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FISH KILL IN THE APIES RIVER: 6th OCTOBER 2000

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1. Introduction and Background

This report relates to the request by Mr L. van Niekerk, a farmer on the banks of the Apies River, to Mr B. Hohls of the Institute for Water Quality Studies. He requested that the IWQS provide assistance in determining the possible cause, or contributing factors, of a fish kill in the Apies River reported on 6th October 2000. Mr J. Daffue of the Gauteng Regional Office of DWAF was informed of the fish kill and notified of the IWQS's intention to assist with the water quality sampling. Mr B. Hohls and Mr H. van Niekerk of the IWQS conducted the investigation on 6th October 2000.

It was reported that fish, including carp and "onderbek" (possibly Silver Labeo) and other fish of varying sizes, had been dying for a number of days prior to the fish kill being reported to the IWQS. It was reported that many dead fish had already been removed from the Apies River prior to the investigation by the IWQS for aesthetic reasons. It was reported that there had not been a fish kill in the river during the preceding number of years.

Water quality samples and physical measurements described in the following sections of the report were taken at the locations indicated in Figure 1. Site 1 corresponds to Mr Van Niekerk's farm where the fish kill was initially investigated, and where the dead fish were evident on 6th October 2000. Site 2 is approximately 1 km downstream of the Rooiwal Water Care Works (WCW) and the Rooiwal power station, and 10 km upstream of Site 1. Site 3 is situated immediately downstream of the Bon Accord Dam. In between Site 2 and Site 3 there are a number of industries, including an abattoir and a plant nursery. Dead fish were only in evidence in the vicinity of Site 1 during the investigation on the 6th October 2000.

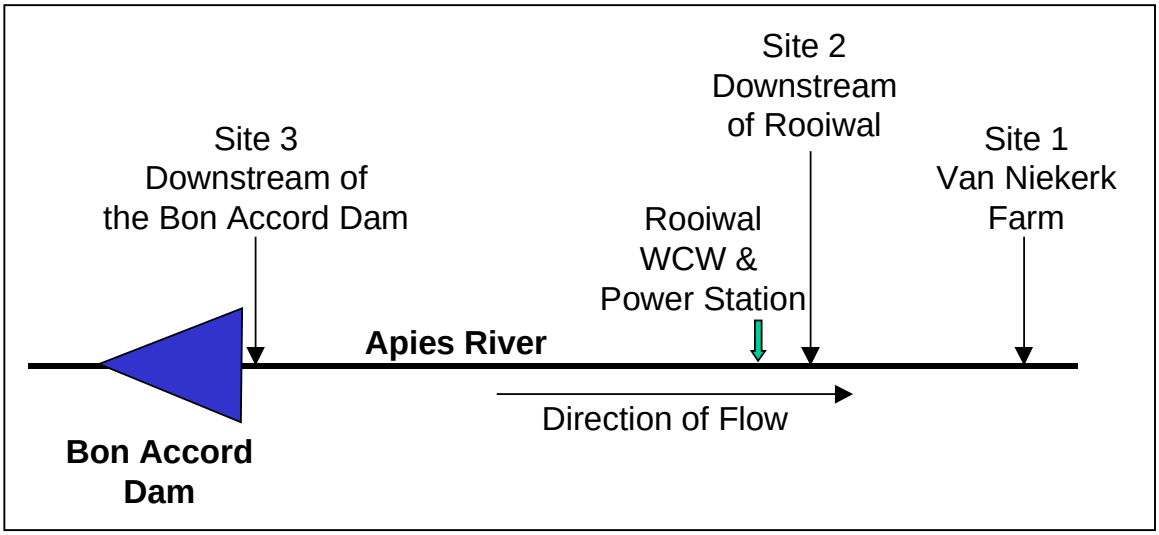

Figure 1. Diagrammatical representation of the sampling sites

Various verbal reports indicated the possibility of the spraying or discharge of a herbicide or pesticide upstream of the fish kill site (unconfirmed) and also of maintenance conducted on the cooling towers at the Rooiwal power station at the time of the fish kill (unconfirmed). It is possible that the herbicide or pesticide containers may have been rinsed in the Apies River.

The following sections outline the sampling and various analyses that were conducted at the three sites. This is followed by conclusions and recommendations made by the authors.

2. Sampling and Analysis Conducted

Various water quality samples were taken at the three sites indicated in Figure 1. These samples were used to determine the concentration of major inorganic constituents, micro inorganic constituents including trace metals, chemical oxygen demand (COD), bacteriology, the presence or absence of algal toxins, a general scan of the organic constituents, and the toxicity of water samples. Temperature, pH and dissolved oxygen readings were taken on site. A large carp that died while the investigation at the Van Niekerk Farm (Site 1) was underway was taken to the pathology laboratory at Onderstepoort for histopathological examination. A sample for organic constituent analysis was only taken at Site 1, not at Sites 2 and 3.

3. Analytical Results and Discussion

Detailed results of the various water quality samples and measurements taken at the three sites are listed in the following sections.

3.1 Physical Measurements

Physical measurements were taken of as many variables as possible with the available field instrumentation, especially of those variables such as temperature, dissolved oxygen, and pH that are prone to change prior to analysis in the laboratory. The values for these variables are listed in Table 1.

Table 1. Measurements taken at the three sites on 6th October 2000

Variable	Site 1. Van Niekerk Farm	Site 2. Downstream of Rooiwal	Site 3. Downstream of the Bon Accord Dam
Temperature (°C)	22.5	22.4	21.5
Dissolved Oxygen (%)	52.2	51.0	51.7
Dissolved Oxygen (mg. $^{-1}$)	4.48	4.44	4.57
pH	8.7	8.3	8.6
Conductivity (mS.m^{-1})	68.0	67.2	51.0
Latitude of sampling site	25° 28’ 47.3” S	25° 32’ 14.6” S	25° 37’ 07.5” S
Longitude of sampling site	28° 15’ 29.9” E	28° 14’ 03.9” E	28° 11’ 44.1” E

Dissolved oxygen concentrations of 80 – 120 % of saturation are considered to constitute the Target Water Quality Range for aquatic ecosystems (DWAF, 1996a). The minimal allowable dissolved oxygen values according to DWAF (1996a) are not less than 60 % for sub-lethal effects and not less than 40 % for lethal effects, respectively. The sub-lethal value relates to the 7-day mean minimum, and the lethal value relates to the 1-day minimum. According to DWAF (1996a), both the 7-day

minimum and the 1-day minimum should be used together. It is stated that the violation of these minimum values is likely to cause acute toxic effects on aquatic biota.

The low dissolved oxygen levels that were recorded at all three sites in the Apies River are indicative of water quality problems and the oxygen levels are low enough to be problematical to the survival of fish. It was unexpected to find such low dissolved oxygen levels, especially when taking into account the turbulent nature of the flow evident in the Apies River in the vicinity of Site 1. Site 1 was characterised by a number of areas containing riffles. Some chemical or biological reaction must have resulted in the dissolved oxygen levels being depleted.

Assuming that the temperature and dissolved oxygen readings taken during the investigation are representative of those during the preceding days and weeks, it is expected that the fish would have been under stress and that would have made them more susceptible to additional stressors.

3.2 Major inorganic constituents

The major inorganic constituents analysed by the IWQS are listed in Table 2, together with aquatic ecosystem guidelines (DWAF, 1996a) where they are available.

Table 2. Major inorganic constituent results (6th October 2000)

Constituent	Site 1. Van Niekerk Farm	Site 2. D/s of Rooiwal	Site 3. D/s of Bon Accord Dam	Aquatic Ecosystem Guideline/ Category
pH	8.4	8.1	8.2	pH change should not be > 5 %
Kjeldahl nitrogen as N	1.47	1.57	1.44	Mesotrophic
Ammonium (NH_4^+) as N	0.17	0.39	0.40	
Nitrate + nitrite as N	4.03	4.05	0.72	Eutrophic at sites 1 & 2, Mesotrophic at 3
Fluoride as F	0.3	0.3	0.2	TWQR ≤ 0.75
Alkalinity as $CaCO_3$	203	199	170	
Sodium as Na	70	67	33	
Magnesium as Mg	17	17	23	
Silicon as Si	9.0	9.1	6.2	
Total phosphate as P	1.107	0.864	0.044	Hypertrophic at sites 1 & 2, Eutrophic at 3
Orthophosphate as P	1.000	0.500	0.043	Hypertrophic at sites 1 & 2, Eutrophic at 3
Sulphate as SO_4	62	60	54	
Chloride as Cl	60	64	47	
Potassium as K	11.4	11.2	6.1	
Calcium as Ca	50	49	42	
EC ($mS.m^{-1}$ at 25 °C)	75.7	74.7	58.6	
Total Dissolved Salts (TDS)	540	531	416	TDS should not change by > 15%

- Concentrations are in $mg.^{-1}$ except for pH and EC

The un-ionised ammonia (UIA or NH_3) concentration was calculated according to the method of Wade (1999) using the NH_4^+ concentration, pH, temperature, and electrical conductivity (EC). The UIA (NH_3) concentration at the Van Niekerk Farm Site (Site 1), the site downstream of Rooiwal (Site 2), and at the site downstream of the Bon Accord Dam (Site 3) were 0.02006 mg. $^{-1}$ NH_3, 0.024351 mg. $^{-1}$ NH_3, and 0.029183 mg. $^{-1}$ NH_3, respectively. The Target Water Quality Range for UIA is less than 0.007 mg. $^{-1}$ NH_3, with the chronic effect value being 0.015 mg. $^{-1}$, and the acute effect value being 0.100 mg. $^{-1}$ NH_3. The concentrations at all three of these sites are, therefore, above the chronic effect value for an aquatic ecosystem (DWAF, 1996a). The UIA concentration was highest at the site downstream of the Bon Accord Dam (Site 3). It was, therefore, possible that the conditions within the impoundment itself, or those upstream of the impoundment, favoured the conversion of ammonium ions to the more toxic un-ionised ammonia. The conversion process is favoured under conditions of higher water temperature and elevated pH.

If the UIA concentrations recorded on the 06th October 2000 are indicative of the situation over the long term, then un-ionised ammonia could have contributed to the fish mortalities. This could be thought of as being sufficient reason for fish dying over a protracted period of time even in the absence of any other factors (such as the low dissolved oxygen levels mentioned previously).

From Table 2 it would appear that there is a point source of nitrogen between Site 3 and Site 2. There was a dramatic increase in the nitrate-nitrite concentration on going from Site 3 to Site 2. The high concentration was also found at Site 1, albeit at a fractionally lower concentration. It is likely that the Rooiwal WCW was a large contributor to the high nitrogen levels evident at Sites 2 and 3.

A progressive increase in total phosphorus and orthophosphate was also evident from Site 3 to Site 1 (Table 2). In contrast to the situation evident with the nitrogen concentrations mentioned above, it is likely that the source of the phosphorus was diffuse.

The Total Dissolved Salt (TDS) concentrations showed a reversal of the trend evident for UIA concentrations. The lowest TDS concentration was recorded downstream of the Bon Accord Dam (Site 3), with a higher TDS at the site downstream of the Rooiwal WCW and the power station (Site 2). The highest TDS concentration was recorded at the Van Niekerk Farm site (Site 1), however, the TDS concentration was only slightly higher than that recorded at Site 2.

3.3 Acute Toxicity Tests

Samples for toxicity analysis were taken at the three sites on the Apies River. The samples taken at all of the sites resulted in 0 % mortality of 17 to 18 day-old *Oreochromis mossambicus* when exposed to 100 % sample concentration for 96 hours. The water samples at these locations could not, therefore, still be considered to be toxic at the time of sampling on 06th October 2000. The fish deaths were, therefore, most likely a result of some contaminant or event that had passed through the system by the time that the sampling was conducted.

3.4 Bacteriological Determinands

Water samples for the determination of faecal contamination were taken at all three of the sites sampled on the Apies River. The samples were analysed for faecal coliform counts, faecal streptococci, and *Escherichia coli* (*E. coli)* (reported as counts per 100 m sample). The faecal coliform to faecal streptococci ratio provides an indication of whether the faecal contamination is of human or animal origin – values greater than 4 indicate contamination of human origin, while values less than 0.7 indicate contamination of animal origin. The results of the bacteriological analyses appear in Table 3.

Table 3. Results of the bacteriological analyses on the samples taken at the three sites

Microbiological Indicator (counts per 100 m)	Site 1. Van Niekerk Farm	Site 2. D/s of Rowel	Site 3. D/s of the Bon Accord Dam
Faecal coliform (FC)	315	1620	20
Faecal streptococci (FS)	555	780	50
Escherichia coli	299	1458	20
FC:FS Ratio	0.568	2.077	0.400

Escherichia coli is not pathogenic to fish (DWAF, 1996b). There are no guidelines for bacteria in aquatic ecosystems. The microbiological levels do, however, provide an indication that human and animal waste is present in the Apies River. This is not unexpected due to the Rooiwal WCW located upstream of Site 2 and the numerous farms adjacent to the Apies River in this area.

There are tentative guidelines for bacteria in the aquaculture guidelines (DWAF, 1996b) that state that aquaculture in domestic waste water has been a practice for a long time, and that in India, for example, fish are cultured in sewage ponds without apparent detrimental effects to the fish. Therefore, it is highly unlikely that bacteria from human origin could have been responsible for the fish kill.

The full-contact recreational guidelines for bacteria (DWAF, 1996c) place the 315 faecal coliform counts per 100 m water sample at the Van Niekerk Farm (Site 1) in the "risk of gastrointestinal illness" category, showing unsuitability of the site for swimming or other full contact recreation. For intermediate contact recreation, this value is within the Target Water Quality Range.

The ratios of faecal coliforms to faecal streptococci were: 0.568 at Site 1; 2.077 at Site 2; and 0.400 at Site 3. This indicates that the microbiological contamination is more likely to be of animal origin at Sites 1 and 3, but more likely of human origin immediately downstream of the Rooiwal WCW and Power Station at Site 2. This confirms what would be expected taking the landuse into account.

3.5 Trace Metal Analyses

Samples for trace metal analyses were taken at each of the three sites in order to determine whether trace metal concentrations could have been at levels that would have resulted in, or contributed to, the fish kill. The results of the analyses appear in Table 4.

Table 4. Trace metal concentrations recorded at the three sites

Constituent (mg. $^{-1}$)	Detection limit	Site 1. Van Niekerk Farm	Site 2. D/s of Rooiwal	Site 3. D/s of the Bon Accord Dam
B – dissolved	0.014	0.081	< 0.014	0.027
Al – dissolved	0.059	< 0.059	< 0.059	< 0.059
V – dissolved	0.005	0.010	< 0.005	< 0.005
Cr – dissolved	0.006	< 0.006	< 0.006	< 0.006
Mn – dissolved	0.003	< 0.003	< 0.003	< 0.003
Fe – dissolved	0.006	< 0.006	0.050	< 0.006
Ni – dissolved	0.009	0.031	< 0.009	< 0.009
Cu – dissolved	0.019	< 0.019	< 0.019	< 0.019
Zn – dissolved	0.005	< 0.005	< 0.005	< 0.005
As – dissolved	0.100	< 0.100	< 0.100	< 0.100
Sr – dissolved	0.003	0.150	0.043	0.139
Mo – dissolved	0.012	< 0.012	< 0.012	< 0.023
Cd – dissolved	0.005	< 0.005	< 0.005	< 0.005
Ba – dissolved	0.003	< 0.003	< 0.003	0.033
Pb – dissolved	0.054	< 0.054	< 0.054	< 0.054

Mr P. Botes (Botes, 2000) indicated that the trace metal concentrations were low and are not likely to have been linked to the fish kill.

3.6 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) provides a measure of the oxygen requirement of organic material present in the water (DWAF, 1996d). A high COD would imply that there is a large amount of organic material present in the water sample. The results of the COD analysis are reflected in Table 5.

Table 5. Chemical Oxygen Demand (COD) concentrations recorded in the samples taken at the three sites

Constituent	Detection limit	Site 1. Van Niekerk Farm	Site 2. D/s of Rooiwal	Site 3. D/s of the Bon Accord Dam
COD (mg. $^{-1}$)	10	36	30	31

Analytical results indicated (Botes, 2000) that the COD concentrations were low and are not likely to have been linked to the fish kill.

3.7 Organic Constituent Analyses

Only one sample was taken for organic constituent analysis, that being at Site 1 where the dead fish were seen during the investigation. One part of the sample was analysed for the presence of algal toxins, Microcystin-LR, Microcystin-RR, Microcystin-YR, and Nodularin using the Enzyme-Linked ImmunoSorbent Assay (ELISA) Microcystin Tube Kit. The limit of detection (LOD) of the ELISA Microcystin kit is 0.5 ppb (µg.ℓ^{-1}). The second part of the sample was extracted with dichloromethane and analysed with a gas chromatograph with a mass selective detector (GC-MS). The compounds were identified using a library search routine and a spectral library containing typical spectra of the compounds. The organic scan indicated the presence of Endosulphan in the sample. An Endosulphan standard was, therefore, used to determine the concentration of the pesticide in the sample.

The results of the various analyses are listed as follows:

1.) The estimated algal toxin concentration was less than 0.5 ppb (µg.ℓ^{-1}).

2.) A 2 µg.mℓ^{-1} Endosulphan standard was also injected. The concentration of Endosulphan in the sample taken at Site 1 can be estimated at less than 1 µg.mℓ^{-1}.

The concentration recorded may not reflect the actual peak concentration since it was already breaking down in the sample when the analysis was conducted. Note that the pesticide Endosulphan is highly toxic to fish. In addition to the Endosulphan, Atrazine, a herbicide, and Phthalates were also isolated in the sample. Phthalates were also present in the blank and would, therefore, most likely be of other origin (such as in the water supply or in the material of the containers used).

3.8 Postmortem *Examination*

Dr E. Du Plessis, of the Pathology Laboratory of the Onderstepoort Veterinary Institute, conducted the histopathological examination on the carp delivered to their laboratories by the IWQS. The results are listed below under the headings used by Dr Du Plessis (Du Plessis, 2000).

Macroscopical Pathological Changes

Severe, generalised congestion, intestinal contents scanty and catarrhal; few gills flukes present on a gill smear.

Histopathological Changes and Morphological Diagnosis

Kidney – Pigmentation, tunica media of medium arteries, moderate.

Intestine – Congestion, lamina propria, multifocal, moderate.

Bacteriological Examinations

No pathogenic bacteria could be cultured.

Discussion

No specific lesions were present to indicate a cause for this fish die-off, including gill lesions indicative of soluble oxygen deficiency. Bacterial presence can be excluded as a cause of death. Further toxicological examinations will hopefully give a better indication of a cause for these mortalities.

At the same time as the above examination, a fish specimen was delivered to the Toxicology Laboratory at Onderstepoort by someone not associated with the IWQS. The herbicide Atrazine was found in the fish tissue of this specimen (Joubert and Basson, 2000).

3.9 Other Possible Factors

Mr J. Daffue (Daffue, 2000) reported that there are pollution problems (including eutrophication problems) upstream of the Bon Accord Dam. This would begin to explain why dissolved oxygen levels are so low in the Apies River, even downstream of the Bon Accord Dam. This may also explain the high UIA concentrations recorded, especially that the UIA levels were highest immediately downstream of the Bon Accord Dam and then progressively improved in the direction of Site 1 (where the UIA concentrations were the lowest of the three sites, albeit still at chronic levels).

Mr Daffue further reported that farmers in the area of the fish kill were complaining of negative growth effects to crops that had been irrigated with water from the Apies River. This is consistent with the presence of Atrazine (a herbicide) in the water sampled.

4. Conclusions and Recommendations

The most obvious cause of the fish kill would appear to be the detectable levels of the pesticide Endosulphan and the herbicide Atrazine in the water. Endosulphan is especially toxic to fish. The source of these toxins is not known. The toxins were already beginning to break down at the time of analysis and it can, therefore, be assumed that their levels had been higher than those recorded.

Un-ionised ammonia (UIA) levels were high enough to result in chronic (long-term) effects on fish. The UIA concentration was surprisingly highest at the site downstream of the Bon Accord Dam (Site 3), with a decreasing gradient to Mr Van Niekerk's Farm (Site 1). It would appear that the conditions within the impoundment itself, or those upstream of the impoundment, favoured the conversion of ammonium ions to the more toxic un-ionised ammonia. These UIA levels, together with the low dissolved oxygen levels recorded, would have made the fish much more susceptible to the pesticides and herbicides than if their levels were more favourable to the well-being of the fish.

It would appear that there is a point source of nitrogen between Site 3 and Site 2 since there was a dramatic increase in the nitrate-nitrite concentration on going from Site 3 to Site 2. The high concentration was also found at Site 1, albeit at a fractionally lower concentration. It is likely that the Rooiwal WCW was a large contributor to the high nitrogen levels evident at Sites 2 and 3.

A progressive increase in total phosphorus and orthophosphate was also evident from Site 3 to Site 1. In contrast to the situation evident with the nitrogen concentrations mentioned above, it is likely that the source of the phosphorus was diffuse.

The effects of the developments between the Bon Accord Dam and the Van Niekerk Farm site, including the effects of the Rooiwal WCW and the Rooiwal power station, can be seen in the increasing salt concentrations that were recorded in the Apies River. There was a significant increase in the TDS concentration at the site downstream of the Rooiwal WCW and the power station (Site 2). There was a further slight increase at the Van Niekerk Farm site (Site 1).

Toxicity tests conducted by the IWQS resulted in 0 % mortality of 17 to 18 day-old *Oreochromis mossambicus*. This indicates that at the time of sampling, the toxin was no longer present at a sufficient concentration to result in mortalities. The conditions in the laboratory would also have resulted in a higher dissolved oxygen level than that evidenced on-site.

Results of the *postmortem* investigation conducted on the carp delivered to the Pathology Laboratory at Onderstepoort, showed that no specific lesions were present to indicate a cause for the fish die-off. It was indicated that bacterial presence could be excluded as a cause of death (Du Plessis, 2000). It was further reported that a fish sample had been delivered to the Toxicology Laboratory at Onderstepoort at the same time by someone not involved with the IWQS investigation. It was found that Atrazine was present in the fish tissue of that specimen (Joubert and Basson, 2000).

In order to address the fish kill adequately, the cause of the elevated UIA concentrations and low dissolved oxygen levels should also be determined. If these factors are improved, then the fish will be in a better position to resist toxins or other factors that threaten their survival.

It is suggested that the Gauteng Regional Office of DWAF, together with the appropriate experts, undertake an information-sharing session with the various land users in the area to inform them of the dangers of insecticides and herbicides to the aquatic ecosystem, and also to their crops. If this information is disseminated to all people managing and using such chemicals, the chance of a similar incident occurring will be decreased.

5. Acknowledgements

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6. References

Botes, P. (2000). Personal communication. Institute for Water Quality Studies, Department of Water Affairs and Forestry. Pretoria, South Africa.

Daffue, J. (2000). Personal communication. Gauteng Regional Office, Department of Water Affairs and Forestry. Centurion, South Africa.

DWAF (1996a). SA Water Quality Guidelines, Aquatic Ecosystems, Department of Water Affairs and Forestry, Volume 7, 1996.

DWAF (1996b). SA Water Quality Guidelines, Agricultural Water Use: Aquaculture, Department of Water Affairs and Forestry, Volume 6, 1996.

DWAF (1996c). SA Water Quality Guidelines, Recreational Use, Department of Water Affairs and Forestry, Volume 2, 1996.

DWAF (1996d). SA Water Quality Guidelines, Domestic Use, Department of Water Affairs and Forestry, Volume 1, 1996.

Du Plessis, E.C. (2000). Personal communication. Pathology Section, ARC Onderstepoort Veterinary Institute. Pretoria, South Africa.

Joubert, J; and Basson, A. (2000). Personal communication. Toxicology Section, ARC Onderstepoort Veterinary Institute. Pretoria, South Africa.

Wade, P. (1999). Calculation of un-ionised ammonia in river water samples. Council for Scientific and Industrial Research, Pretoria, South Africa. Exact reference not known.