Changes in haematological characteristics of a bony fish, *Tilapia guineensis* (Bleeker, 1862), exposed to common pesticides in the Niger Delta Wetland, Nigeria

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ABSTRACT

This study is aimed at examining the effect of pesticide on a predominant fish in the Niger Delta of Nigeria. The effects of cypermethrin and chlorpyrifos on *Tilapia guineensis* juveniles were evaluated using haematological parameters (erythrocyte and leucocyte numbers and haematocrit) of the fish. The fish was exposed to varying sub-lethal levels of the two pesticides (cypermethrin and chlorpyrifos) at different concentrations (0.0006, 0.00125, 0.0025, and 0.005 ppm) for 8 weeks to assess the effects of the pesticides on haematological status of the fish. The erythrocyte, leucocyte, and haematocrit of *T. guineensis* exposed to sub-lethal concentrations of the two toxicants declined with concentration. The variations between treatments observed for erythrocyte, leucocyte, and haematocrit were significant for both pesticides. The variations between cypermethrin treatments were $F = 9.63 > P = 0.45_{0.05}$, $F = 5.08 > P = 0.008_{0.05}$, and $F = 11.03 > P = 0.002_{0.05}$ for leucocyte, erythrocyte and haematocrit, respectively. Chlorpyrifos showed similar trend of $F = 5.89 > P = 0.04_{0.05}$, $F = 9.04 > P = 0.004_{0.05}$, and $F = 8.03 > P = 0.0009_{0.05}$ for the same parameters. In addition, chlorpyrifos depressed concentrations of erythrocyte, leucocyte and haematocrit more than cypermethrin. Histological changes were observed in the erythrocyte of the fish. These conditions were more severe in the higher concentrations of the pesticide.

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RÉSUMÉ

CHINDAH, A. C., SIKOKI, F. D. & VINCENT-AKPU, I.: *Changements de caractéristiques hématologiques d'un poisson plein d'arêtes,* Tilapia guineensis *(Bleeker, 1862), exposé aux pesticides communs dans les marécages deltaïques du Niger, au Nigéria.* Cette étude est visée à étudier l'effet de pesticide sur un poisson prédominant dans le delta du Niger au Nigéria. Les effets de cypermethrin et chlorpyrifos sur les juvéniles de *Tilapia guineensis* étaient évaluès en utilisant les paramètres hématologiques (nombres d'érythrocyte et leucocyte et l'hématocrit) du poisson. Le poisson était exposé aux différents niveaux sub mortels des deux pesticides (cypermethrin et chlorpyrifos) aux différentes concentrations (0.0006, 0.00125, 0.0025 et 0.005 ppm) pour 8 semaines pour estimer les effets de pesticides sur l'état hématologique du poisson. L'érythrocyte, leucocyte et hématocrit de *T. guineensis* exposés aux concentrations sub mortelles des deux toxiquants reduisaient avec concentration. Les variations entre les traitements observées pour érythrocyte, leucocyte et hématocrit étaient considérables pour les deux pesticides. Les variations entre les traitements cypermethrins étaient $F = 9.63 > P = 0.45_{0.05}$, $F=5.08 > P= 0.008_{0.05}$, et $F = 11.03 > P= 0.002_{0.05}$ respectivement pour leucocyte, érythrocyte et hématocrit. Alors que chlorpyrifos montrait la même tendance de $F = 5.89 > P = 0.04_{0.05}$, $F = 9.04 > P = 0.004_{0.05}$ et $F = 8.03 > P = 0.0009_{0.05}$ pour les mêmes paramètres. En plus, chlorpyrifos diminuait les concentrations d'érythrocyte, leucocyte et hématocrit que cypermethrin. Les changements histologiques étaient observés de l'érythrocyte du poisson. Ces conditions étaient plus sévères dans les concentrations plus élevées du pesticide.

Introduction

Adverse effects of pesticides and their residues on non-target organisms had not been seriously considered in Nigeria (Victor & Ogbeibu, 1986). Pesticides are often applied directly on agricultural land, primarily to control pest and improve crop yield to meet the high demand for food needed by Nigeria's fast-growing populations. Two pesticides, cypermethrin and chlorpyrifos, constitute about 75 per cent of the crop pesticides used in most farms in the Niger Delta region (NDES, 2001). These pesticides are either applied in the field or get into the environment by accidental spills of pesticides and careless discarding of pesticide containers; thereby finding their way finally into the aquatic environment either through storm water run off and or as aerosols carried by wind (Victor & Ogbeibu, 1986; Dieter, Duffy & Flake, 1996).

Pesticides have been reported to have negative ecological consequences on biota and the non-living environment at large. On biota, the effect on physiological processes (Yasuno, Hatakayama & Miyashita, 1980; Baticados & Tendencia, 1991; Bookhout, Costlow & Monroe, 1976, 1980; Seikai, 1982) has been reported. Then, reproductive failures attributed to pesticides have also been reported by Scorge (1980), Bosveld *et al.* (1993), and Bosveld *et al.* (1995). De Silva & Ranasinghe (1989) and Dieter *et al.* (1996) further reported the effect of pesticides on fish mortality. Finally, pesticide-related instability in the ecosystem function has been reported by Bostveld *et al.* (1993), Bostveld *et al.* (1995), and Hanazato & Kasai (1995). These pesticides in the aquatic environment could lead to bioaccumulation in fish and biomagnification of the pesticides in man.

In Nigeria, similar studies have centred mainly on crude oil and petroleum product toxicity on aquatic organisms (Sikoki & Enajekpo, 1991; Oladimeji & Onwumere, 1987; Omoregie, Ufodike & Keke, 1990; Dambo, 1993; Omoregie, 1995; Chindah, Braide & Nduaguibe, 2001). Studies on pesticide toxicity are scanty, despite several decades of its introduction and wide use and magnitude (Victor & Ogbeibu, 1986; Chindah, Sikoki & Vincent-Akpu, 2000).

Therefore, this study aimed at determining the effect of major pesticides (cypermethrin and chlorpyrifos) used against soil insects of field and vegetable crops on circulatory fluids of a common Tilapine fish species (*Tilapia guineensis*) prevalent in the Niger Delta waters. Biochemical indicators of environmental contamination have potential use as sensitive and early warning indicators of long-term detrimental effects. Several ecotoxicological studies have implied that haematological changes such as increased levels of plasma enzymes occurred in some vertebrates after exposure to pesticides (Abdo *et al.*, 1983; Abou-Donia, 1990; Lapadula, Johannsen & Abou-Donia, 1990).

Materials and methods

Tilapia guineensis of almost uniform standard length (7.4 $\pm$ 1.3 cm) and mean weight of 9.0 $\pm$ 1.2 g were collected with hand net from their natural habitat at upper reach of the Bonny estuary at Elechi creek and acclimatized in holding tanks for 2 weeks before the experiment. The holding tanks were aerated, cleaned, and water changed daily. The fish were fed with feed pellets at the rate of 10 per cent of their biomass per day given in two rations.

Studies were determined by renewal static bioassay. All experiments were conducted in 10 rectangular glass aquaria (25 cm × 60 cm × 25 cm) containing 20 l of brackish water from upper reach of the Bonny estuary at Elechi creek to which 0.0006, 0.00125, 0.0025, 0.005 and 0.00 ppm (control), concentrations of cypermethrin and chlorpyrifos were separately prepared.

Ten glass aquaria were used with two replicates per treatment. A group of 10 fish were carefully placed into each replicate tank of five different concentrations (0.0006, 0.00125, 0.0025, 0.005 and 0.00 ppm). The experiments were conducted at room temperature (28 °C), tanks aerated, and fish were fed during the experiment.

During the experiment, freshly prepared test solutions were added to maintain the level of the water in the tank, and waste accumulated on the aeration filters were systematically removed and replaced with new ones. The test solution was changed once weekly and the tanks cleaned (Reish & Oshida, 1986). Fish and water quality parameters (temperature, *p*H, salinity, conductivity, alkalinity, and dissolved oxygen) of the test solution were monitored during the experiment, using the methods explained in APHA-AWWA (1980). Every 2 weeks, two fish specimen were killed and blood was collected, using appropriate size needle and syringe that were rinsed with EDTA, from vessels below the anal fin to determine the various haematological parameters (Wedemeyer & Yasutake, 1977). Blood films were made for each fish sample. The cells were observed under binocular microscope and photographed.

The significant differences among means were tested with 2-way analysis of variance (ANOVA).

Results

Fig. 1 to 3 show the changes recorded in leucocyte numbers of *Tilapia guineensis* exposed to different doses (0.0006, 0.00125, 0.0025 and 0.0050 ppm) of cypermethrin and chlorpyrifos. Leucocyte values were observed to decrease with increasing concentrations of the toxicant pesticides, while the values for fish in the control tank maintained relatively uniform leucocyte cell count with a mean of 9998.2 ± 2.0 cell/mm^{3f}. The mean leucocyte values after 8 weeks of exposure to varying concentrations of 0.006, 0.00125, 0.0025 and 0.0050 ppm for cypermethrin were 9964.8 ± 37.7, 9892.2 ± 63.6, 7349.2 ± 1542.1, and 6844.6 ± 1787.1 cell/mm^3; and for chlorpyrifos were 9820.7 ± 94.7, 7681.7 ± 1343.9, 6560.6 ± 1950.5, and 6234.6 ± 2113.5 cell/mm^3, respectively (Fig. 1 and Table 1).

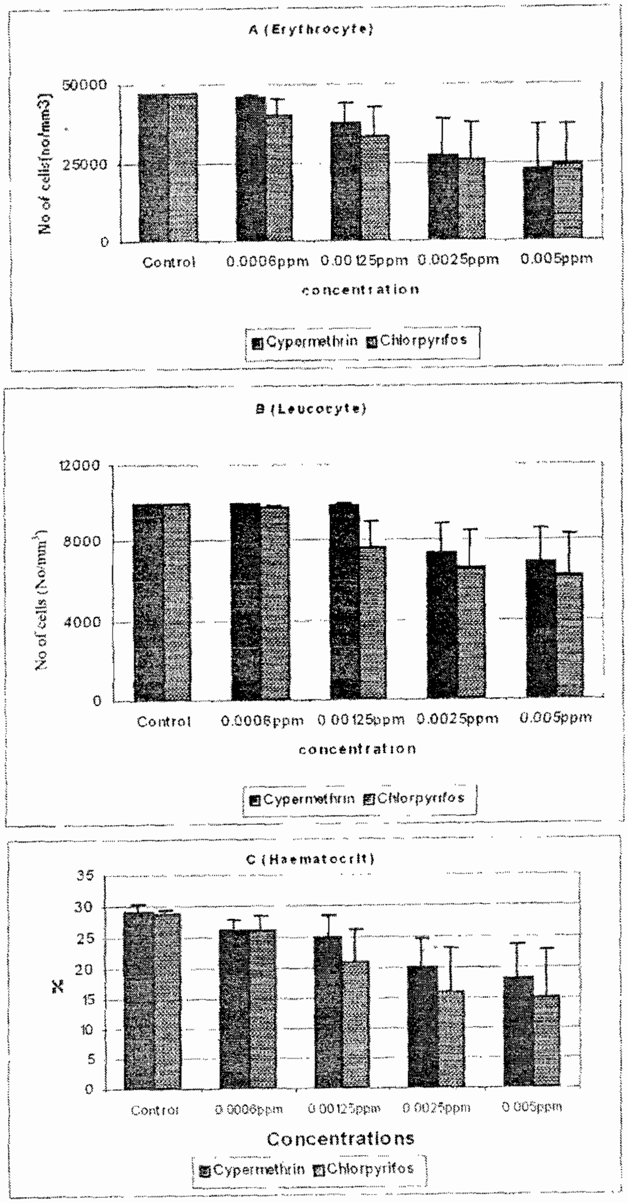

Fig.1-3. Changes in haematological features (erythrocyte, leucocyte, and haematocrit) after 8 weeks exposure to cypermethrin and chlorpyrifos.

The variation between treatments in leucocyte count for cypermethrin ($F = 9.63 > P = 0.45_{0.05}$) and chlorpyrifos ($F = 5.89 > P = 0.004_{0.05}$) were statistically significant.

Similarly, the erythrocyte values in the control tank had narrow variation (47100 - 47150) and treatment tanks showed steady declining values after 8 weeks of exposure (Fig. 2 and Table 2). The mean values after 8 weeks of exposure to cypermethrin at 0.006, 0.00125, 0.0025 and 0.0050 ppm were 45550 ± 897.3, 37950 ± 5827.6, 27730 ± 11243.6, and 23320 ± 13855.5 cell/mm^3; and for chlorpyrifos 40090 ± 5016.6, 33550 ± 8856.8, 26230 ± 11797.4, and 24470 ± 12879.4 cell/mm^3, respectively. These declining variations in erythrocyte cell count were significant for cypermethrin (ANOVA, $F = 5.08 > P = 0.008$) and chlorpyrifos (ANOVA, $F = 9.04 > P = 0.004_{0.05}$).

In addition, for fish exposed to the two toxicants, the erythrocyte (cells) changed in size and shape with increasing concentration (Plates 1 and 2). At 0.0025 ppm of the two toxicants, the cells looked sloppy and defaced (Plates 1 and 2). Plate 2 shows the severity of the effect (0.005 ppm) the cells becoming polymorphic and the nuclei faint.

The haematocrit values (%) showed no marked variation after 8 weeks of fish exposure in the control experiment (Fig. 3 and Table 3). However, fish in treatment tanks at the different concentrations (0.006, 0.00125, 0.0025, and 0.0050 ppm) showed decreasing values of 26.1 ± 1.8, 25.1 ± 3.3, 20.1 ± 4.6 and 18 ± 5.9 per cent for cypermethrin; and 26.1± 2.4, 20.8 ± 5.3, 15.88 ± 7.3 and 14.89 ± 7.8 per cent for chlorpyrifos. The variations between treatments for cypermethrin (ANOVA, $F = 11.03 >$

TABLE 1

Average Leucocytes (no/mm^3) of T. guineensis *After 8 Weeks of Exposure to Various Concentrations of Cypermethrin and Chlorpyrifos*

Concentration (ppm)	*Weeks* 0	2	4	6	8
0.000	10000(9995)	10000(9989)	9995(9998)	9998(9998)	9998(9995)
0.0006	10000(9998)	9981(9789)	9980(9783.5)	9961(9761)	9902(978I)
0.00125	10000(9998)	9891(7534.5)	9870(7241)	9866.5(7091)	9834(6544)
0.0025	9998(9998)	7184(6021)	7004(5992.5)	6459(5588)	6101(5203.5)
0.005	9998(9995)	6522(5667)	6012(5215)	5981.2(5196)	5709.5(5100)

*(Chlorpyrifos in bracket)

TABLE 2

Average Erythrocytes (no/mm^3) of T. guineensis *After 8 Weeks of Exposure to Various Concentrations of Cypermethrin and Chlorpyrifos*

Concentration (ppm)	*Weeks* 0	2	4	6	8
0.000	47100(47150)	47100(47150)	47100(47100)	47150(47150)	47150(47100)
1.0006	47150(47150)	45250(42500)	45050(40100)	45150(35550)	45150(35150)
0.00125	47150(47150)	39500(37100)	36250(31000)	35000(27500)	31850(25000)
0.0025	47100(47150)	27250(22550)	23750(22000)	21550(20950)	19000(18500)
0.005	47100(47100)	22500(21450)	18750(20950)	11700(18500)	16500(15950)

*(Chlorpyrifos in bracket)

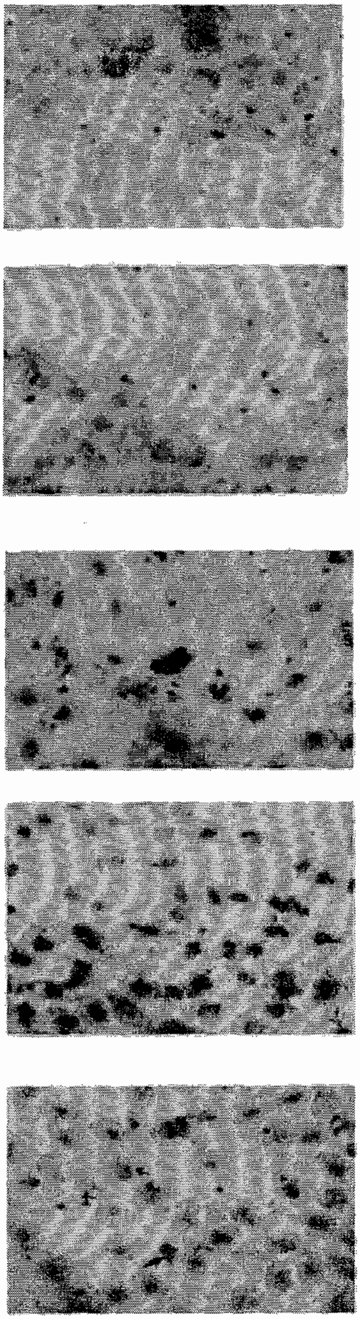

Plate 1. Changes in erythrocyte after 8 weeks exposure to various concentrations of cypermethrin.

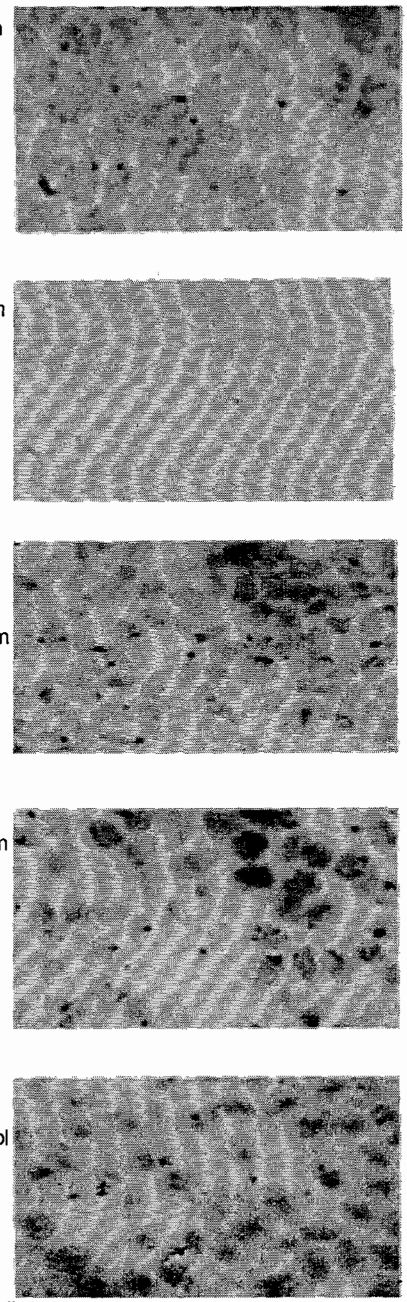

Plate 2. Changes in erythrocyte after 8 weeks exposure to various concentrations of chlorpyrifos.

TABLE 3

The Average Haematocrit (%) of T. guineensis *After 8 Weeks of Exposure to Various Concentrations of Cypermethrin and Chlorpyrifos*

Concentration (ppm)	*Weeks* 0	2	4	6	8
0.000	30(29)	30(29.5)	29.5(28.5)	28(28.5)	27.5(28)
0.0006	28(28.5)	28(27.5)	25.5(27)	25(25)	24(22.5)
0.00125	29.5(28)	27(23.5)	25(20)	23(18.5)	21(14)
0.0025	27.5(28.5)	20.5(15.9)	19.5(13)	17.5(11.5)	15.5(10.5)
0.005	28(28.5)	18(13.5)	16.5(12.5)	15(10)	12.5(9.75)

*(Chlorpyrifos in bracket)

$P = 0.0002_{0.05}$) and chlorpyrifos (ANOVA, $F = 8.03 > P = 0.0009_{0.05}$) were significant.

In all, the concentrations of erythrocytes, leucocytes, and haematocrit were relatively depressed more by chlorpyrifos than cypermethrin.

Discussion

The differences in the fish haematological constituents (erythrocytes, leucocytes, and haematocrit) between fish in the control and those exposed to the different pesticides (cypermethrin and chlorpyrifos) declined with decreasing concentrations of the pesticides. This observation indicated that fish haematological constituent was damaged even at a low concentration of 0.005 ppm, and severe damage was caused with increasing concentration of the pesticides.

Similarly, the effects of the two pesticides showed a consistent decline pattern in the sequence of leucocyte > erythrocyte > haematocrit, indicating the blood component that was most affected. The two pesticides had possibly the same functional and perhaps physiological effect on the fish species. In addition, the results suggest that chlorpyrifos is more potent than cypermethrin.

Reductions in haematological parameters in fish as observed in this study have been reported on fish exposed to refined hydrocarbon products (Omoregie *et al.*, 1990; Sikoki & Enajekpo, 1991; Singh, Srivastava & Srivastava, 1992). The observed significant reduction in the erythrocytes and leucocytes implies that the blood cells are destroyed or blood production is reduced which could result in anaemia and leukopenia (Dixon & Dick, 1985; Wedemeyer & Yasutake, 1977). In addition, these changes in erythrocytes and leucocytes may induce changes in osmoregulation such as decreased plasma osmolarity which is associated with a rise in blood volume and tissue water content, suggesting increased influx of water through the gills from changes in gill permeability to water, a failure of electrolyte regulatory mechanisms (Scheck, 1990; Weber & Spieler, 1994).

The probable explanation for the observed destruction of blood cells may be the effect of the pesticides on some receptor enzymes (Arbelin & Litman, 1981). For instance, β Adenoreceptor (βARs) and muscarinic cholinergic receptor (MChRs) are known to mediate in several functions in brain and peripheral tissues of fish, which include heart rate and contractility (Ask, Sten-Larsen & Helle, 1980), decreased resistance in gill vasculature (Wood, 1974), and increased Na^+/H^+ antiporter activity in red blood cells. Thus, the increase or decrease in these endogenous neurotransmitters can affect up or down regulation in the receptor number and quality of the fish blood cells. These changes may adversely impact and alter the normal blood cell physiology and or homeostasis (Reid, Lebras & Perry, 1993).

This scenario may in part be responsible for the decrease in blood cells in fish, or for changes in gill vasculature through βARs that is responsive to endogenous adrenergic neurotransmitters which cause vasodilatation and thus, an increase in blood flow which facilitates the uptake of hydrophobic chemicals such as PAH (Sijm, Verterne & Part, 1994). The decrease in receptor number after stressor response is caused by exposure to the toxicants. This would be similar, albeit a different mechanism, to the down regulation of brain MChRs that occurs in rainbow trout after carbaryl exposure. An alternative explanation could be that the decreased number of receptors is a consequence of generalized toxicity of the fish.

An analogous reckoning on the severity in the decline of cells with increasing exposure of the pesticides implies the need to ensure greater care in the use and management of pesticides. The implication is that it may be responsible for survival and perhaps declining yield observed in the region (Chindah *et al.,* 2001). Furthermore, the potency of the pesticides on blood cells, which is shown by the depurated cells, also suggests the negative effect of the pesticide on the body fluid (Cheah, Avault & Grave,1980; Singh & Narain, 1982; Stein *et al.*, 1992; Kersting & van Wijngaarden, 1992).

Conclusion

The study indicated that chlorpyrifos and cypermethrin had impacted on the fish haematological parameters measured. Similarly, based on the comparable impact, chlorpyrifos seems to have greater effect on haematological parameters than cypermethrin. Consistently, the severity of impact increases with pollutant concentration and the relative impact seems very consistent in the order of leucocyte > erythrocyte > haematocrit for each treatment.

In the light of the findings, the use of erythrocyte, leucocyte, and haematocrit is adequate in detecting and monitoring possible effects of sublethal doses of pesticides in the environment. It may be adequate as a surrogate for more complex and expensive analyses of pesticide effects in route-monitoring programmes.

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