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Determination of the acute toxicity of mine tailings from Nussir ASA to the marine alga Skeletonema costatum, the marine copepod Tisbe battagliai and the polychaete Arenicola marina

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REPORT

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Abstract

The acute toxicity of mine tailings from Nussir ASA have been determined. The concentrations of the mine tailings ranged from 100% v/v to 1% v/v and the mine tailings were diluted with a clean reference sediment obtained from NIVA's Marine Research Station in Solbergstrand plus a reference sediment control and were mixed with clean reference seawater at a ratio of 1:10 v/v mine tailings:water. Three different species were used for the toxicity assessments, representing different trophic levels in the environment, comprising of the alga *Skeletonema costatum*, the copepod *Tisbe battagliai* and the polychaete worm *Arenicola marina*. The copepod and algal tests were performed on overlying water taken from the test system prior to commencement of the polychaete worm test. There were no effects observed on the algae at any concentration up to and including 100% v/v. There was a slight effect (35% mortality) observed in the *T. battagliai* test at the 100% v/v concentration and a 50% effect (mortality) at the 100% concentration in the *A. marina* test. Therefore the Lowest Observed Effect Concentration (LOEC) for the mine tailings is 100% v/v and the No Observed Effect Concentration (NOEC) for this study is 32% v/v.

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2. *Tisbe battagliai*	2. *Tisbe battagliai*
3. *Skeletonema costatum*	3. *Skeletonema costatum*
4. Gruveavgang	4. Mine tailings

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Determination of the acute toxicity of mine tailings from Nussir ASA to the marine alga *Skeletonema costatum*, the marine copepod *Tisbe battagliai* and the polychaete *Arenicola marina*

Preface

This study has been conducted by staff from the Section for Ecotoxicology and Risk Assessment, Norwegian Institute for Water Research (NIVA). The authors acknowledge the contribution of Ailbhe Macken during the study.

Oslo, April 2011

Adam Lillicrap

Contents

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2 Introduction

The acute toxicity of mine tailings to the marine alga *Skeletonema costatum*, the marine copepod *Tisbe battagliai* and the polychaete *Arenicola marina* was carried out at NIVA Gaustadalléen 21, 0349, Oslo, Norway at the request of Nussir ASA. The tests were performed according to methods detailed in OSPAR guidelines for testing of contaminated marine sediment and aimed to determine the acute toxicity of the mine tailings at three 3 different trophic levels. This included a sediment consumer (*A. marina*), a primary producer (*S. costatum*) and a primary consumer (*T. battagliai*). The mine tailings were all supplied by Nordic Mining ASA. The exposure dates for the studies were between 28th March and 9th April 2011.

3 Materials and methods

3.1 Test substances

The mine tailings used for the investigations were supplied by Nussir ASA and were prepared in the form of a mixture of mine tailings and clean reference sediment with appropriate concentrations of the flocculating agent Magnafloc 10. The mine tailings/sediment mixture were prepared at NIVA. The Magnafloc 10 was prepared as a 100 mg/L stock solution in freshwater prior to testing. The mine tailings/sediment mixtures were prepared in 1 kg batches in each replicate of each test concentration. Each concentration had 2 replicates, and the concentration range tested was 1.0, 3.2, 10, 32 and 100% v/v plus a reference sediment only control. The concentration range of mine tailings were diluted with clean reference sediment obtained from nearby Jeløya, in the outer Oslofjord. The Magnafloc was added to the sediment mix at different quantities as indicated in Table 1. The amount of Magnafloc used was based on the quantities used by Nussir ASA annually (i.e. 30g Magnafloc 10/tonne/year). Subsequently, 10 L of freshwater was added to each mine tailings/sediment mix, stirred well and then allowed to settle over night before the overlying water was removed and discarded. The addition of the freshwater and Magnfloc 10 was to simulate the industrial process of mineral retrieval. Following removal of the overlying freshwater water, 10 L of reference seawater (obtained from the marine field station at Solbergstrand at a depth of 60m) was added to the mine tailings/sediment mixture. The mine tailings/sediment mixtures were thoroughly homogenised and left to stand a further 5 hours to reach equilibrium between the substances and the water and for the particles to settle. This resulted in each replicate containing 10 L of seawater and 1 kg of mine tailings/sediment mixture. After settling,

approximately 2 L of the overlying water from each replicate were decanted using an adapted siphon tube for use in the algae and the copepod tests. The remaining mine tailings/sediment and seawater preparations were used for the polychaete worm test.

Table 1. Volumes of Magnafloc stock solution to be added to the sediment mix

Concentration of sediment mix (%)	Volume of Magnafloc stock to be added (ml)	Final conc of Magnafloc added to sediment mix (mg/kg)
Control	0	0
1	3.0	0.30
3.2	9.6	0.96
10	30	3.0
32	96	9.6
100	300	30

3.2 Test organisms

The test organisms used for the test was the marine algae *Skeletonema costatum*, the marine copepod *Tisbe battagliai* and the polychaete worm *Arenicola marina*. The copepods (approximately 7 days old) and algae (NIVA-strain BAC 1) were from continuous cultures, maintained at NIVA. The polychaete worms were obtained from Green Blue Fish ltd, 3 St. Colme Road, Dalgety Bay, Dunfermline Ky11 9lh, Scotland and each worm was between 1 and 5g in weight.

3.3 Dilution water

The dilution water for the study was natural seawater taken from a depth of 60 m from within the outer Oslofjord. Seawater taken from this source is well characterised and is used routinely for culturing and testing purposes at NIVA. The freshwater used for the initial application of the process chemicals was standard lab tap water.

3.4 Algal toxicity test

The algae growth inhibition test was performed according to International Standard ISO 10253: Water Quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. The algal toxicity test was performed using the overlying water removed from each replicate of each concentration. The overlying water was filtered to remove any biological or particulate material and spiked with a growth medium concentrate. All batches were inoculated with *S. costatum* from an exponentially growing laboratory culture and incubated in glass flasks on a shaking table at 20 °C under continuous illumination from fluorescent tubes providing approximately 75 µM m^{-2} s^{-1} of photosynthetic active radiation (PAR). The test was performed with three replicates from each of the individual test replicates from each concentration. The cell densities were determined using an electronic particle counter (Coulter Multisizer) after approximately 24, 48 and 72 hours. The growth rate of each culture was calculated and expressed as a percentage of the growth rate of control cultures in untreated overlying water.

3.5 Acute toxicity to the copepod *Tisbe battagliai*

An acute toxicity test was performed on the copepod *Tisbe battagliai* according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda*, *Crustacea*). Copepods that were approximately 7 days old, were added to the overlying water in plastic microplates containing approximately 5 ml test solution/well. There were 4 exposure wells, each with 5 animals used for the 2 replicates of each test concentration (40 animals per test concentration). The number of survivors were determined after 24 and 48 hours and the no and low observed effect concentrations (NOEC/LOEC)

were calculated. The study was carried out in a temperature controlled room (20°C) and with a photoperiod of 16:8 (light:dark) cycle.

3.6 Acute toxicity to the polychaete worm *Arenicola marina*

An acute toxicity test was performed on the polychaete worm *Arenicola marina* in accordance with the PARCOM guideline (Thain and Bifield, 1994). Five individual worms (weighing between 1 and 5 g each) were removed from a holding tank and added to each replicate test vessel containing sediment and overlying water. Observations for dead animals were made on a daily basis and any dead organisms were removed. Any dead worms observed within the first 24 hours of the test were taken out and replaced with fresh worms. These worms were not included in the calculation of mortalities. At the end of the study, the sediment was sieved and the numbers of live and dead worms counted in each test replicate and a NOEC and LOEC were calculated. The animals were not fed during the exposure and the overlying water was aerated continuously throughout the study. The toxicity test was carried out in a temperature controlled room (15°C) and in the dark.

3.7 Physico-chemical parameters

The following parameters, pH, dissolved oxygen and temperature were measured on the excess overlying test solutions at the start of the studies. At the end of the studies, the pH and dissolved oxygen were measured in the overlying waters of the copepod and polychaete worm tests and only the pH was measured in the excess solutions from the algal test. The temperature was measured daily in the algae and copepod tests and twice in the polychaete worm test.

3.8 Quality Assurance

All data and subsequent reports have been subject to internal quality assurance within NIVA.

3.9 Archiving

All raw and electronic data will be archived for a minimum period of 5 years.

4 Results

4.1 Physical parameters

The physical parameter and water quality data for the study are shown in Table 2 of the appendices. In summary, the pH and the dissolved oxygen (DO) content of the overlying water at the start of the test ranged between 7.79 and 8.01 and between 6.5 and 7.3 (mg/L) respectively. At the end of the study, the pH and DO were measured, and in the polychaete worm test, the parameters ranged between 7.61 and 7.81 and between 7.0 and 8.1 (mg/L). In the *T. battaglia* test, the parameters ranged between 7.99 and 8.12 and between 8.0 and 8.2 (mg/L) respectively and in the algal test, the pH ranged between 8.48 and 8.75. The light intensity of the algae test was 62 µmol $m^{-2}s^{-1}$. The water quality was within the range expected for the study and none of the analytes present would have affected the outcome of the results.

4.2 Biological data

The effect data from the ecotoxicity studies are presented within the appendices. The data from the copepod test indicated that at the highest test concentration (100% v/v) there were slight effects with reduced survival of 35% mortality. There were slightly more significantly effects in the polychaete worm test with 50% mortality observed at the end of the study. There were no effects seen at any

lower concentrations than 100% v/v in both these tests. Furthermore, in the polychaete worm test, 80% of the surviving worms in the 100% v/v test concentration were observed to be on the surface of the mine tailings indicating an avoidance mechanism. In the algal study, there were no effects on growth up to and including the 100% v/v test concentration.

5 Conclusion

There were effects observed at the top concentration of 100% v/v mine tailings from Nussir ASA in the copepod and the polychaete worm test. The Polychaete worm test resulted in 50% mortality at the 100% v/v test concentration and the LC50 may be expressed as 100%. Furthermore, the Low Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC) may be expressed as 100% and 32% v/v respectively for the 2 tests. This indicates that at the maximum concentration of mine tailings released, there may be some indications of effects in the environment.

6 References

International Standard ISO 10253: Water Quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*.

International Standard ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*).

Thain, J.E. and Bifield, S. (1993). A sediment bioassay using the polychaete *Arenicola marina*. Test guideline for PARCOM sediment reworker ring-test. MAFF Fisheries Laboratory, Burnham-on-Crouch, Essex, UK.

Appendices

Table 2. Physical parameters of the over lying water

Conc (%)	ON		OFF				
			Worms		Algae	Tisbe[a]	
	DO (mg/L)	pH	DO (mg/L)	pH	pH	DO (mg/L)	pH
Control A	7.1	8.01	7.5	7.61	8.72	8.09	8.11
Control B	7.3	7.96	7.0	7.72	8.75		
1.0 A	7.0	7.94	7.2	7.81	8.61	8.24	8.12
1.0 B	7.0	7.82	7.3	7.83	8.70		
3.2 A	7.0	7.88	7.7	7.83	8.67	-	-
3.2 B	7.1	7.92	7.6	7.83	8.63		
10 A	7.1	7.93	7.8	7.83	8.67	-	-
10 B	7.1	7.93	7.7	7.83	8.65		
32 A	7.2	7.93	8.0	7.83	8.62	-	-
32 B	7.0	7.93	7.8	7.83	8.48		
100 A	6.5	7.79	8.1	7.85	8.65	8.01	7.99
100 B	7.0	7.89	7.5	7.78	8.60		

[a] Parameters measured on pooled samples as the volume was too small to measure for each independent replicate

Table 3. Algal toxicity data

Hours:	**Day 1**	**Day 2**	**Day 3**	**G. rate**
	30	49	72	Day 3
Conc. (%)	mill./l	mill./l	mill./l	d^{-1}
Control A	69	339	1150	1.82
	88	330	1146	1.82
	74	324	1208	1.84
Control B	96	406	1859	1.98
	104	451	1869	1.99
	80	385	1385	1.89
1 A	83	365	1582	1.93
	100	400	1890	1.99
	85	359	1576	1.93
1 B	97	470	1385	1.89
	95	445	1360	1.88
	80	340	1147	1.82
3.2 A	97	507	1716	1.96
	84	354	1482	1.91
	78	336	1608	1.94
3.2 B	83	305	1748	1.96
	85	352	1602	1.93
	82	318	1489	1.91
10 A	93	462	1852	1.98
	87	352	1601	1.93
	94	444	1851	1.98
10 B	87	497	2304	2.06
	86	362	1682	1.95
	87	440	2101	2.03
32 A	93	455	1901	1.99
	94	385	1916	1.99
	89	382	1830	1.98
32 B	85	347	1401	1.89
	85	362	1355	1.88
	80	351	1334	1.87
100 A	85	378	1820	1.98
	86	355	1668	1.95
	90	492	1752	1.96
100 B	85	501	2065	2.02
	74	355	1622	1.94
	86	342	1641	1.94

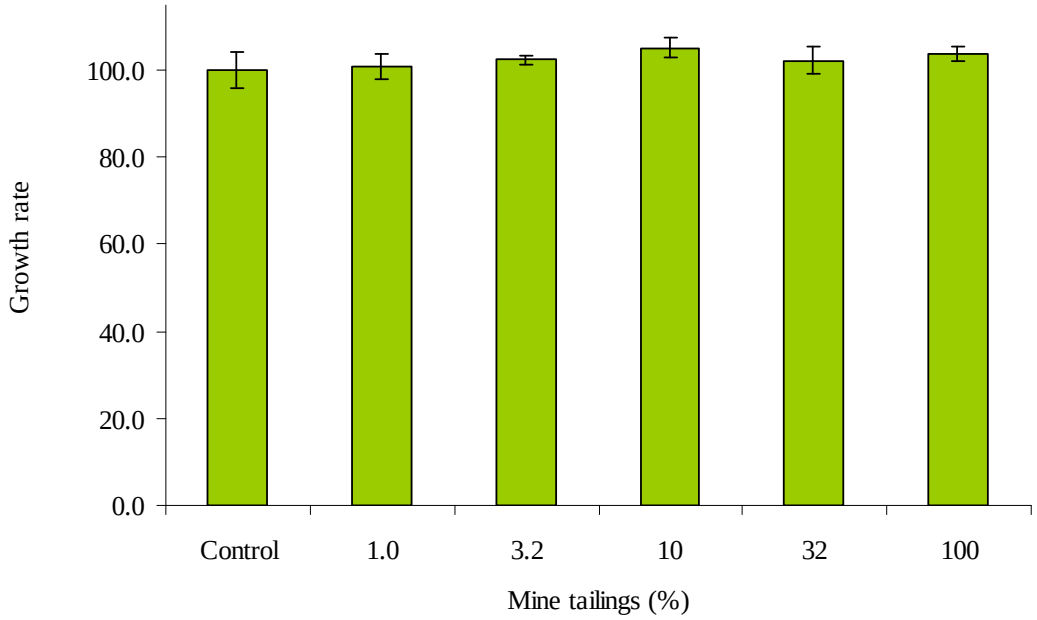

Figure 1. Algal growth data as a percentage of control

Table 4. Mortality data

Conc (%)	*Tisbe battagliai*			*Arenicola marina*		
	No. dead at 24 hours	No. dead at 48 hours	% mortality	No. alive at end of test	No. dead at end of test	% mortality
Control A	0	0	0	5	0	10
Control B	0	0		4	1	
1.0 A	0	0	0	5	0	0
1.0 B	0	0		5	0	
3.2 A	0	0	0	5	0	0
3.2 B	0	0		5	0	
10 A	1	1	5	4	1	10
10 B	0	0		5	0	
32 A	0	0	0	5	0	0
32 B	0	0		5	0	
100 A	3	5	35	0	5	50
100 B	0	2		5*	0	

* 4 remaining live worms were observed to be present on the surface of the mine tailings

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