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EFFECT OF SOME PESTICIDES ON BACTERIAL INDICES OF POLLUTION

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INTRODCTION:

Bateriolgical parameters applied to assess water include, total viable counts, faecal coliforms and faecal strepto cocci previous studies showed the significant correlation batwenn faecal pollution and pereviously given parameters (Geldreich, et al., 1968; Geldreich and Kenner, 1969, and Geldreich, 1970).

Any factor which masks the presence of faecal pollution and /or effect the survival of such indicators organisms other than natiral aquatic condition, will lead to a wrog decision with regard than natural aquatic condition, will lead to a wrong decision with reagard to the bacteriological quality of water. Consequently the effect of organic pecticide residues on bacteriological parameters need to be investigated. Meanwhile, the effect of such organic compounds on bacterial population will be linked with persistence of the parent compounds and /or theri degradation products. organic herbicides exert inhibitory effect on quatic algal growth, photosynthetic activity and could change the natural balane between algal species as well as between algae and other

intervals according to the type of herbicide Flasks of atrazine herbicide were examined twice weekly, while monuron was examined weekly, The samples were withdrawn under aseptic condition to prevent any contamination.

The following bacteriological procedures were employed:

1- total viable count using poured plat technique (p.C):

From a wall mixed water sample, 1 ml inocula (from ten fold serial dilutions) were placed in 4 sterile petridishes. Molten plate - count agar maintained at 48°Cwas poured in 15 ml amounts into each plat through mixing of contents was carried out and plates were left on bench of about 30 minutes after which duplicate plates were incubated at 22°C Counting of the resulting growth was carried out 38-hr - the mean figure of reading obtained was recorder to repersent the bacteraial count per 1ml of the sample. the plate count ager medium used for pouring contained yeast extract 0.25%, tryptone 0.5%, glucose 0.1% and agar 1.5% (pH 7.0)

2- Most total coliform density (MPN-index):

The determination of MPN- index for total coliform by the "Multiple tube fermentation technique" was carried out by using 3 decinal dilutions from each sample, 10 ml, 1 ml and 0.1 ml, in 5 replicate tube of Macconkey broth from each inoculum.

The medium contained pepton 2%, sodium chloride 0.5%, sodium taurocholate 0.5% lactose 1% and neutral red

as indicators (pH. 7.4). A triple strength medium was used for the 10ml inocula, distribution 10 ml in each tube. Other wise, only single strength of Macconkey broth was used for 1 ml and 0.1 ml incula. All tubes were provided with Dhrham fermentation tubes. Incubation was at 37°C for 48-hr (± hr), after which reading were taken. A positive reaction was indicated by acid and gas production. The tables of ***Swarrop (1951)*** were used or MPN- index derivations according to number of positive and negative tubes in each replication. To account for MPN- index exceeding 2400, moredecimals in five replicates were incorporated to atest water sample. The MPN reading were derived from Swaroop's tale and computed for the last 3 decimals beginin with five positive tube through multiplication of the ordinary index by the middle dilution gradient of such particullar combination.

3- Faecal streptococci density (MPN- index):

Determination of the MPN-= index of faecal strepto-cocci in Nile water was carried out by using 3 decimal concentrations (10 ml, 1 ml and 0.1 ml) from a sample each in 5 replicate tube containing buffered azide glucose glycerol broth (tryptose 2% dextrose 0.5% dipotassium hydrogen phosphate 0.4% monopotassium phosphate 0.15% sodium chloride 0.5%, sodium azide 0.05%, bromocresol purple 0.0015% pH was fixed at 6.9 and 5 ml of glycerol was added to 1000 ml of mediam. Double strength of azide dextrose broth was used for the 10 ml inocula and single strength for the 1 ml and 0.1 ml, and incubation carried out at 37°C for

48h (± 2h). A positive reaction was indicated by acid production. The table of Swaroop (1951). Were used for derivation of the MPN- index per 100 ml of water. From all azide dextrose broth possitive tubes, showing turbidity after 48hrs, three loopfulls of growth were transferred to ethyl violet azide broth tube (Bacto- tryptose 20 gm, Bacto-Dextrose 5 gm, Dipotassium phosphate 2.7 g, Monopotassium phosphate 2.7 gm, sodium chlordie 5 gm, sodium Azidde 0.4 gm, and ethyl violet 0.00084 pr liter.

Postive tubes (showing a purple buttom) within each replicate were recorded and the corresponding MPN- index was derived from the computation table of Swaroop.

For each of the studied compounds, a set of three flasks, 5-liters each, were sterilized. Each set of flasks included, a flask containing 4-liter of Nile River water from (Benha branch), asecond flask containing 4-liter of the river water fortiied with settled sewage (10ml.L) and the thrid flask contained sterilized river water (by autoclaving).

Each of the studied compounds was introdued to aset of the flasks so that the initial concentration of each herbicide was 5 mg/L. A fine stream of air bubbles was passed through the aqueous media to keep microflora in aerobic conditions. All flasks were kept at room temperature (25±2) and the pH value of the water adjusted to 7.5± 0.3 by addition 1 ml/L of the phosphate buffer used for the BOD test ***(APHA, 1985).***

Aliquots of water sample (100 ml) were withdrawn from each flask for the detrmination a residual concentration of the

herbicides. Frequency of sampling was according to the rate of change takeing place. WHen biodegradation proceeded and the herbicide concentration was nearly exhausted, a second dose was added to the specific flask.

Analytical procedures:

The heribicide was determined by using liquid- liquid extraction procedure. A known volume of water sample (100 ml) was extracted 3 times with 50 ml portions of 15% methylen chloride in hexane. The combined extracts were dehydrated with anhydrous sodium sulphate and concentrated to 2 ml by gentle evaporation under vaccum. Clean up was followed according to ***EPA Method (1984).***

Residues of atrazine were identified and determined using a varian aerograph 3700 GLC equiped with a flame ionization detectors (FID). Analysis were performed using a glass column (2 mx 0.4 cm i.d) packed with 5% OV-17 on chrmosorb W. 80/100 mesh. The flow rate of nitrogen (Carrier gas) hydrogen and air were 20,20 and 200 ml/min. The column, injector and detectr temperatures were 190°C, 230°C and 250°C respectively. Determination of phenylamides and their corresponding aniline derivatives was carried out by the colorimetric method as given ***(El-Dib, 1971; El-Dib and Ali 1972)***

RESULTS AND DISCUSSION

1.Effect of atrazine:

Response of bacterial population to atrazine is present in tables (2) and (3). Results of total viable counts revealed some fluctuation in numbers during the 8th days of the first addition of atrazine. However, a steady increase in total viable counts, was recorded during the succedding period that lasted for 40 days. As the concentration of Atrazine decreased by the end of the first addiion, the total viable counts decreased. The same pattern of variation of total viable counts was observed in case of Nile River water seeded with sewage. The observed limited reduction in bacterial counts indicates that atrazine exerted low toxicity to bacteria. Moreover, increase of atrazin to 10 mg/L, during the second and third addition, was associated with further increase in bacterial density. Such a trend of variations in total counts tend to show that bacterial in Nile river and sewage have the capacity for adaptation and possibel use of atrazine. Woth regard to total coliforms, results presented clearly show that colifrom density, in presence of atrazine was higher as compared with the control. Moreover, coliforms in Nile water doses with atrazine, with or without sewage seed, was able to survive for an extended period of time exceeding the control. Variation in faecal streptococci density of Nile water due to addition of atrazine in presented in. Results attained reveal a minor change in density due to the addition of atraizin to Nile water as compared with control.

Biodegradation of Atrazine:

Variation of atrazine concentration, dosed in Nile river water, with time is shown in Tables (3) and (4). Results obntained show that Atrazin maintained its chemical stability in Nile river water for about 15-days, wherease in the presence of sewage microflora, atrazine persistence was reduced to 8 days. There after, the herbicide concentraion gradually decreased. Atrazin biodegradation amounted to 40% within 21 days when doses in Nile river water. In presence of sewage microflora, atrazine disappeared during the same previous period of time. The rate of biodegradation increased during the second and third addition of atrazine even after the increase of the herbicide to (10 mg/L). Such an increase in degradation rate indicates the presence of adapted microflora capable to utilize the herbicide as a food substrate nutrients.

Effect of Monuron:

The response of bacterial population studied to monuron is shown in Tables (5) and (6).

Results of total viable bacterial conunts showed the growth pattern of bacterial population of Nile water., the number of cells increased from 10^4 to 10^7/ 100 ml within 42 days and nearly maintained that value during the stationary phase which extend for 10^o days.

In the presence of monuron, bacterial density increased from 10^4 cells/ 100 to 10^5 cells within 35 days. Later a

gradual decrease in total vable counts was recorded. Nile river water seeded with sewage, showedd a steady increase in bacterial counts which reach the maximal value of $7x10^{11}$ cells/ 100 ml after 38 days. That was followed by a statioonary phase which extended for 10 days, wherby the toal vialble counts decreased to 10 cell/100ml. The toal viable bacterial counts increased in presence of Monuron. Such findings arre in good agreement with that reported by comper and ***Shively (1968).*** They observed an intial decrease in bacterial population of fresh water containing some selected heribcides. ***Comper and Shively (1974)*** attributed the rapid growth of bacterial to a selective enrichment for those organism capable to utilize the hrbicides as a source of carbon. The effect of Monuron on total coliform density, found in Nile river water, is presented in Tables 6 and 7. MPN. index of total coliform in Nile river water smaples that severed as a control, nearly maintained a steady level for 22 days, then declined and finally dissapeared after 38 days. Addition of monouron to the river water lead to an obvious reduction in total coliforms which disappeared withing 29 days. However, in the Nile river water seeded with sewage, coliform density was considerably high and disappeared after 38 days. The effect of monuron on MPN index of faecal streptococci control sample of Nile water, reflected a normal growth pattern of faecal strepto-cocci which persisted for 35 days. In presenc of monuron results showed that faecal streptocci were not affected by herbicides.

Biological Degradation of Monuron:

Monuron was found to maintain its chemical stability in Nile river water for 19 days bnefore biodegradation was observed (Tables 5,7) However monuron degradation proceeded at an accelrated rate especially where sewage microflora were added. Under such condition was affected within. Aniline derivatives were liberated in the solution during the degradation of monuron (Table 6 and 7). The concentration of anilin derivatives increased by time which indicate that their degradation in Nile river water was slow. According to the presented results, it could be observed that biodegradation of Monuron coincide with the increase in total viable bacterial counts. That clearly indicated that Monuron and its metabolite were utilized by bacterial as a food substrate. Meanwhile, coliforms and faecal streptococci were subjected to the of Monuron for two weeks.

DISCUSSION

The present study concentrates on the effect of studied compounds on bacteria indicative of pollution parameters selected were total viable bacterial counts, total faecal coliforms and total faecal streptococci.

Results in this study showed the atrazine and monuron maintained their chemical stability in the aquatic environment for 2 or 3 weeks.

Consequently, bacterial population indicative of pollution were subjected to the direct effect of the studied compouned during the first phase of the investigation. As a genral trend, atazine only show an inhibitory effect on bacterial population leading to a temporary decrease in total viable counts. However of monuron, inhibitory effects was reported with respect to total viable bacterial. Morever, progressive increase in bacterial counts recorded as microflora of river Nile water get adapted for the utilization of studied organics as food substrate. According to the present studdy, atrazine and monuron did not exert direct inhibitory or toxic effects on faecal coliforms and streptococci. Slight reduction in number of coliforms found in Nile water in presence of monuron was obsrved. However in presence of sewage seed, coliforms were not affected. ***Ferebee and Guthrie (1973)*** reported that diuron caused a reduction in a total bacterial counts wherease bacterial number increased in presence of paraquate. ***Guthrei et al., (1974)*** reported that diuron (at a concentration of 14 mg/L) changed that natural bacterial population balance in aquatic ecosystem. ***Sykes (1963)*** reported that multiplication of bacteria in aquatic environment depend on several factors such as nutrients, type of bacterial and temprature. Decease in the concentration of the compound dose in Nile river water was attributed to biodegradation since the pervious studies by ***El-Dib and Aly, 1976*** have indicated the chemical stability of anilide herbicides at pH values normally encountered in natural surface water. Biodegradation of triazines and

phenylurea herbicides was reviewed and the pathways of their degradation was shown ***(Mac. Rae and Alexander, 1965, Keerney and kaufman, 1965, kaufman, et al., 1965 and kaufman, 1967).***

In the present study the release of aniline derivatives during the course of biodegradation of atrazine and monuron was confirmed and quantitatively determined. As the river water and sewage micro- flora get adapted to the degradation of the pesticides, rate of libreation of anilines into the water was considerally accelerated.

The study of aromatic degradation shows that the breakdown of many of these compounds is subject to tightly controlled regulations ***(Dagley, 1971; Ornston, 1971; and Clark and Ornston, 1975)***.This control can take place by means of enzyme induction and enzyme repression. Many enzymes are present in the cell in trace amounts under normal metabolic cnditions, but in the presence of specific enzyme inducers, the amount of enzyme is increased at least, ten fold. The process is known as enzyme induction ***(Lehninger, 1975).*** In the process known as enzyme repression, bacterial normally produce a group of enzymes synthesizing some compounds, However, very little work has been carried out on the control of enzymes responsible for the degradation of specific aromatic cmpounds in the natural environment ***Darby and Ruber (1971).*** There is the possiblity that bacteria will mutate to give cells having capable to attack the herbicides.

In general, aquatic organisms will be exposed to adverse effect of the studiied herbicides during the lag period and the rate of biodegradation will be a limiting factors controlling the time of exposure.

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Table (1)

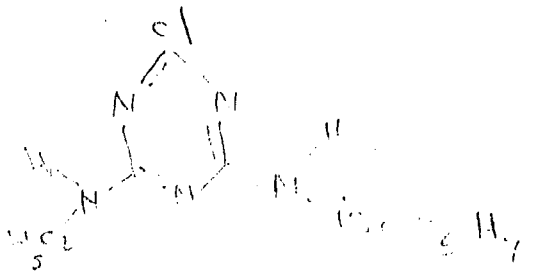
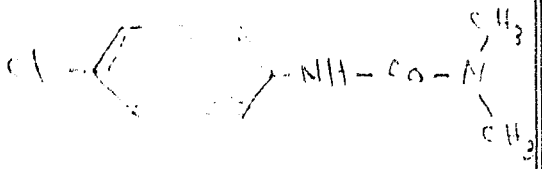
Common name	Chemical name	Structure
atrazine	2-chloro-4 ethylamino-6-isopropylamino 1,3,5 - traiazine.	
Monuron	3-(4-chlorophenyl) -1,1-dimethyl urea.	

Table (2) : Bacterial density of Nile water

Time / days	Total bacterial count at 22ºC/100 ml	MPN - Index of	
		Total coliform	Faecal streptococci
1	9.7×10^5	9.2×10^2	3.9×10^1
8	7.1×10^5	9.1×10^2	2.2×10^1
13	6.5×10^5	1.7×10^2	2.2×10^1
15	5.1×10^5	0.45×10^2	0.75×10^1
20	4.8×10^5	0.23×10^2	0.35×10^1
25	3.9×10^5	0	8
32	3.1×10^5	0	5
40	2.8×10^5	0	0
48	2.3×10^5	0	0
55	1.3×10^5	0	0
60	0.45×10^5	0	0
68	0.42×10^5	0	0

* means control for atrazine.

Table (3) : Bacterial density of Nile water in presence of atrazine

Time Day	Conc. of Atrazine	% of degradation	Total bacterial count of 22oC / ml	MPN Total coliform	MPN Faecal strepto cocci
1st addition (5 mg /L)					
1	5.0	0	1.4×10^5	9.0×10^2	2.2×10^1
10	5.0	0	1.2×10^5	3.6×10^2	2×10^1
15	5.0	0	1.1×10^5	2.5×10^2	1.8×10^1
17	4.5	10	0.9×10^2	1.3×10^2	1.3×10^1
20	4.10	18	0.6×10^5	1.0×10^2	0.95×10^1
21	4.0	20	0.4×10^5	0.72×10^2	0.65×10^1
22	3.0	40	0.3×10^5	0.60×10^2	0.35×10^1
2 nd addition (10 mg / L)					
22	10.0	0.0	5.1×10^5	0.65×10^2	0
24	9.6	4	4.8×10^5		
28	9.10	9	3.1×10^5	0.43×10^2	0
30	8.6	14	3.0×10^5		
36	7.4	26			
40	6.5	35			
45	5.2	48	2.2×10^5	0.11×10^2	0
47	4.8	52	2.0×10^5		
50	4.3	57	1.8×10^5	0.04×10^2	0
3 rd addition (10 mg / L)					
50	10	0			
55	9.2	8			
57	9.0	10			
60	8.6	14	2.5×10^5	4	0
63	7.4	26	1.88×10^5	4	0
65	6.0	40			
68	5.0	50	1.5×10^5	0	0
69	3.0	70	1.0×10^5		
70	1.8	82	0.7×10^5		

Table (4) : Bacterial density of Nile water seeded with sewage in presence of atrazine .

Time(days)	Conc. of Atrazine	% of Degradation	Total bacterial count at 22oC / 100 ml	MPN - index of Total coliform	MPN - index of Faecal streptococci
1st addition (5 mg / L)					
1	5.0	0	4.4×10^8	1.5×10^5	1.7×10^3
5	5.0	0	3×10^8	1.1×10^5	1.5×10^3
10	4.8	2	2.3×10^8	1.0×10^5	1.0×10^3
15	4.0	10	1.7×10^8	0.5×10^5	0.7×10^3
17	3.2	18	1.5×10^8	0.45×10^5	0.5×10^3
20	2.1	58	1.3×10^8	0.4×10^5	0.4×10^3
21	2.0	60	1.1×10^8	0.3×10^5	0.3×10^3
2nd addition (10 mg/L)					
22	10	0	11.0×10^7	3×10^5	3×10^2
24	9.10	9	9.4×10^7	2.6×10^5	2×10^2
28	8.0	20	9.0×10^7	2.4×10^5	1.0×10^2
30	7.2	28	8.4×10^7	2.1×10^5	8
35	6.2	38	8.0×10^7	2×10^5	
40	4.3	57	7.3×10^7	1.8×10^5	8
45	2.5	75	6.5×10^7	1.3×10^5	2
47	1.6	84	5.4×10^7	1.0×10^5	2
50	1.5	85	5×10^7	0.7×10^5	
3rd addition (10 mg/L)					
50	10	0	5×10^7	7×10^5	
55	9.0	10	4.1×10^7	3×10^5	5
57	7.0	30	3.9×10^7	1.2×10^5	0
59	6.0	40	3.3×10^7	4	
60	5.1	49	2.9×10^7	4	0
63	3.7	63	2.1×10^7		0
65	1.4	86	2.0×10^7	0	0
67	0.4	96	1.9×10^7	0	0
69	0.4	96	1.3×10^7	0	0
70	0.4	96	1.0×10^7		

Table (5) : Bacterial density of Raw water* .

Time day	Total bacterial count at 22oC / 100 ml	MPN - index of	
		Total coliform	faecal streptococci
1	6.8×10^5	7.7×10^2	3.8×10^1
3			2.9×10^1
6			2.5×10^1
9	4.0×10^5	5.1×10^2	2.4×10^1
13	3.5×10^5	3.0×10^2	1.9×10^1
15			
22	3.3×10^5	$2.1\ 10^2$	1.8×10^1
25			
27	2.6×10^5	7.0	0
30			
32		0	0
35	2.5×10^5		
45	2.2×10^5	0	0
50	2.1×10^5		
55	2.1×10^5	0	0
60	1.7×10^5	0	0
68	1.3×10^5		
74	1.1×10^5	0	0
78	0.8×10^5	0	0
85	0.6×10^5		
90			
95	0.5×10^5	0	0
105			

* means control for anilide studies

Table (6) : Bacterial density of Nile water in presence of Monuron.

concentration time day	Monourn	Aniline	% of degradation	Total bacterial count at 22oC / 100 ml	MPN - index	
					Total coliform	faceal streptococci
1	5	-	00	6.8×10^5	1.1×10^2	3.8×10^1
4	5	-	00	6.3×10^5		
5	5	-	00	6.1×10^5	1.0×10^2	1.6×10^1
8	5	-	00	5.8×10^5		
10	5	-	00	5.6 c 10^5	0.9×10^2	1.1×10^1
13	5	-	00	5.3×10^5		
15	5	-	00	5.2×10^5	0.8×10^2	1.0×10^1
19	4.6	0.4	00	4.8×10^5		
22	4.2	0.6	8%	4.5×10^5	0.7×10^2	2
26	4.0	0.7	12	4.1×10^5		
29	3.7	0.8	14	3.9×10^5	0	8
33	3.1	1.0	16	3.6×10^5		
38	3.1	1.2	20	3.3×10^5	0	0
42	3.0	1.3	24	3.1×10^5		
48	2.8	1.4	26	2.8×10^5	0	0
62	2.5	1.8	28	2.1×10^5		
68	2.1	1.9	36	1.9×10^5	0	0
72	2	2.0	38	1.8×10^5		
76	1.8	2.1	40	1.7×10^5	0	0
83	1.8	2.2	42	1.5×10^5		
90	1.7	2.2	44	1.4×10^5	0	0
95	1.6	2.3	46%	1.2×10^5		
105	1.4	2.3	46%	0.8×10^5	0	0

Table (7) : Bacterial density of Nile water seeded with sewage in the presence Monuron.

time day	concentration		% of degradation	Total bacterial count at 22oC/ 100 ml	MPN of	
	Monuron	Aniline			Total coliform	Faceal strepto-cocci
1	5	-	0	9.0×10^8	4.6×10^5	2.4×10^3
4	5	-	0	8.9×10^8	4.3×10^5	$2.3 \times$
5	5	-	0	8.7×10^8	3.7×10^5	2.1
8	5	-	0	8.5×10^8	2.6×10^5	1.6
10	5	-	0	8.4×10^8	2.3×10^5	1.5
13	5	-	4	8.3×10^8	8.3×10^5	
15	4.8	0.2	16	8.0×10^8	1.92×10^5	1.1
19	4.2	0.6	20	7.8×10^8	0×10^5	
22	4.0	0.65	24	7.3×10^8		0
26	3.9	0.8	28	7.1×10^8	0	
29	3.8	0.9	34	6.8×10^8		0
33	3.4	1.0	36	6.1×10^8	0	
38	3.2	1.1	40	5.8×10^8		0
42	3.0	1.2	42	5.3×10^8	0	
48	2.9	1.3	46	5.2×10^8		0
50	2.7	1.4	50	4.7×10^8	0	
58	2.5	1.5	56	4.5×10^8		0
62	2.2	1.6	60	4.4×10^8	0	
65	2.0	1.9	64	4.2×10^8		0
68	1.0	2.0	68	4.1×10^8	0	
72	1.0	2.1	80	3.8×10^8		0
76	1.0	2.30	84	3.1×10^8	0	
83	0.8	2.50	90	2.1×10^8		0
90	0.6	2.8			0	

M. M. Hazaa

تأثير بعض المبيدات على دلائل التلوث البكيترى

محمود هزاع

كلية العلوم ببنها – قسم النبات

استهدف البحث لدراسة وتتبع بقاء بعض المبيدات مثل التريازين والفنيبل يوريا وذلك لمعرفة وتقييم الدور الذى تلعبه الكائنات الدقيقة فى تكسير هذه المواد.

وقد أختبر قياس العد الكلى للبكتريا، العد الاحتمالى لبكتيريا القولون الكليه والبرازيه، والعد الاحتمالى للبكتريا السبحيه البرازيه.

وأوضحت النتائج بقاء كل من الأترازين والمونورون ثابته فى البيئة المائية لمدة تراوحت بين ٢-٣ أسابيع. كما أن المادتين لم يكن لهما تأثير مثبط مباشر على بكتريا القولون البرازيه والبكتريا السبحيه البرازية. بالنسبه لبكتريا القولون الكليه فقد انخفضت أعدادها انخفاضا بسيط نسبيا فى مياه نهر النيل إلا أنها لم تتأثر بالمواد المدروسة فى وجودها فى مياه المجارى. كما أظهرت النتائج أن الكائنات الدقيقة الموجودة فى البيئة المائية يحدث لها نوع من الأقلمة حيث تقوم بعملية التكسير الحيوى لمادتى الدراسة يؤكد ذلك وجود مشتقات الانيلين التى أمكن رصدها نتيجة لهذا التكسير الحيوى لكل من الاترازين والمونورون.