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Distribution of Propoxur in Water, Sediment and Fish From Warri River Niger Delta, Nigeria

[Nijerya'da Warri Nehri Nijer Deltasındaki Suda, Tortuda ve Balıklarda Propoxur Dağılımı]

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ABSTRACT

Objective: This paper presents the first attempt to quantify the levels and distribution pattern of propoxur in surface water, sediment and fish (Chrysichthys furcatus and Tilapia zilli).

Materials and methods: The samples were collected from three stations (Ovwian, Ekakpamre and Ovu) of Warri River in the Western Niger Delta of Nigeria in 2006 during the dry (January-April) and wet seasons (May–August). A total of 96 samples made up of 24 samples each for water, sediment and fish were analyzed in this study. The pesticide levels were analyzed using high performance liquid chromatography to elucidate its distribution in various environmental compartments.

Results: Residue levels in the matrices ranging from below detection limit-0.97 mg/L in water, between below detection limit-2.01 μg/gdw in sediment, 0.01-6.90 μg/gdw in Chrysichthys furcatus and below detection limit-0.24 μg/gdw in Tilapia zilli. From this result, decreasing order of occurrence of the pesticide is as follow; sediment>fish>water. Spatial variations occurred with downstream stations having statistically higher concentrations in all matrices at $p<0.05$. Seasonal variations occurred with higher concentrations in dry season for water and sediment only, while the fish species had higher concentrations in wet season.

Conclusion: The observed values were above the ecological benchmarks (0.01mg/L) recommended by Nigeria Environmental Protection Agency, an observation that calls for regular monitoring of the Niger Delta water bodies.

Key Words: Bioaccumulation, benchmarks, Chrysichthys furcatus, Niger Delta, propoxur, sediment, surface water, Tilapia zilli, Warri

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ÖZET

Amaç: Bu çalışma propoksurun yüzey suyunda, tortu ve balıklardaki (Chrysichthys furcatus ve Tilapia zilli) dağılımı ve miktarını gösteren ilk araştırmayı ortaya koymaktadır.

Gereç ve Yöntemler: Örnekler Nijerya'nın Batı Nijer deltasında bulunan Warri Nehri'nin üç farklı bölgesinden (Ovwian, Ekakpamre ve Ovu) 2006 yılının kuru (Ocak-Nisan) ve yaş (Mayıs-Ağustos) dönemlerinde elde edilmiştir. Su, tortu ve balıkların herbiri için 24 adet olmak üzere toplam 96 adet örnek toplanmış ve incelenmiştir. Pestisit düzeyleri yüksek performanslı sıvı kromatografisi kullanılarak analiz edilmiştir.

Bulgular: Kalıntı düzeyleri su için tespit sınırlarının altı ile 0.97 mg/L arasında, tortu için tespit sınırlarının altı ile 2.01 μg/gdw arasında, Chrysichthys furcatus için 0.01-6.90 μg/gdw ve Tilapia zilli için ise tespit sınırlarının altı ile 0.24 μg/gdw arasında bulunmuştur. Bu sonuçlardan yola çıkarak pestisit dağılımı tortu>balıklar>su şeklindedir. Akıntı yönündeki bölgelerde pestisit derişimi bütün örneklerde daha yüksek bulunmuştur ($p<0.05$). Mevsimsel değişiklikler kuru geçen mevsimde suda ve tortuda propoksur derişiminin yüksek olmasına; yaş olan mevsimde ise balık türlerinde derişimin artmasına neden olmaktadır.

Sonuç: Çalışmadan elde edilen bulgular Nijerya Çevre Koruma Dairesi tarafından belirlenen ekolojik kriterlerin (0.01mg/L) üzerindedir. Bu nedenle Nijer deltasındaki suyun düzenli olarak takip edilmesi gerekmektedir.

Anahtar Kelimeler: Biyobirikim, kriterler, Chrysichthys furcatus, Nijer Deltası, propoksur, tortu, yüzey suyu, Tilapia zilli, Warri

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Introduction

Pesticides are substances or mixtures of substances intended for preventing, destroying, repelling or mitigating any pests, including not only insects but also other animals, unwanted plants, fungi or microorganisms .Intensive agricultural practices often include the use of these chemicals to enhance crop yields. However the improvement in yield is sometimes concomitant with the occurrence and persistence of pesticide residues in soil and water (1). Pesticides may reach the soil through direct application to the soil surface, incorporation in the top few inches of soil, or during application to crops (2). Pesticides can enter ground water resources and surface run-off during rainfall, thereby contributing to the risk of environmental contamination.

Warri River is a source of potable and recreational water as well as a source of cheap, affordable protein in the form of fish. Pesticide pollution to the lake is therefore likely to pose a danger to both aquatic organisms and humans. Generally it is believed that the contaminants taken in by aquatic organisms are from water, rather than from their food, and may vary with seasonal variation in contaminant availability within the water column (3). Fish act as non-polar media that can absorb hydrophobic organic chemicals within the water column. Since birds and humans consume fish, this makes fish good biomonitors for xenobiotic pollutants. The ingestion of foods contaminated with persistent lipophilic pesticides can result in the accumulation of these pesticides in humans.

Propoxur (a carbamate) is used throughout the world as insecticides, herbicides, nematocides, acaricides, fungicides, rodenticides, avicides, and bird repellants. These compounds are applied in a wide variety of habitats including agricultural lands, forests, rangelands, wetlands, residential areas, and commercial site. It is a non-systemic insecticide that was introduced in 1959 (4).The pesticide is highly toxic to non-target, beneficial species such as bees. In insects, these chemicals block the production and action of cholinesterase, an essential nervous system enzyme. These materials quickly paralyze the nervous systems of insects. It is also highly toxic to crustaceans, aquatic insects and worms (5). It is not used on food crops; it is used against mosquitoes in outdoor areas, for flies in agricultural settings, for fleas and ticks on pets, as an acaricide, on lawns and turf for ants, on flowering plants, and in private dwellings and public buildings. It is also used as a mollusicide, a chemical that kills.

Warri is a major navigable channel of the Niger Delta, southern Nigeria. It takes its origin from around Utagba Uno and flows through zones of freshwater swamps, mangrove swamps, and coastal sand ridges. It is a relatively large water body which stretches within latitudes $5^{0}21'$ - $6^{0}00'$ N and longitude $5^{0}24'$ - $6^{0}21'$E, covering a surface area of about 255 square km with a length of about 150km (6-8). It drains various tributaries and empties into the brackish Forcados that in turn empties into the Atlantic Ocean (Figure 1).

Researchers have detected pesticides residues in Nigerian water. The detected pesticides include diedrin, propoxur, heptachlor, endosulfan, aldrin, DDT and PCB. Many of these pesticides have also been detected in sediments, aquatic plants, and fish (9). Until recently, the adverse effects of pesticides and their residue on non-targeted organisms have not been seriously considered in Nigeria. The use of pesticide is on increase both for promotion of the farmland and for fishing. The present study was initiated to determine the temporal and spatial distribution of propoxur in water discharged from this primarily agricultural watershed located in suburb of Warri. The pesticide is registered for use in the watershed. However, information regarding actual use by individual land owners and managers within the watershed was not available. There are no documented reports on residual levels of pesticides in the Western Niger Del-

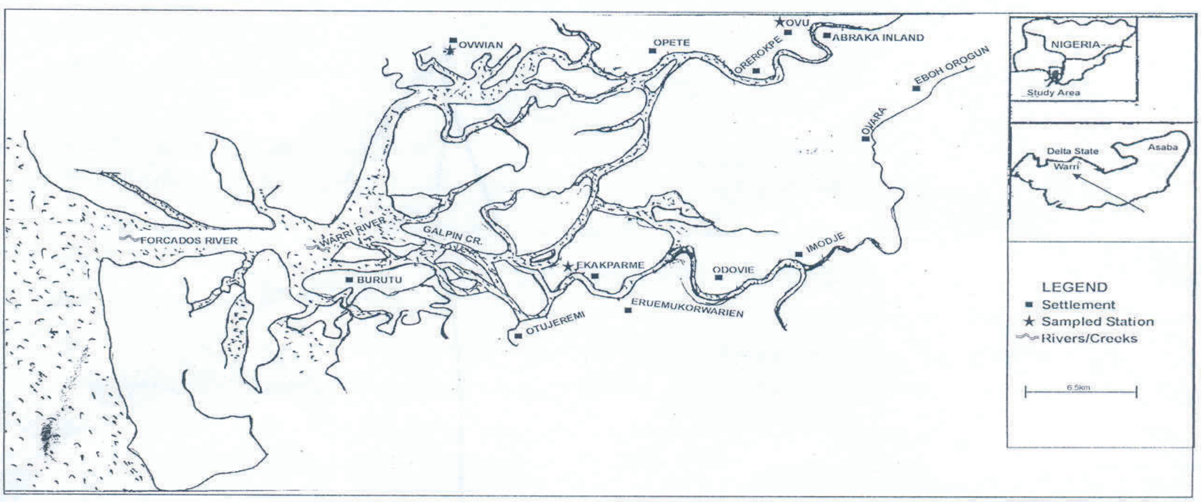

Figure 1. Map of entire Warri River, Source: Ministry of land and survey, Asaba Nigeria

ta region. To the author's knowledge this investigation represents the first evaluation of the pesticide concentrations in Water, sediment and fish samples from the Warri River. Therefore, this study apart from providing baseline information; it will also provide bases for ecological risk assessment of pesticides use in Western Niger Delta and Nigeria in general.

Materials and Methods

Study Area and Sampling Schedule

The study area is the Warri River with stations established at Ekakpamre, Ovwian and Ovu. Ekapamre and Ovwian stations were chosen to reflect possible sources of pesticides contamination while Ovu Station served as control. Two dominant fish species were selected for this study; *C. furcatus* and *T.zilli*. Catchments of intensive agricultural use are drained into the stations without any control. Sampling was conducted during the dry (January-April) and wet seasons (May-August) and a total of 96 samples made up of 24 samples each for water, sediment and fish were analyzed in this study

Collection of Samples

The water was sampled by grab method into 2.5 L amber bottles, which had been pre-washed with distilled water and dried. Each water sample was treated with 1 g mercuric chloride, and mixed for 5 minutes to kill microorganisms that could degrade the pesticides. The sterilized water samples were kept in icebox containing wet ice during the sampling trip and later stored in a refrigerator at 4 ^{0}C after sampling trip prior to extraction.

Sediment samples were taken from the positions where an accumulation of fine–texture substrate took place. The upper 2 cm of bed sediment at each site were collected with a teflon-coated spoon and wrapped in aluminum foil (five samples of each of surface water and sediment from each station were collected for analysis). Samples were immediately stored on ice (<6hrs) after collection and stored at 4 ^{0}C in the laboratory until analysis.

Six samples each of *Chrysichthys furcatus* and *Tilapia zilli*, weighing 2.5 and 1.2 kg respectively from each station were captured, wrapped in aluminum foil, kept at 4 ^{0}C in refrigerator and analyzed within 1 week of sampling.

Chemical Analysis

The extraction of propoxur from water samples was done by Solvent-solvent extraction method. Two litres of water was transferred into a separatory funnel and pH measured. 50 ml of 0.2 M disodium hydrogen phosphate buffer was added to the sample, and pH adjusted to 7 by adding drops of 0.1 M sodium hydroxide and HCl solutions. The neutralised sample was treated with 100 gr sodium chloride to salt out the pesticides from the aqueous phase. 60 ml triple distilled dichloromethane was added and shaken for two minutes while releasing pressure. The sample was allowed to settle for 30 minutes to enhance separation of the phases. The extractions were repeated twice using 60 ml portions of dichloromethane, the extracts combined and cleaned by passing through florisil column. The clean extracts were concentrated on a rotary evaporator to near dryness and reconstituted in HPLC hexane to 5 ml. The final samples were analysed by high performance liquid chromatography

Extraction of propoxur from sediment and fish samples was performed according to the method described by the Steinwandter et al. (10). Sediment samples (20 gr) were extracted three times with 50 ml of 1:1 hexane/acetone using ultrasound, and to remove interferences the pooled extracts were concentrated, washed, and purified by gel permeation chromatography using toluene as eluent (11). The purified extract was analyzed by high performance liquid chromatography.

Fish sample was examined individually and 192 individual analyses were performed. Before the extraction, the bones were removed. A commercial meat chopper was used to macerate the fillet while still partially frozen, as this makes the skin more brittle. Each analysis required about 50 grams of the edible part of the fish, which was then homogenized and macerated in a metallic blender at high speed with three consecutive volumes (150, 100, and 100 ml) of petroleum ether. The extracts from each portion were combined in a 250-ml volumetric flask and made up to volume. A portion (25 ml) of the combined extract was used to determine the extractable fat content, while 200 ml was evaporated to near dryness on a rotary evaporator. The residue in the flask was then transferred with three 1-mL portions of acetonitrile–dichloromethane mixture (25/75 v/v) to a centrifuge tube and frozen for 24 hours. The frozen extract was centrifuged in a Heraeus Biofuge 22R centrifuge (Heraeus Instruments GmbH, Germany), operated at 15 000 rpm and 0 ^{0}C, to precipitate the fat. The supernatant was transferred to a weighed flask. The precipitation step was repeated and the supernatants were combined. The combined extract, equivalent to 20 gr of fish tissue, was loaded onto a column of 20 gr florisil .The florisil was topped with 4 gr of anhydrous Na_2SO_4. The column was eluted with HPLC methanol.

Sample preparation for Analysis

Chemicals/Reagents: Methanol (HPLC analytical grade), Propoxur (98.5 % purity), which were used as internal standard in HPLC analysis were obtained from chemical Service, West Chester, U.K.

Equipment: Cecil high performance Liquid Chromatography (HPLC) system comprised of CE 1200 High performance variable wavelength monitor and CEII00 liquid chromatography pump, UV detector with variable wavelength and stainless steel column (C_{18} Reverse phase) packed with Octasilica vacuum pump and ultrasonic check.

One milligram per gram stock solutions of the internal standard was prepared by measuring 0.1 gr of propoxur standard into 100 ml volumetric flask. A small quantity of methanol was then added to the volumetric flask to dissolve the standard. Distilled water was then used to fill the flask to 100 ml mark. The following concentrations (80, 60, 40,20 and 10) mg/gr were later prepared from the 1 mg/gr stock solution. Then from the 1 mg/gr concentrations, lower concentrations up to 0.01 mg/gr were prepared.

Activation of the HPLC System

The target wavelength for the analyses was determined by UV/visible spectrometer. A small quantity of the stock solution was diluted with methanol and maximum absorption wavelength of 202 nm was determined by scanning. The instrument wavelength was then set at 202 nm, with a sensitivity of 0.05 nm and a flow rate of 1 ml/min. The instrument was purged to remove air and charge the column. Purging was conducted using a washing solution of 30 % methanol and 70 % distilled water.

Helium gas was bubbled into the solution to degassing the mobile phase. The mobile phase was then injected into the instrument and allowed to run through the system for 20 minutes.

Determination of Retention Time for Internal Standard

The internal propoxur standard was injected into the instrument to determine the retention time. A series of concentrations ranging from 0.025 ppm to 100 ppm where then injected. There were no peaks when solvents and blanks were chromatographed, before the samples were analyzed under the same condition. Known standards, of propoxur were also chromatographed, the retention time were used to quantified the concentrations of propoxur present in the sample .Using this approach, the retention time for the propoxur standard was 4.21 minutes.

Pesticide Analysis

Each sample residue was dissolved in 1 ml of methanol. The extracted residue was then loaded and injected into the valve of the chromatograph system. The resulting chromatograph for each sample was printed out. The retention times noted, concentrations determined and recorded.

Data Analysis

The data were summarized separately for each sampled station using Descriptive statistics (means, range, and histogram). The student's t-test and one way analysis of variance (ANOVA) was used to test for the level of significance at 0.05 level of probability for the seasons and the stations respectively.

Results

Results of spatial and seasonal variations in propoxur concentrations in surface water, sediment and fish (C. furcatus and T. zilli) from Warri River are presented in Table 1 with further illustration in Figures 2-5 (gdw = gram dried weight).

Propoxur in Surface Water

The mean propoxur concentrations in the surface water were; 0.44_µg/L (Ovwian), 0.04 µg/L (Ekakpamre). However, the pesticide was not detected in samples

Table 1. Propoxur concentrations during the dry and wet seasons in (a) River water (b) Fine-particle sediments and (c) Fish species in three named sites on the Warri River, Niger Delta, Nigeria sampled monthly from January to August, 2006. Means are based on the monthly observations. ND = Not Detectable.

		Surface water(µg/gdw		Sediment(µg/gdw)		*C. furcatus*(µg/gdw)		*T .zilli*(µg/gdw)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Dry season	Ovwian	0.62±0.65	0.05-1.36	0.93±0.06	0.67-1.28	1.79±0.23	1.07- 2.33	0.04±0.03	0.02-0.08
	Ekakpamree	0.04±0.03	0.01-0.08	0.18±0.09	0.02-0.22	1.22±0.04	1.00-1.45	0.03±0.01	0.01-0.04
	Ovu	ND		ND		ND		ND	
Wet season	Ovwian	0.09±0.08	0.01-0.12	0.42±2.11	0.31-0.79	4.44±0.32	3.12-6.90	0.19±0.29	0.10-0.26
	Ekakpamree	ND		0.11±0.18	0.07-0.41	2.47±0.17	1.98-3.02	0.04±0.06	0.02-0.06
	Ovu	ND		ND		ND		ND	

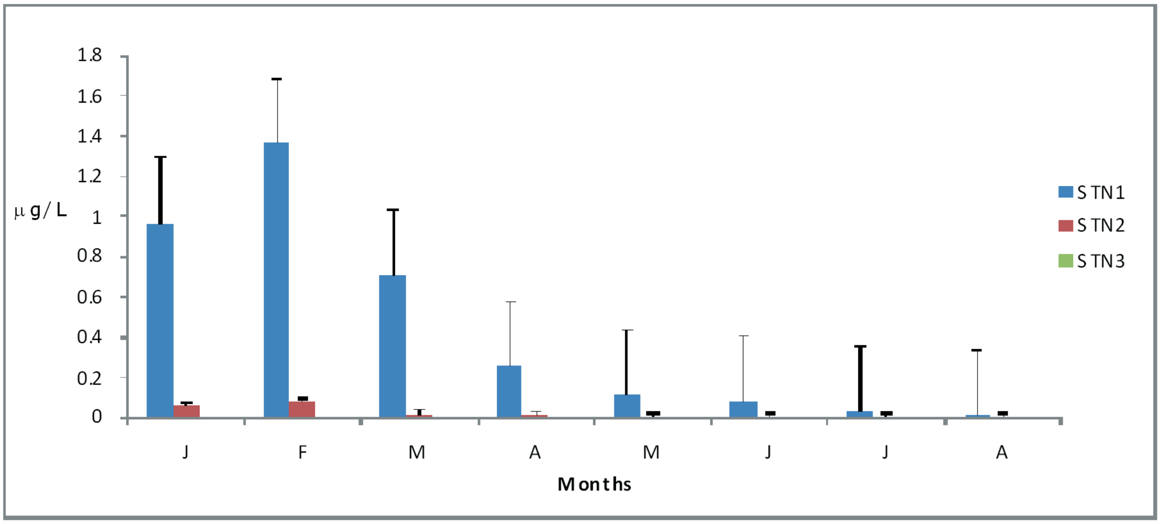

Figure 2. Surface water propoxur concentrations (mg/L) in Warri River, Niger Delta, Nigeria. Monthly concentrations ± standard error .STN = station

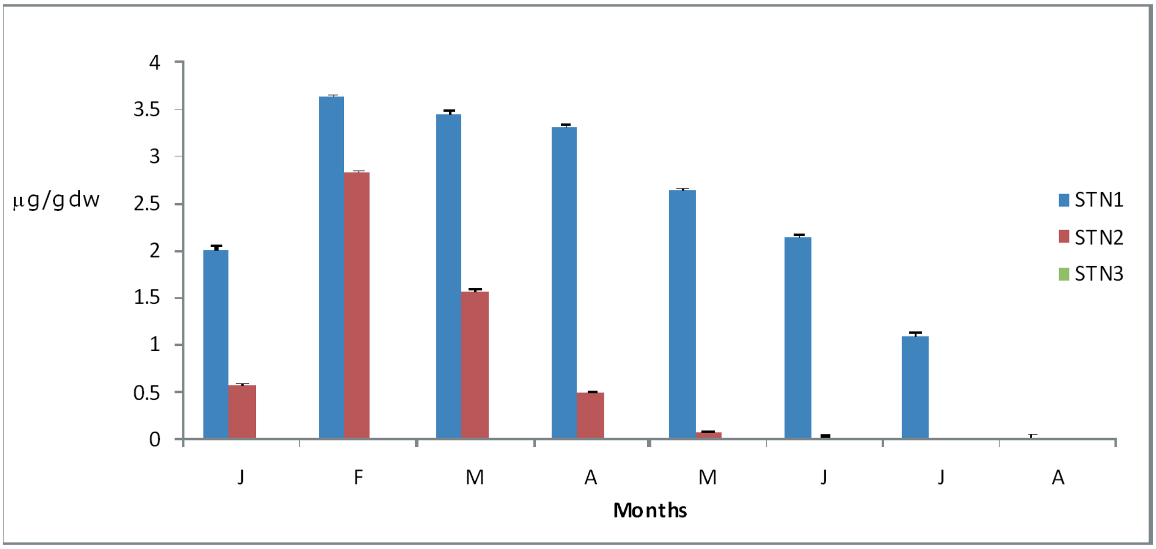

Figure 3. Sediment propoxur concentrations (mg/gdw) in Warri River, Niger Delta, Nigeria. Monthly concentrations + standard error. STN = station

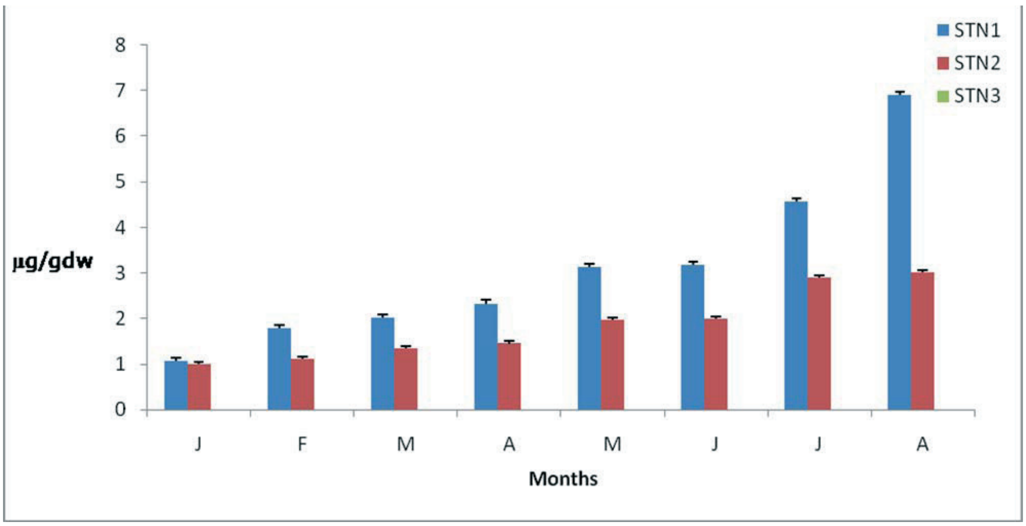

Figure 4. *Chrysichthys furcatus* propoxur concentrations (mg/gdw) in Warri River, Niger Delta, Nigeria. Monthly concentrations ± standard error. STN = station

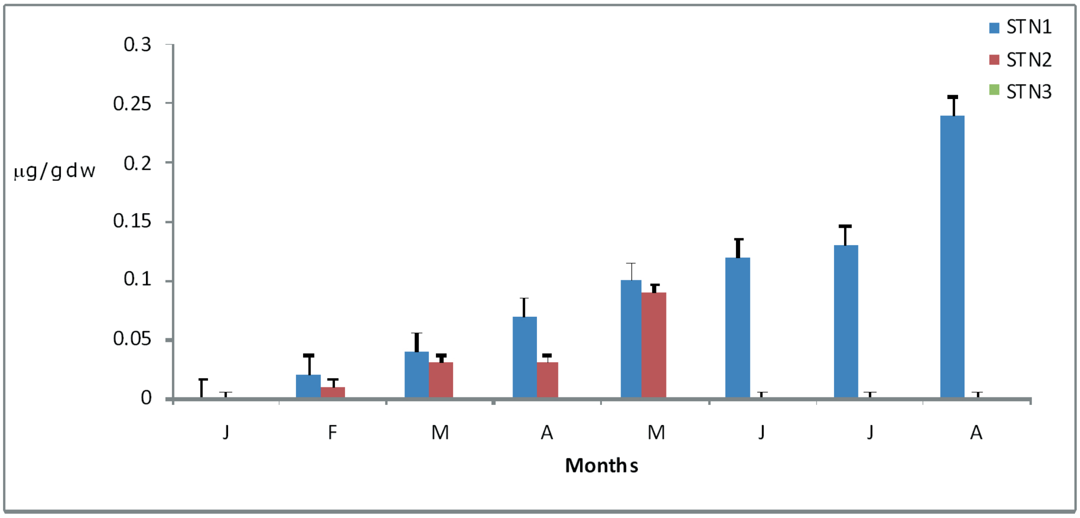

Figure 4. *Tilapia zilli* propoxur concentrations (mg/gdw) in Warri River Niger Delta, Nigeria. Monthly concentrations ± standard error. STN = station

collected at station 3. The mean value for dry and wet seasons were; 0.62 µg/L and 0.09 µ/L (Ovwian), 0.04 µg/L and ND (Ekakpamre). The results show that the pesticide concentrations were significantly different in the seasons at $P < 0.05$ (F=4.31).

Propoxur in the Sediment

The mean propoxur concentrations in the sediment were; 1.33 µg/gdw (Ovwian), 0.15 µg/gdw (Ekakpamre), the pesticide was not detected in samples collected at station 3 (Ovu). The mean seasonal values were 0.93 µg/gdw and 0.42 µg/gdw (Ovwian), 0.42 µg/gdw and 0.18 µg/gdw (Ekakpamre).The concentrations varied significantly in time and space at $p < 0.05$ (F = 5.43).

Propoxur in Chrysichthys furcatus

The mean propoxur concentrations in this fish were; 3.12 µg/gdw (Ovwian), 1.73 µg/gdw (Ekakpamre). The pesticide was not detected in samples collected at station 3 (Ovu). The mean values for dry and wet seasons were 1.07 µg/gdw and 4.25 µg/gdw (Ovwian), 1.03 µg/gdw and 2.11 µg/gdw (Ekakpamre). No significant different in the concentration of the pesticide both in time and space

Propoxur in Tilapia zilli

The mean concentrations of the pesticide were 0.29 µg/gdw (Ovwian), 0.05 µg/gdw (Ekakpamre). However, the pesticide was not detected in samples collected at station 3. The mean values for dry and wet seasons were 0.04 µg/gdw and 0.54 µg/gdw (Ovwian), 0.01 µg/gdw and 0.07 µg/gdw (Ekakpamre). The pesticide concentrations varied significantly both in time and space at $p < 0.05$ (F = 0.17).

Discussion

This study reported the occurrence of varying concentrations of propoxur in surface water, sediment and fish (C. furcatus and T. zilli) samples from designated stations of the Warri River.Propoxur was detected in all the stations and matrices even from the reference station. Concentrations of propoxur in sediment were much higher than concentrations in water. In general, these results support that concept of sediments acting as a sink for pollutants. The observation corroborated the finding of Voss and Embrey where they reported higher concentrations of dichlorvos and malathion in bottom sediment of a small streams toxicity/pesticide study in selected small streams in King and Snohomish Counties, Washington (12).

Higher concentrations of propoxur detected in the fish (C. furcatus) from Ovwian and Ekakpamre Stations indicated the long-term effect of agriculture and other human activities. Schmiit and Linder reported similar result during the biomonitoring program of pesticides residues in United State fresh water fish, sediment and water and observed that the concentrations of pesticides compounds detected in fish tissue from eastern Iowa stream and rivers were as a result of long term effect of previous human activities (13).

Concentrations of propoxur were higher sediment in than C. furcatus indicating possible poor bioaccumulation and easy elimination in the fish (14). Propoxur levels varied significantly between the two fish species (C. furcatus and T. zilli) at $p < 0.05$ (F= 4.65). This may be attributed to lipid content, size and species (15). The bottom detritus and predatory feeder C. furcatus had propoxur residues significantly higher than the herbivorous T. zilli. This variation is probably due to different feeding and living habits. Predatory fishes might bioaccumulate the pesticide by eating other fishes and the constant contact of the fish with the sediment allows their continuous exposure to pesticides. Kent and Johnson found that Utah sucker (Castomus ardens) a bottom feeder, contained the highest level of pesticide compounds in American Fall Reservoir (16).

Higher values of propoxur were recorded in the dry season in surface water and sediment samples which is consistent with the observation of Osibanjo et al. and may be attributed to the planting season (dry season) when farmers treat their farms before planting (8). Most of the crops grown in these areas (Ovwian and Ekakpamre) are seasonal and include leafy vegetable such as Amaranthus species, fluted pumpkin and bitter leaf. Other crops include tomatoes, okra, sweet pepper, garden eggs and tuber crops like cassava. The concentrations of the propoxur observed in the water and sediment during the rainy season could be as a result of the inactivity during the period in the area.

Lower concentrations of propoxur were observed during the dry season. The pesticide concentrations in (C. furcatus) were 53 % higher in the raining season than concentrations in the dry season. This could be attributed to; different living habits of the fishes between the seasons, breaking down of the propoxur in environment and absorption of the pesticide through the skin. According to Leight et al. the accumulation of organic contaminants in the tissue of aquatic organisms is a complex function of the physicochemical properties of the contaminants, its distributions in the aquatic system, the feeding behaviour and metabolism of the aquatic organism (17).

The concentrations of propoxur increased towards the downstream direction with Ovwian station having highest concentrations. The upstream station (Ovu) was comparatively low with the pesticide contamination; this condition may be due to very low to absence of agricultural activities in the area

Conclusion

This study presents the first site-specific data on propoxur concentrations in the Warri River of Niger Delta, Nigeria. It also provides a platform for developing regulatory measures to control contamination of aquatic environments in this region. The obtained results show clear evidence of propoxur pollution in studied River. This is against the background that propoxur levels in Warri river samples from Ovwian, Ekakpamre and Ovu stations exceeded Federal Environmental Protection Agency (FEPA) recommended limit of < 0.01_μg/L

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