Water Technology Research Group

Trinity College, University of Dublin, Dublin 2 Ireland

ENVIRONMENTAL IMPACT OF ACID MINE DRAINAGE ON THE AVOCA RIVER: Acute Toxicity Experiments in the Field Using Macro-invertebrates

C. Byrne and N.F. Gray

Water Technology Research
Technical Report: 18
Tigroney Press

Acknowledgements:

This work was funded by EU Contract: EV5V-CT93-0248 *Bio-rehabilitation of the acid mine drainage phenomenon by accelerated bioleaching of mine waste*

First published November, 1994 (ISBN 1-872220-19-3)

This e-version: Published by Tigroney Press on behalf of the Water Technology Research Group, Trinity College, University of Dublin.

June, 2017

ISBN 978-1-912290-11-6

EXECUTIVE SUMMARY

Field toxicity experiments were conducted in the Avoca River during August and September 1994 in order to evaluate the toxicity of acid mine drainage (AMD) to a number of selected macro-invertebrate species. The main objective of the experiment was to design and evaluate a field toxicity method for rivers receiving AMD.

An experimental flow-through chamber was designed and tested in the field It was found to produce reliable and reproducible results. Three test macro-invertebrates were evaluated *Gammarus duebeni, Ephemerella ignita* and *Baetis rhodani*. Only the former species proved both robust and sensitive enough for toxicity assessment work using the chambers. Three replicates were used at each test site comprising of twenty individuals per replicate (five per chamber). Large specimens of a small size range were used to eliminate size/age effects. Mortality at the control site was acceptable at <10%. The difficulty encountered in using the more sensitive insects may be overcome by collecting animals in early spring. However, their smaller size may make them more suitable for laboratory-based experiments.

A significant toxicity effect was only recorded within the mixing zone of the river, with the completely mixed water at Avoca Bridge showing no significant toxicity. From the water data, the AMD had only a moderate impact on the river during the experimental period. Using the mean AMD index this varied from 92.4 over the experimental period at the upstream control site to 90.3 and 93.5 below Avoca bridge and above the IFI plant respectively. Water quality in the mixing zone is very variable depending on flow characteristics, but with the area clearly severely impacted. In the mixing zone the AMD index varied from 71.3 to 95.6 over the same experimental period.

While the field toxicity tests indicated that only the mixing zone was impacted, routine biological surveillance has shown the impact to be on a more extensive scale with the entire river below the mines (12km) severely damaged. These results suggest that factors other than water toxicity are responsible for the elimination of species in the lower Avoca, although the water quality may result in long term chronic toxicity.

INTRODUCTION

Field toxicity experiments were carried out in August and September 1994 on the Avoca River to evaluate the toxicity of the water to selected macroinvertebrate species. This was considered necessary to determine if water toxicity was sufficient to explain the impact of AMD on the stream community. Field experiments had the advantage of being realistic without the problem of maintaining experimental conditions (e.g. temperature, oxygen, pH, metal adsorption, etc.) as would be necessary in the laboratory. Also, field experiments provided a spatial pattern of water toxicity on the Avoca River. Disadvantages of field experiments included the difficulty in determining the exact factors, conditions and concentrations of toxicants causing the toxic impact due to natural fluctuations in the field. Other disadvantages included the high manpower cost involved in the experiment and the possibility of difficult field conditions for execution of the experiment (especially access to experimental chambers in high water level conditions).

SELECTION OF SPECIES FOR EXPERIMENTS

Species for experiments were based on several criteria including (i) ecological relevance (species should preferably be indigenous or at least occur in similar ecosystems), (ii) availability in sufficient numbers for the experiment (iii) sensitivity to toxicants (iv) ease of handling and (v) ease of identification.

Three species were chosen, none of which completely satisfied all of the above conditions. The crustacean *Gammarus duebeni* was chosen because of the documented sensitivity of *Gammaridae* to toxicants and their subsequent widespread use in toxicity studies (Pascoe *et al.,* 1994), they were also easy to handle and were plentiful in the nearby River Aughrim sub-catchment. *Gammarus duebeni* was only found in very low numbers in the Avoca River upstream of AMD discharges. As mentioned previously, calcium levels in the Avoca River were not sufficient to support a good population. However, for short term toxicity studies this was not considered to be a problem as low calcium would have a long-term effect on the life-cycle of the animal (i.e. growth and moulting). This was confirmed by the observed high survival rate of *Gammarus duebeni* in uncontaminated water from the Avoca River. Two species of mayfly *(Ephemeroptera), Ephemerella ignita* and *Baetis rhodani,* were chosen because of their high representation in the Avoca River and

hence their ecological relevance. They were present in sufficient numbers for the experiments and both genera (*Ephemerellidae* and *Baetidae)* have been noted for their sensitivity to toxicants (Warnick & Bell, 1969; Buikema & Voshell, 1993).

METHODOLOGY

Collection of animals

The animals were collected from riffle areas using a pond-net sampler. Animals were treated as recommended by APHA (1992). They were placed in buckets with stream water and transferred to a laboratory nearby within thirty minutes to minimise stress. The animals were then transferred to plastic holding tanks using large bore pipettes. The tanks contained freshly collected water from the Avoca River to allow acclimatisation. The water was constantly aerated and temperature rises kept to a minimum by placing in a cool room. Netting was placed over the tanks to minimise disturbance and some leaf litter was placed in each to provide shelter and food (*Gammarus duebeni* could feed on the decaying leaves and associated periphyton, *Ephemerella ignita* and *Baetis rhodani* could feed on the periphyton). Survival was checked after 24 hours to ensure that the holding conditions were not causing significant mortality. A 100% survival was observed for *Gammarus duebeni. Ephemerella ignita* and *Baetis rhodani* showed greater than 10% mortality. Some mayflies emerged during the holding period. Late summer was not ideal for toxicity experiments with mayflies as many have already emerged or are continuing to emerge. Early spring, approximately February to April, would be better, ensuring adequate numbers, eliminating the problem of emergence and possibly reducing mortality due to stress.

Field experimental Procedure

Chambers for holding the animals instream were constructed by modifying small plastic pipe chambers used by Dr. Normal Allot (ESU, TCD) in a toxicity experiment with salmonid eggs. The chamber consisted of a 13 cm length of 5 cm diameter Wavin^{TM} pipe with a plastic disc sealing the bottom and a screw-on cap at the top. Two circular windows (approximately 3.5 cm diameter) were cut in the side of the chamber opposite each other. This allowed water to flow directly through the chamber. Nylon mesh (1 mm mesh size) was fixed with non-toxic Rocafix^{TM} heat sealant to the inside of the chamber to prevent the invertebrates from escaping. To anchor the chambers instream PVC Marley^{TM} clips were screwed onto wooden

blocks which were then secured to 150 mm thick concrete blocks using wire. These blocks were positioned instream on relatively flat areas of substrate. The chambers could then be secured to and detached from the Marley clips quickly and easily.

In the laboratory animals were placed into chambers using large bore pipettes in a predetermined sequence to ensure random allocation of animals in the experiment. An equal size range of animals was maintained for all experimental treatments to minimise size/age effect. The chambers were placed in basins with shallow water freshly collected from an uncontaminated site on the Avoca River and constantly aerated. When the allocations were complete the chambers were capped and transported to the experimental sites immediately. The basins containing the chambers were partially flooded to ensure that the animals were constantly submerged. The chambers were quickly clipped in place and adjusted so that the stream current passed straight through the windows. Each block held four chambers, two on either side, which together constituted one sample unit or replicate. Three blocks were placed at each site, therefore, there were three replicates per site. Because of the small size of the chambers the number of animals in each was kept at five. Therefore, there were twenty animals per replicate and sixty per site.

The experimental blocks were placed in glide areas with similar current velocities and not exceeding 70 cm depth to ensure constant accessibility. The substrate was generally gravel/cobble. The base of each chamber was approximately 1 to 3 cm from the substrate bottom. The experiment was carried out over a four day (96 hour) period. At the end of this period the chambers were retrieved and sorted in the field while kept submerged. The number of survivors and mortalities were counted. Mortality was determined by the failure of the animals to respond to physical stimulus. Single water samples were taken from each site in the proximity of the blocks at the start and finish of the experiment. These samples were analysed for pH, conductivity, sulphate, copper, cadmium, iron and zinc. Current velocity in front of each block and 10 cm from the substrate bottom was also measured on these occasions.

RESULTS OF ACUTE TOXICITY EXPERIMENTS

Preliminary trial toxicity experiment

Initially the experimental protocol was tested to evaluate its performance (22/8/94 to 26/8/94). Three sites were chosen based on their contamination status determined from impact on the faunal communities and chemical monitoring data (Table 1).

Table 1. Sites used for the preliminary trial toxicity experiment

Site No.	Site	Location of blocks	AMD pollution status
1	Above White Bridge (Control)	Midstream, spaced in a row	Uncontaminated
4	Below Avoca Bridge	Near east bank, spaced in a row	Moderate contamination
5	Above IFI plant	Near west bank, spaced in a row	Low contamination

Two blocks were placed at each site with eight experimental chambers between them. Each chamber was considered as a replicate in this instance. Seven specimens of *Gammarus duebeni* were placed in each of four replicates and likewise with *Ephemerella ignita.* Two replicate chambers for each species were placed on each block.

Table 2 represents the results for the preliminary trial experiment. It can be seen that in several chambers there were some animals missing at the end of the test period. It was unclear whether this was due to animals escaping or dying and disintegrating. To compensate for this the survival rate was calculated for only those animals remaining in the chambers.

Table 2. Results of the preliminary acute toxicity test for *Gammarus duebeni* and *Ephemerella ignita*

Site	Site No.	Replicate	No. Alive	No. Dead	No. Missing	% Survival*	Mean % Survival
Gammarus duebeni							
Above White Bridge	1	1	7	0	0	100	88.7
(Control)		2	6	0	1	100	
(Size range = 11-		3	5	2	0	71.4	
15 mm)		4	5	1	1	83.3	
Below Avoca Bridge	4	1	5	2	0	71.4	76.8
(Size range = 11-		2	6	1	0	85.7	
15 mm)		3	5	1	1	83.3	
		4	4	2	1	66.7	
Above IFI plant	5	1	7	0	0	100	79.8
(Size range = 11-		2	5	1	1	83.3	
15 mm)		3	3	3	1	50	
		4	6	1	0	85.7	
Ephemerella ignita							
Above White Bridge	1	1	3	3	1	50	72
(Control		2	4	2	1	66.7	
(Size range = 8-6mm)		3	6	1	0	85.7	
		4	6	1	0	85.7	
Below Avoca Bridge	4	1	3	3	1	50	65.5
(Size range = 8-6mm)		2	4	3	0	57.1	
		3	5	1	1	83.3	
		4	5	2	0	71.4	
Above IFI plant	6	1	3	4	0	42.9	61.9
(Size range = 8-6mm)		2	4	2	1	66.7	
		3	4	2	1	66.7	
		4	5	2	0	71.4	

*Survival was calculated form the animals remaining in the chambers, missing animals were excluded from the calculation

The survival for each species did not differ significantly between sites (One-way ANOVA was not significant, percentages were arcsine transformed). The high mortality for *Ephemerella ignita* at the control site (mean = 28%) was considered unacceptable and highlighted the problem of using sensitive insects such as mayflies for toxicity tests. In standard laboratory based toxicity test procedures it is recommended that if mortality is greater than 10% in controls the test should be discarded (APHA, 1992). Regarding *Gammarus duebeni* it was felt that increasing the number of animals per replicate would increase the resolution of the bioassay and improve the calculated survival at the control site. Water chemical analysis showed that sulphate, conductivity, copper, zinc and iron were higher and pH lower at Avoca and above the IFI plant than at the control site (Above White Bridge) (Table 3).

Table 3. Results of water analysis from sites used in the preliminary trial toxicity test

Site	Site no.	Sampling occasion (dates)	pH	Cond-uctivity µS/cm	Sulphate mg/l	Cu mg/l	Cd mg/l	Fe mg/l	Zn mg/l	AMDI
Above White Bridge (Control)	1	Start (22/8/94)	7.00	81	6	0	0	0.05	0.005	97.79
		Finish (26/8/94)	6.32	49.6	5	0	0.01	0.13	0.060	91.31
Below Avoca Bridge	4	Start (22/8/94)	6.0	135	34	0.05	0	1.26	0.72	79.01
		Finish (26/8/94)	4.9	71.3	15	0	0	0.53	0.33	81
Above IFI plant	5	Start (22/8/94)	6.5	139	30	0	0	0.39	0.45	89.20
		Finish (26/8/94)	6.3	80.0	14	0	0	0.39	0.24	91.31

where AMDI = acid mine drainage index

Main acute toxicity experiment

The main experiment, carried out over a four day period (96 hours) from 13/9/94 to 17/9/94, consisted of five sites (Table 4), including the control site, with three replicates per site made up of twenty *Gammarus duebeni* per replicate (five per chamber). Twenty specimens of *Baetis rhodani* (five per chamber) were included in the sample chambers as *Gammarus duebeni* at three sites to assess the future use of this species in toxicity work. These sites included Sites 1, 2 and 4 (Above White Br., Tigroney leachate outfall and Avoca). Site 5 above the IFI plant had only two replicates due to a shortage of experimental chambers. When animals were allocated to chambers it was discovered that some animals had escaped due to breached areas where the mesh had been sealed. These breached chambers were excluded. However, after the experiment several animals were missing and were presumed to have escaped. Survival rate was thus calculated from the remaining animals. It is

advisable that for future assays using the chambers the seals should be carefully examined and only large animals be used.

Table 4. Sites used for the main toxicity experiment

Site No.	Site	Location of blocks	AMD pollution status
1	Above White Bridge (Control)	Midstream, spaced in a row	Uncontaminated
2	Below Tigroney leachate outfall	Near east bank, 10 m below input in plume	Severe contamination
3	Above Ballymurtagh leachate outfall	Near west bank, 5 m above input	Severe contamination
4	Below Avoca Bridge	Near east bank, spaced in a row	Moderate contamination
5	Above IFI plant	Near west bank, spaced in a row	Low contamination

Mortality of *Gammarus duebeni* at the control site was acceptable averaging at approximately 10%. The results indicated that survival was significantly lower at Sites 2 (Tigroney leachate outfall) and 3 (above Ballymurtagh leachate outfall), the most severely contaminated sites, than at all other sites (Table 5 and Figure 1). A one-way ANOVA and *post-hoc* Scheffe's MCT showed this to be statistically significant at an overall protection level of 5% (Table 6). Survival at sites 4 (Avoca) and 5 (Above the IFI plant) were not significantly different from the control site (Site 1 - Above White Bridge). The interpretation of these results was that water at sites 2 and 3 was sufficiently toxic to cause significant mortality but was not sufficiently toxic further downstream at sites 4 and 5.

Specimens of *Gammarus duebeni* which were still alive at the sites showing significant mortality were observed to be noticeably sluggish in movement and response to stimulus in contrast to specimens at other sites which were more active.

Table 5. Results of the main toxicity experiment for *Gammarus duebeni* showing percentage survival

Site No.	Site	Size range (mm)	Replicates			Mean
			1	2	3	
1	Above White Bridge (Control)	8-17	94	87.5	89.5	90.3
2	Below Tigroney leachate outfall	8-17	20	26	44	30
3	Above Ballymurtagh leachate outfall	8-17	25	50	63	46
4	Below Avoca Bridge	8-17	85.5	92	86.7	88.1
5	Above IFI plant	8-17	92	94	-	93

Figure 1. Percent survival of *Gammarus duebeni* at sites on the Avoca River. o represents the mean. The horizontal lines represent the standard deviation.

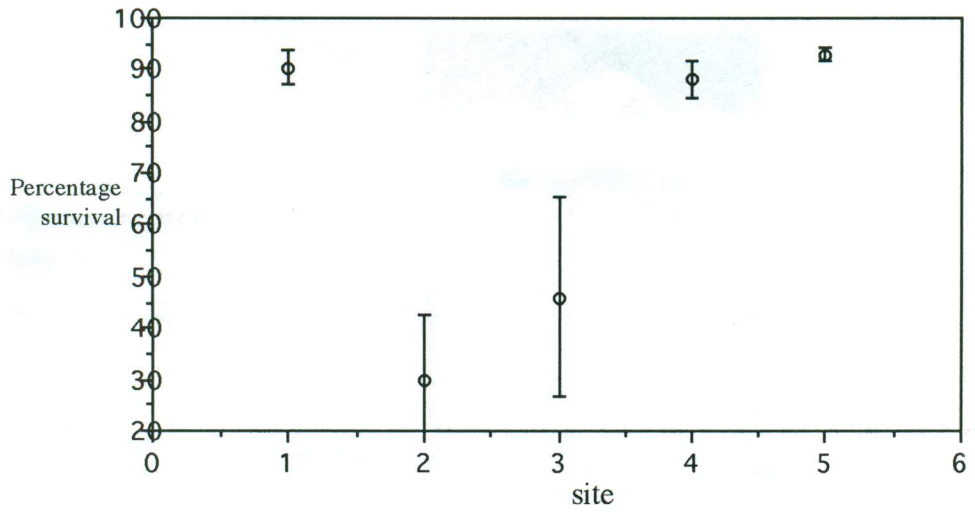

Table 6 Results of an ANOVA and Scheffe's MCT for percent survival of *Gammarus duebeni* at sites on the Avoca River (Data were arcsine transformed)

		F = 22.83		p = <0.001 ***		
Mean	**Site**	**1**	**2**	**3**	**4**	**5**
90.3	**1**					
30	**2**	*				
46	**3**	*	-			
89.4	**4**	-	*	*		
93	**5**	-	*	*	-	

Note:* indicates samples that were significantly different at an overall 5% level
*** statistically significant at the 0.1% level

The experiment was unsuccessful for *Baetis rhodani* as significant numbers of this small species had escaped through the 1 mm mesh and those remaining showed highly variable mortality between replicates. A smaller mesh could be employed to eliminate escapees but this would easily become blocked with debris. However, despite the failure of this experiment it was observed that all the remaining specimens of *Baetis rhodani* at site 2 (Tigroney leachate outfall) were dead while a proportion of those at the other two sites (site 1 - above white Bridge and site 4 - Avoca) were still alive. Therefore, there would appear to be good reason to investigate toxicity to this species in the future. Mayflies, like many insects, are known to be difficult to handle in experiments and culturing and handling techniques need to be developed.

Water chemical parameters including conductivity, copper, iron, zinc and sulphate were higher and pH lower below the control site (Table 7). However, the measured values at the start and finish of the experiment did not indicate that water at sites 2

and 3, which showed significant mortality, were excessively polluted. This may indicate that fluctuations were occurring unmonitored during the experimental period. From long term monitoring data this would seem to be the case. Mortality may have been caused by episodic pulse releases of AMD.

CONCLUSIONS AND RECOMMENDATIONS

The impact on the Avoca river predicted by faunal community surveillance (Byrne and Gray, 1994) was not fully supported by the toxicity experiments. Surveillance indicated impact at all sites below where AMD entered the river, whereas toxicity results indicated that only sites in the proximity of leachate inputs were impacted. It would be reasonable to suggest that factors other than water toxicity were responsible for the impact on the fauna further downstream. Sediment toxicity or substrate compaction may cause the substrate to be uninhabitable to the fauna. In view of this sediment toxicity experiments should be done. Indeed the indiscriminate depletion of all taxa would suggest that conditions are unfavourable for all species rather than causing the elimination of sensitive forms and proliferation of tolerant forms.

For future toxicity experiments it is recommended to use *Gammarus duebeni*. Large specimens of a small size range should be used to eliminate size/age effect. The experimental chambers should be checked rigorously before the experiment for breaches in the chamber seals. Water chemical parameters should be monitored more intensively over the experimental period to detect episodic pulses of AMD causing mortality. Laboratory based experiments using *Gammarus duebeni* may be used to investigate dose-response relationships, thus enabling prediction of toxicity from contaminant concentrations. The difficulty of using sensitive insects in toxicity experiments may be overcome by collecting animals in early spring (February to April) and performing laboratory based experiments.

Table 7 Results of water analysis from sites used in the main toxicity test

Site	Site no.	Sampling occasion (dates)	Current velocity (m/s)	pH	Cond-uctivity µS	SO4 mg/l	Cu mg/l	Cd mg/l	Fe mg/l	Zn mg/l	AMDI
Above White Bridge (Control)	1	Start (13/9/94)	0.65 (0.55-0.77)*	6.36	44	4	0	0	0.27	0.05	93.44
		Finish (17/9/94)	0.57 (0.5-0.74)	5.78	62	4	0	0	0.18	0	91.31
Below Tigroney leachate outfall	2	Start (13/9/94)	0.61 (0.5-0.77)	6.74	57	8	0	0	0.29	0.19	95.60
		Finish (17/9/94)	0.52 (0.4-0.6)	4.57	127	46	0.02	0	1.8	1.42	71.31
Above Ballymurtagh leachate outfall	3	Start (13/9/94)	0.56 (0.52-0.6)	6.29	71	15	0	0	1.04	0.33	87.11
		Finish (17/9/94)	0.5 (0.42-0.55)	5.37	72	16	0	0	0.73	0.25	83.01
Below Avoca Bridge	4	Start (13/9/94)	0.47 (0.23-0.57)	6.52	61	10	0	0	0.45	0.27	93.44
		Finish (17/9/94)	0.6 (0.57-0.62)	5.64	66	11	0	0	0.44	0.18	87.11
Above IFI plant	5	Start (13/9/94)	0.31 (0.0-0.33)	6.59	61	8	0	0	0.39	0.195	95.60
		Finish (17/9/94)	0.34 (0.3-0.34)	6.3	72	11	0	0	0.29	0.11	91.31

* Denotes range of current velocity readings

REFERENCES

APHA, American Public Health Association. "*Standard methods for the examination of water and waste water.*" Joint publication of American Public Health Association, American Water Works Association and Water Pollution Control Federation. Metals. Editors A.E. Greenberg, L.S. Clesceri, and A.D. Eaton. Eighteenth Edition. Washington D.C., 1992.

Byrne, C.F. and Gray, N.F. (1994) *Environmental impact of acid mine drainage on the Avoca River: Effect of habitat types on biological monitoring of the macroinvertebrate fauna.* Technical Report, EU Contract: EV5V-CT93-0248, Trinity College, Dublin

Buikema, A.L. Jr. & Voshell J.R. Jr. "Chapter 10. Toxicity studies using freshwater benthic macroinvertebrates." *Freshwater biomonitoring and benthic macroinvertebrates,* 344-398. Editors D.M. Rosenberg and V.H. Resh. First edition. New York and London: Chapman and Hall, 1993.

Pascoe, D., Kedwards, T.J., Maund, S.J., Muthi, E. & Taylor, E.J. Laboratory and field evaluation of a behavioural bioassay - the *Gammarus pulex* (L.) precopula separation (GaPPS) test. *Water Research* **28, No. 2** (1994): 369-372.

Warnick, S.L. & Bell, H.L. "The acute toxicity of some heavy metals to different species of aquatic insects" *J. Wat. Poll. Cont. Fed.* **41** (1969): 280-284.