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FULL PAPER

Pesticide/Herbicide pollutants in the Kafue river and a preliminary investigation into their biological effect through catalase levels in fish

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Abstract

The study determined the types of pesticide/herbicide pollutants in water, sediment and fish from the Kafue River. A preliminary investigation of the oxidative stress from these pesticides/herbicides was also assessed by measurement of catalase activity. Water, sediment and fish samples were collected upstream, midstream and downstream the Kafue river in Chingola, Kitwe, Kafue National Park and Kafue Town. Water, sediment and fish muscle were sampled and analysed for pesticides using Gas chromatography. For catalase activity fish liver samples only were examined. The pesticides/herbicides detected in all samples collectively included : Heptachlor, pp'-DDE, Cypermethrin, Chlordane, Toxaphene, Terbufos, Kelthane, Endosulfan, Dieldrin, pp'-DDD, pp'-DDT, Atrazine, Disulfoton, d-trans-Allethrin and Endrin. On the other hand, catalase activity was detected in all fish liver samples from all sites. Its levels increased significantly from Chingola upstream to sites downstream with highest being in Kafue town. This study therefore, demonstrates that there is widespread contamination of the Kafue River with pesticides/herbicides. It also demonstrates that organochlorides are found throughout the river especially in fish samples. The spectrum of pesticides/herbicides was much wider in fish probably due to bioaccumulation. It was also observed that fish are subjected to oxidative stress as determined by catalase levels. The stress is more pronounced downstream where the catalase levels were significantly higher than Chingola. The observation that more pesticide varieties are

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also found downstream may suggest a likely causative effect of the pesticides on oxidative stress although this needs further investigation. This study further tentatively highlights the potential dangers of these agro-related substances to dependants of the Kafue River water body and the need to carry out risk assessments and thereafter institute corrective measures to help reduce contamination and adverse effects.

Key Words : Pesticides, Catalase, Kafue River, Zambia

Introduction

As the Kafue River flows through the heart of Zambia, it passes through major mining and agricultural areas. Some studies have been carried out to assess the influence of mining on water quality[9,10]. However, very few such studies have been done to investigate the influence and types of agro-related substances such as pesticides and herbicides on the river and its resources despite the river passing through agricultural areas identified by the Environmental Council of Zambia (ECZ) to use a lot of pesticides/herbicides[2]. This information is long overdue because in the past couple of years, Zambia has seen a 100% increase in the sale and use of pesticides and herbicides[2].

A study[7] around Kitwe and Kafue reported toxic levels of pesticides and heavy metals and the fish near these places were found to have had many pathological abnormalities of the livers and reproductive organs with reduced hatching frequencies. The report attributed the observed abnormalities to the pollutants. Unfortunately, no further studies have been conducted to further ascertain the type of pesticide pollutants in the river and their biological effect.

In this study we determine the types and levels of pesticide pollutants in water, sediment and fish in the river. We further do a preliminary assessment of the biological effect of these pollutants on the water's common inhabitants, fish, through Catalase activity, which is a biomarker of oxidative stress.

Materials and Methods

Sampling sites

In order to cover as much of the length of the river as possible, samples were collected upstream, mid stream and downstream the river. The sites chosen were near those places identified by the ECZ to have used most of the bulk of pesticides and herbicides in the last couple of years[2]. In the upstream area, two towns Chingola and Kitwe ; in midstream, Kafue National Park ; and downstream, Kafue town were selected. These places also support a lot of fishing activities.

Field sampling protocol and samples

Four sampling trips were done in a space of 2 years (March to November of each year) between the years 2003 and 2005. These are the months when fishing is permitted and fishing sites are accessible. Although a variety of fish species were caught at each site we only concentrated on one species found at all the sampling sites and this was *Serranochromis angusticeps*. This fish species is localised mainly in lagoons, slow flowing tributaries and quiet backwaters mostly within or adjacent to aquatic vegetation therefore its unlikely that they can move greater distances at upstream or downstream areas in fast flowing water even though it is possible that they may migrate between the sites in each of the

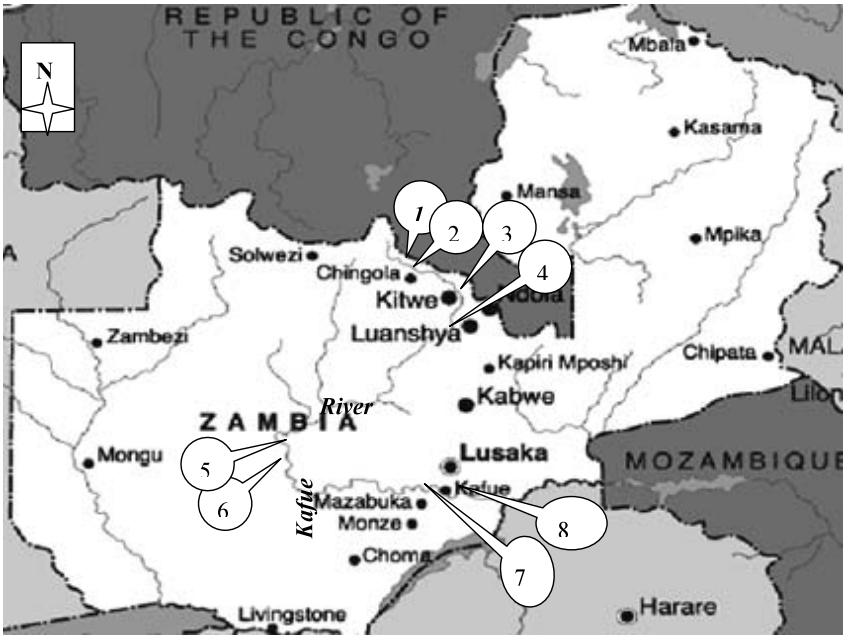

Figure 1. Map of Zambia showing sampling sites along the Kafue river; 1 Chimfunshi, 2 Lukobeko, 3 Chipata, 4 Kumasamba, 5 Hook bridge, 6 Highland, 7 Kasaka, 8 Kafue Bridge

towns sampled. The fish spawns in late December hence our sampling was done outside the spawning period[13]. Ten fish were sampled per sampling site during each trip. Each fish was weighed, opened up and the muscle tissue (fish fillet including the belly flap representing the commonly consumed parts) collected, packaged and preserved. The liver tissues were also collected separately in polythene bags and immediately preserved in liquid nitrogen. Water and sediment were also collected from the same sampling sites about 2 meters from the edge of the river. Free flowing surface water (about a litre) was sampled whereas the sediment (about 1 kg) was collected using an Ekman Grab Sediment Sampler mostly in the mornings and late afternoons during the same month for each trip. The water and sediment samples were kept-cooled at 4 ℃ until testing.

Determination of the Pesticides

Standards (AccuStandards-USA) were lined up for the commonly used pesticides groups (i.e. organochlorides, organophosphates, pyrethroids, and carbamates). For compounds with congeners, the total values are reported except where stated. The pesticides/herbicides were tested by Gas Chromatography (Hitachi 263-50) with a 2m OV17 packed glass column and electron capture detector (ECD-Hitachi) at the School of Veterinary Medicine, University of Zambia. The method used to extract the pesticides was that of Luke M. A. and Doose G. M[6]. To clean up the extracts the method of Luke M. A et. Al[5] was used. For fish only muscle was used for the analysis. This study did not target fat even though this is an important sample in bioaccumulation studies of pesticides. Edible parts of fish in Zambia were targeted as a build up of data for future studies on health implications of consuming fish from the river.

Catalase Analysis

Catalase was measured in liver samples

that had been preserved in liquid nitrogen. Analysis was done at Rand Afrikaans University (RAU) in Johannesburg, South Africa. The activity was measured in duplicate using the method of Cohen et al[1] with some modifications. Exactly 0.5 g of liver sample was homogenised in 1 ml of 0.01 M ice-cold phosphate buffer, pH 7.0. The homogenate was centrifuged at 10000 rpm for 10 minutes at 4 ℃ and supernatant was used in the determination of catalase activity. For the measurement of enzyme-catalysed decomposition of H_2O_2, 10 µl of sample supernatant was added to sample wells, 10 µl distilled water to blank wells and 103 µl of 0.01 M ice cold phosphate buffer pH 7.0 to standard wells of a 96 well microplate. Then 93 µl of ice-cold 6 mM H_2O_2 was added to sample and blank wells only and vortex mixed. The mixture was incubated for 3 minutes at room temperature after which 19 µl of H_2SO_4 were added to all wells and vortex mixed to stop the reaction. Undecomposed H_2O_2 was measured by reacting it with 130 µl of 2 mM $KMnO_4$ that was added to all wells and absorbance was read on a microplate reader at 492 nm within 30-60 seconds from the addition of $KMnO_4$. Under the conditions described, the decomposition of H_2O_2 by catalase follows first-order kinetics as given by the equation : $k=\log(So/S3)x\ 2.3/t$ where, k is the first-order reaction rate constant and is being used as a measure of catalase activity, t is the time interval (3 minutes) over which the reaction was measured, *So* is the substrate concentration at time zero (standard absorbance-blank absorbance) and *S3* is substrate concentration at 3 minutes (standard absorbance-sample absorbance).

Calculations

The calculations of means and standard deviations and comparisons between sites (using ANOVA followed by Fisher's) were performed using statistical StatView 4.5 J (Abacus). The significance of results was ascertained at $P<0.05$.

Results

Pesticides detected in water, sediment and fish

Fish sizes/weight varied greatly at the different sampling sites. The average fish weights were 123.5 ± 50.99 g in Chingola, 46.9 ± 38.35 g in Kitwe, 225 ± 83.24 g in Kafue National Park and 191.3 ± 161.08 g in Kafue town.

The pesticide detection limit was 0.001 ppm and recovery rates were between 75% in fish and 85% in sediment and water spiked at 0.01 ppm. Levels of individual pesticides varied at different sites. It was not appropriate from the water results to pin point one site as having the highest concentration because of the dynamic nature of an aquatic system especially that a number of pesticides were being determined. Heptachlor, pp'-DDE and Cypermethrin were common at all sites. In addition to the above Kitwe had Chlordane and Toxaphene while Kafue National Park had Terbufos. The details of pesticides detected and probable amounts at each sampling site are shown in Table 1.

The variety of pesticides detected in sediment did not differ significantly from those in water samples. As in water, pp'-DDE and Cypermethrin were found at all sites ; Kelthane was found in Chingola, Kitwe and Kafue National Park ; Heptachlor only in Chingola ; Endosulfan in Chingola and Kitwe ; dieldrin only in Chingola ; pp'-DDD at all sites except Kafue Town ; Chlordane in Kafue National Park ; Disulfoton in Kitwe and Kafue town ; and Atrazine was only detected in sediment samples from Chingola. Detailed list of those detected and amounts are shown in Table 2.

A much wider variety of pesticides were detected in fish samples. pp'-DDT, pp'-DDE,

Cypermethrin and Disulfoton were common to fish at all sites. Chlordane was present in Kitwe, Kafue National Park and Kafue Town ; Kelthane in Kitwe and Kafue National Park ; pp'-DDD at all sites except Kafue Town. Furthermore, Kafue Town had additional types of pesticides not found at the upstream sites that included : d-Allethrin, Endrin, Atrazine, Deltamethrin and Endosulfan. Table 3 gives details of the pesticides detected and their amounts at the different sites.

There were also no observed significant differences in the concentrations of particular pesticides at different sites for particular samples. However the spectrum of pesticides detected was wider for fish samples downstream in the Kafue Town sampling area compared to the other sites.

Catalase Activity

The fish in the Chingola area had the lowest catalase activity with an average reaction rate constant of 3.05 ± 2.21. The levels kept rising downstream up to Kafue Town where the rate became 9.69 ± 0.56. Statistically there was a significant difference ($P < 0.05$) between Chingola and the areas downstream namely Kafue National Park and Kafue town. There were no significant differences ($P > 0.05$) between Kitwe and Kafue town, Kafue National Park and Kafue town and between Chingola and Kitwe. Figure 2 below shows the different catalase levels at the difference sampling sites.

Discussion

Pesticides detected

In this study Heptachlor, pp'-DDE, Cypermethrin, Chlordane, Toxaphene and Terbufos were detected in water samples. Although Heptachlor, pp'-DDE and Cypermethrin were found at all sites, total Chlordane and total Toxaphene, both organochlorides, were only detected in Kitwe and Ter-

Table 1. Pesticides detected in water samples at the different sampling areas

Sampling		Concentration (μg/ml)
Area (Sites)	Pesticide Identified	Mean ± SD
Chingola (Chimfunshi, Lukobeko)	Heptachlor	0.156 ± 0.024
	pp'-DDE	0.003 ± 0.001
	Cypermethrin	0.010 ± 0.001
Kitwe (Chipata, Kumasamba)	Heptachlor	0.094 ± 0.006
	Chlordane	0.058 ± 0.010
	Toxaphene	0.050 ± 0.017
	pp'-DDE	0.003 ± 0.006
	Cypermethrin	0.050 ± 0.001
Kafue National Park	Heptachlor	0.056 ± 0.027
(Hook Bridge, Highland Camp)	Terbufos	0.003 ± 0.001
	pp'-DDE	0.003 ± 0.001
	Cypermethrin	0.059 ± 0.011
Kafue Town	Heptachlor	0.054 ± 0.004
(Kasaka, Kafue Bridge)	pp'-DDE	0.004 ± 0.002
	Cypermethrin	0.018 ± 0.001

Values are expressed as Mean ± SD (n=10)

Table 2. Pesticides detected in sediment samples at the different sampling sites

Sampling		Concentration (μg/g)
Area (Sites)	Pesticide Identified	Mean ± SD
Chingola (Chimfunshi, Lukobeko)	Heptachlor	0.036 ± 0.008
	Kelthane	−[a]
	Endosulfan	−[a]
	Dieldrin	−[a]
	pp'-DDD	−[a]
	pp'-DDE	0.006 ± 0.001
	Cypermethrin	0.107 ± 0.002
	Atrazine	0.019 ± 0.001
Kitwe (Chipata, Kumasamba)	Kelthane	0.005 ± 0.002
	pp'-DDE	0.004 ± 0.001
	pp'-DDT	0.005 ± 0.001
	Endosulfan	0.003 ± 0.001
	Disulfoton	0.041 ± 0.001
	Cypermethrin	
Kafue National Park	Kelthane	0.042 ± 0.001
(Hook Bridge, Highland Camp)	Chlordane	−[a]
	pp'-DDE	−[a]
	pp'-DDD	−[a]
	pp'-DDT	−[a]
	Cypermethrin	−[a]
Kafue Town	Disulfoton	0.067 ± 0.002
(Kasaka, Kafue Bridge)	pp'-DDT	0.001 ± 0.001
	pp'-DDE	−[a]
	Cypermethrin	0.086 ± 0.003

−[a] represents unavailable data. Values are expressed as Mean ± SD (n=10)

bufos, an organophosphate, only at Kafue National Park. In the sediment, Kelthane, Endosulfan, Dieldrin, pp'-DDD, pp'-DDT that are organochlorides ; Atrazine a triazine ; and Disulfoton an organophosphate were detected in addition to those reported in water. What was striking was the common presence of DDT or its derivatives at all the sampling sites and the widespread presence of organochlorides in general. Furthermore, fish samples, had more pesticide types detected over and above those reported in water and sediment. These included d-trans-Allethrin, a pyrethroid and Endrin, an organochloride both within the Kafue area. This may be explained by the fact that the Kafue area has a wider agricultural and industrial base and thus uses more varieties of pesticides compared to the other town. The town is immediately downstream to the largest sugar plantation in the country, which obviously uses a lot of chemicals in its production process. The fact that these pesticides were more evident in fish than water may be due to two factors : Firstly that the dilution effect may have reduced the pesticides to levels below the detection limit in water. Sec-

Table 3. Pesticides detected in fish muscle at the different sampling sites

Sampling		Concentration (μg/g)
Area (Sites)	Pesticide Identified	Mean ± SD
Chingola (Chimfunshi, Lukobeko)	pp'-DDT	0.040 ± 0.002
	Cypermethrin	0.068 ± 0.011
	Disulfoton	0.020 ± 0.001
	pp'-DDE	0.017 ± 0.010
	pp'-DDD	0.014 ± 0.001
Kitwe (Chipata, Kumasamba)	Kelthane, Chlordane	−[a]
	pp'-DDE	0.01 ± 0.001
	pp'-DDT	0.018 ± 0.002
	pp'-DDD	0.015 ± 0.002
	Cypermethrin	0.018 ± 0.002
	Disulfoton	0.046 ± 0.003
Kafue National Park	Kelthane, Chlordane	−[a]
(Hook Bridge, Highland Camp)	pp'-DDE	0.024 ± 0.012
	pp'-DDD	−[a]
	pp'-DDT	0.017 ± 0.010
	Cypermethrin	0.06 ± 0.002
	Disulfoton	0.034 ± 0.013
Kafue Town	d-Allethrin, Chlordane	−[a]
(Kasaka, Kafue Bridge)	pp'-DDE	0.021 ± 0.010
	pp'-DDT	0.019 ± 0.001
	Endrin,Heptachlor	−[a]
	Atrazine, Disulfoton	−[a]
	Deltamethrin	0.033 ± 0.013
	Endosulfan	0.087 ± 0.012
	Cypermethrin	0.012 ± 0.001

−[a] represents unavailable data. Values are expressed as Mean ± SD (n=10)

ondly, fish have a tendency to bioacummulate compounds such as pesticides and thus it becomes easier to detect such compounds as concentrations become higher.

A number of critical observations are highlighted by this study : Firstly that the Kafue river is constantly contaminated by a number of pesticides throughout its flow in Zambia. Secondly that the range of pesticides that are polluting the environment are better assessed using fish in which they bioaccummulate and are easily detectable than in water and sediment. Lastly, because fish are part of the common food chain to humans in Zambia, food safety implications need to be investigated.

Furthermore, the presence of DDT or its derivatives and the great variety of organochlorides in water and fish at all sampling sites is a worrying observation. DDT use is generally prohibited in Zambia but its use was widespread in the 1970s and early 1980s during tsetse fly control campaigns thus its possible that it may have remained in the

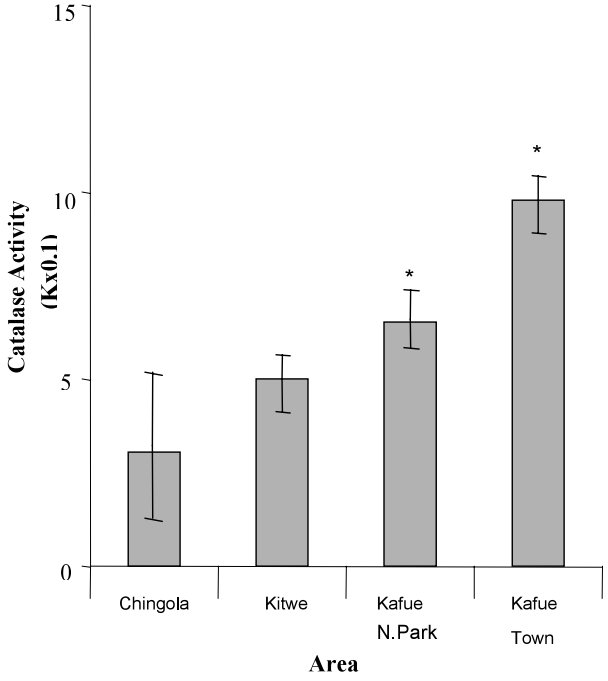

Figure 2. The mean catalase concentration in liver samples at the different sampling sites collected from 10 fish per site. Values are expressed as Mean ± SD (n=10).

*represents a significant difference ($P<0.05$) compared to the Chingola area.

environment long after that. We however suspect that the levels observed are a result of current usage of the pesticide as it finds its way into the agricultural industry illegally due to the country's poor pesticide/herbicide regulatory mechanisms coupled with the recent increase in mosquito spraying that is suspected to use DDT and other organochloride compounds. The dangers that these pesticides pose to both aquatic inhabitants and dependents of the Kafue River including humans are enormous so detailed studies to assess risk and then institute corrective measures should be expedited.

Catalase

Catalase is mainly a peroxisomal enzyme, and it is possible that an elevation of catalase activity reflects peroxisomal proliferation rather than antioxidant defence partly explaining why catalase was detectable in fish at all sampling sites. However, catalase activity is frequently used as a biomarker to assess oxidative stress in biomonitoring programs in the aquatic environment[4, 11, 12]. The preliminary results of this study seem to indicate that it may also be used in the Kafue River for biomonitoring purposes. This is especially so because the catalase levels were observed to progressively increase significantly ($P<0.05$) downstream where the pesticide/herbicide variety became broader as the river passed through the agricultural areas in the heart of Zambia. Although this study does not adequately test the association of this increase to any individual pesticide or the combined effect of pesticides in general the tendency to increase as observed downstream where the variety of pesticides increased suggests a possible relationship. This is in agreement with previous studies that have associated pesticide contamination with oxidative stress[4, 11, 12]. It should also be noted that since the concentrations of individual pesticides did not increase significantly downstream it is speculated that the oxidative stress is more a result of the combined effect of the different pesticides as is observed when the spectrum of pesticides increased. It is still however possible that the pesticides that were observed downstream but were absent upstream may be the ones that are influencing the most stress. Further investigations are therefore required to clarify these observations.

It should also be noted that a number of other physiological conditions can influence catalase levels in a similar manner as pesticides do in fish. As such a number of other oxidative stress biomarkers such as superoxide dismutase (SOD), xanthine oxidase (XOD) and glutathione redox cycle enzymes etc are available and would need to be determined if

the role played by pesticides in this particular case is to be clarified. A previous study[8] has shown that a combination of the above enzymes provides a more rational use of oxidative stress biomarkers in aquatic ecosystem pollution biomonitoring. It is therefore hoped further studies will explore this aspect by testing a suite of enzymes that measure oxidative stress.

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