range	0.002-0.083	0.004-0.022	0.002-0.138	0.001-0.032	0.001-0.019	0.001-0.048
	Frequency	100	100	96.25	100	100	87.5
γ-HCH (Lindane)	Mean	0.002	0.002	0.004	0.001	0.001	0.002
	Range	0.001-0.005	0.001-0.005	0.001-0.039	0.0004-0.003	0.001-0.002	0.001-0.005
	Frequency	100	85.7	30	90	60	22.5
Heptachlor & Hep. epoxide	Mean	0.006	0.009	0.011	0.001	0.0015	0.008
	Range	0.0003-0.009	0.009-0.009	0.0003-0.061	0.001-0.001	0.001-0.002	0.008-0.008
	Frequency	50	7.1	12.5	20	20	2.5
Aldrin & dieldrin	Mean	0.003	0.001	0.004	0.0007	0.002	0.003
	Range	0.001-0.009	0.001-0.002	0.001-0.009	0.0006-0.001	0.001-0.003	0.0003-0.008
	Frequency	40	21.4	35	40	20	15
Endrin	Mean	0.008	0.001	0.013	0.001	0.002	0.006
	Range	0.001-0.016	0.0002-0.001	0.001-0.121	0.001-0.002	0.0007-0.003	0.002-0.011
	Frequency	50	50	30	40	50	12.5
HCB	Mean	0.001	0.0006	0.004	0.001	0.001	0.004
	Range	0.0004-0.004	0.0002-0.001	0.0003-0.017	0.0006-0.002	0.0006-0.003	0.0002-0.022
	Frequency	100	71.4	98.75	100	70	92.5

a = on fat basis

derivative (100%) with the highest amounts, more than 80% of the DDT complex in all tissue samples indicating the continuous degradation of DDT to the less toxic and more persistent derivative as reported or studied by Fries et al., (1972) and Hayes, (1975); who reported that DDE is more resistant to metabolic degradation than DDT in animals and man. Also, DDE is found in almost all the living organisms because of its strong affinity with body fat (Jensen and Jasson, 1976).

In spite of the known information about the prohibition use of DDT in Egypt along the last fifteen years according to the authorities report, the continuous use of the acaricide dicofol (Kelthane®) replaced DDT as the primary source of environmental DDE and contains as much as 0.6 % p,p´ and o,p´-DDT (Camoni et al., 1983); this indicates the continuous contamination of the environment by DDT and DDE.

Edwards, (1973) reported that DDT has an average half life in the soil of about 3 years, and 5-10% of the amounts applied still remains 10 years after the application, while Nash and Woolson, (1967) stated that as much as 40% of DDT were still present 17 years in a Maryland

Table 2. Mean, maximum values and frequency (f) of individual organochlorine pesticide residues detected in buffaloes and cattle fat samples collected from Assiut Governorate.

Pesticides	Animal species					
	Buffaloes			Cattle		
	mean	max.	f	mean	max.	f
p,p'-DDT	0.010	0.037	81.3	0.006	0.013	25
o,p'-DDT	0.017	0.120	8.8	0.021	0.035	5
p,p'-DDE	0.183	0.727	100	0.105	0.758	100
p,p'-DDD	0.020	0.068	93.8	0.006	0.018	27.5
DDT complex	0.213	0.869	100	0.108	0.758	100
α-HCH	0.004	0.026	50	0.004	0.026	72.5
β-HCH	0.028	0.126	96.3	0.015	0.048	77.5
γ-HCH	0.004	0.039	30	0.002	0.005	22.5
δ-HCH	0.002	0.003	10	ND	ND	0
HCH isomers	0.030	0.138	96.3	0.017	0.048	87.5
Heptachlor	ND	ND	0	0.004	0.004	2.5
Hept. epoxide	0.011	0.061	12.5	0.004	0.004	2.5
Total heptachlors	0.011	0.061	12.5	0.008	0.008	2.5
Aldrin	ND	ND	0	ND	ND	0
Dieldrin	0.004	0.009	35	0.003	0.008	15
Aldrin & Dieldrin	0.004	0.009	35	0.003	0.008	15
Endrin	0.013	0.121	30	0.006	0.011	12.5
HCB	0.004	0.017	98.8	0.004	0.022	92.5

ND = not detected.

soil after the application; which means a permanent source of pollution with DDT. On the basis of ERL of 5 mg/kg total DDT complex residues in animal tissues, all the analyzed samples were prooved to he below the limit.

Total hexachlorocyclohexane isomers (alpha, beta, gamma and delta) were detected at the same frequency as DDT in liver and muscle samples and with less frequency in fat samples. Detected residues of total HCH isomers were almost high in buffaloe than in cattle tissues especially in adipose tissue and liver samples. This may be attributed to the body fat condition, which was noticed during sample's collection and almost seemed that cattle contain more fat in its carcasses. Spence et al., (1990) recorded that the reduction in BHC residue levels occurred by redistribution and dilution throughout the increased body fat.

α- and β-HCH were the most frequent isomers detected in all tissues of both animals, but β-HCH constituted more than 80% of the total HCH residues in all the analyzed samples (table 2). All the investigated tissues were below the ERL's (2 ppm) for total HCH isomers and lindane.

Beta isomer was the most pronounced HCH isomer detected in all tissue samples of buffaloes and cattle, which agreed with Posgay

et al., (1980) and constituted more than 80% of the total HCH residues in all tissues. This isomer is the most persistent and slowly eliminated from the body (Pfeilsticker, 1973) and has the ability to accumulate in fat tissues 10-30 times than lindane (Heeschen, 1980), but it is not the most hazardous one (Scholz et al., 1985). Its predominant occurrence in the tissue samples indicate the continuous degradation of the more toxic alpha and gamma isomers (Dogheim et al., 1988).

Lindane (gamma isomer of HCH) which is used alone as a pesticide and also is the most toxic isomer of this group was always detected in insignificant amounts. Almost no differences in the residual amounts of lindane were observed between the different tissues of the two animal sorts.

α-HCH was also more frequent in tissue samples as β-HCH but with very low values. This may be attributed to the possible use of technical HCH that contained a large proportion of α-HCH or the external treatment of animals with HCH based veterinary preparations, which could be ingested or absorbed through skin (Harper, 1980). Gamma-HCH transforms to the alpha and delta isomers, which are of 4 and 50 times less insecticidal activity, respectively (Newland et al., 1969).

Taking into consideration that the increase in HCH total isomers was mainly due to the beta isomer which is only a minor component in the technical HCH pesticide (Pfeilsticker, 1973). It might be concluded that these exceeding amounts could be attributed to the degradation process of the other isomers giving elevated amounts to the most persistent and relatively least hazardous beta isomer. Jensen, (1983) stated that a- and g-HCH isomers may isomerize into the b-isomer in the living organisms .

A sum of heptachlor and heptachlor epoxide defined as total heptachlors were detected in liver, muscle and adipose tissue samples at low frequencies and levels but they could not be detected (table 2). Higher residual amounts of heptachlors were almost detected in buffaloe tissue samples. Adipose tissue samples expressed the highest levels of total heptachlors' residues in contrast to other tissue samples due to the storage of these compounds in the animal fat (Rusoff et al., 1963 and Bruce et al., 1965).

Wilkinson et al., (1964) indicated that toxic residues mostly heptachlor epoxide, will persist in the soil for as long as 9 years, the possibility thus exists for contamination of crops from these fields. However, it was translocated from the soil into certain crops (Bruce and Decker, 1966), subsequently stored in the fat of dairy cows (Rusoff et al., 1963).

Referring to the ERL´s of the (CCPR, 1990) presence of 0.2 mg/kg of heptachlor and its epoxide in animal tissue, it could be revealed that all detected residues in buffaloes and cattle tissues were below the permissible limit.

Aldrin residue definition includes dieldrin residues as well. Inspite of dieldrin being an insecticide by itself it is also resulting as a degradation product from aldrin application. A statement that was mentioned by Bann et al., (1956) that dieldrin is the form of which aldrin usually being stored in fats.

The magnitude of aldrin and dieldrin residues revealed low levels and few frequencies in liver, muscle and adipose tissue samples of both animals.

Aldrin residues were absent almost from all samples as the total residues resulted mainly from dieldrin (table 2). All analyzed buffaloes and cattle tissue samples contained aldrin and dieldrin residues below the extraneous residue limit.

Concerning solubility and tendency to accumulate in fatty tissues (Kiigemgi et al., 1958 and Humphrays, 1988), endrin was detected at higher amounts in adipose tissues of buffaloes and cattle but still at low frequencies.

As far as human safety is concerned, endrin is the most hazardous pesticide from the cyclodiene group. The extraneous residue limit of endrin in animal tissues is 0.1 mg/kg (CCPR, 1990). All liver and muscle samples from buffaloes and cattle were safe for human consumption where endrin residues were absent or below the permissible limits. Only one fat sample from (1.3%) buffaloe out of 80 exceeded the ERL. Cattle adipose tissue never exceeded the limit.

The fungicide, industrial product and by-product HCB, has since the early 1970s been recognized as an environmental contaminant comparable to DDT and PCBs in industrialized countries (Acker and Schulte, 1970 and Stijve, 1971). On the other hand, HCB contamination does not seem to be a serious problem in Egypt, since only very low residue levels were found which never exceeded the permissible limits. The increase in its frequency in this study may indicate a recent exposure of our environment .

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متبقيات المبيدات العضوية الكلورينية في أنسجة الجاموس والأبقار في محافظة أسيوط

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*قسم الطب الشرعى والسموم – كلية الطب البيطرى ـ جامعة أسيوط ** الهيئة القومية للسيطرة على الأمراض المعدية – وزارة الصحة – مودلنج – النمسا ، *** المعمل المركزى للمبيدات – معهد البحوث الزراعية – وزارة الزراعة – الدقى – القاهرة

إن تزايد المخاطر البيئية من جراء استخدام المبيدات الحشرية فى الأغراض الزراعية وفي الطب البيطرى أدى إلى تلوث العديد من المزروعات والأغذية الحيوانية مما ساعد على زيادة هذه المركبات فى أنسجة الحيوانات ومنتجاتها . وهذا دفعنا إلى إجراء هذه الدراسة لاستبيان مستوى تلوث أنسجة الجاموس والأبقار فى مدينة أسيوط ببعض المبيدات الحشرية العضوية الكلورينية .

وقد تم فى هذه الدراسة قياس مستويات مركبات ال د.د.ت.ومركبات سادس كلوريد الهكسان الحلقية بالإضافة إلى مركبات الهبتاكلور والهبتاكلورأبوكسيد ومركبات الالدرين والديلدرين والاندرين ومركبات سادس كلوريد البنزين وذلك فى أنسجة عدد ١٦٤ عينة . وقد استخدم جهاز الفصل الغازى المزود بكاشف إليكترونى (GC-ECD) لهذا الغرض .

أوضحت النتائج وجود كميات قليلة جداً من المركبات الكلورينية فى كبد وعضلات العينات المفحوصة والتى لاتزيد عن الكميات المسموح بها دولياً بواسطة المنظمات العالمية (ERL) بينما أظهرت عينات الدهن وجود كميات عالية نسبياً ولكن نادراً ما تكون أعلى من الكميات المسموح بها عالمياً .

كما أظهرت النتائج أن مشتقات ال د.د.ت.وسادس كلوريد الهكسان الحلقية مجتمعة وسادس كلوريد البنزين كانت أعلى ظهوراً يليها مركبات الالدرين والديلدرين والاندرين والهبتاكلور أبوكسيد كما أظهرت عينة واحدة فقط من دهن الجاموس (١,٣٪) زيادة فى كمية الاندرين عن الكميات المسموحة بواسطة منظمة الصحة العالمية ومنظمة الأغذية والزراعة عام ١٩٩٠ ومن ثم فإن الفحص الدورى والمستمر لتواجد المبيدات الحشرية فى الأغذية والمنتجات الحيوانية يعد من الامور الهامة التى يجب أن تؤخذ فى الاعتبار لتدارك أخطارها.

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GEOELECTRICAL PROSPECTING FOR GROUND WATER AND SUBSURFACE GRAVEL OCCURRENCES IN THE SOUTHERN PART OF EL-SALHAIYA PLAIN, ISMAILIA GOVERNORATE

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ABSTRACT :

The southern part of El-Salhaiya plain extends parallel to the Ismailia irrigation canal for a distance of about 40 km., covering an area of about 600 km^2 . It is one of the most important reclamation and agricultural projects in Egypt. The geologic and hydrogeologic history of this area concluded that the Quaternary ground water aquifer has an appreciable thickness, besides the presence of subsurface gravel bodies with reasonable thicknesses, depths and lateral extension. Twenty Schlumberger vertical electrical soundings (VES) were measured, covering an area in a grid pattern system in order to evaluate the ground water and gravel occurrences. This geoelectrical surveying has detected a fresh water horizon ranging in thickness from 8 to 38 meters where the recommended depths and sites for drilling water wells are given. On the other hand, the delineated thicknesses of the subsurface gravel deposits, with different grain sizes, vary from 10 to 45 meters occurring at depths of approximately 10 meters where an exploitation map is proposed .

INTRODUCTION :

The serious increment of population in Egypt requires directing our attention towards reclamation projects taking into account that these projects should depend, mainly, on the ground water and not on the Nile water. El-Salhaiya plain is one of these large projects. The present piece of work studies only the southern part of this plain which extends

parallel to the Ismailia irrigation canal for a distance of about 40 km and goes northward for about 15 km, covering an area of about 600 km^2 . This part of the plain comprises El-Qassasine city and the associated villages Fig.(1).

PURPOSE OF STUDY :

The purpose of the study has been directed to cover the following topics :

1- Prospecting for ground water, their conditions; depths; qualities and recommending sites for drilling productive wells.
2- Prospecting for subsurface gravel deposits, their depths; thicknesses; grain sizes; approximate volumes of each grain size and recommending sites for carrying out quarrying activities.

GEOLOGICAL AND HYDROGEOLOGICAL CONDITIONS :

The southern part of El-Salhaiya plain varies in altitude between 20 and 40 meters above sea level, surrounded by low lands from all sides Fig.(1). It is mainly composed of a clastic unit, varying in thickness from 200 to 250 meters of quartz sands and gravels, intercalated with clay lenses. These sediments are assigned to early Pleistocene age and they form the main ground water aquifer in the eastern Nile delta (El-Shazly et.al. 1975, Said 1981 and Zoetbrood 1984). It was also believed that this clastic unit might represent the final depositional phases of the Prenile when its water flow became minimum and its far tributaries suffered great deficiency in their water supply. The surface gravel associations represent the earliest channel-fills and vary in size from granules (2-4 mm) and pebbles (4-64 mm) to cobbles (> 64 mm) which are suitable for concrete manufacture, water wells casing and foundation purposes (El-Fawal & Shendi 1991). The coarse grain size characterizing the plain sediments can reflect good hydraulic properties and therefore good environment for ground water movements and accumulations (Geriesh 1989).

Structurally, the study area is a part of the traditional tectonic zone between the Gulf of Suez taphrogeosyncline and the unstable shelf of the northern part of Egypt (Said, 1981). NNW-SSE and NE-SW faulting are young and affected the quaternary sequence, whereas, the WNW-ESE faulting affected clearly the underlying Miocene rocks (El-Heiny 1981 and Said 1991). However, the ENE-WSW faults configurate a number of parallel morphotectonic basins with highs in-between. These basins controlled the depositional regime, thickness and configuration of the overlying quaternary sediments and can be considered as a good environment for ground water accumulation.

The clastic unit in the southern part of El-Salhaiya plain is classified as the main ground water aquifer which is of unconfined type and underlined by Miocene sequence of marine origin. Sometimes, clay lenses may exist forming confined conditions. The thickness of the sandy aquifer increases gradually from

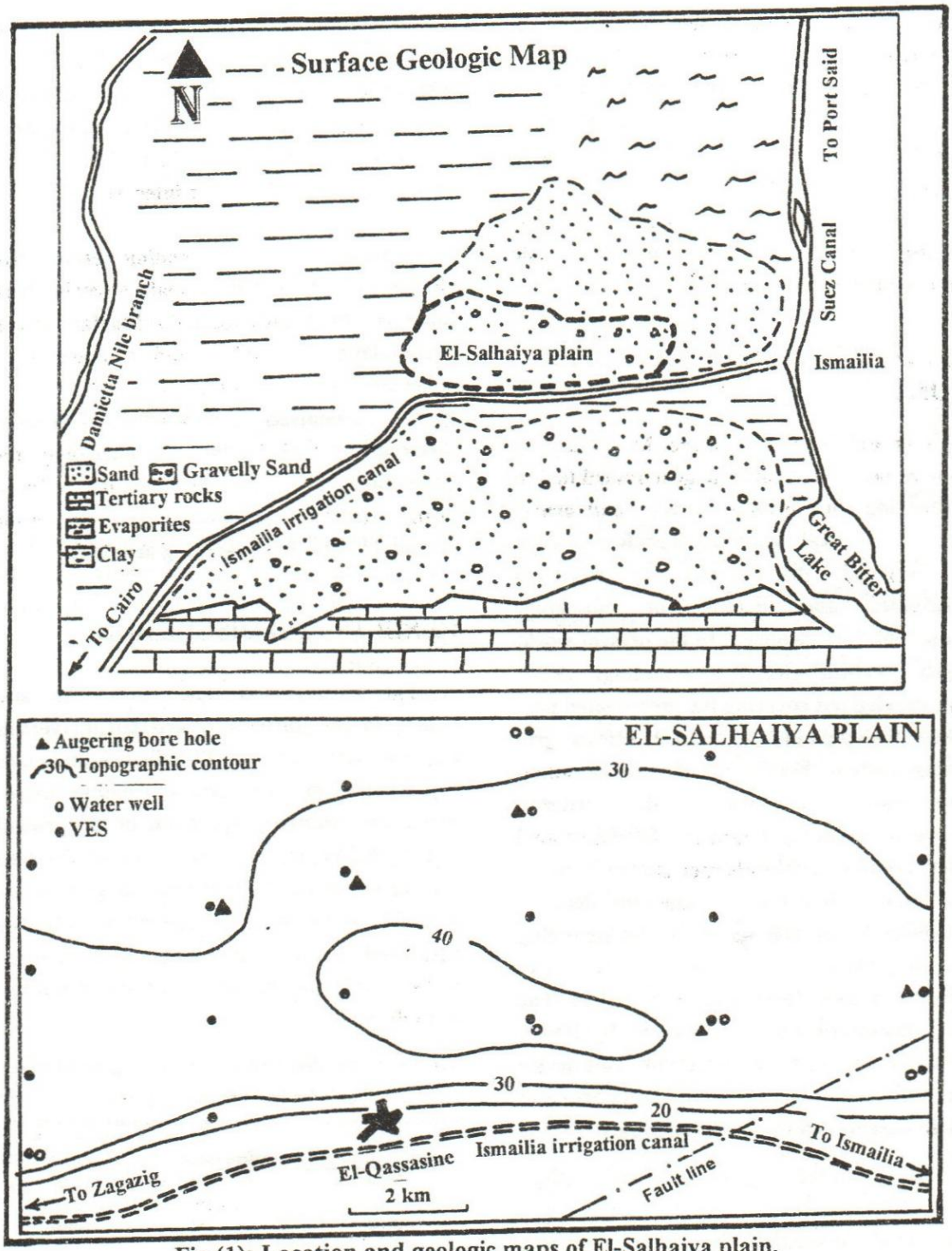

Fig.(1): Location and geologic maps of El-Salhaiya plain.

south to north due to the general tilting of the underlying Miocene rocks . The aquifer recharge from rain water doesn't exist due to the rare rainfall. However, Wadi El-Tumilate branch might have played a significant role in forming and recharging the ground water in the area because it has acted during its evolution history as a distributary for the Nile delta branches (Gereish 1994).

FIELD AND LABORATORY OPERATIONS :

It is well known that the D.C. resistivity method provides a valuable geophysical tool in prospecting for ground water and gravel deposits. Vertical sounding is preferred when the vertical distributions of electrical resistivities and depths of subsurface formations are required. In the present work, twenty vertical electrical soundings (VES) were carried out covering the investigated part of El-Salhaiya plain in a relatively regular grid pattern system Fig.(1). Six of these soundings were measured near water wells in order to deduce a resistivity spectrum for the ground water aquifer. Schlumberger electrode layout was used with a current electrode distance (AB-distance) of 600 meters. This spreading was long enough to penetrate the water bearing horizon for a few tens of meters. The field measurements were taken by RSP-6 resistivity and self potential unit. This device was designed to measure direct current resistivities and spontaneous potentials.

The calculated apparent resistivity values were then plotted on log-log papers and interpreted manually by curve matching technique through using two and three layer master curves of Mundry & Homilius (1979). Thereafter, the obtained true resistivities and depths were used as an initial input for a computer software of Velpen (1988) to get more accurate and reliable interpretation.

Depending on the sounding results, five sites were anticipated to make augering bore holes reaching the expected subsurface gravel accumulations (Fig.1) and samples were collected. These samples were subjected to grain size analysis using a system of sieves of different meshes trying to determine the percentage and approximate volume of each grain size as an initial step to decide reasonable sites for quarrying activities.

RESULTS AND DISCUSSION :

The interpreted true resistivities and depths to the different geoelectrical layers in the southern part of El-Salhaiya plain are represented in the form of contour maps. First. the resistivity spectrum of the ground water aquifer in the area could be deduced relying on the results of the soundings near the available water wells in compatible with the measured water salinity values from these wells. The expected spectrum values are listed in table (1).

Table (1): Resistivity spectrum of the ground water aquifer in El-Salhaiya plain.

Average water salinity (ppm)	True resistivity range (Ωm)	Assumed water quality
2500	50 - 100	salt
1300	100 - 200	brackish
1000	200 - 300	fresh

The geoelectrical results with respect to ground water occurrences and subsurface gravel deposits will be discussed as follows: the concerned clastic unit in the studied part of El-Salhaiya plain could be divided into three main geoelectrical layers which are :

1- The top layer varies in thickness from 1.5 to 6 meters and has a true resistivity ranging between 60 and 400 Ωm (Figs. 2&3). The great variation in resistivity values could be attributed to lithologic changes (i.e. variations in sand/clay ratio) and water content due to irrigation processes. The high resistivity values represent high percentage of gravel and pebble content. These deposits can be exploited by open quarrying operations. Accordingly, two sites are recommended (i.e. sites A & B, Fig.3) to exploit these deposits where, their true resistivity reaches as high as 500 Ωm. However, the removal of such deposits will improve the physical properties of the land for agricultural purposes.

2- The second geoelctrical layer has a true resistivity ranging between 140 and 1500 Ωm (Fig.4) and its thickness varies from 5 to 45 meters (Fig.5). This layer is treated as the main source of the subsurface gravel occurrences. The great variation in its true resistivity is mainly due to a change in gravel/sand ratio where the resistivity values increase towards the increment of this ratio. Accordingly, three sites are recommended for quarrying of gravel deposits (i.e. sites A,B & C, Fig.6). Site (A) is located to the east of El-Qassasine city and is easily accessible from El-Qassasine - Ismailia asphaltic road. The other two sites are located to the north of El-Qassasine city and they lie directly at the asphaltic road of El-Qassasine - El-Qantara (Fig.6). These three sites are anticipated according to the following reasons:

a- Their high values of true resistivities which could be attributed to the presence of considerable percentage of gravels accumulations.
b- Their great thicknesses which reach as high as 45 meters (Fig.5).
c- Relatively deep water table which leaves these sediments in dry conditions, reasonable for quarrying operations.

3- The third geoelectrical layer represents the ground water aquifer in El-Salhaiya plain. Depending on the vertical variation in its true resistivity, this layer could be divided into two zones which are:

a- Upper fresh water zone which has true resistivities varying from 100 to 500 Ωm (Fig.7) and thicknesses between 6 and 38 meters (Fig.8). It is noticed that the resistivity values decrease toward the Ismailia irrigation canal in opposite to what is expected. This means that the Ismailia canal has no direct recharging effect on this shallow water aquifer. However, the depth to this water zone increases northward (Fig.9); reflecting variations in ground topography. It is recommended that, drilling of wells should be directed towards the sites where there is a great thickness of this water zone (Fig.8). According to the resistivity spectrum of the ground water aquifer in the area, this zone is classified as fresh water aquifer which may have water with low salinity (1000 ppm, table 1).

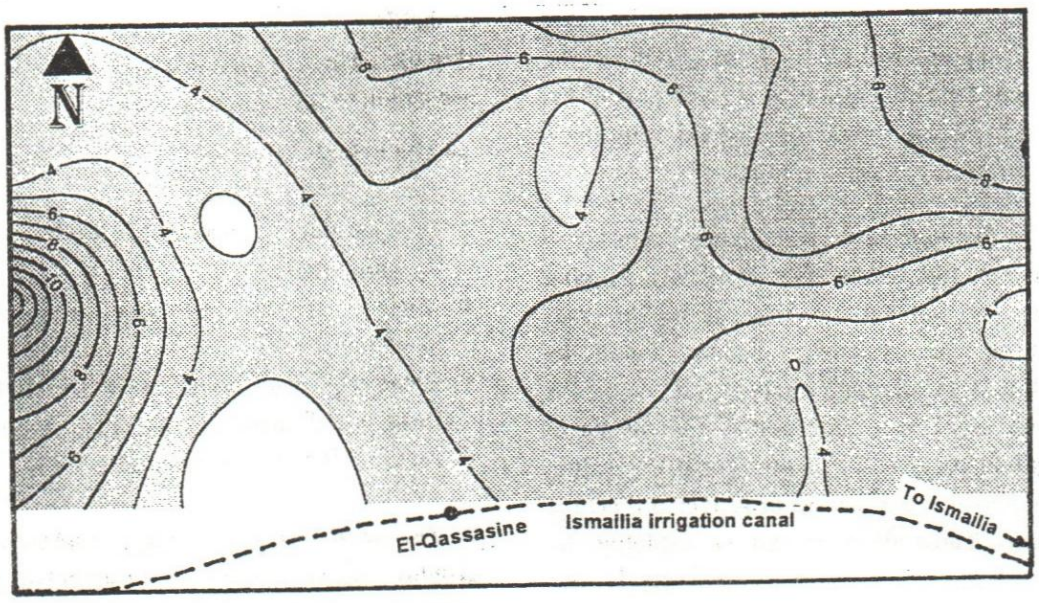

Fig.(2): Thicknesses of the surface geoelectrical layer (in meters)
(Depths to the subsurface gravel deposits)

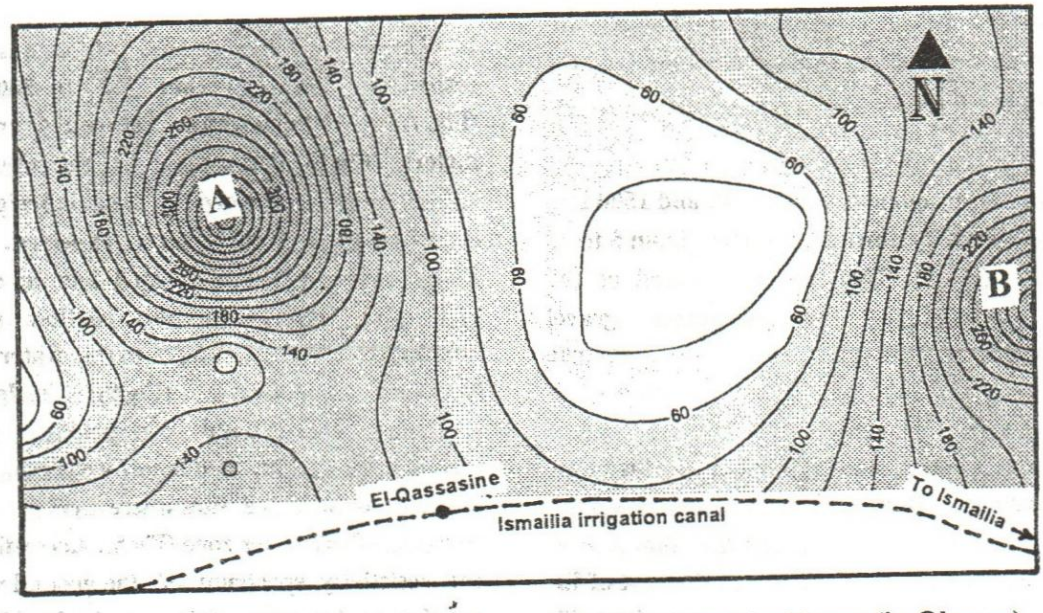

Fig.(3): True iso-resistivity map of the surface geoelectrical layer (in Ohm.m)

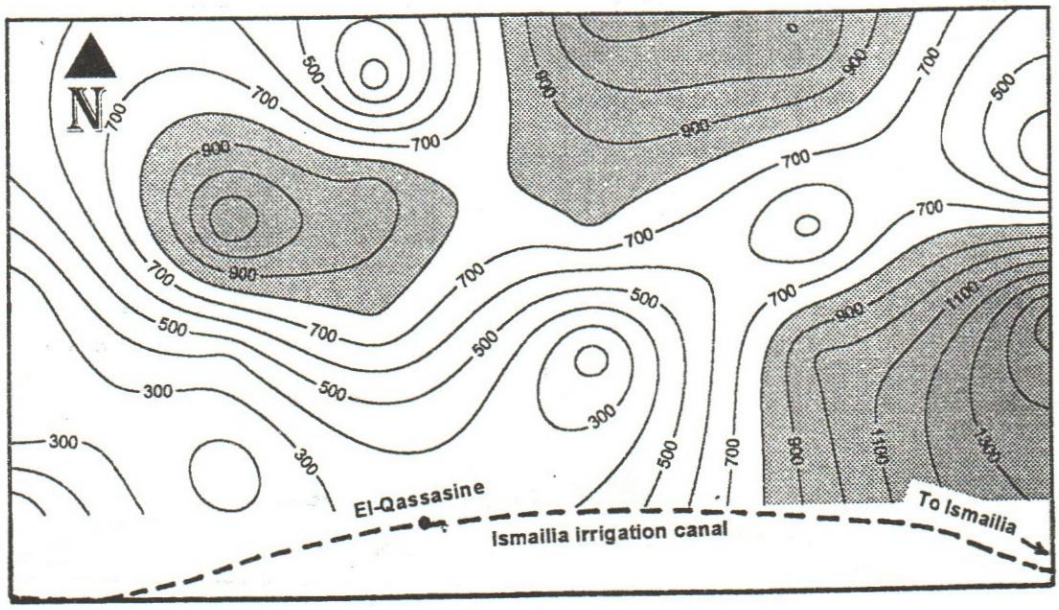

Fig.(4): True isoresistivity map of the subsurface gravel deposits (in Ohm.m)

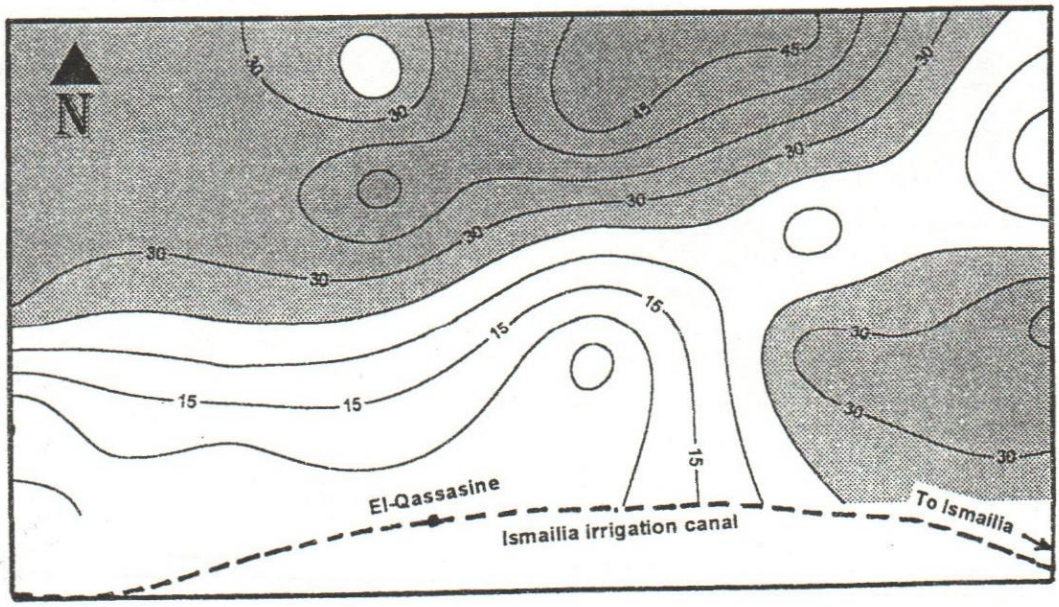

Fig.(5): Thicknesses of the subsurface gravel deposits (in meters)

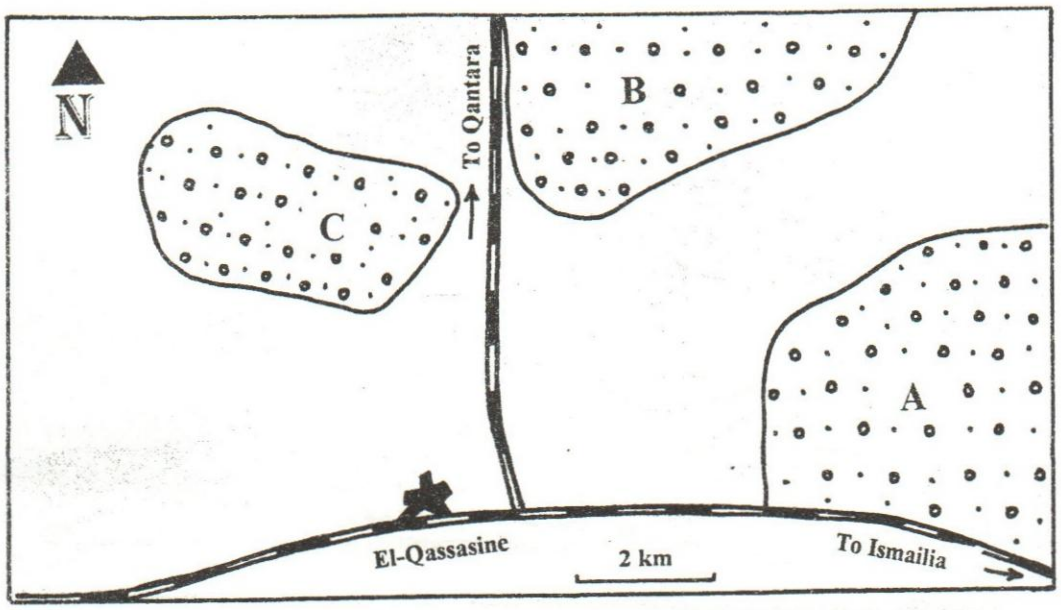

Fig.(6): Expected subsurface gravel bodies in El-Salhaiya plain

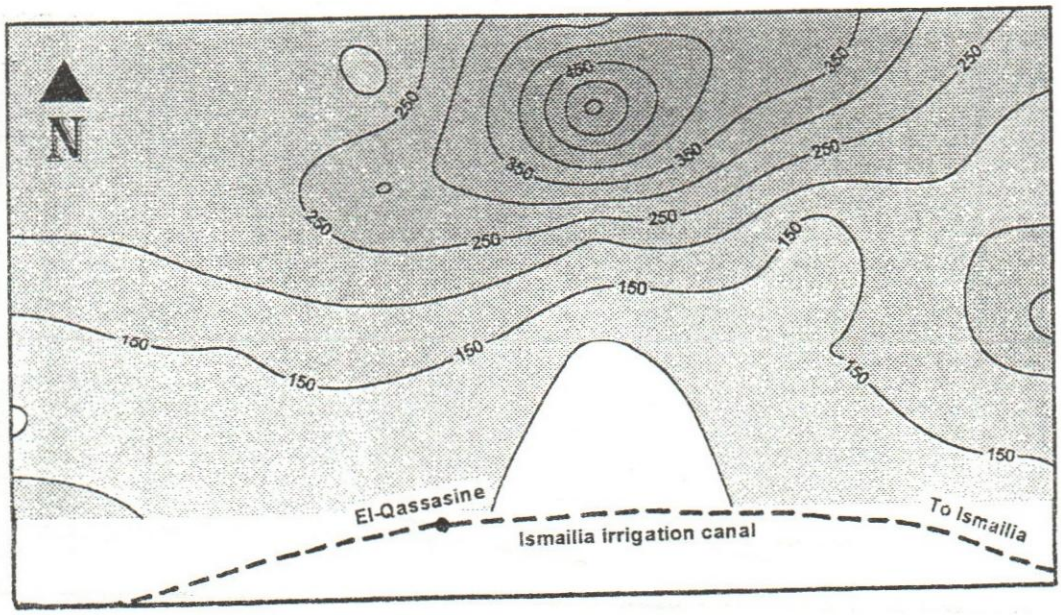

Fig.(7): True iso-resistivity map of the shallow fresh water aquifer (in Ohm.m)

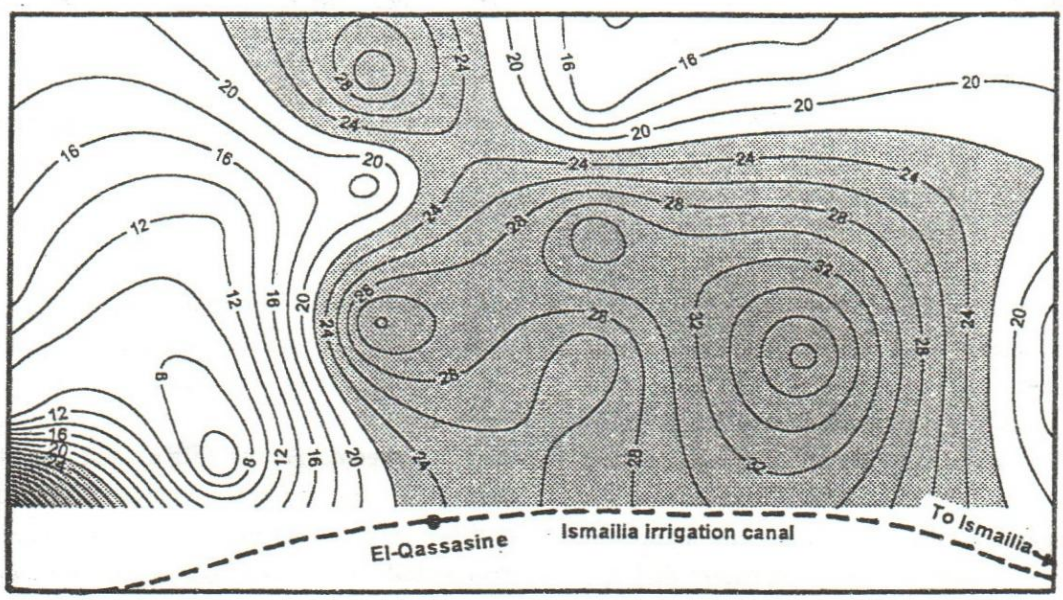

Fig.(8): Thicknesses of the shallow fresh water aquifer (in meter).

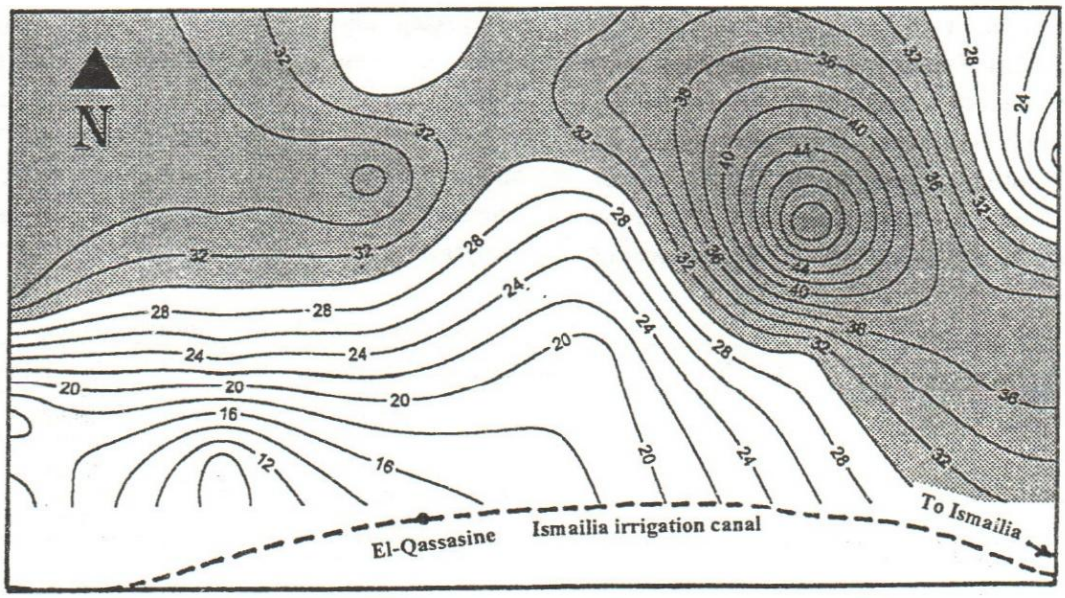

Fig.(9): depths to the shallow fresh water aquifer b.g.l.(in meter)

b- Deep brackish to salt water zone which has true resistivity ranging between 10 and 45 Ωm Fig.(10) and depths starting from 25 meter to 80 meter below ground surface (Fig.11). The ground water salinity in this zone reaches about 2500 ppm as measured from the water wells and it could be classified as brackish to salt water aquifer. Depths of water in this aquifer should control drilling water wells. It is also noticed that the true resistivity of this zone decreases in the part of El-Salhaiya plain to the west of El-Qassasine city but increases in the eastern part (Fig.10). This observation may explain the effect of the Ismailia canal in recharging the deep ground water aquifer in the eastern part of the plain rather than the western part due to the following reasons:

a- The increase of clay/sand ratio in the western part of the plain which may prevent the intrusion of canal water into the land.

b- The opposite occurs in the eastern part of the plain where there is an increase in the gravel+sand/clay ratio besides the presence of major fault near the village of El-Mahsama Fig.(1) which may facilitate the movement of fresh water from the canal towards the concerned ground water aquifer.

APPROXIMATE VOLUME ESTIMATION OF THE SUBSURFACE GRAVEL SEDIMENTS :

According to the interpreted true resistivities of the second geoelectrical layers, three subsurface gravel bodies are expected (i.e. A,B &C, Fig.6). Thereafter, five augering boreholes were done in these zones, reaching the interpreted depths (Fig.6) and samples were taken for grain size analyses. From the results of this analyses, only three fractions were considered, namely gravels, granules and pebbles (2-64 mm in diameter), very coarse sand (1-2 mm) and coarse sand (0.5-1 mm) according to Wentworth scale 1922. The interpreted thicknesses and the approximate areas of these gravel bodies were used to estimate the approximate total volume as well as the volume of each grain size (table 2) taking into account the percentage of each fraction as deduced from the grain size analyses.

Table (2): Approximate volume of each grain size in the expected subsurface gravel bodies.

Body number	A.T. (m)	A.T.S.A. (m^2)	A.T.V. (m^3)	% & V.G.	% & V.V.C.S.	% & V.C.S.
A	29	16000	464,000	49%	19%	28%
				22 7,360	88,160	129,920
B	31	13000	44,000	14%	26%	53%
				6160	11,440	23,320
C	33	11000	363,000	32%	9%	25%
				116,160	32,670	90,750

A.T. = Average thickness in meter.
A.T.S.A. = Average total surface area in km^2.
A.T.V.= Average total volume in m^3.
% & V.G. = Percentage and volume of gravels in m^3.
% & V.V.C.S.= Percentage and volume of very coarse sand in m^3.
% & V.C.S. = Percentage and volume of coarse sand in m^3.

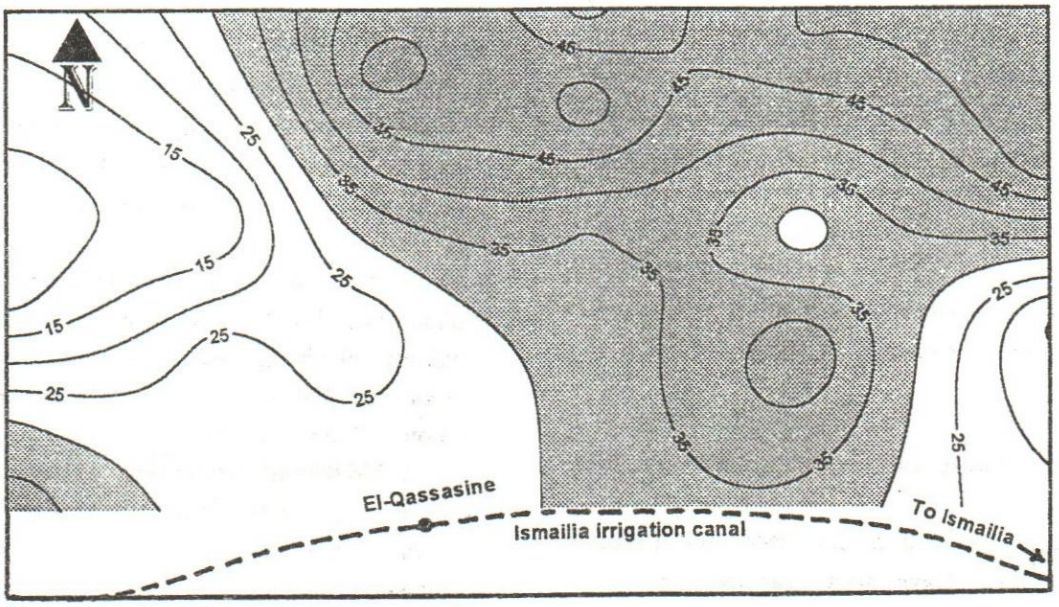

Fig.(10): True iso-resistivity map of the deep salt water aquifer (in Ohm.m)

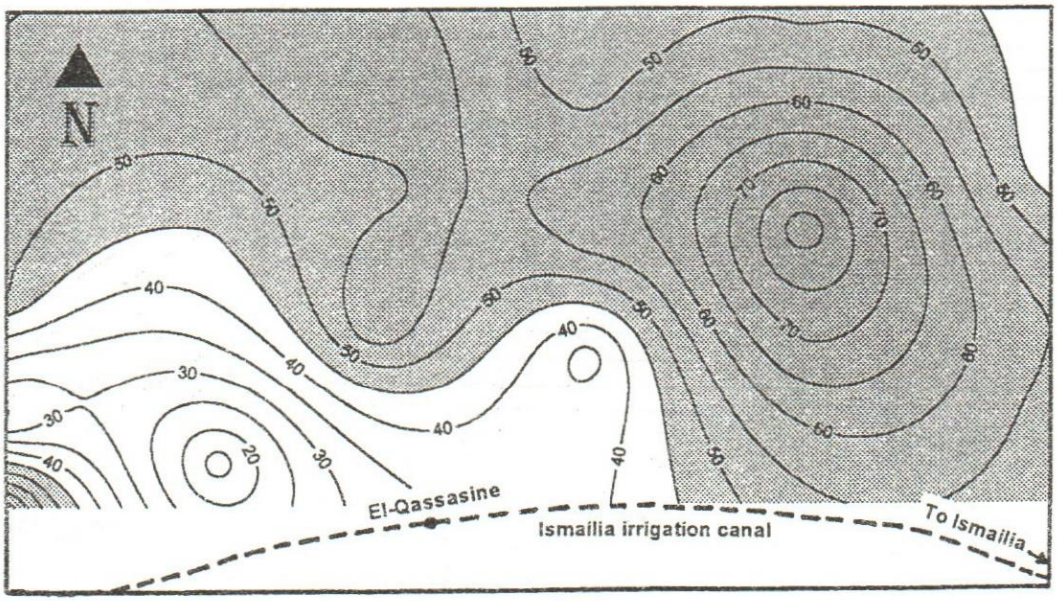

Fig.(11): Depths to the deep salt water aquifer b.g.l. (in meters)

The numbers in the above table indicate that the three subsurface gravel bodies give approximately a total volume of about 350,000 m^3 of gravels, about 132,000 m^3 of very coarse sand and about 244,000 m^3 of coarse sand. These bodies can be arranged according to their importance as follows: A, C and B. Their locations are easily accessible through El-Qassasine - Ismailia asphaltic road and El-Qassasine - El-Qantara asphaltic road (Fig.6).

CONCLUSION AND RECOMMENDATIONS :

Interpretation of the geoelectrical soundings which have been carried out in the southern part of El-Salhaiya plain could guide us to the following conclusion:

1- Except the southwestern corner of the studied area, El-Salhaiya plain is recommended for drilling water wells ranging in depth from 30 to 80 meters below ground level (Fig.12).
2- Three sites were recommended for gravel quarrying Fig.(6). The total approximate volume of the gravels in the three sites is about 350,000 m^3 , the very coarse sand is about 132,000 m^3 and the coarse sand is about 244,000 m^3. These types of sediments are mostly used for concrete constructions, casing water wells and other foundation purposes.

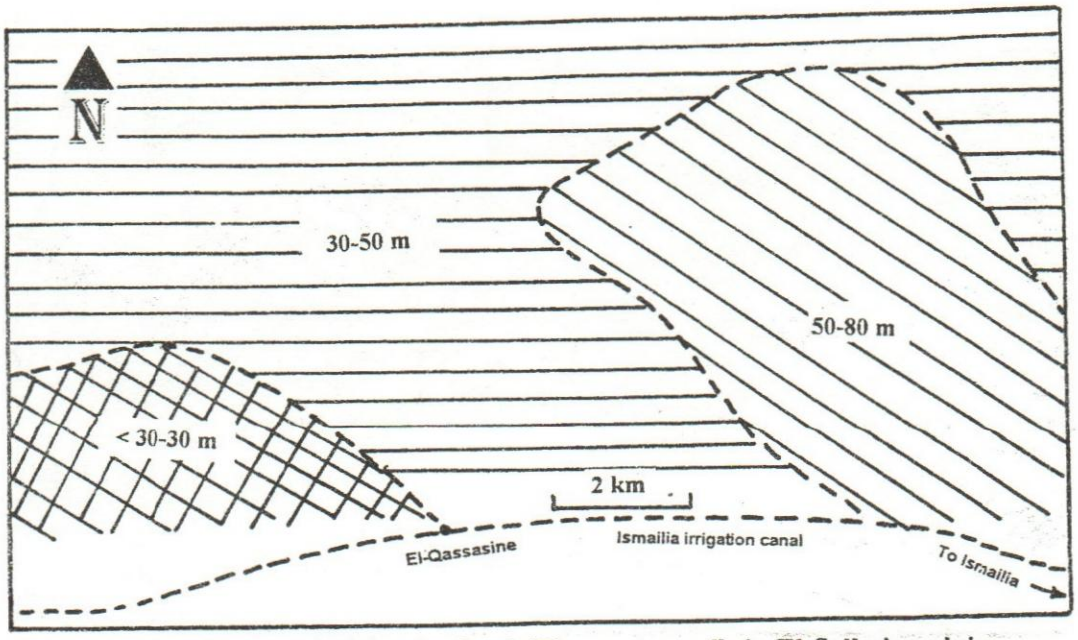

Fig.(12): Recommended depths for drilling water wells in El-Salhaiya plain

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تنقيب جيو كهربى عن المياه الجوفية وتواجدات الحصى التحت سطحية فى الجزء الجنوبى من سهل الصالحية – محافظة الإسماعيلية

العربى هندى شندى

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يمتد الجزء الجنوبى من سهل الصالحية لمسافة أربعين كيلو متراً موازياً لترعة الإسماعيلية ومغطياً مساحة قدرها حوالى ستمائة كيلو متر مربع ، ويقع غرب مدينة الإسماعيلية . تعتبر هذه المنطقة أحد أكبر مشروعات استصلاح واستزراع الأراضى فى مصر . ومن الناحية الجيولوجية فإن المنطقة مغطاه بطبقة سطحية من الرمال والحصى مختلطة ببعض ترسيبات الطين فى شكل عدسى ، ويتراوح سمك هذه الطبقة من الرمال والحصى ٢٠٠ إلى ٢٥٠ متر وتتبع عصر البليستوسين ، وتعتبر خزان المياه الجوفية الرئيسى فى شرق دلتا نهر النيل . وقد تم إجراء دراسات جيوفيزيائية حلقية بطريقة الجس الكهربى الرأسى على منطقة الدراسة من خلال تنفيذ عدد عشرين جسة عميقة بتوزيع شلمبرجير بحيث تغطى المنطقة فى نظام شبكى بغرض :

١- استكشاف تواجدات المياه الجوفية وحساب أعماقها وسمك الطبقة الحاملة لها ومن ثم تحديد أماكن لحفر آبار إنتاجية فى استصلاح واستزراع المنطقة .
٢- استكشاف تجمعات الحصى التحت سطحية وحساب أعماقها ، وكذلك أحجامها ومن ثم تحديد أماكن لاستغلال تلك الرواسب .

وقد توصلت هذه الدراسة إلى أن المنطقة الجنوبية من سهل الصالحية تحتوى على مياه جوفية عذبة تتجمع فى النطاق الرسوبى المحصور بين عمقى ٣٠ إلى ٨٠ متراً ، وبالتالى لا ينصح بحفر آبار مياه يتعدى عمقها ٨٠ متراً حتى نتجنب تداخل المياه المالحة من الخزان الجوفى العميق . كما حددت الدراسة ثلاثة مواقع لاستغلال الحصى عن طريق عمليات التحجير حيث يبلغ الحجم الكلى لترسيبات الحصى فى تلك المواقع حوالى ٧٢٦٠٠٠ متر مكعب تصلح لعمليات الخرسانة وتبطين آبار المياه ورصف الطرق وغيرها من عمليات التشيد والبناء .

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CHLORINATED HYDROCARBON PESTICIDE RESIDUES IN SMALL RUMINANTS AND CAMEL FAT IN ASSIUT, EGYPT

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ABSTRACT :

A total of 80 fat samples (40 goats, 20 sheep and 20 camels) were collected from Assiut Governorate slaughter houses and analyzed for determination of chlorinated hydrocarbon pesticides residue levels (α, β, γ, and δ-hexachlorocyclohexane (HCH), p,p'-DDT and some analogues (p,p'-DDE, p,p'-DDD and o,p'-DDT), aldrin and dieldrin, heptachlor and heptachlor epoxide, endrin and hexachlorobenzene (HCB) by using GC-ECD.

The main chlorinated hydrocarbon contaminants found in all analyzed samples of goat and sheep were β-HCH, p,p'-DDE and p,p'-DDT and p,p'-DDE in camel followed by p,p'-DDD, α-HCH, HCB and endrin. Heptachlor and aldrin could not be detected in sheep and camel fat but recorded in few goat samples. Total HCH isomers averages were 0.01 ± 0.007, 0.006 ± 0.007 and 0.004 ± 0.003 ppm in goat, sheep and camel fat, respectively, while the mean values of total DDTs were 0.0116 ± 0.008, 0.0122 ± 0.008 and 0.0024 ± 0.0011 ppm .

The obtained results showed a decrease in the levels of the investigated pesticides. The changes were related to the prohibitions and restrictions applied on the usage of these compounds. The detected residue levels were compared to Extraneous Residue Limits (ERL's) released by the Codex Committee on Pesticide Residue (CCPR) of FAO/WHO in 1994. Health hazards were also discussed.

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INTRODUCTION :

A number of chemicals are used in modern agriculture for producing food commodities as cheaply and efficiently as possible to meet world-wide food demands. Despite obvious benefits, occasional misuse of such chemicals has resulted in over intoxication of animals and accumulation of residues in feeds and foods. Introduction of chlorinated hydrocarbon insecticides in the 1940s created the potential for serious residue problems. All these synthetic pesticides are fat soluble, rapidly absorbed, stored in fatty tissues and slowly secreted (Hansen and Lambert, 1987). These compounds represented by hexachlorocyclohexane (HCH), chlorocyclodienes (aldrin, dieldrin, endrin, heptachlor and heptachlor epoxide), DDTs, and the fungicide hexachlorobenzene (HCB) accumulate in the body fat of animals continuously exposed to them through spraying of the environment or feed contamination. However, the toxic implications of such residues in humans are not completely understood, since they may be unsafe even at low daily intakes as they accumulate in the fat tissue and may induce changes in biotransformation of the endogenous and exogenous compounds (Concon, 1988).

Chlorinated hydrocarbons have been used extensively as pesticides and insecticides in Egypt since 1950a as in the other parts of the world . Although their use was officially benned in 1980s (Dogheim et., 1996b) , surveys carried out in Assiut and other Egyptian Governorates (El-Shafei, 1988; Dogheim et al., 1988; and Salem et al., 1990, 1991,1995, 1996a and 1996b) showed a widespread contamination of chlorinated hydrocarbon residues (HCH, DDTs, HCB) in buffaloes and cattle tissues, raw milk, mother's milk and fresh water fish .

Since meat is an important source of pesticide residues in the human diet, this paper reports the results of analyses of goat, sheep and camel carcasses fat generally consumed by human beings in Assiut Governorate.

MATERIALS AND METHODS :

Eighty perinephric fat samples (40 goats, 20 sheep and 20 camels) were collected randomly during January-June 1995. Adipose tissue samples were obtained from carcasses prepared for human consumption in Assiut Governorate slaughter houses. The samples were placed in aluminium foil, transferred to the laboratory, minced and frozen until analysis.

Apparatus :

* Gas chromatograph- Carlo Erba MEGA HRGC 5330 with ^{63}Ni ECD and split-splitless injector and column, HP ULTRA1 50m X 0.2mmX0.33um was used. Temperature programming: start at 80°C hold 1 min., increase to 200°C at 30°C/min., hold 3 min., increase to 220°C at 1°C/min., hold 10 min., increase to 250°C at 25°C/min., hold 15 min. Conditions: injector 250°C; detector 320°C; carrier gas (helium) 250 kPa; makeup-gas nitrogen 300 kPa.

Reagents:

* All the reagents used were for pesticide residue's analysis:- Dichlormethane, iso-Octane and Petroleum ether.
* Sodium sulphate anhydrous (heated for one night at 500 °C before use).
* Florisil (grain size 0.15-0.25 mm; 60-100 mesh) Merck 12518: was heated for one night at 550°C and cooled in a desiccator and mixed with distilled water (3%): i.e. 97 gm florisil +3 gm distilled water. The flask was closed and shaked immediately by hands for 5 minutes. Finally, it was shaked for 20 min. by a shaking machine and left for 10-12 hours before use. It must be used within 3 days.

NB: Florisil stayed more than 48 hours without use, must be heated at least for 5 hours or one night at 130 °C, then prepared as mentioned above before further use.

Pesticide reference standards:

Alpha-HCH (Supelco Nr. 4-8493), Beta-HCH (Supelco Nr. 4-8494), Gamma-HCH (Supelco Nr. 4-9049) and delta-HCH (Supelco Nr. 4-8495), p,p'-DDE (Supelco Nr. 4-9017), p,p'-DDD (Supelco Nr. 4-9009), o,p'-DDT (Ehrenstorfer P1111), p,p'-DDT (Supelco Nr. 4-9019), Heptachlor and heptachlor epoxide (Supelco Nr. 4-9041 and 4-9042), Aldrin and dieldrin (Supelco Nr. 4-9000 and 4-9024), Hexachlorobenzene (Supelco Nr. 4-8508) and endrin (Supelco Nr. 4-9032). Standard solution of reference materials were prepared in petroleum ether.

Extraction of adipose tissue samples:-

Tissue extraction and clean-up was performed according to Anonymous, (1988) and Stijve and Cardinale, (1974) that previously used by Salem, (1993).

A 10 gm sample was mixed with 30 gm granular sodium sulphate anhydrous in a porcelain mortar until formation of a homogeneous mass. The homogenate was transferred into a wide mouth Erlenmeyer flask and petroleum ether was added to the mixture in the flask until 2 cm above its surface, the mortar was rinsed with it and , transferred into the flask. The flask with its contents was kept for one night. Petroleum ether was filtered into a round evaporatory flask that was previously weighed. The first flask, insoluble material and filter paper was rinsed with petroleum ether 3 times. Petroleum ether was evaporated by rotatory evaporator at maximum 40 °C. The flask with fat was weighed again, and the fat content of adipose tissue was calculated. One gram of this pure fat was used for clean-up (florisil column).

A chromatographic column 300×22 mm was filled with nearly 70 ml petroleum ether. 25 gm deactivated florisil were added, topped with 15 gm sodium sulphate anhydrous. All petroleum ether was withdrawn until 1 cm above the column packing. The fat sample (1 gm) was dissolved and transferred into the column with nearly 10 ml petroleum ether. Pesticide residues were eluted with 300 ml pet. ether/dichloromethane (8:2 , v:v) . The elute

was collected in a 500 ml evaporator flask and evaporated by rotatory evaporator at 40°C, transferred quantitatively into another 50 ml special round flask and evaporated again until 1 ml remained, which was evaporated under stream of nitrogen. Residues were transferred into a 5 ml volumetric flask by iso-octane for GC. All residue levels were not corrected with its recoveries (table 4).

RESULTS :

The levels and the frequencies of various chlorinated hydrocarbon residues found in each animal are shown in tables 1, 2 and 3. Results presented are expressed as ppm on a fat basis (mg/kg of extractable fat).

DISCUSSION :

β-BHC, p,p'-DDE and p,p'-DDT were the only chlorinated hydrocarbon pesticides determined in all goat and sheep samples analyzed plus p,p'-DDE in camel fat. Overall, the frequency of detection of chlorinated hydrocarbon residues in our samples was high since all samples contained at least more than two different pesticides. In general, camel appeared to be less contaminated. This study indicated also that the concentrations of all pesticides were dramatically decreased more than thirty folds; in some pesticides (HCH) reached one hundred folds in comparison with previous studies (El-Shafei, 1988 and Salem, 1993).

Table(1) : Mean, range values (ppm) and frequency of chlorinated hydrocarbon pesticide residues detected in goat fat collected from Assiut. (Number of analyzed samples= 40) .

Pesticide	mean	min.	max.	median	90th percentil	frequency	F%
α-HCH	0.0003 ± 0.0002	0.0001	0.0007	0.0003	0.006	36	90
β-HCH	0.008 ± 0.006	0.002	0.029	0.0061	0.016	40	100
γ-HCH	0.001 ± 0.0015	0.0001	0.0053	0.0002	0.0034	36	90
δ-HCH	0.0006 ± 0.0007	0.0001	0.0028	0.0003	0.0015	30	75
Total HCH	0.010 ± 0.007	0.0021	0.0311	0.0075	0.0198	40	100
p,p'-DDE	0.010 ± 0.0123	0.0021	0.0621	0.0057	0.0162	40	100
p,p'-DDD	0.0014 ± 0.0011	0.0004	0.0036	0.0009	0.003	37	92.5
o,p'-DDT	0.0011 ± 0.0008	0.0005	0.003	0.001	0.0019	21	52.5
p,p'-DDT	0.002 ± 0.0017	0.0003	0.006	0.0016	0.004	40	100
Total DDTs	0.0116 ± 0.008	0.0041	0.0661	0.0086	0.0204	40	100
Heptachlor	0.0004 ± 0.0001	0.0002	0.0005	0.0003	----*	8	20
H. epoxide	0.0017 ± 0.0017	0.0003	0.0062	0.0012	0.0041	30	75
T. heptachlors	0.0017 ± 0.0017	0.0003	0.0062	0.0012	0.0041	30	75
Aldrin	0.0003 ± 0.0004	0.0001	0.0012	0.0004	----*	6	15
Dieldrin	0.0005 ± 0.0004	0.0003	0.0011	0.0006	0.0011	25	62.5
Ald. & Dield.	0.0006 ± 0.0004	0.0004	0.0015	0.0008	0.0012	25	62.5
Endrin	0.001 ± 0.0006	0.0003	0.0024	0.0009	0.0016	26	65
HCB	0.0005 ± 0.0003	0.0001	0.0011	0.0003	0.001	36	90

* Number of positive samples less than 10

Table(2) : Mean, range values (ppm) and frequency of chlorinated hydrocarbon pesticide residues detected in sheep fat collected from Assiut. (Number of analyzed samples= 20) .

Pesticide	mean	min.	max.	median	90th percentil	frequency	F%
α-HCH	0.0012 ± 0.002	0.0001	0.007	0.0002	0.0023	19	95
β-HCH	0.0035 ± 0.003	0.0015	0.013	0.0029	0.0042	20	100
γ-HCH	0.0002 ± 0.0001	0.0001	0.0003	0.0001	0.0003	12	60
δ-HCH	0.0014 ± 0.0014	0.0002	0.003	0.0011	----*	8	40
Total HCH	0.006 ± 0.007	0.0016	0.024	0.0032	0.0078	20	100
p,p'-DDE	0.009 ± 0.007	0.0025	0.0232	0.0065	0.0159	20	100
p,p'-DDD	0.0009 ± 0.0005	0.0005	0.0017	0.001	0.0015	20	100
o,p'-DDT	0.0007 ± 0.0007	0.0003	0.002	0.0005	0.0008	12	60
p,p'-DDT	0.0022 ± 0.0021	0.0003	0.0074	0.0013	0.004	20	100
Total DDTs	0.0122 ± 0.008	0.0068	0.0321	0.0093	0.017	20	100
Heptachlor	ND	ND	ND	ND	ND	0	0
H. epoxide	0.0004 ± 0.0001	0.0003	0.0006	0.0004	0.0005	10	50
T. heptachlors	0.0004 ± 0.0001	0.0003	0.0006	0.0004	0.0005	10	50
Aldrin	ND	ND	ND	ND	ND	0	0
Dieldrin	0.001 ± 0.001	0.0004	0.0026	0.0006	0.0016	12	60
Ald. & Dield.	0.001 ± 0.001	0.0004	0.0026	0.0006	0.0016	12	60
Endrin	0.0006 ± 0.0004	0.0003	0.0014	0.0007	0.0013	14	70
HCB	0.0007 ± 0.0014	0.0001	0.0043	0.00024	0.001	18	90

* Number of positive samples less than 10 , ND = not detected.

Table(3) : Mean, range values (ppm) and frequency of chlorinated hydrocarbon pesticide residues detected in camel fat collected from Assiut. (Number of analyzed samples= 20) .

Pesticide	mean	min.	max.	median	90th percentil	frequency	F%
α-HCH	0.0004 ± 0.00036	0.0001	0.0011	0.0002	0.0005	16	80
β-HCH	0.0043 ± 0.0024	0.0012	0.0083	0.0035	0.0062	18	90
γ-HCH	0.0002 ± 0.0001	0.0001	0.0003	0.0002	0.0003	14	70
δ-HCH	0.0003 ± 0.0	0.0003	0.0003	0.0003	----*	4	20
Total HCH	0.004 ± 0.003	0.0013	0.0088	0.0038	0.0063	20	100
p,p'-DDE	0.001 ± 0.0004	0.0005	0.0016	0.0008	0.0014	20	100
p,p'-DDD	0.001 ± 0.0006	0.0003	0.0021	0.001	0.0012	14	70
o,p'-DDD	0.0005 ± 0.00	0.0005	0.0005	0.0005	----*	2	10
p,p'-DDT	0.0008 ± 0.0005	0.0004	0.0015	0.0006	0.001	14	70
Total DDTs	0.0024 ± 0.0011	0.0005	0.004	0.0023	0.0035	20	100
Heptachlor	ND	ND	ND	ND	ND	0	0
H. epoxide	0.0004 ± 0.0001	0.0003	0.0006	0.0004	----*	8	40
T. heptachlors	0.0004 ± 0.0001	0.0003	0.0006	0.0004	----*	8	40
Aldrin	ND	ND	ND	ND	ND	0	0
Dieldrin	0.0006 ± 0.0004	0.0005	0.0012	0.0005	0.001	10	50
Ald. & Dield.	0.0006 ± 0.0004	0.0005	0.0012	0.0005	0.001	10	50
Endrin	0.0005 ± 0.0003	0.0003	0.0011	0.0006	0.0008	10	50
HCB	0.0001 ± 0.00015	0.0001	0.0004	0.0002	0.0003	14	70

* Number of positive samples less than 10 , ND = not detected.

Table (4) : Recovery percent and limit of quantitation.

Pesticides	Spiked conc. (mg/kg)	Recovery percent	Limit of quantitation (mg/kg)
HCH alpha	0.02	90	0.0001
HCH beta	0.1	86	0.001
HCH gamma	0.02	89	0.0001
HCH delta	0.02	76	0.0001
DDE p,p	0.1	83	0.0002
DDD p,p	0.1	85	0.0003
DDT o,p	0.1	80	0.0004
DDT p,p	0.1	91	0.0002
Heptachlor	0.01	100	0.0001
Hep. epoxid	0.1	84	0.0003
Aldrin	0.01	83	0.0001
Dieldrin	0.1	85	0.0003
Endrin	0.1	84	0.0003
HCB	0.01	97	0.0001

According to studies carried out in many countries, i.e., Egypt (El-Shafei, 1988 and Salem, 1993), Iran (Hashemy-Tonkabony, 1981); Iraq (Al-Omar et al., 1985); Ireland (Harper, 1980) and Spain (Herrera et al., 1994), the pesticides most commonly found in the ruminants were lindane, the isomers of HCH, DDT and its metabolites (DDE and DDD), dieldrin and heptachlor epoxide. HCB and HCH gamma and alpha isomers were the most frequently detected pesticides in Germany (Knoeppler, 1976) and in Austria (Jarc, 1980).

Total hexachlorocyclohexane isomers (alpha-, beta-, gamma- and delta-HCH) were found in all analyzed fat samples. Its mean values were 0.01±0.007, 0.006±0.007 and 0.004±0.003 ppm in goat, sheep and camel fat, respectively. Goat fat contained the highest levels of total HCH isomers. This may be attributed to the body fat condition. Spence et al., (1990) recorded that the reduction in HCH residue levels occurred by redistribution and dilution throughout the increased body fat. β-HCH was most frequently detected isomer of the four HCH isomers (α, β, γ and δ), 100% in goat and sheep and 90% in camel fat, followed by α-HCH and γ-HCH (lindane), then delta isomer.

As shown in tables 1, 2 and 3, the mean values of total HCH isomers found in all fat samples were mainly due to the beta isomers, which might correspond to the concept of possible isomerization of alpha and gamma isomers to the beta isomer (IPCS, 1991 and 1992). β-HCH showed levels distinctly higher than those reported for the other three isomers, (α, γ and δ-HCH) in the investigated animals. Such findings were reported by Salem (1993), Salem et al. (1995) and Dogheim et al. (1988, 1990, 1991 and 1996a) . β-HCH

may occur in meat at higher levels because of its great persistence, which far exceeds that of the other isomers (Hecht, 1988). This isomer is the most persistent and slowly eliminated from the body (Pfeilsticker, 1973) and has the ability to accumulate in fat tissues 10-30 times than lindane (Heeschen, 1980), but not the most hazardous (Scholz et al., 1985).

Lindane (gamma isomer of HCH) which is used alone as a pesticide and also is the most toxic isomer of this group is always detected in insignificant amounts. Almost no differences in the residual amounts of lindane were observed between the different animal sorts.

α-HCH was also more frequent in fat samples as β-HCH but with very low values except in some samples of sheep. This may be attributed to the possible use of technical HCH that contained a large proportion of α-HCH or the external treatment of animals with HCH based veterinary preparations, which could be ingested or absorbed through skin (Harper, 1980). Gamma-HCH isomerizes to the alpha and delta isomers, respectively 4 and 50 times less insecticidal (Newland et al., 1969).

Animal exposure to HCH can occur through the use of lindane or technical HCH as a dipping chemical to control scab mostly in sheep (Sumner, 1984). The distribution pattern of HCH isomers indicates that such residues might result from previous exposure to lindane (> 90% γ-isomer) and to Gamaxan, the HCH mixture of isomers (< 70% α-isomer, 15% γ- isomer and 10% β-isomer) sold in Cairo (Dogheim et al., 1996a).

All the detected levels were below the ERL (2 ppm) for total HCH isomers and lindane released by the Codex Committee on Pesticide Residue (CCPR) of FAO/WHO in 1994. Also all the investigated samples were found to contain residues far below the EC level of safety (1 ppm) for total HCH, (100 ppb) for β-HCH and 0.2 ppm for α-HCH (Herrera, et al., 1994).

The overall pollution of goat, sheep and camel with DDT and its metabolites, especially of the p,p' form, was found to be very low, averaging 0.0116, 0.0122 and 0.0024 ppm, with a maximum of 0.0661, 0.0321 and 0.004 ppm, respectively. Total DDTs were presented in all adipose tissue samples with p,p'-DDE. This could be attributed to the high solubility and tendency of DDT and its metabolites to accumulate and stored in fatty tissues (WHO, 1979 and 1989).

The metabolite p,p'-DDE made up the greatest fraction (0.01, 0.009, and 0.001 ppm) in goat, sheep and camel fat, respectively, followed by p,p'-DDT (0.002, 0.0022 and 0.0008), indicating the continuous degradation of DDT to the less toxic and more persistent derivative. This was shown by Fries et al., (1972) and Hayes, (1975); who reported that DDE is more resistant to metabolic degradation than DDT in animals and man. Also, DDE is found in almost all the living organisms because of its strong affinity with body fat (Jensen and Jansson, 1976).

In spite of the known information about the prohibited use of DDT in Egypt at the last

15 years according to the authorities report, the continued use of the dicofol (Kelthane®) replaced DDT as the primary source of environmental DDE and contains as much as 0.6 % p,p´ and o,p´-DDT (Camoni et al., 1983). This indicates the continuous contamination of the environment by DDT and DDE.

On basis of ERL of 5 mg/kg total DDTs (CCPR, 1994) residues in animal tissues, all the analyzed samples were below the limit.

Contamination with cyclodienes was quit low, except for a high frequency of some pesticides. Heptachlor and aldrin could not be detected in sheep and camel. The residue averages of this group were very low as shown in tables 1, 2 and 3. This group as a whole shows levels in samples usually below 0.020 ppm on a fat basis as reviewed by Ruiter, (1985). This limited extent of contamination by the cyclodiene group is in accordance with the figures given by El-Shafei, (1988) and Salem, (1993) in meat of various species and Dogheim et al., (1996b) and Salem et al., (1995) in fish as a result of their use being banned in Egypt.

Wilkinson et al., (1964) indicated that toxic residues mostly heptachlor epoxide, will persist in the soil for as long as 9 years, the possibility thus exists for contamination of crops from these fields. However, it was translocated from the soil into certain crops (Bruce and Decker, 1966), subsequently stored in the fat of dairy cows (Rusoff et al., 1963). Referring to the ERL´s of the (CCPR, 1994) of 0.2 mg/kg of heptachlor and its epoxide in animal tissue, it could be revealed that all detected residues were found below the permissible limit.

Aldrin residue definition includes dieldrin residues as well. Inspite of dieldrin being an insecticide by itself it is also resulting as a degradation product from aldrin application. A statement that was mentioned by Bann et al., (1956) that dieldrin is the form of which aldrin usually being stored in fats. The magnitude of aldrin and dieldrin residues revealed low levels and few frequencies in adipose tissue samples of all animals. Aldrin residues were absent in all sheep and camel samples as the total residues resulted mainly from dieldrin (tables 2 and 3). All the analyzed samples contained aldrin and dieldrin residues below the extraneous residue limit.

As far as human safety is concerned, endrin is the most hazardous pesticide from the cyclodiene group. Its average was very low in comparison with the previous data recorded by Salem, (1993) The extraneous residue limit of endrin in animal tissues is 0.1 mg/kg (CCPR, 1994). All fat samples never exceeded the permissible limit.

Hexachlorobenzene has been used as a seed treatment fungicide. It is a by-product of some chemical processes, and has been known to cause human poisoning (Cam and Nigogosyan, 1963). It was translocated in foods and human tissues from non-agricultural sources such as industrial high-temperature processes involving chlorine and production of organic solvents (Heinish, 1985 and Vogelgesang, 1986). Recently, Salem, (1993)

and Salem et al., (1995) detected HCB with frequencies more than 90% in buffaloe and cattle fat and fresh water fish samples collected from Assiut with low values. Thus, the rate of contamination with HCB in the food-chain is possibly extraordinarily high. Therefore, it was not surprising to find such widespread HCB contamination (90%) of goat and sheep samples and (70%) of camel samples.

HCB, has since the early 1970s been recognized as an environmental contaminant comparable to DDT and PCBs in industrialized countries (Acker and Schulte, 1970 and Stijve, 1971). On the other hand, HCB contamination does not seem to be a serious problem in Egypt, since only very low residue levels were found in all analyzed samples (tables 1, 2 and 3), far below the current EC-MRL (maximum residue limit) of 0.2 ppm. Ruiter, (1985) reviewed that accumulation ratios (tissue level to feed level) for this fungicide are 10 to 30 in beef and poultry, and ratios of 8 to 11 were observed in adipose tissue of swine. The increase in its frequency in this study may indicate a new exposure of our environment to this compound.

From the summary of the data obtained, it was shown that, among the various chlorinated hydrocarbon pesticides, total HCH and total DDTs were the most abundant in all type of meat considered. This probably the consequence of their persistence and could also indicate a fraudulent use. It is worth nothing that the mean levels of these pesticides in different meat were well below the extraneous residue limits set up by CCPR of FAO/WHO and EC authorities.

The persistence of these pesticides and their metabolites in the environment means that much of the materials used for control of insect-borne diseases and elimination of agricultural pests still contaminates soil and water. In this respect, it should be mentioned here that Macklad et al., 1990 and Dogheim et al., 1992 detected organochlorine pesticide residues in water samples collected from the River Nile and its branches. Dogheim et al., (1996a) detected also higher levels of HCH isomers, DDTs, Dieldrin and heptachlor in ground water, followed by River Nile water and tap water. They found also that soil contained higher levels than water, especially for DDTs in appreciable amounts that could be an important source of contamination.

It is advisable to continuously monitor the degree of chlorinated hydrocarbon pesticides contamination in meat and meat products. This is due to their high levels of consumption. The presence of chlorinated hydrocarbon pesticides contamination is still real, although very moderate in extent.

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متبقيات المبيدات الكلورينية العضوية في دهن المجترات الصغيرة والجمال بأسيوط - جمهورية مصر العربية

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يعد الفحص الدورى المستمر للأغذية واحداً من الأهداف القومية الهامة التى تقوم بها الجامعة كمؤشر وقائى لحماية البيئة والمستهلكين من أخطار التلوث ، وبما أن لمتبقيات المبيدات باللحوم وما يتخللها من دهون خطورة على المستهلكين ، فقد تم تحليل ثمانين عينة دهن (٤٠ ماعز و٢٠ أغنام و٢٠ جمال) والتى تم جمعها بصورة عشوائية من مجازر أسيوط خلال الفترة من يناير إلي يونيه ١٩٩٥ ؛ لاستبيان مدي إحتوائها علي متبقيات المبيدات الكلورينية العضوية بواسطة جهاز كروماتوجرافيا الغاز (GC-ECD) . تم الكشف عن مشتقات مبيد الهكساكلوروسيكلو-هكسان(الفا ، بيتا ، جاما ودلتا) ومركبات الدي دي تي (بارا بارا وأورثو بارا دي دي تي ، دي دي إي و دي دي دي)والهبتاكلور والهبتاكلور إبوكسيد والألدرين والديلدرين والإندرين وكذلك الهكساكلوروبنزين.

أوضحت نتائج التحليل إحتواء جميع العينات علي أكثر من مبيد ، حيث احتوت عينات الماعز والأغنام علي مشتق بيتا لمبيد الهكساكلوروسيكلوهكسان والدي دي تي والدي دي إيوكذلك عينات الجمال علي الدي دي إي . كما كان تواجد مبيد الهكساكلوروبنزين ومشتقي الفا وجاما لمبيد الهكساكلوروسيكلوهكسان ملحوظاً في جميع الحيوانات.

وكانت مستويات المبيدات المتواجدة بالعينات منخفضة بالمقارنة مع الدراسات التي تمت علي الجاموس والأبقار في المنطقة ذاتهاأو في المحافظات الأخري . وقد كان مستوي مجموع مركبات الدي دي تي ٠,٠١١٦ ، ٠,٠١٢٢ و ٠,٠٠٢٤ جزء في المليون ، بينما كان مجموع مشتقات مبيد الهكساكلوروسيكلوهكسان ٠,٠١ ، ٠,٠٠٦ و ٠,٠٠٤ جزء في المليون في دهن الماعز والأغنام والجمال علي التوالي وتمثلت هذه المبيدات في جميع العينات بالمبيد ذاته أو أحد مشتقاته.

ويتبين من النتائج المتحصل عليها أنه لم يتعد أي من المبيدات المتواجدة بالعينات الحدود المسموحة والمقررة من الهيئات العالمية مثل لجنة الكودكس التابعة لمنظمة الصحة العالمية والأغذية والزراعة التابعة لهيئة الأمم المتحدة وكذلك الاتحاد الأوروبى بما يفسر ظاهرة ارتفاع الوعى الثقافى البيئى وقلة تعرض المجترات الصغيرة والجمال لمثل هذه المبيدات .

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GASEOUS POLLUTANTS: 1- OZONE

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REVIEW ARTICLE

INTRODUCTION :

Community air pollution is a problem that is as old as civilization, and may date back to prehistoric cultures. Investigation of the effects of air pollutants on human health has followed a multidisciplinary approach using animal toxicology, epidemiology, controlled human exposure studies and, more recently, molecular and cellular biology. Air pollutants may, in addition to other responses, cause lung damage, inflammatory responses, impairment of pulmonary host defenses, acute changes in lung function and respiratory symptoms as well as chronic changes in lung cells and air ways. The focus of this review is on the human health hazards of pollutants as determined through controlled human exposure studies and emphasizes the deleterious responses that have been shown with ozone.

Ozone (O_3), a reactive species of oxygen, is an important natural constituent of the atmosphere (Cadle and Allen, 1970). Background levels of ozone in the lower atmosphere may reach 0.1 ppm and are modified by geographic elevation, solar radiation and climatic conditions (National Research Council, 1977). Since some effects of ozone are radiomimetic (Brinkman and Lambert, 1958; Brinkman et al., 1964), its action may be enhanced in the presence of ionizing radiation from background and or man-made sources (Borek and Mehlman,

1983). While stratospheric ozone spares the earth from excess solar ultraviolet radiation (National Research Council, 1977); high levels of ozone in the environment are toxic and result in health hazards to man (WHO, 1979 and Lee et al., 1983).

Ozone is one of the most powerful oxidizing substances. It is formed in the troposphere by the action of sunlight on nitrogen dioxide. Direct emission of ozone into the atmosphere as a result of industrial activity is only very limited. Ozone is an important component of photochemical smog and its formation in the atmosphere depends to a large extent on the absolute and relative concentrations of volatile organic substances on the one hand and nitrogen oxides on the other. The maximum natural background concentration, expressed as the average over a period of 24 hours, is 120 μgm^{-3} (0.06 ppm), the 50% values lying between 40 And 60 μgm^{-3} (Feron et al., 1996).

On a global level, the main concern with ozone is the reduction in its concentration in the upper atmosphere. The well-publicized ozone "hole" which occurs over the Antarctic (but now detected at high latitudes in the Northern Hemisphere), is caused by the degradative effects of Chlorofluorocarbons (CFCs) on ozone molecules. CFCs are released from aerosol containers, from the coolants in domestic refrigerators when they are broken up or leaked, and from foam packaging. The ozone layer absorbs ultraviolet light so that one hazard associated with the thinning of this ozone layer, is an increase in the rates of skin cancer. Environmentally, it has been suggested that the increased radiation could decrease photosynthesis of phytoplankton in the Antarctic (Walker et al., 1996).

TOXICOLOGY :

Ozone is an extremely reactive oxidant molecule. Its toxicity is complex because of the large number of biological systems that can be affected and the variety of effects that can result from ozone interactions with cellular components (Lee et al., 1983). The toxic effects of ozone are manifested upon its inhalation and absorption in the lungs. The pulmonary system is therefore the primary target for ozone toxicity though extrapulmonary effects have been recognized and documented (Stockinger, 1965; Goldstein, 1979; Borek and Mehlman, 1983).

The levels at which ozone toxicity becomes evident are influenced by variety of parameters. These include; genetic factors, (species, airway anatomy, stage of development) as well as host factors such as pre-existing disease state, age, dietary and hormonal status and cellular protective systems. The latter, which directly or indirectly suppress the oxidative damage induced by ozone , serve as important determinants in establishing the consequences of ozone health effects (WHO, 1979; Borek and Mehlman, 1983).

While the exact molecular mechanisms of ozone toxicity are unknown; a number of pathways have been proposed which suggest that ozone damage is in part produced via free

radical mechanisms (Pryor, 1976; Borek and Mehlman, 1983). The peroxidation of polyunsaturated fatty acids (Chow et al., 1981; Goldstein et al., 1969) and oxidation of thiols, amines and proteins (Mudd et al., 1969; Mudd and Freeman, 1977) which produce free radicals have been implicated in ozone-induced damage in pulmonary and extrapulmonary sites (Borek and Mehlman, 1983).

(A) PULMONARY EFFECTS OF OZONE :

Ozone is an air pollutant and a major component of photochemical smog. It is a respiratory irritant producing irritation of the upper airways and high concentration may even produce fatal pulmonary edema in both humans and experimental animals.

In rodents, approximately 50% of the ozone present is removed from the inhaled air in the nose. The highest tissue concentrations, in both man and experimental animals, are found in the transitional area between the bronchioles and the alveoli. Exposure to ozone causes damage to all parts of the respiratory tract. The effects exerted are dependent mainly on the concentration. At relatively low concentrations (400 μgm^{-3}), effects are observed mainly in the lung, while at higher concentrations (800-1600 μgm^{-3}) the nasal mucosa is also affected. The effects range from reversible interference with pulmonary function, increased enzymatic activity, reduced resistance to pulmonary infections, proliferation of type II pneumocytes, hyperplasia and metaplasia of the respiratory epithelium in the nose, to permanent pulmonary fibrosis. Clinical and epidemiological studies in man have suggested that exposure to concentrations between 160 and 340 μgm^{-3} resulted in respiratory complaints such as coughing, dry throat, chest pain and tightness of the chest (Feron et al., 1996).

1- Morphology:

Toxicological research on the effects of ozone in laboratory animals indicated that the types and distribution of lesions in the respiratory tract following short- or long-term ozone exposure largely depend on the following variables: (1) Lung morphometry which differs among species (American Thoracic Society, 1983), (2) Location of sensitive cells (Stephens et al., 1974a, b) and (3) Junction between conducting airway and gaseous exchange areas (Schrieder and Raabe, 1981).

Scheel et al (1959) reported that short term exposure to ozone resulted in pulmonary edema, hemorrhage and inflammation. Damage to respiratory tract epithelia can be seen in various species exposed to 0.2 ppm ozone and higher. The sites of damage include the trachea (Cavender et al., 1977; Schwartz et al., 1976), bronchi (Castleman et al., 1973), bronchioles (Castleman et al., 1980), alveolar ducts and alveoli (Stephens et al., 1974b; Schwartz et al., 1976). Fibrosis and enhanced collagen synthesis have been observed following long-term exposure to ozone at doses of 0.1 ppm and higher (Boorman et al., 1980; Last et al., 1979; Moore and Schwartz, 1981; Plopper et al., 1978).

The sensitivity of lung tissue to ozone-induced damage varies with cell type and location. The ciliated cells in the airway passages and the squamous alveolar epithelial cells (type I cells) are the most sensitive to ozone (Stephens et al., 1974a, b; Schwartz et al., 1976; Mellick et al., 1977; Castleman et al., 1980). Type II alveolar epithelial cells are more resistant to ozone and in fact serve as progenitor cells which differentiate into type I cells during the process of repair of ozone lesions (Evans et al., 1976). Morphological changes following ozone exposure also differ with the state of the animal and are modified by an altered nutritional or immunological status (U.S. Environmental Protection Agency, 1978). For example, rats maintained on vitamin E-deficient diets tend to develop more morphological lesions as compared to those maintained on vitamin E-supplemented diets (Plopper et al., 1978; Chow et al., 1981).

2- Pulmonary function:

Changes in the pulmonary function have been observed in a variety of species following short- and long-term exposure to ozone (National Research Council, 1977; Lee et al., 1983). Short-term exposure (1-2 hr) in experimental animals produces rapid, shallow breathing, increasing pulmonary resistance (Lee et al., 1977, 1979) and increases in residual volume, closing volume, closing capacity (Inoue et al., 1979). Long-term exposure (0.2 ppm for 4-6 weeks) resulted in an increased lung distensibility at high lung volumes (Raub et al., 1983; Bartlett et al., 1974). A longer period of ozone exposure (up to 0.8 ppm for 3-12 months) shows more severe consequences resulting in increased pulmonary resistance, impaired airway stability and lung distensibility suggesting a development of lung fibrosis (Wegner et al., 1982).

Studies of airway reactivity following ozone exposure indicate a hyperactive state resulting from a disruption of the respiratory epithelium and a sensitization of the underlying nerves to chemical and mechanical stimuli (Nadel, 1977; Lee et al., 1979). This sensitization may be an underlying factor in the reflex broncho-constriction and the rapid shallow breathing observed following ozone exposure; conditions which are enhanced if exposure takes place during exercises (Lee et al., 1979; Folinsbee et al., 1978). Epithelial damage induced by ozone has also been implicated in the enhanced allergic reactions to inhaled foreign proteins (Osebold et al., 1980).

Chronic exposure to ozone causes pulmonary arterial lesions that result in thickening of the arterial walls. Ultrastructural changes in the alveolar capillaries have also been found (Van Vleet et al., 1991).

3- Inhibition of enzymatic systems:

A large number of enzymes are inhibited following ozone exposure. The cytochrome P-450 enzyme system, important in carcinogen and drug metabolism, is inhibited in hamsters (Palmer et al., 1971) and rabbits (Goldstein et al., 1975) following short exposure to ozone

(0.75 - 1.0 ppm). The inhibition of cytochrome P-450 activity increases the hazard of ozone exposure due to the decrease in detoxification of inhaled chemicals including pneumotoxicants and carcinogens. The inhibition of prostaglandin synthetase, a membrane bound enzyme, has also been reported (Menzel et al., 1976). The decreased lung cholinesterase activity which has been observed following ozone exposure (Gordon et al., 1981) could result in elevated acetylcholine concentration in the bronchial muscle that end by enhancement of bronchial concentrations following a given stimulus.

4- Lung protein synthesis:

The action of ozone on pulmonary protein synthesis falls into two general categories: (a) an effect on the synthesis of collagen and structurally related proteins, which is directly related to ozone-associated lung fibrosis; (b) an action on glycoprotein synthesis and mucous secretion, which affects their role in protecting underlying cells from ozone toxicity and in removing adventitiously inhaled particles (Mehlman and Borek, 1987).

Continuous exposure to ozone for 7 days (0.5 - 0.8 ppm) results in a significant enhancement of collagen synthesis and precursor protein, but a negligible effect is observed when animals are exposed to 0.2 ppm (Hussain et al., 1976). Exposure of rats to 0.5 ppm ozone for 1-3 weeks results in a linear dose-response relationship for pulmonary biochemical and histological responses (Last et al., 1979). The inhibitory effect of ozone on the synthesis and secretion of mucus glycoprotein synthesis in tracheal explants varied with the species studied (Last and Kaizu, 1980).

5- Tolerance:

Many studies indicated that preexposure to low concentrations of ozone renders animals less sensitive to the damaging action of a second dose of exposure (Matzen, 1957; Mendenhall and Stockinger, 1959; Fairchild, 1967; U.S. Environmental Protection Agency, 1978). Frager et al (1979) showed that the protective effect of preexposure to ozone exerts its action on mucociliary clearance of foreign particles. Preexposure of animals to 0.8 ppm ozone for 3 days resulted in a marked reduction in the delay of mucociliary clearance, which lasted for 1 week.

Tolerance does not impart protection from all forms of lung injury (U.S. Environmental Protection Agency, 1978). Preexposure of animals to ozone at levels of 0.3 ppm prevents edema but affords only a partial protection from infection upon challenge with infectious agents (Coffin and Gardner, 1972a) and has little effect on cell renewal upon a second exposure (Evans et al., 1976).

At a cellular level, tolerance induced by preexposure to 0.5 ppm ozone protects against pulmonary edema, but has little effect on the cytotoxic effects caused by a second exposure to ozone as measured by a reduction of enzyme activity, enhanced inflammation and altered macrophages (Alpert and Lewis, 1971; Gardner et al., 1972). Rats preexposed to 0.75 ppm ozone for 3 days followed by secondary

challenge to 4.0 ppm ozone for 8 hours, showed no dramatic effects on a variety of biochemical parameters in lung tissue (Chow et al., 1976).

(B) EXTRAPULMONARY EFFECTS OF OZONE :

1-Central nervous system and behavioral effects :

Rats and mice exposed to low levels of ozone (0.5 ppm) suffered from a depression of motor activities . Limited data on the effects of ozone on the enzymes in the central nervous system indicate variability depending on the studied enzyme. For example, catechol-O-methyl transferase is decreased while the levels of monoamine oxidase are increased (Trams et al., 1972). Despite reports of dizziness, throat and nose irritations and visual impairment in humans exposed to ozone; there is limited information on ozone-induced changes on animal behavior (Tepper et al., 1983).

2- Effects of ozone on the immune system :

The immunotoxicological data on ozone indicate marked impairment of the pulmonary host defense mechanisms (Graham and Gardner, 1985). Mice exposed to 0.1 ppm ozone show decreased host resistance to bacterial challenge (Coffin and Gardner, 1972b). Additionally, ozone has been shown to increase the incidence of pulmonary infections induced by a number of other pathogenic organisms, including streptococcus sp., Pasteurella haemolytica and Mycobacterium tuberculosis (Graham and Gardner, 1985). The increased susecptibility may relate to ozone induced impairment in alveolar macrophage function. Studies supporting this assumption demonstrated that exposure to ozone can significantly decreases the number of alveolar macrophages (Gardner and Graham, 1976), impairs the phagocytic ability of alveolar macrophages (Devans et al., 1985) and decreases the ability of macrophages to secret reactive oxygen intermediates (Amoruso et al., 1981) and interferon (Shingu et al., 1980). Ozone-induced systemic immune dysfunction has also been demonstrated (Aranyi et al., 1983) and cannot be ruled out as an addition ional factor which impairs host defense. Alterations in rabbit alveolar macrophage production of arachidonic acid metabolites (increased prostaglandin E2, PGE2) following in vitro and in vivo ozone exposure have been also reported (Driscoll, 1986; Schleshinger and Driscoll, 1987). These authors suggested that increased PGE2 production represents a potential mechanism for the impaired alveolar macrophage function consistently observed in ozone-exposed animals. Non immunological mechanisms may contribute to decreased host resistance. Ozone-induced impairment in mucuciliary clearance and increased mucous secretion could result in an accumulation of pathogenic organisms (Kenoyer et al., 1981; Last et al., 1977). Lung inflammation occurs in humans exposed to extremely low level of ozone (Koren et al., 1989).

3- Hematological effects:

The effects of ozone exposure on hematological endpoints has been investigated in many in vivo and in vitro studies (U. S. Environmental Protection Agency, 1978; Borek and Mehlman, 1983). The morphological and biochemical parameters observed in hematological studies serve as useful indicator for ozone in different species (Chow et al., 1975). Exposure of red blood cells (RBCs) to ozone resulted in some changes including increased fragility, potentiation of complement-dependent membrane damage (Goldstein et al., 1974), formation of Heinz bodies (Menzel et al., 1975), inhibition of Na^+/K^+-ATPase and spherocytosis (Koontz and Heath, 1979) RBCs from humans exposed to 0.5 ppm ozone for 2 hrs showed enhanced glutathione and glucose-6-phosphate dehydrogenase activity (Buckley et al., 1975), while that from rats and monkeys at the same dose and conditions failed to show such changes (Chow et al., 1975). Oxidation of intracellular glutathione has been observed in human RBCs exposed to 0.84 ppm ozone for 2 hr (Freeman and Mudd, 1981) supporting earlier studies by Menzel et al., (1972) which showed a decrease in RBCs glutathione following long-term exposure of rats to 0.5 ppm ozone for 23 days.

In addition to the effect of ozone on RBCs, other changes have been detected in serum of animals to ozone. These include decreased albumin and enhanced levels of globulins in rabbits (P›an and Jegier, 1976) and increased lysozyme activity (Chow et al., 1974) and prostaglandin levels in rats (Giri et al., 1980).

4- Endocrine effects:

The endocrine system is adversely affected by ozone inhalation. Morphological changes are observed in the parathyroid after a single exposure to ozone (Atwal, 1974). Reduced levels of thyrotropin and thyroid hormones were shown in rats exposed to 1 ppm ozone for 24 hr (Clemons and Garcia, 1980), suggesting changes in hypothalamic function. Hormones have been recognized as playing a role in modulating ozone toxicity (Stockinger, 1965; Borek and Mehlman, 1983). A protective effect has been observed by removing the thyroid, hypophyseal or adrenal glands (Fairchild, 1963; Fairchild and Graham, 1963), suggesting that hormones such as thyroxin may potentiate the toxic action of ozone (Fairchild and Graham, 1963).

5- Reproductive effects:

The reproductive and teratogenic effects of ozone have been investigated. Brinkman et al., (1964) reported from his studies on mice, that prenatal exposure of mice to 0.2 ppm ozone can reduce infant survival. Progeny of dams exposed to 1 ppm ozone during late gestation showed slower growth rate and retarded early reflex development (Kavllock et al., 1980).

6- Genotoxic effects:

The genotoxic effects of ozone have been predicted from its radiomimetic character. Free radicals produced upon decomposition of ozone in water, such as OH^- radical, are similar to those produced by ionizing radiation and thought to play a role in some of its

genotoxic actions (Borek, 1982; Borek and Troll, 1983).

Fenter (1962) showed an induced chromosomal aberrations in human fibroblasts due to exposure to 1960 μg ozone m^{-3}. Different results were obtained when human lymphocytes were exposed to ozone both in vivo and in vitro (Gooch et al., 1976). No effect was shown in hamster and mouse peripheral lymphocytes exposed in vitro to 1 ppm ozone or less than 5 hr (Gooch et al., 1976). In contrast, significant chromosomal aberrations were found in hamster peripheral lymphocytes when exposed to ozone under similar previous conditions (Tice et al., 1978; Zelac et al., 1971a).

Data on sister-chromatid exchange (SCE) induced in human cells by ozone at 1 ppm or less are also conflicting. A dose-related increase in SCE was observed in WI-38 diploid cells exposed in vitro; but exposure of human subjects to ozone showed no significant enhanced SCE in their blood lymphocytes (Guerrero et al., 1979).

7- Carcinogenic effects of ozone:

The role of ozone in carcinogenesis is unclear (Stockinger, 1965). Some of its actions as a powerful oxidant and producer of free radical might render it suspect in playing a role in carcinogenesis and mutagenesis (Borek and Mehlman, 1983). Radiation, a complete carcinogen (Borek, 1982), produces chromosomal aberrations in synergistic fashion with ozone (Zelac et al., 1971b). These may play a role in the neoplastic process. Free radicals produced by oxidants damage to DNA (Lesko et al., 1980) and modify cellular genetic integrity, could lead to carcinogenic events.

The role of free radicals in carcinogenesis is seen by the fact that scavengers of free radicals, such as catalase, inhibit oncogenic transformation in vitro by radiation and chemicals (Borek and Troll, 1983). In addition, dietary factors such as selenium enhance the breakdown of peroxides in the cells exposed to radiation and chemicals and prevent oncogenic transformations (Borek and Biaglow, 1984).

MECHANISMS OF OZONE TOXICITY :

The toxicity of ozone depends upon its oxidative properties.

(1) Ozone acts by initiating peroxidation of polyunsaturated fatty acids (PUFAs) present in the cell membrane. The peroxides and secondary reactive oxygen species which ensue produce their toxicity by damaging the integrity of the cell membrane and other cellular molecules, Fig.(1) (Goldstein et al., 1969; Chow and Tappel, 1973; Mudd and Freeman, 1977; Borek and Mehlman, 1983; Pryor et al., 1983; Witz et al., 1983; Mehlman and Borek,1987).

(2) Ozone exerts its toxicity by oxidation of compounds of low-molecular weight like those containing thiol, amine, aldehyde and alcohol functional groups and by oxidation of proteins. Both soluble peptides, such as glutathione and

protein in lipid bilayers provide potential targets for ozone action. Protein modification takes place via oxidation of amino acid side groups (Mudd et al., 1969; Freeman and Mudd, 1981). So, the two mechanisms of ozone toxicity may be interrelated. Peroxidation of PUFAs gives rise to water-soluble products such as aldehydes, peroxides and hydroxy radicals (Pryor, 1976; Pryor et al., 1983,) which diffuse into the cytosol and initiate oxidation of amino acids and proteins, Fig. (2) (Borek and Mehlman, 1983; Mehlman and Borek, 1987).

Direct oxidation of amino acids and proteins by high ozone levels or oxidation by secondary reaction products of PUFAs peroxidation can inhibit a variety of cellular protective systems. These include glutathione, a scavenging thiol, glutathione peroxidase, superoxide dismutase and catalase, which detoxify peroxides (Mustafa et al., 1983), enzymes which supply reducing cofactors such as glucose-6-phosphate dehydrogenase (DeLucia et al., 1972) and antiproteases (Johnson, 1980), which play a role in the inhibition of ozone-mediated leakage and edema (Borek and Mehlman, 1983). Both thiols and enzymes may be restored metabolically to control levels or rebound to higher protective levels following intermittent or continuous ozone exposure (Chow and Tappel, 1972, 1973; DeLucia et al., 1972; Mustafa and Lee, 1976).

The degree to which ozone reacts with proteins is determined by the presence of ozone-susceptible amino acids at their active sites (Mudd et al., 1969; Mudd and Freeman, 1977; Freeman and Mudd, 1981) and the location of the amino acids in the tertiary structure of the protein (Boyer, 1972). For example, cystein, methionine and tryptophan are very susceptible to ozone (Mudd et al., 1969) and the oxidation of tryptophan produces peroxides (Meiners et al., 1977) which are toxic and give rise to other reactive toxic oxygen species (McCord and Fridovich, 1978).

The hypothesis that lipid peroxidation is the primary factor in ozone-mediated toxicity has its strongest support in the findings that vitamin E, a dietary antioxidant, is a powerful protective agent against ozone toxicity (Chow et al., 1981; Chow, 1983; Mustafa, 1975). Its effectiveness is further enhanced by other antioxidants such as ascorbic acid and butylated hydroxytoluene (Fletcher and tappel, 1973).

Peroxidation of PUFAs by ozone results in the generation of fatty acid hydroperoxides. These are destroyed by glutathione peroxidase-consuming glutathione. Oxidized glutathione is reduced by glutathione-reductase consuming NADPH. Thus, the loss of glutathione following ozone exposure (Freeman and Mudd, 1981) promotes lipid peroxidation indirectly through the inhibition of glutathione peroxidase or by direct oxidation and depletion of glutathione. Chow and Tappel (1973) suggested that peroxides formed via lipid peroxidation induce glutathione peroxides and this in turn induces enhanced levels of the enzymes required to supply reducing factors such as NADPH to

glutathione peroxidase. Vitamin E suppresses spontaneous formation of lipid peroxides. The supplementation of vitamin E in the diet would therefore decrease the utilization of glutathione peroxidase and maintain a high level of protection in the cell. There is a cross function between various antioxidants. Selenium, an essential factor in selenium-dependent glutathione peroxidase and an inducer of the enzyme (Borek and Biaglow, 1984), prevents vitamin E deficiency and increases the transport and utilization of the vitamin (Menzel, 1970; Borek and Mehlman, 1983).

Following exposure to high levels of ozone, the relative importance of PUFAs peroxidation and the oxidation of proteins and compounds of low molecular weight depends on many factors. These include (a) membrane composition of PUFAs and proteins, which determine ozone accessibility and degree of interaction and damage, (b) enzymatic pathways to decompose peroxides, (c) pathways to generate thiols and (d) the presence of antioxidants (vitamin E, vitamin C and selenium) to prevent peroxide formation and to partake in scavenging free radicals arising from secondary reactions (Pryor et al., 1983; Borek and Mehlman, 1983).

CONCLUSION :

Ozone, a potent toxic agent, exerts its effects in a rapid fashion and only a small amount of ozone is needed in each lung cell to intiate damage and cytotoxicity. The lipid peroxidation theory is an attractive one to explain ozone action. The reaction is rapid and is propagated through cascading events mediated via free radicals (Pryor et al., 1983; Borek and Mehlman, 1983). Direct oxidation of proteins which regulate membrane functions could alternatively produce some forms of cytotoxicity through cell leakage and edema. The powerful protective effect of vitamin E and other antioxidants supports lipid peroxidation as a primary toxic action of ozone.

In vitro studies suggested that ozone toxicity is due to oxidative damage of membrane lipids and cellular sulfhydryls and by inactivation of key enzymes involved in cellular metabolism. Ozone affects the respiratory epithelium from the nasal cavity to the alveoli. Ciliated and type I epithelial cells are injured, while mucous production and mucous cell membranes are increased, Fig. (3) (Haschek and Witschi, 1991).

Ozone is a toxic potent oxidant. In the body ozone reacts to produce reactive free radicals. These species become involved in destructive oxidative processes, particularly reaction with SH groups and lipid per-oxidation. This is a process in which the C = C double bonds in unsaturated lipids are attacked by free radicals and undergo chain reactions in the presence of O_2, resulting in their oxidative destruction. Species rich in sulfhydryl groups, such as metallothionine acts as antidotes to ozone poisoning (Manahan, 1992).

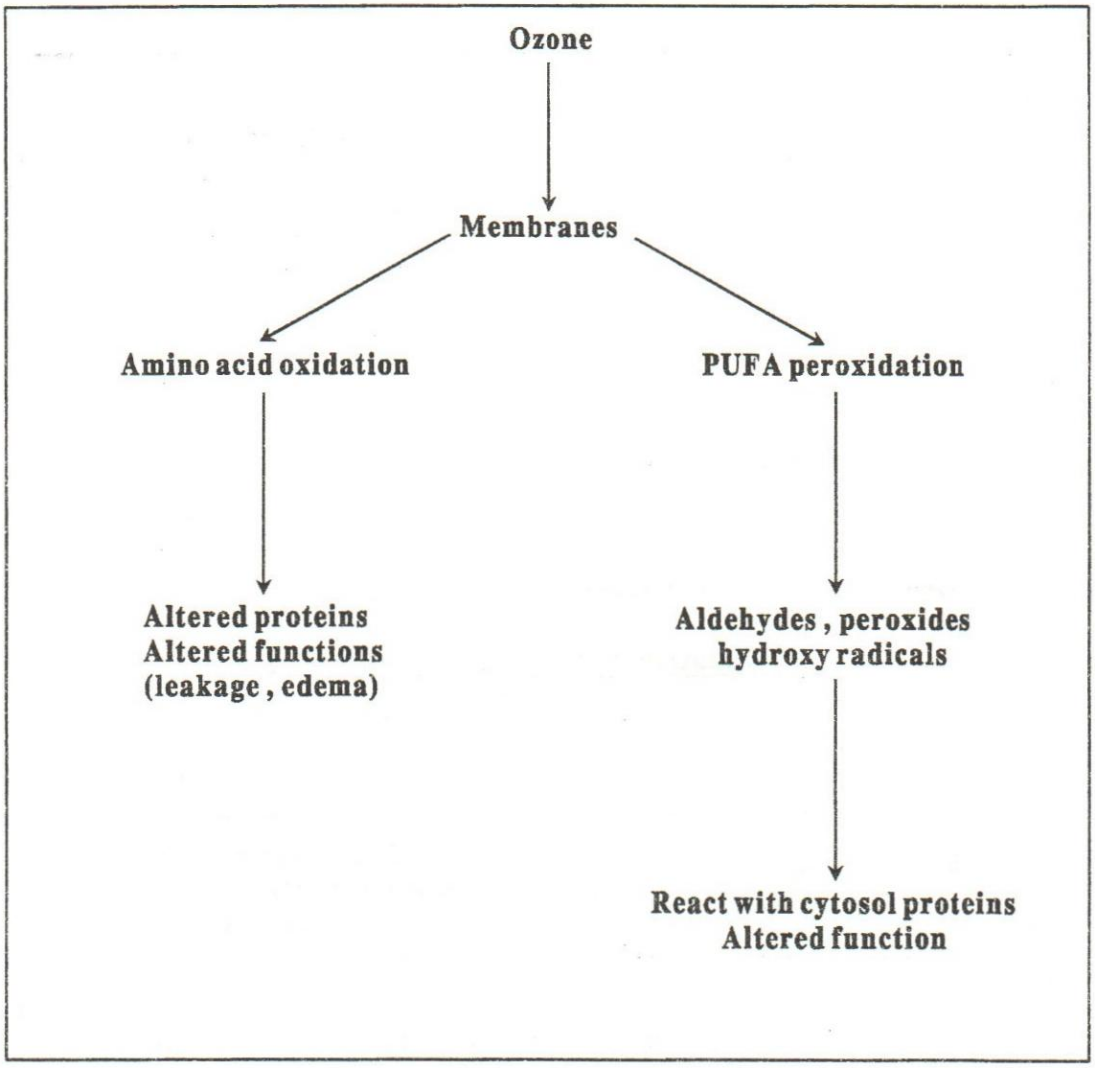

Fig.(1): The effects of ozone on the cell membrane as a primary site of its toxicity which suggest that ozone damage to membranes is in part induced via free radical processes (Mehlman and Borek, 1987).

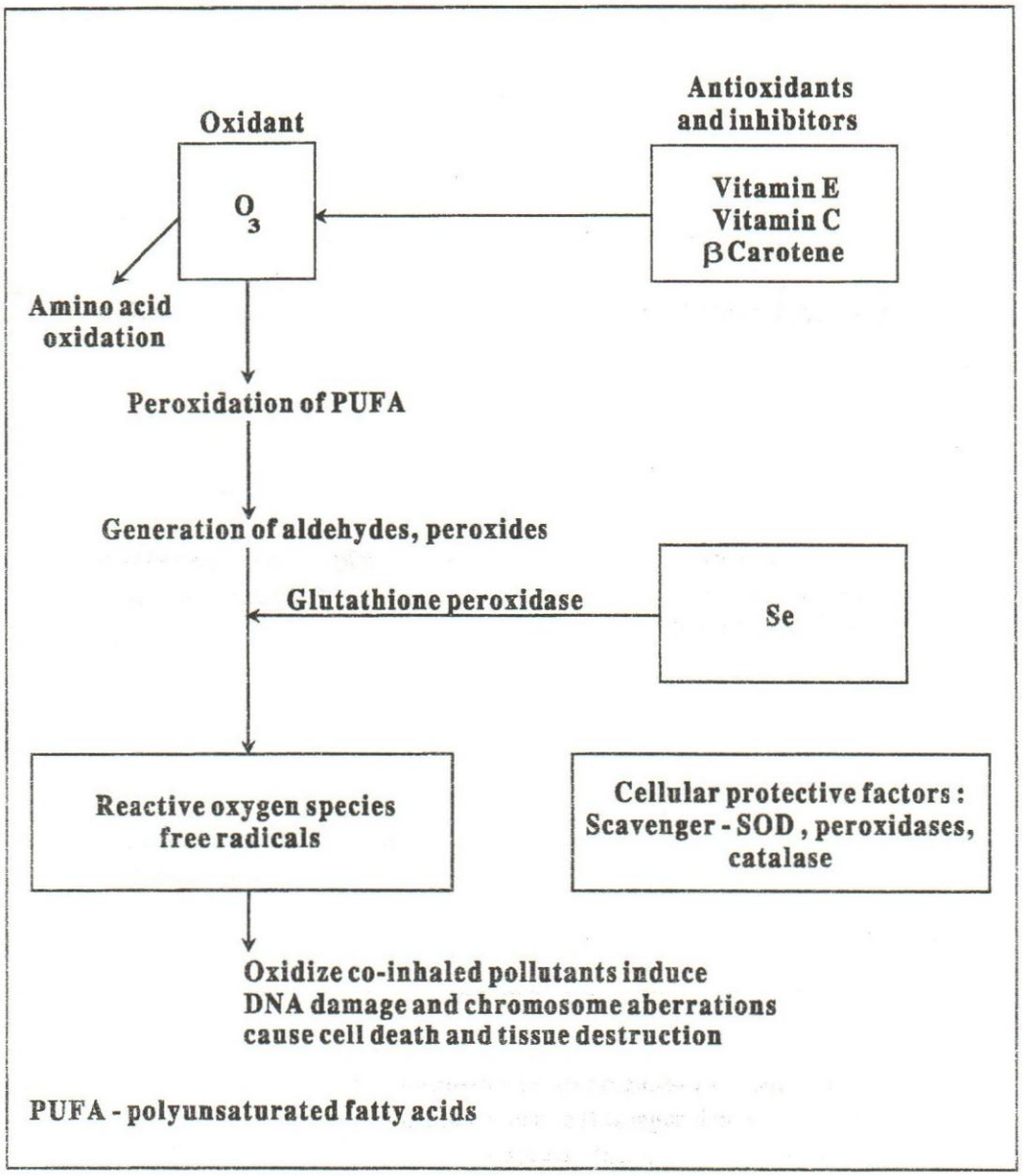

Fig.(2): Mechanisms of ozone toxicity (1) Initiation peroxidation of polyunsaturated fatty acids which present mainly in the cell membrane. (2) Oxidation of proteins via oxidation of amino acid side groups (Mehlman and Borek, 1987).

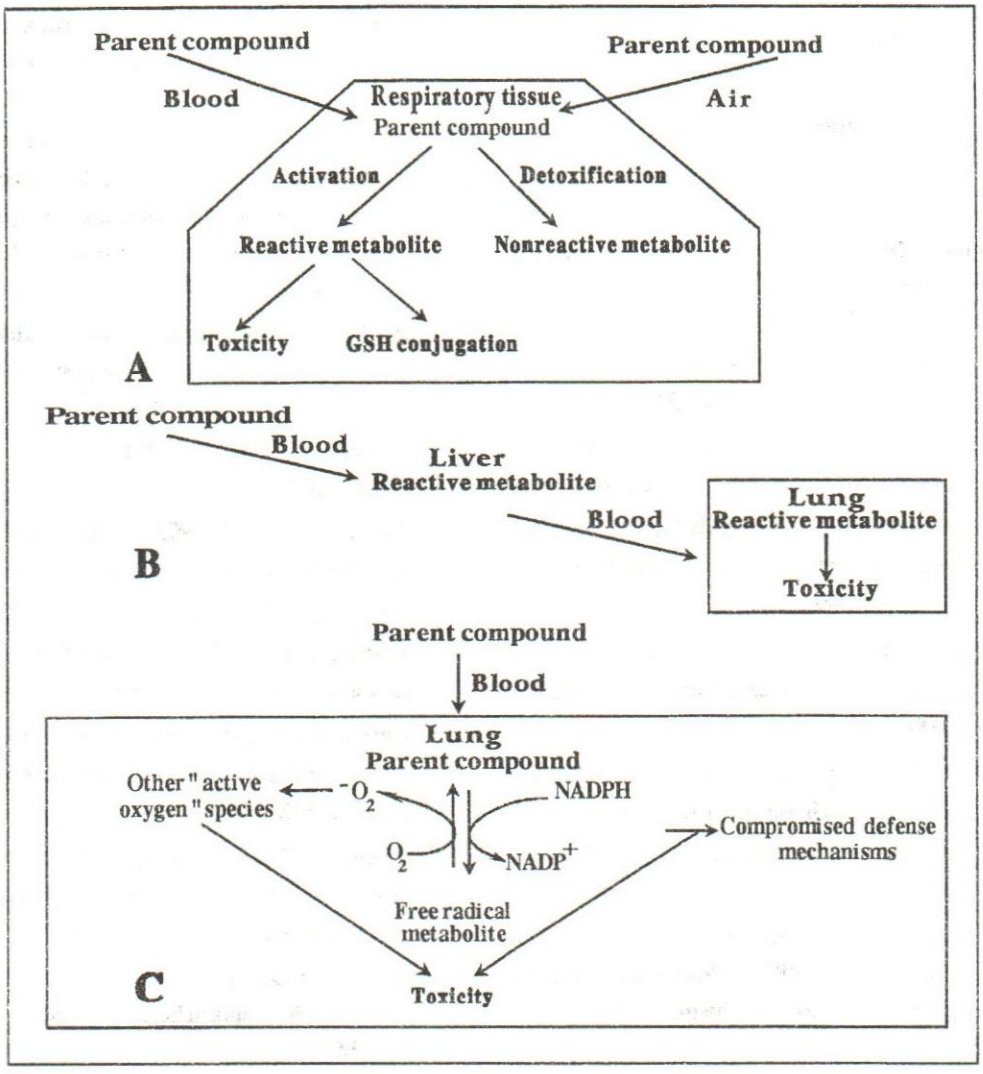

Fig.(3): Mechanisms of respiratory injury involving metabolic activation. (A) In situ metabolic activation of parent compound. (B) Activation of parent compound in liver, metabolite produces toxicity in lung. (C) Parent compound undergoes cyclic reduction/oxidation, indirectly inducing toxicity (Haschek and Witschi, 1991).

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الملوثات الغازية : ١ - الأوزون

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يعد التلوث من أهم المشاكل البيئية التى تواجه العالم اليوم . ويعتبر الأوزون واحداً من أهم الملوثات الغازية إذ أنه أحد مركبات الأكسجين النشطة ، والذى إذا زادت نسبته فى الهواء الجوى عن ٠,١ جزء فى المليون يحدث بعضاً من الآثار السامة .

وقد تم فى هذه المقالة إظهار تأثيرات الزيادة فى تركيزات الأوزون فى الهواء المحيط بنا على أجهزة الجسم المختلفة كما يلى :

أولاً - تأثيرات الأوزون على الجهاز التنفسى :

مثل : تأثير على الشكل الظاهرى للجهاز التنفسى ، تأثير على وظائف الجهاز التنفسى ، تثبيط بعض انزيمات الجهاز التنفسى ، تثبيط تكوين البروتين داخل أنسجة الرئتين .

ثانياً - تأثيرات الأوزون على الأجهزة الأخرى :

مثل : الجهاز العصبى ، الجهاز المناعى ، الجهاز الدموى ، الغدد الصماء ، الجهاز التناسلى ، تأثير على الكروموسومات ، تأثيرات مسببة للسرطان .

ومما لا يفوتنا أن الأوزون يعمل عن طريق :

١- تنبيه أكسدة الأحماض الدهنية المتعددة الغير مشبعة التى توجد فى جدر الخلايا .

٢- أكسدة المركبات الخلوية ذات الوزن الجزيئى البسيط والتى تحتوى على المجموعات الوظيفية.

مما سبق يتبين لنا أن الأوزون من الملوثات السامة التى تعمل عن طريقة الأكسدة ، ولذلك يجب أن نتوخى الحذر من التعرض لمثل هذه المواد ، وأن نعمل جاهدين بكل ما أوتينا لتلاشى جميع الأخطار التى قد تنجم عن انتشار مثل هذه الملوثات فى بيئتنا ، من خلال تقنيات علمية .