RESULTS

Virus Concentration Assay

Table (5) Comparison between nitrocellulase and glass microfibre filters for concentration of coxsackievirus type B4 in Experimentally infected effluents.

Membrane type	Without Conditioning*		After Conditioning**	
	PFU/100 ml	% of recovey	PFU/100 ml	% of recovey
Nitrocellulose 0.45 um poresize (scheicher+schuell)	1.35×10^4	30	1.98×10^4	40
Glass microfibre filter (Gf / A) (whatman)	1.14×10^4	20	1.85×10^4	40

* Initial concentration is 4.5×10^6 PFU/ 100 ml

** Conditioning means the addition of $AlCl_3$ and adjustment of pH at 3.5

Table (4) showes that no great difference in Nitrocellulose membrane with and without conditioning where the percentage of recovery were 40% and 30% respectively. In case of glass microfiber filter the percentage of recovery were 40% and 20% respectively. Between Nitrocellulose and Glass microfiber after conditioning the percentage of virus recovery were the same, so we started the concentration process with nitrocellulose membrane because its availability.

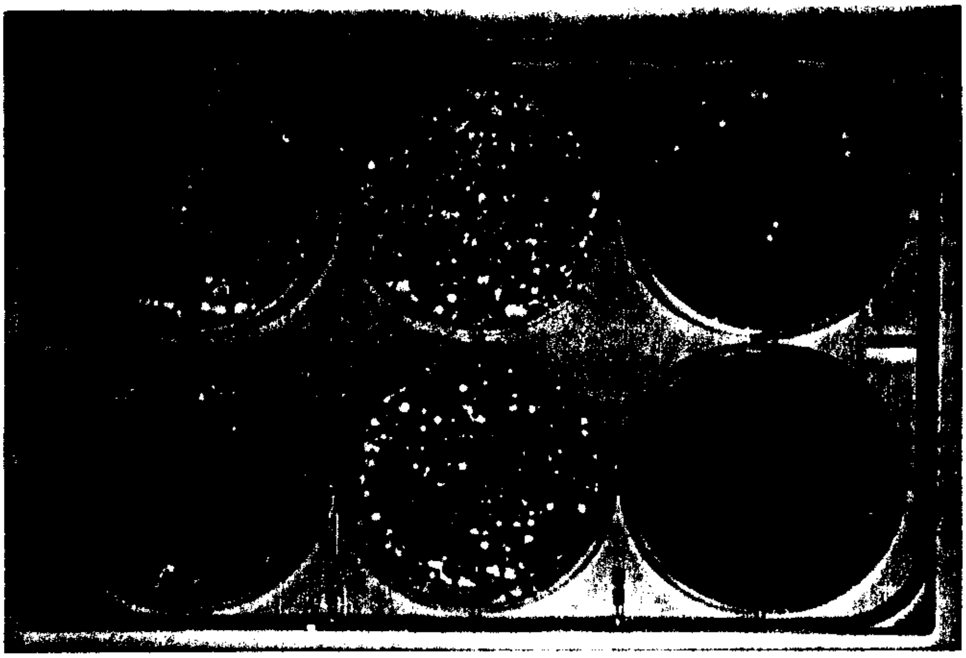


Fig (3) : Plaque Assay Titration Of Coxsackie B4 Virus Concentrated By Filtration Through

(A) Nitrocellulose Menbrane 0.45 μm Pore Size :

1- Before Conditioning, 2- After Conditioning

(B) Glass Microfiber Menbrane :

1- Before Conditioning, 2- After Conditioning

Well No (3) Represent Coxsackie B4 Viruse Initial Seed , & Well No (4) Is Control BGM Cells.

Virus isolation in cell cultures

Table (6) Effect of concentrated wastewater samples from Aerated facultative pond effluent (AeE) on cell cultures.

Site of Samples	Date oF Sampling	Effect On Cell Cultures (CPE +/-)		
		BGM	VERO	C_636
Aerated facultatitve pond Effeuent (AeE)	5/91	+	+	-
	6/91	+	+	-
	7/91	+	+	-
	8/91	+	+	-
	9/91	+	+	-
	10/91	+	+	-
	11/91	+	+	-
	12/91	+	+	-
	1/92	+	+	-
	2/92	+	+	-
	3/92	+	+	-
	4/92	+	+	-

Table (7) Effect of concentrated wastewater samples from Maturation pone effluent (ME) on cell cultures.

Site of Samples	Date oF Sampling	Effect On Cell Cultures (CPE +/-)		
		BGM	VERO	C_636
Matursation pond Effeuent (ME)	5/91	+	+	-
	6/91	+	+	-
	7/91	+	+	-
	8/91	+	+	-
	9/91	+	+	-
	10/91	+	+	-
	11/91	+	+	-
	12/91	+	+	-
	1/92	+	+	-
	2/92	+	+	-
	3/92	+	+	-
	4/92	+	+	-

Table (8) Effect of concentrated wastewater samples before Maturation pond effluent (ME) on cell cultures.

Site of Samples	Date oF Sampling	Effect On Cell Cultures (CPE +/-)		
		BGM	VERO	C_636
Before Maturation pond Effeuent (ME)	5/91	+	+	-
	6/91	+	+	-
	7/91	+	+	-
	8/91	+	+	-
	9/91	+	+	-
	10/91	+	+	-
	11/91	+	+	-
	12/91	+	+	-
	1/92	+	+	-
	2/92	+	+	-
	3/92	+	+	-
	4/92	+	+	-

Table (9) Effect of concentrated wastewater samples After Maturation pond effluent (ME) on cell cultures.

Site of Samples	Date oF Sampling	Effect On Cell Cultures (CPE +/-)		
		BGM	VERO	C_636
After Maturation pond Effeuent (ME)	5/91	+	+	-
	6/91	+	+	-
	7/91	+	+	-
	8/91	+	+	-
	9/91	+	+	-
	10/91	+	+	-
	11/91	+	+	-
	12/91	+	+	-
	1/92	+	+	-
	2/92	+	+	-
	3/92	+	+	-
	4/92	+	+	-

Tables (6,7,8,9). shows that BGM and vero cell lines were sensitive for enteroviruses isolation and BGM cells were sensenitive and speed than vero for virus isolation, but C_636 cells were found not sensitive.

Identification of virus isolates.

Table (10) Virus type of the CPE induced Samples in Aerated facultative pond Effluent (AeE)

Date of Sampling	Virus type					
	PVI	PVII	PVIII	$COX.A_6$	$COX.B_4$	Rotavirus
5/91	-	-	+	+	-	-
6/91	-	-	+	-	+	-
7/91	-	-	+	-	+	-
8/91	-	+	+	-	+	-
9/91	-	-	+	-	+	-
10/91	+	-	-	-	+	-
11/91	-	+	+	-	-	-
12/91	+	-	+	+	-	-
1/92	+	-	-	-	+	-
2/92	+	+	+	-	-	-
3/92	-	-	+	-	-	-
4/92	+	-	+	-	-	-

Table (11) Virus type of the CPE induced Samples in Maturation Pond Effluent (ME)

Date of Sampling	Virus type					
	PVI	PVII	PVIII	COX.A_6	COX.B_4	Rotavirus
5/91	-	-	+	+	+	-
6/91	-	-	+	-	-	-
7/91	-	+	+	-	+	-
8/91	-	-	+	-	-	-
9/91	-	-	+	-	+	-
10/91	+	-	+	-	+	-
11/91	-	-	+	-	-	-
12/91	-	-	+	+	+	-
1/92	+	-	+	-	-	-
2/92	+	+	+	-	-	-
3/92	-	+	+	-	-	-
4/92	+	+	-	+	-	-

Table (13) Virus type of the CPE induced Samples After Maturation pond Effluent (ME)

Date of Sampling	Virus type					
	PVI	PVII	PVIII	COX.A6	COX.B4	Rotavirus
5/91	-	+	+	-	+	-
6/91	-	-	+	-	+	-
7/91	-	+	+	-	+	-
8/91	-	+	+	-	+	-
9/91	+	-	-	+	+	-
10/91	-	-	+	-	+	-
11/91	-	-	-	+	+	-
12/91	-	-	+	-	-	-
1/92	-	-	+	-	-	-
2/92	-	-	-	+	-	-
3/92	-	-	+	-	-	-
4/92	+	-	-	-	-	-

Tables (10,11,12,13) shows that poliovirus type III was dominant in sludge (37.2%) followed by coxsackievirus type B4 (21.5%), Polioviurs type I (16.6%), poliovirus type II (13.7%), Coxsackievirus A6 was detected in (10.7%) of the investigated samples, while Rotavirus was not detected in all samples.

A

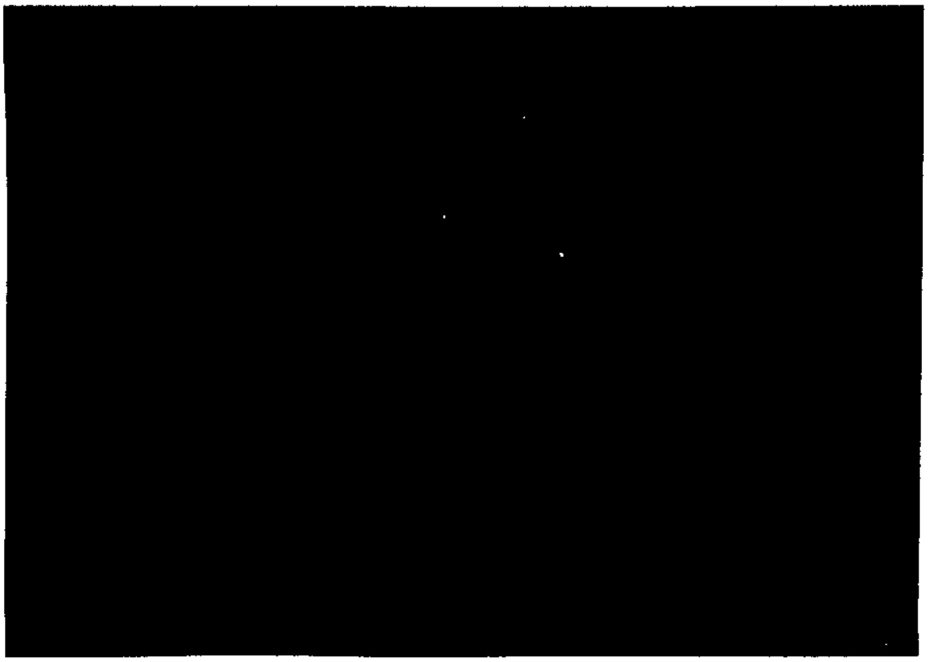


Fig (4) : DOT-Enzyme Linked Immunosorbent Assay (DOT-ELISA) For CPE-Induced Samples At The Third Passage In BGM Cells. Positive Dots Appear In Brown Color.

(A) Positive Samples For Poliovirus Type I.

(b) " " " Poliovirus Type II.

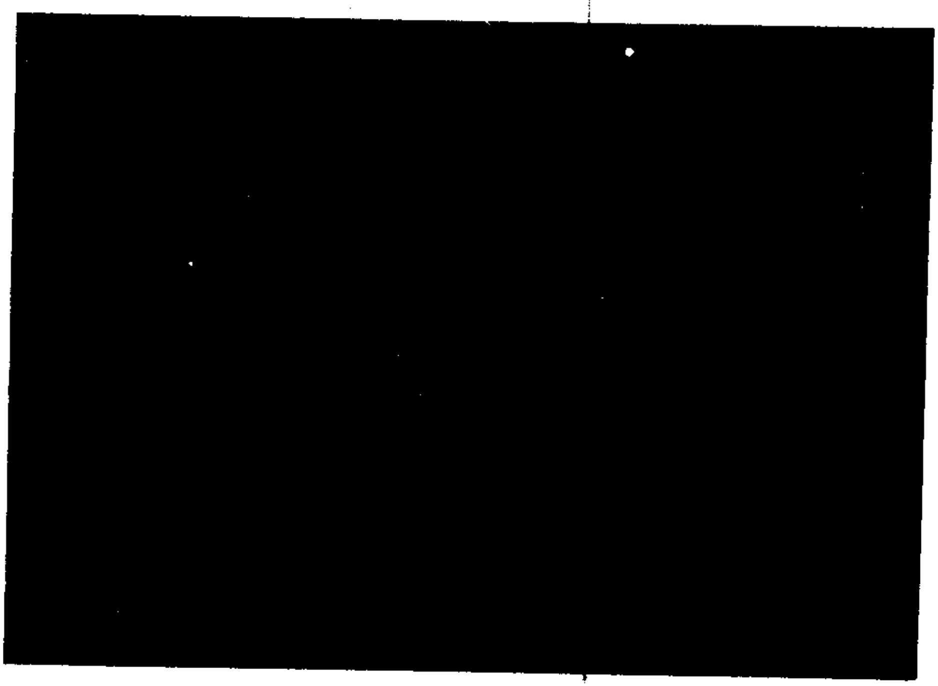
(c) " " " Poliovirus Type III.

(d) " " " Coxsackievirus Type A6.

(e) " " " Coxsackievirus Type B4.

B

C



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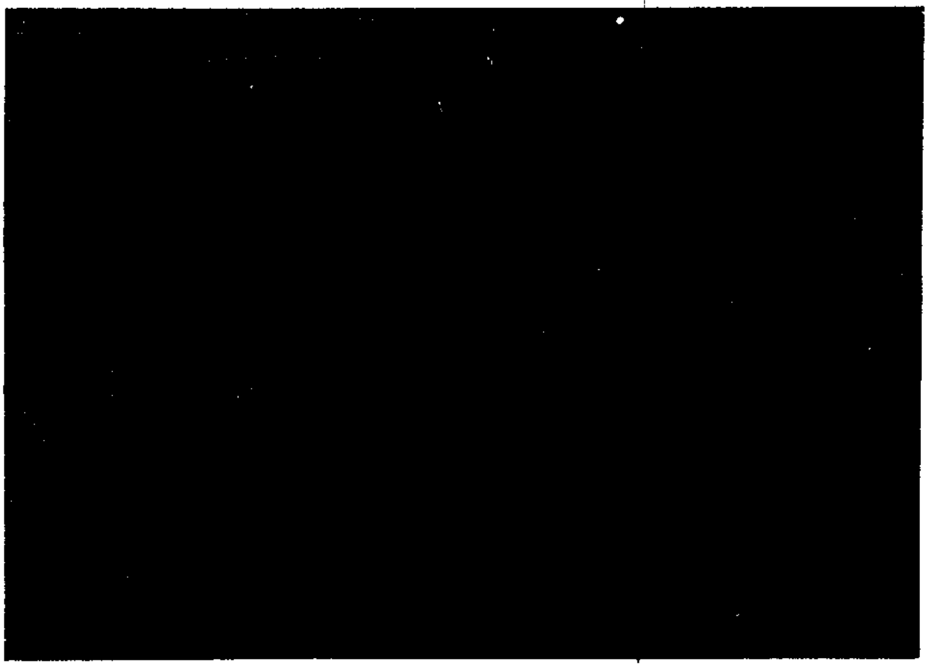

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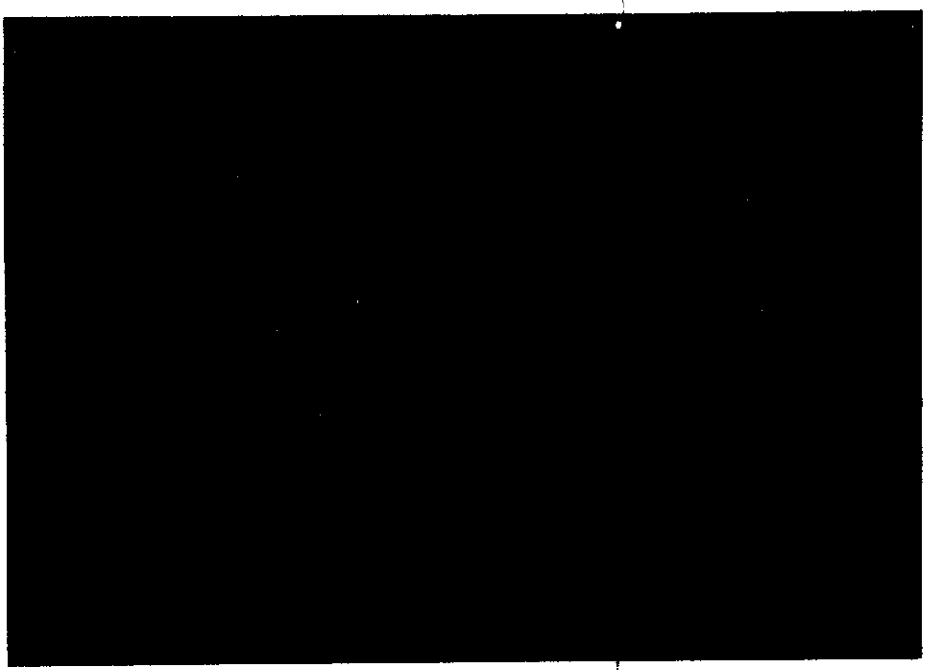

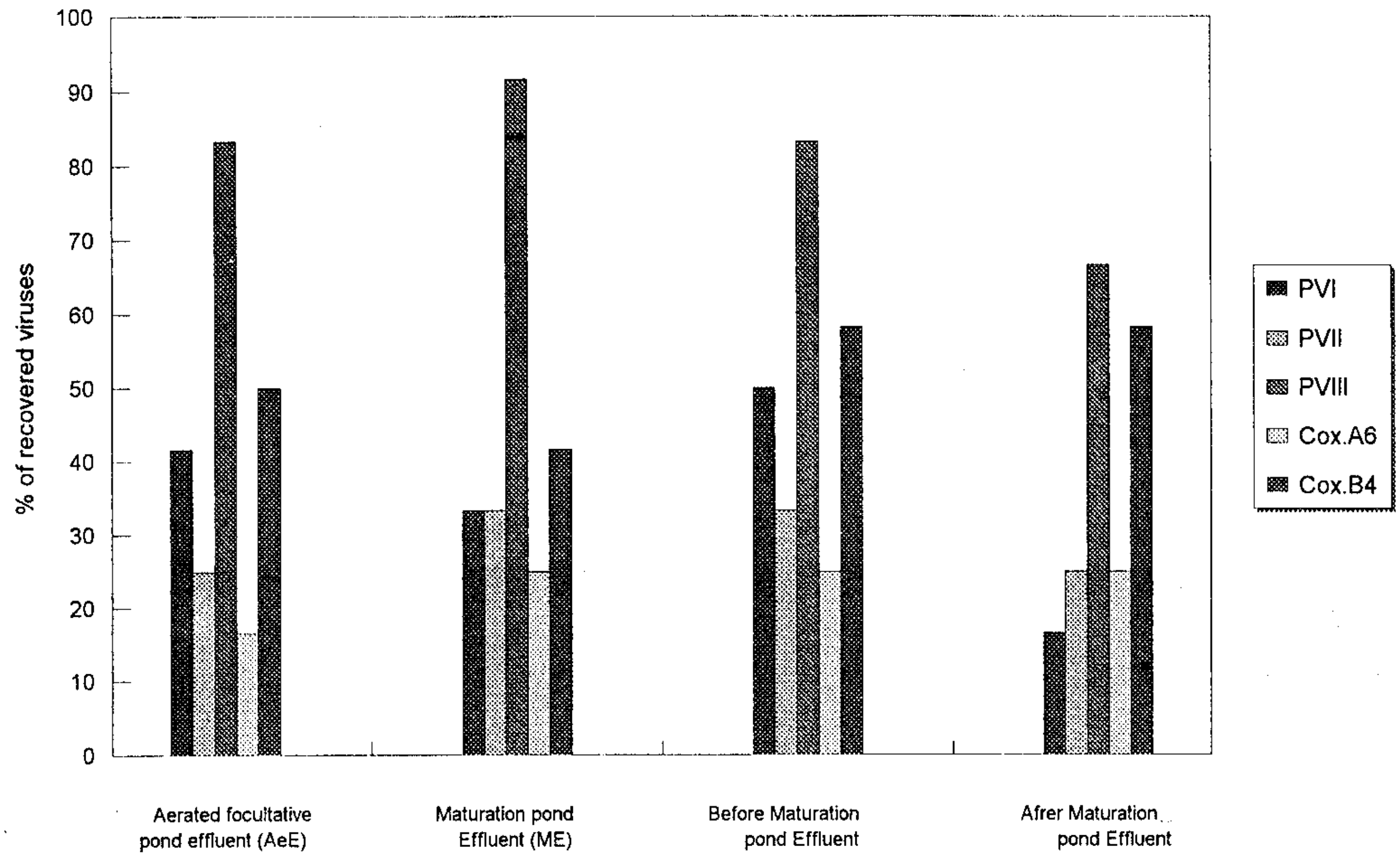

Fig (5) : Enteroviruses detected in four sites of wastewater treatment plant at Mit Mazah W.W.T.P.

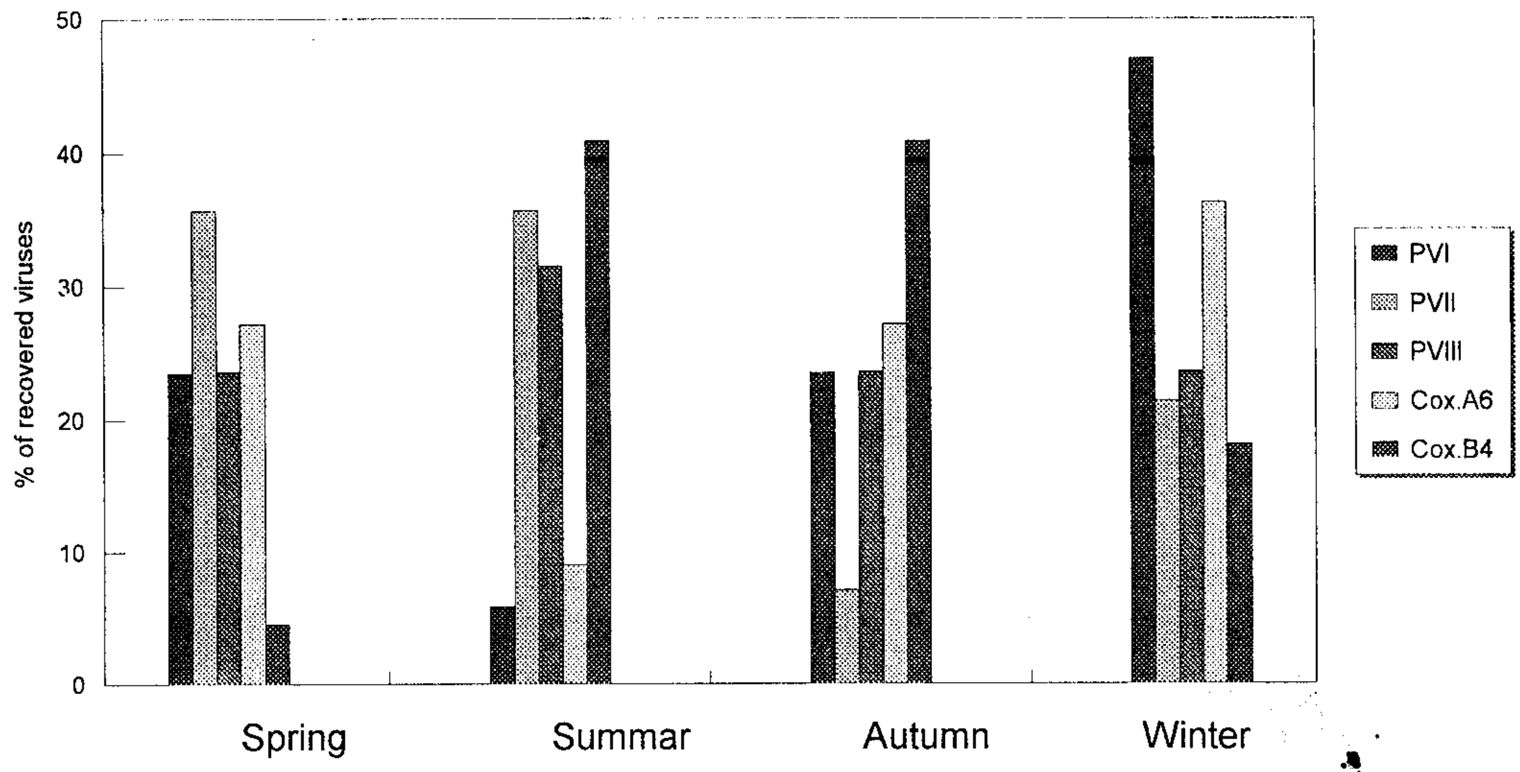

Fig (6) : Seasonal variation of enteroviruses detected in wastewater treatment plant (Mit Mazah).

Assay of Disimfection

Table (14) Effect of chlorine disinfection on polio II virus seeded wastewater effluent.

chlorine dose	Virus count *	
	CPE (+/-)	virus count (PFU /ml)
5	+	over
10	+	130
15	+	10
20	0	0
25	0	0
30	0	0

* Polio II virus (initial seed is 7 X 10^{7} Pfu / ml)

Table (15) Effect of chlorine disinfection on Coxsackievirus Type B_4 seeded wastewater effluent.

chlorine dose	Virus count *	
	CPE (+/-)	virus count (PFul /ml)
5	+	2160
10	+	620
15	+	540
20	+	320
25	+	20
30	-	0

*Coxsackie virus type B_4 (initial seed is 4.5×10^6 PFU / 100 ml)

Table (14,15) Shows the amount of chlorine that inactivated Poliovirus Type II (Salk strain) was 20 mg/L (Residual chlorine 1.9 mg/L) where as needed for inactivation of Coxsackie virus B_4 was 30 mg/L (Residual chlorine is 2.3 mg/L)

Table (16) Effect of Ozone disinfection on polio II virus seeded wastewater effluent.

Ozonation time (min)	Virus count	
	CPE (+/-)	virus count (PFU /ml)
9	+	250
11	+	210
13	+	140
15	+	80
17	0	0
19	0	0
21	0	0

Table (17) Effect of Ozone disinfection on Coxsackie virus Type B_4 seeded wastewater effluent.

Ozonation time (min)	Virus count	
	CPE (+/-)	virus count (PFU /ml)
9	+	920
11	+	420
13	+	230
15	+	20
17	+	10
19	+	20
21	+	10

Table (16,17) Shows the doses of ozone used for poliovirus II inactivation was 3/9 mcg/L/h, 17 min, whereas for Coxsackievirus B_4 over 21 min.

Table (18) Effect of u.v exposure time for disinfection of Polio II virus in seeded wastewater

Exposure time(sec)	Virus count *	
	CPE (+/-)	virus count (PFU /ml)
5	+	over
10	+	130
15	+	10
20	-	0
25	-	0
30	-	0

Table (19) Effect of u.v exposure time for disinfection of Coxsackie B_4 virus seeded wastewater

Expoure time (sec)	Virus count *	
	CPE (+/-)	virus count (PFU /ml)
5	+	over
10	+	280
15	+	30
20	-	0
25	-	0
30	-	0

Table(18,19) Shows that U.V exposure time for disinfection of poliovirus II and Coxsackievirus B_4 were the same 20 sec for seeded effluent samples.

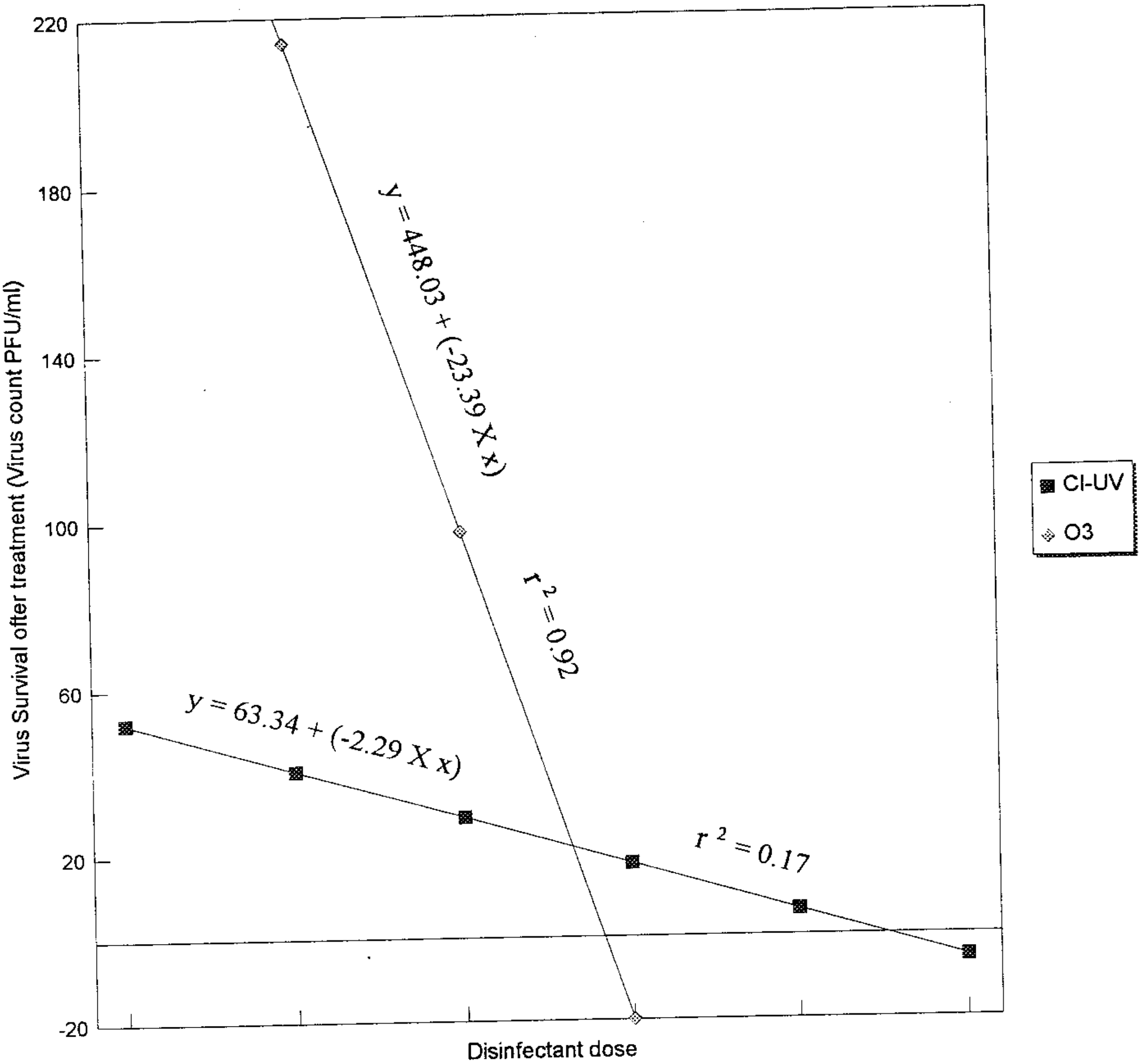

Fig (7) : Regression Correlation between Polio II virus count and different Disinfectant Dose

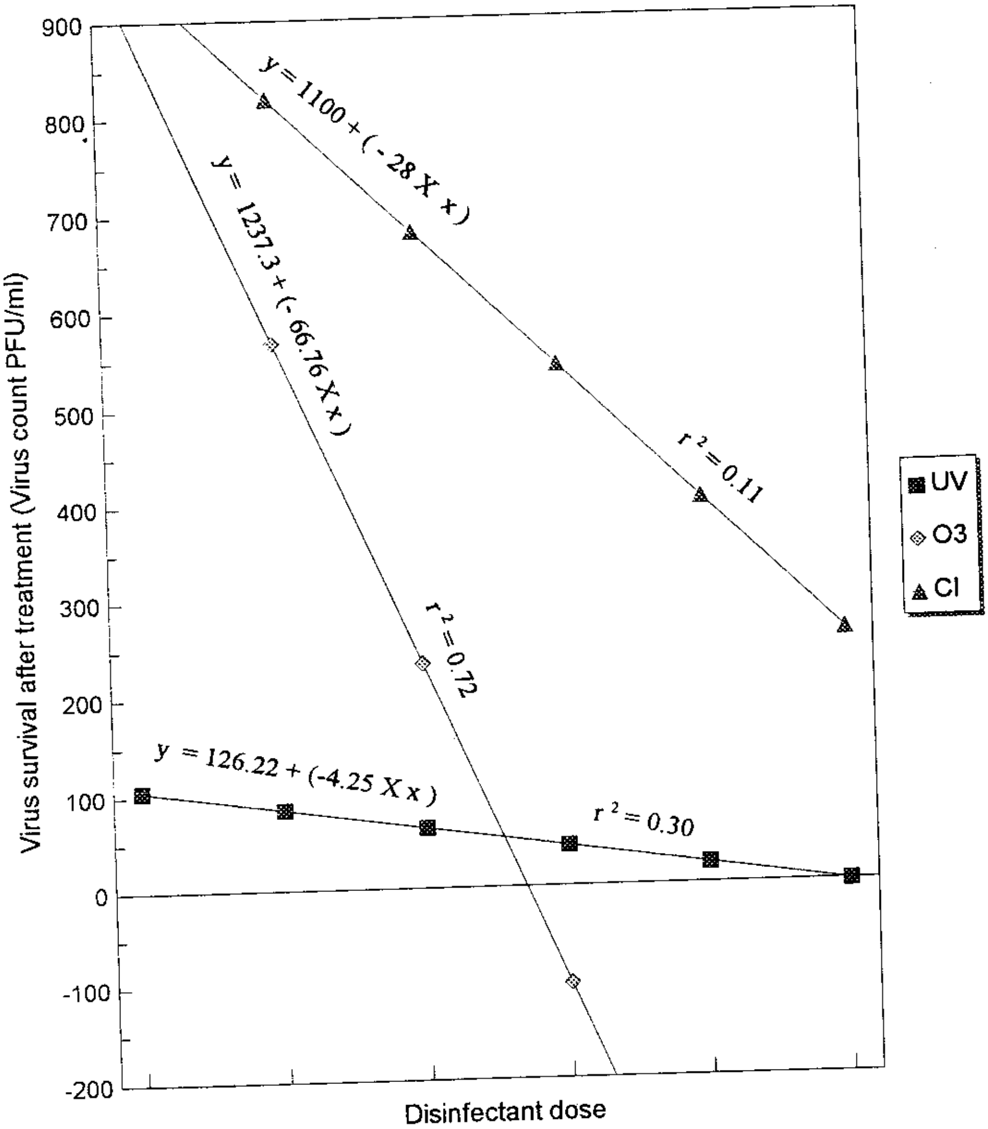

Fig (8) : Regression Correlation between Coxsackie virus type B4 Count and different disinfectant Doses.

Fig (7,8) : Revealed high regression coeffiecient, this mcans that ozone is the most suitable disinfctant for coxsackievuns type B4 and polio virus type II where the regession cefficient reached to 0.72 for cox. B4 and 0.92 for PVII.

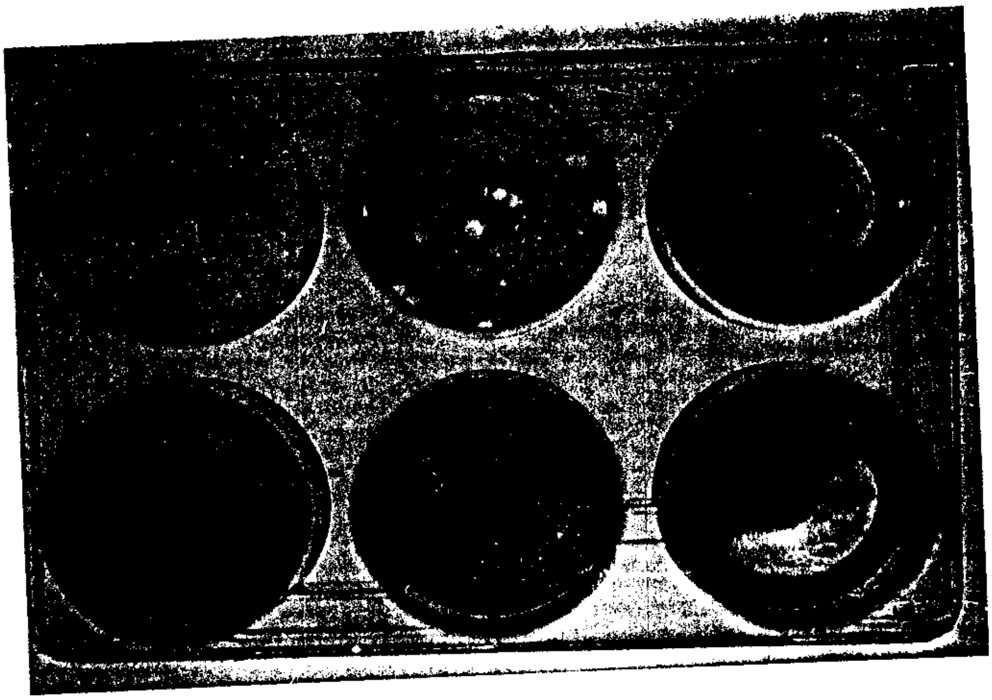

Fig (9) : Effect Of Chlorine Disinfection On Poliovirus Tupe II (Salk Strain). At 20 mg Chlorine / L For 30 min, Complete Inactivation Of The Virus was done.

Table (20) Selected chemical - physical water quality data for four samples locations

Parameters	Sampling Locations			
	Aerated facultative pond Effeuent (AeE)	**Matur ation pond Effeuent (ME)**	**Before Matur ation pond Effeuent (ME)**	**After Matur ation pond Effeuent (ME)**
Water temperature	018.900	021.100	019.200	018.500
Hydrogen ion (PH)	007.600	007.900	008.000	007.800
Turbidity NTU	900.000	891.000	205.000	283.000
Electric conductivity μmosh/cm	050.000	061.000	189.000	312.000
Nitrite mg/LN	000.006	000.065	000.008	000.328
Nitrate mg/LN	000.480	000.500	000.360	000.490
Ammonia mg/LN	049.840	050.210	048.530	050.025

selected chemical-physical water quality data for four samples location :-

The physicochemical parameters (water temperature, Hydrogen ion PH, Turbidity, and Electric conductivity) was detected. No clear change was observed in temperature and PH value between different sites. High electric conductivity was recorded in After maturation pond effluent, reached to 312 M mosh/cm, high reduction in turbidity value between different sites where it reached to 900 and 891 in Aireated facultative pond effluent (AeE) and Maturation pond effluent (ME) while reached to 205 and 283 in before Maturation pand effluent and After Maturation pond effluent. Also table (21) showed that Ammonia and Nitrate values ranged between 48.5-50.2. For Ammonia, and ranged between 0.36-0.50 for Nitrate. high Nitrate value was detected at After Maturation pond effluent where it reached to 0.328. while in other sites it ranged between 0.006-0.065

Table (21) The population of aquatic insects collected in different seasons

Species	Season				
	sping	summer	Autumn	Winter	Total
Order : Hemiptera					
Family: Belostomatidae					
Sphaerodema urinator	25	160	140	30	355
Limnogeton Fieberi Mayer	17	19	10	3	49
Family: Nepidae					
Ranatra vicina sign	0	3	20	2	25
Family : Gerridae					
Gerris aegyptiacus	0	3	1	0	4
Order : Coleoptera					
Family : Hydrophilidae					
Amphiops lucidus	9	13	29	5	56
Family : Dytscidae					
Cybister tripunctatus africanus	5	8	3	2	18
Hydaticus leander Rossi	3	7	0	0	10
Sternolophus solieri cast	18	10	3	4	35
Canthydrus notula Er.	1	3	3	0	7
Order : Diptera					
Family : Culicidae					
Aedes sp	37	0	0	5	42
Anopheles sp	0	0	11	19	30
Culex pipens	60	7	28	30	125
Order : Odonata					
Family : Aeschnidae					
Anax imperator leach	30	72	19	3	124
Family : Coenagriondae					
Ischnura seneglensis Ramb	30	42	13	8	93
Enallagma Vansomereni pinhey	16	20	3	0	39
Family : Libellulidae					
Brachythemis leucosticata	15	8	14	12	49
Crocothemis erythraea	15	6	3	5	29
Total	281	381	300	128	1090
Percentage	25.77	34.95	27.52	11.75	

The population of aquatic insects collected in different seasons:-

The results in table (20) showed that all sampling location at Mit Mazah treatment plant were strongly dominated by four Taxonomic orders Hemiptera, Diptera, Coleoptera, and Odonata. Order Hemiptera, Family Belostomatidae (*Spharerodema urinator* sp, *Limnogeton Fieberi Mayer* sp) represent 100% of the sample in spring, while in the summar represent 96%, and Family Nepidae (*Ranatra vicina sign*) 2%, Family Gerridae (*Gerris aegyptiacus sp*) 2% also. In Autumn Family Belostomatidae represent 87%, while *Ranatra Vicina sign* 12%, and *Gerris aegyptiacus* 1%. In winter *SPhearodema urinator sp*, and *Limnogeton Fieberi Mayer* represent 94%, while *Ranatra vicina* 6%. In spring Order Coleoptera present in two Families Family Hydrophilidae (*Amphiops lucidus*) 25% , while in summer it was 32% , 76% in Autnmn, and 45% in winter, while Family Dytiscidae (*Cybister tripunctatus africanus, Hydaticus leander Rossi, Sternolophus solieri cast,* and *Canthydrus nodula Er.*) represent 75% in spring, 68% in summar, 24% in Autumm, and 55% in winter. order Dipter represent in Family Culicidae (*Aedes Sp, Anopheheles Sp,* and *Culex pipens*) it was 100% in all season. order Odonata it was manifasted in three families Family Aeschnidae (*Anax imperator leach*) 28% of the sample in spring, while family Coenagriondae *Ischnura seneglensis Ramas, Enallagma vansomereni* pinhey 43%, And family Libellulidae represent 28% it was *Brachythemis leucosticata*, and *Crocothemis erythraea,* family Aeschnidae in summar was 19%, 36% in Autumn, and 11% in winter. While family Coenagrionidae represent 42% of the sample in summar, 31% in Autumn, and 28% in the winter, and family Libellulidae were present in 9% from the sample in summer, 33% in Autumn, and 61% in winter As shown in Fig (10)

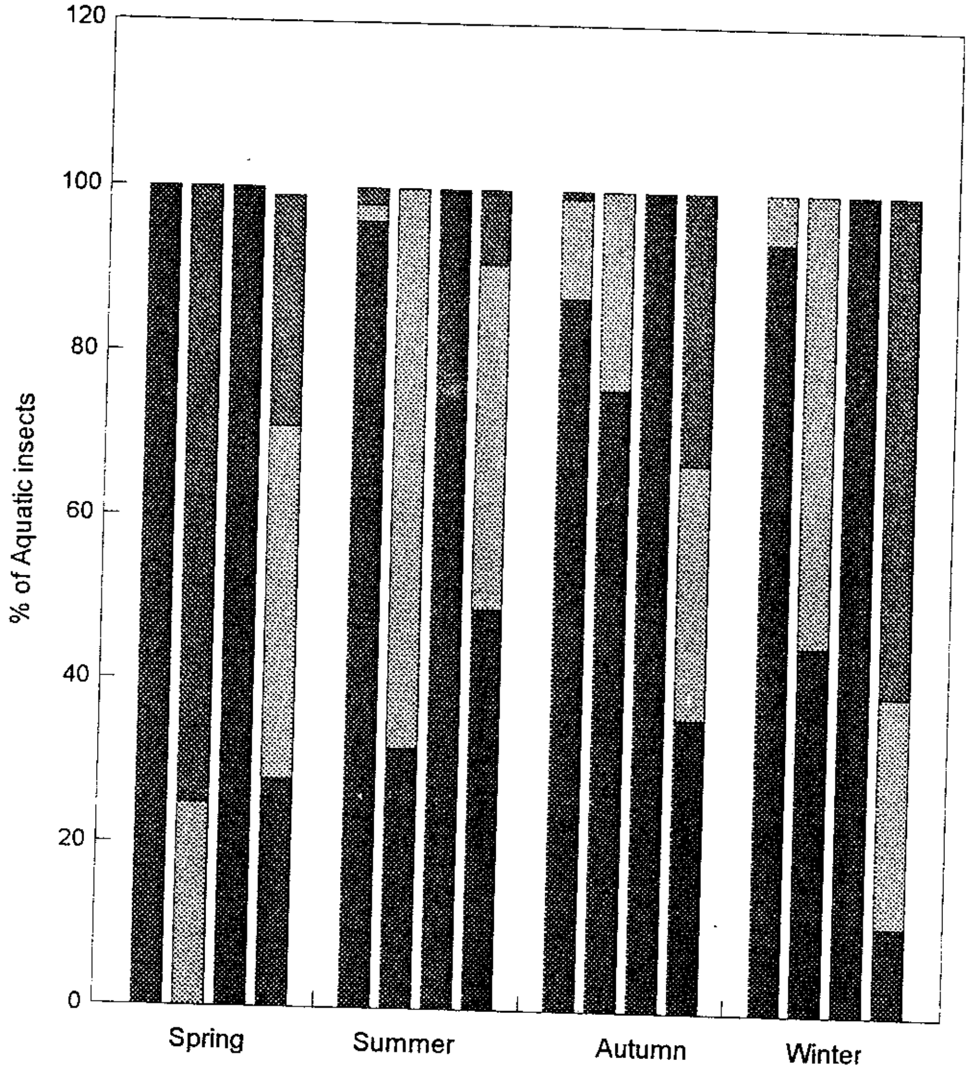

Fig (10) : Population of aquatic insects collected in different seasons

a- .Order Hemoptera (Family : Belostomalidae, Nepidae, and Gerridae)

b- Ordr Colesptera (Family : Hydrophilidae, and Dytscidae)

c- Order Diptera (Family : Culicidae)

d- Order Odonata (Family : Aeschnidae, Coenagriondae, and Libellulidae)

Fig (12) : *Limnogeton Fieberi Mayer.*

Fig (13) : *Ranatra Vicina Sign.*

Fig (14) : *Gerris Aegyptiacus Put.*

Fig (15) : *Amphiops Lucidus.*

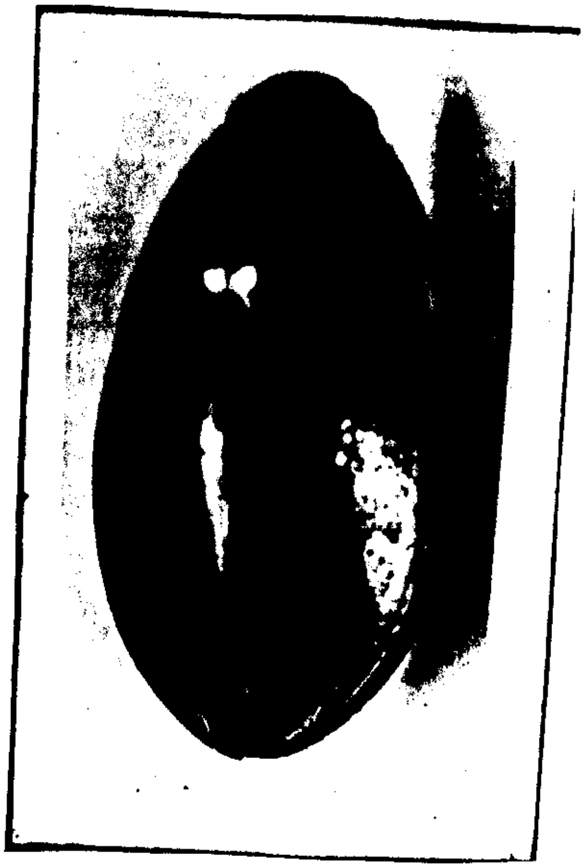

Fig (16) : *Cybister Tripunctatus Africanus.*

Fig (17) : *Hydaticus Leander Rossi.*

Fig (18) : *Sternolophus Solieri Cast.*

Fig (19) : *Canthydrus Notula Er.*

Fig (20) : *Culex Pipens.* *Aedes SP.* *Anopheles SP.*

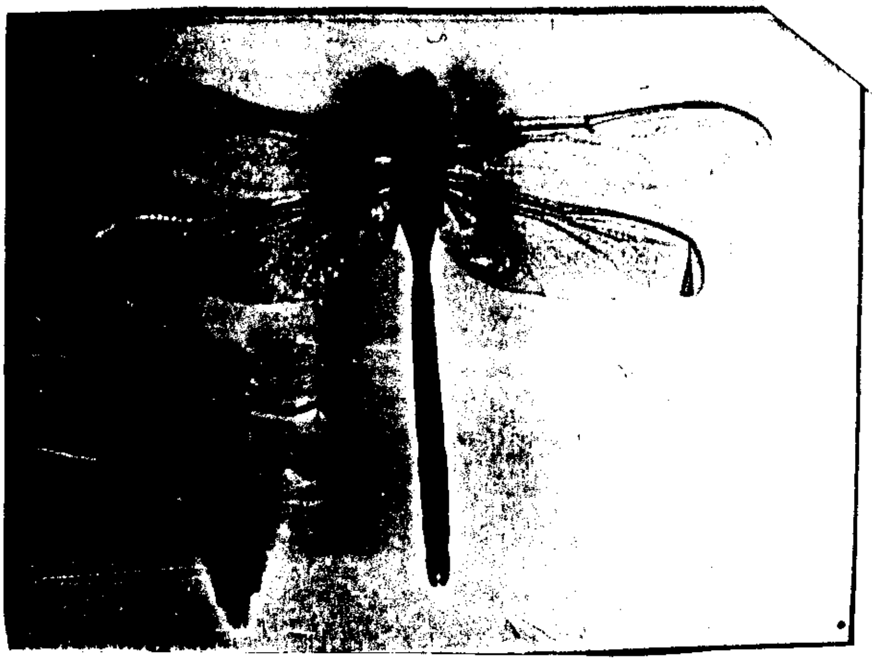

Fig (21) : *Anax Imperator Leach.*

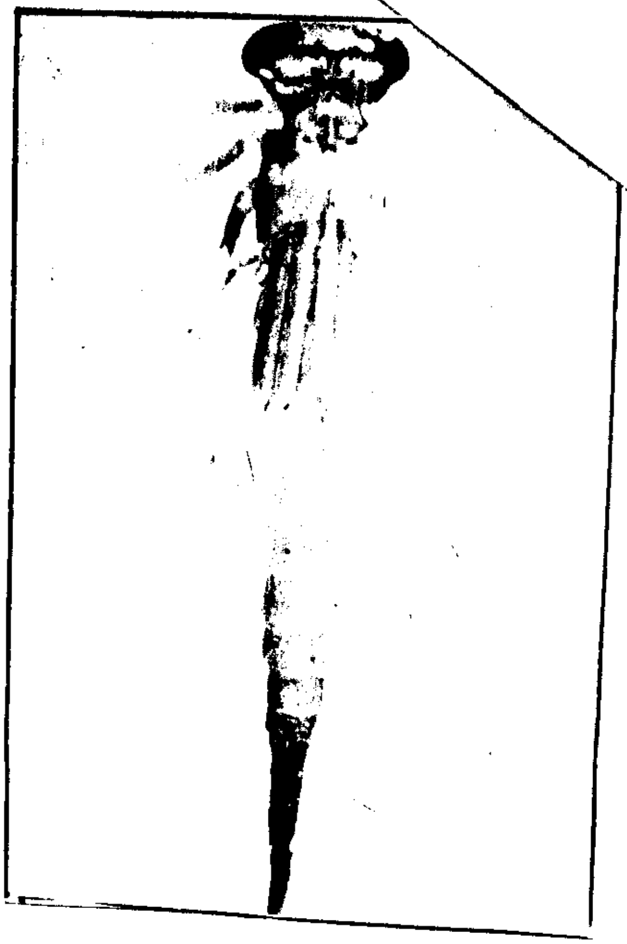

Fig (22) : *Ischnura Senegalensis Ramb.*

Fig (23) : *Enallagma Vansomereni Pinhey.*

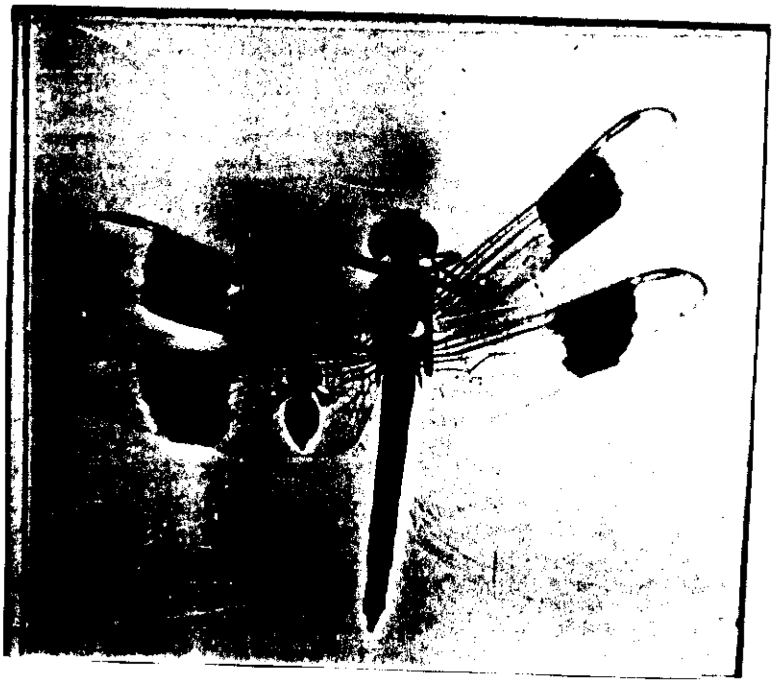

Fig (24) : *Brachythemis Leucosticata Burm.*

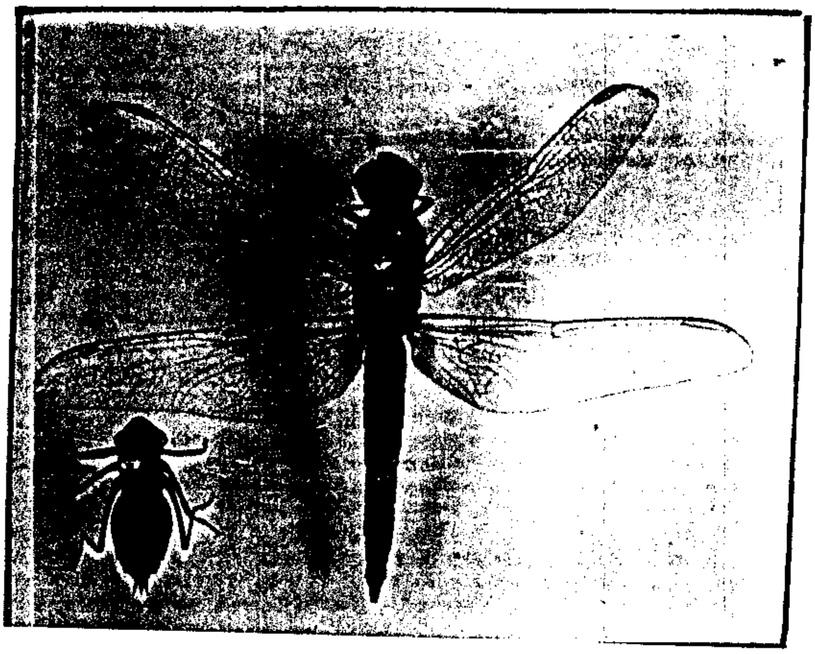

Fig (25) : *Crocothemis erythraea Brulle.*