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Recolonisation of mine tailing by meiofauna in mesocosm and microcosm experiments

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ABSTRACT

The Batu Hijau copper/gold mine in Sumbawa, Indonesia processes ore at approximately 130,000 tpd and discharges tailing via a submarine pipeline to depths below 3000 m at the base of a submarine canyon. The study investigated recolonisation of tailing by meiofauna and its dependence on subsequent accumulation of natural sediment. Microcosm and mesocosm scale experiments were carried out using two tailing and two control samples, the latter comprising defaunated and unaffected natural sediment. All test materials were similar in physical and chemical respects, except for the higher copper concentration in the tailing. The abundances of meiofauna colonising defaunated controls and both tailing samples increased from zero to levels statistically indistinguishable from natural unaffected controls after 97 and 203 days. Colonisation was well established in tailing from freshly mined ore after 40 days, and in oxidized tailing from stockpiled ore after 65 days, and was not dependent on settled natural material.

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1. Introduction

PT Newmont Nusa Tenggara (PTNNT) operates the Batu Hijau copper/gold mine in Sumbawa, Indonesia in which ore is processed at a rate of approximately 130,000 tpd. The tailing residue is discharged to the deep ocean floor from a pipeline, with its terminus located at 125 m depth at the head of a steep submarine canyon (the Senunu Canyon), some 3.2 km offshore of the southwest coast of Sumbawa (Fig. 1). Below the pipe exit, the steep gradient of the canyon facilitates tailing flow as a density current to its final deposition at depths below 3000 m. The mine started operation in 1999 and is expected to continue until 2023. There are several other similar examples of disposal of tailing to deep ocean, mainly in the Indo-Pacific archipelagic nations, but also the Black Sea and the Mediterranean (Jones and Gwyther, 2000; Spitz and Trudinger, 2008).

The present study investigated benthic recolonisation in tailing in order to validate predictions of benthic recovery that were made in the project's environmental impact statement (Dames and Moore, 1996). Meiofauna (metazoans between 32 and 500 µm) were used in these experiments as they provide a rapid and sensitive measure of recolonisation potential because of their high

abundance, short generation times and association with the sediment throughout their life cycle (Coull and Chandler, 1992; Thiel, 1992). Meiofauna have been widely used in similar investigations of benthic community responses to contamination or physical disturbance under field and experimental conditions (Coull and Chandler, 1992; Somerfield and Warwick, 1996; Austen and Somerfield, 1997; Austen et al., 1994; Austen and McEvoy, 1997; Schratzberger and Warwick, 1998; Boyd et al., 2000; Schratzberger et al., 2000a, b; Schratzberger et al., 2004, 2006).

The present experiments examined the species and communities that recolonised barren tailing material, representative of the final settled tailing surfaces at the end of the life of the mine, and in sediment naturally settling on top of the tailing.

1.1. Objectives

The experiments compared the abundance, diversity and community composition of meiofauna colonising tailing and two controls, comprising natural sediment (with natural meiofauna), and defaunated natural sediment.

The objectives of the study were to determine whether:

1. The tailing is colonised by meiofaunal organisms.
2. Colonisation is dependent on natural material accumulating and burying the tailing during the experiment.

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higher copper content due to lower process recoveries. Sample of TB material was obtained from test processing at the metallurgical laboratory.

Two control materials were used. Control negative (CN) consisted of surface sediment collected by box corer from the test site in Benete Bay and defaunated by deep-freezing for 24 h. Control positive (CP) consisted of surface sediment collected by box corer from the test site and used directly in the experimental chambers (i.e., natural sediment with live meiofauna).

2.1. Experimental design

The experiments were conducted at microcosm and mesocosm scales. The microcosms were made from 450 mm lengths of perspex tubes of internal diameter 50 mm, which were sealed at the base. The mesocosms were constructed from open pvc half-drums of internal diameter 60 cm and height 60 cm.

The microcosm tubes were held in four racks, each holding three replicates of each of the four test materials, and one empty tube to act as a sediment trap (to measure sediment accumulation over time). The tubes were filled with test material (to 15 cm depth). The four mesocosm drums were half-filled (to 30 cm depth) with approximately 90 l of TA, TB, CN and CP material respectively. A plastic marker was glued to the inside of each mesocosm drum to mark the surface of the test material at the start of the experiment and to compare the level at the end. The test chambers were each sealed with temporary lids to prevent turbulent loss of material when lowered to the seabed, where divers attached the racks and mesocosm drums to pre-laid concrete weights (Fig. 2). The experiments were set-up on 12 August 2005 and sampled after intervals of 40, 65, 97 and 203 days.

2.2. Sampling

Divers sampled the mesocosms *in situ* on the seabed by taking three sub-samples from each of the four experimental mesocosm drums, using a 3 cm diameter syringe barrel inserted to 10 cm depth into the sediment. Once the syringe was extracted from the sediment, the open end was capped to prevent any loss of the sample on return to the vessel. The three sub-samples were considered as independent replicates to give an assessment of variation, on the basis that the mesocosm area was sufficiently large (827 cm²) to represent natural fine-scale distribution adequately. The sample was extruded from the syringe and each 2 cm horizon preserved in 4% formaldehyde and Rose Bengal stain for separate sorting and identification. Samples were also taken from the Tailing A and B mesocosms for analyses of metal and total organic carbon (TOC) concentrations.

The microcosm rack pre-allocated for recovery was located by divers and each tube capped to prevent any loss of material during its recovery. A 10 cm core was taken from each of the three replicates of the four test materials, using a purpose-built, pvc corer of 1.8 cm diameter, and each 2 cm horizon was cut and preserved separately, as for the mesocosm samples. The remaining sediment in the three replicate tubes (after meiofauna samples were collected) was combined and collected for analysis of sediment metal, toxicity characteristic leachate procedure (TCLP) metals and TOC content. Metal concentrations in the samples were analysed using atomic absorption spectrometry methods, and TOC using high temperature combustion standard method (see APHA, AWWA, WEF, 2005).

The material in the sediment trap was transferred to a measuring cylinder and allowed to settle for one hour before measurement of settled height. On day 40, this material was preserved for analysis of its meiofauna species composition, in order to identify the range of species potentially able to colonise the experimental treatments.

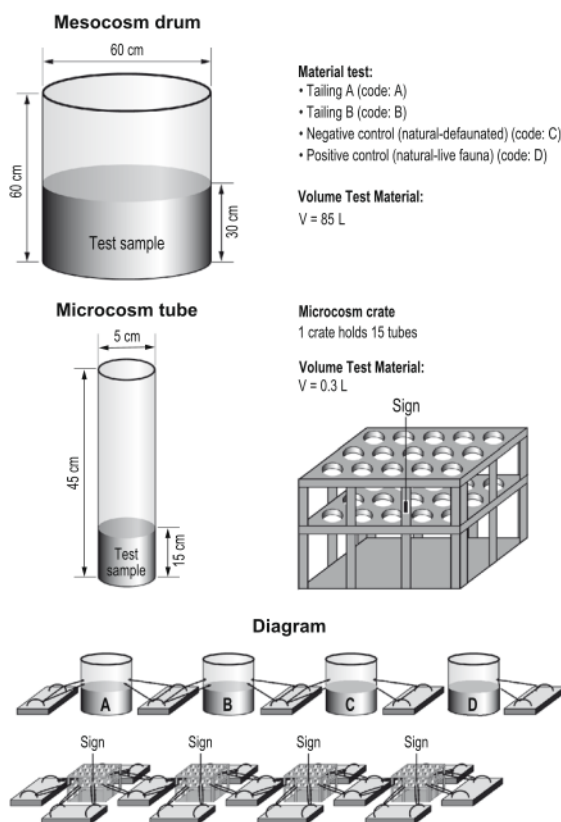


Fig. 2. Experimental design of mesocosms and microcosms.

2.3. Sample sorting

The formalin-preserved, stained samples were washed gently in filtered fresh water then poured through a metal sieve with 0.5 mm mesh size in order to remove any macrofauna and large particles. The passed sediment was then washed again on a sieve with mesh size 32 μm to remove fine silt and clay. Meiofauna in sediment retained on this last sieve was extracted using methods described in Higgins and Thiel (1988). The stained fauna were sorted and counted into major taxa and numbers were standardized into numbers per 10 cm² surface area for each of the horizons.

2.4. Statistical analysis

Univariate, one-way analyses of variance (using SYSTAT) tested for significance of differences between the mean abundances and the mean numbers of taxa in the four treatments (CP, CN, TA, TB) and at each of the four sampling time intervals.

Multivariate analyses were also used to compare the meiofaunal communities colonising the four different treatments and time intervals using the multi-dimensional scaling (MDS) ordination and analysis of similarity (ANOSIM) routines of PRIMER, developed by Plymouth Marine Laboratory, UK, (Clarke, 1993; Clarke and Warwick, 1994; Clarke and Gorley, 2001). Fourth root transformation of numbers was used to reduce the importance of the most and least abundant taxa in the analyses. Samples with zero counts were omitted from the ordination process. Clustering was done on the basis of the Bray–Curtis similarity measure. The non-metric MDS ordination produced a two-dimensional 'map' of the sites,

in which similarity is represented by the closeness of samples. One-way ANOSIM technique was used to test for differences in community structure between the treatments at each time interval and also to assess changes in the meiofaunal communities over time.

3. Results

3.1. Sediment grain size

The grain size distributions of the four test materials (taken from the mesocosms) are given in Fig. 3, and show that particle size ranges were generally similar in both the control (CN and CP) and tailing (TA and TB) material. The majority of size fractions fall between the sand IV to silt VII fraction (0.2 mm–5 µm), but in the tailing, the range extended to include slightly coarser sand III fraction (0.2–0.5 mm). However, in most cases, the median diameters fell between sand IV and sand V (0.2–0.05 mm).

3.2. Sediment metal concentration

The concentrations of metals in the test sediments taken from mesocosms at the start of the experiment are given in Table 1. The tailing and control test sediments were differentiated mainly by the concentrations of copper. Over the course of the experiment, concentrations were between 600 and 1100 mg/kg in TA, around 900 mg/kg in TB, and between 30 and 50 mg/kg in the control material (Fig. 4).

Results of tests of mobility of metals under weak acid conditions (TCLP procedures) found detectable levels only for copper (1.1 mg/l and 1.7 mg/l for TA and TB) and zinc (0.15–0.47 mg/l in TA and TB). All other metals were below detection.

3.3. Natural sedimentation

It was anticipated that the narrow microcosm tubes would act as sediment traps and gradually collect natural sediment, but that

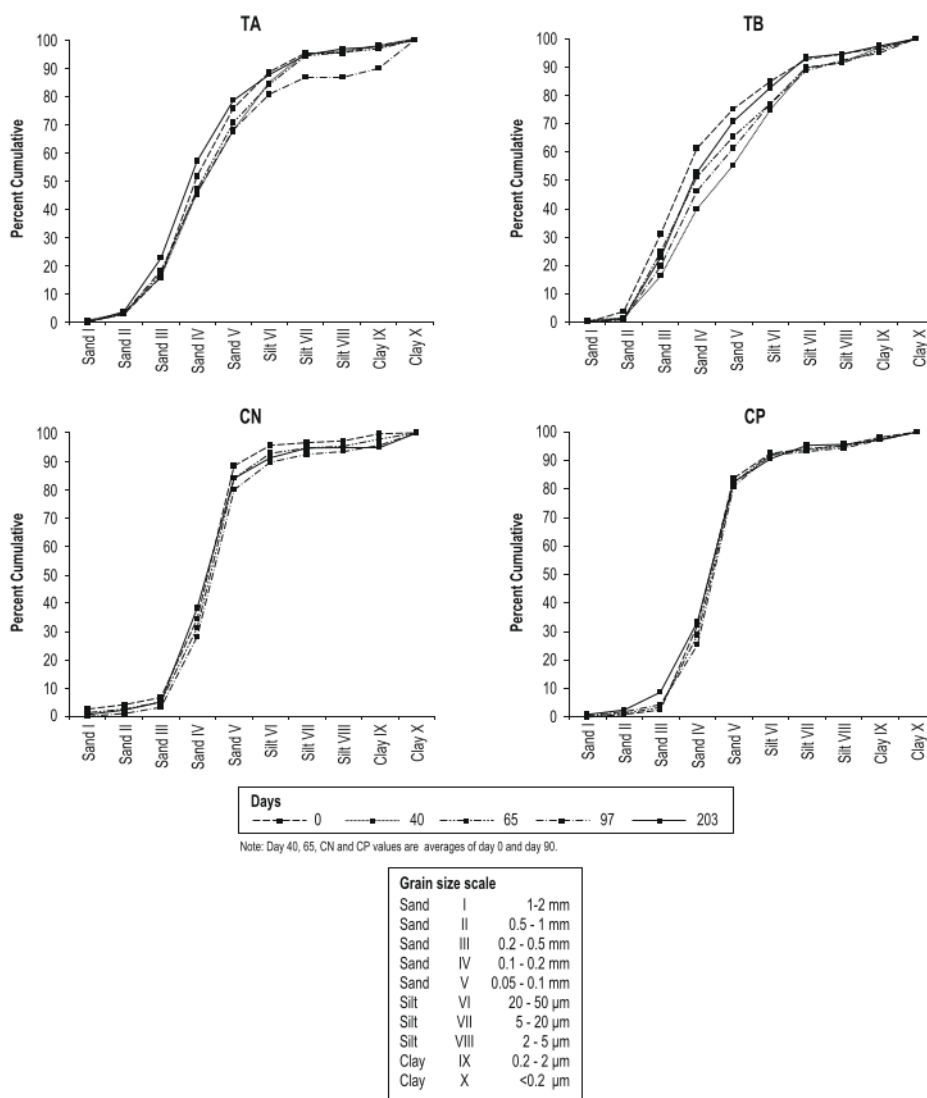


Fig. 3. Grain size distribution of CN, CP, TA and TB sediments.

Table 1
Analysis of metal concentrations in test samples.

Analysis	DL ^a	CP	CN	TA	TB
<i>Total metals</i>					
Al	6	25,600	26,100	10,200	20,900
As	1	2	2	2	1
Cd	0.1	ND	ND	ND	ND
Cr	1	10	8	11	8
Cu	0.2	31.1	31.0	1,110	944
Fe	1	21,400	19,400	26,300	33,700
Pb	1	ND	ND	ND	5
Mn	0.2	175	180	390	265
Hg	0.001	0.015	0.031	0.020	0.005
Se	1	ND	ND	ND	ND
Ag	0.4	ND	ND	ND	ND
Zn	0.1	18	31	53	77

^a DL = detection limit. Units of mg/dry kg (total metals).

in the much wider mesocosm drums, most sediment would continue to drift in suspension. The depth of accumulation of sediment in the (initially) empty microcosm tube at each sampling period is given in Table 2, and averaged a little over 2 cm per month for the first 3 months, decreasing thereafter. In contrast, there was no perceptible accumulation of any sediment in the mesocosm drums at the end of the experiment, as indicated by the level markers fixed to the drum walls, so the surfaces comprised test material throughout the duration of the experiments.

3.4. Total organic carbon

The concentrations of TOC in both TA and TB samples were initially about half those in the CP and CN samples (see Fig. 5). In the microcosms, TOC gradually increased over the duration of the experiment (particularly in the TB) and reached similar levels to CN and CP after 203 days. No similar increase was observed in the tailing mesocosms (apart from one anomalously high value in TA after 40 days). This is consistent with the lack of accumulation of drifting material in the mesocosms compared with the narrower microcosm tubes.

3.5. Colonisation

Table 3 compares the assemblages of meiofauna in the samples taken from the study area at the start of the experiment with those

Table 2
Sedimentation rate in microcosm tubes.

No	Date of recovery	Day	Settled height (cm)	Rate of settlement (cm/day)
1	12-Aug-05	0	0	
2	20-Sep-05	40	2.7	0.068
3	15-Oct-05	65	4.8	0.074
4	17-Nov-05	97	7.1	0.073
5	03-March	203	8.3	0.041

in the drift material that had settled in the (initially) empty settlement tube after 40 days. Some variance between grab samples 1–3 is evident but the numerical dominance of nematodes over harpacticoid copepods is apparent. The settled drift material contained a similar range of taxa, but with a reversed numerical dominance of harpacticoid copepods over nematodes.

The average numbers of individuals and numbers of taxa (+standard errors) observed in the samples recovered after 40, 65, 97 and 203 days are given in Figs. 6 and 7 for mesocosms and microcosms, respectively. In the mesocosms, the average abundance of meiofauna in the CP samples declined from the initial (natural) level of around 900/10 cm² (Table 3) to 570/10 cm² at 40 days, thereafter stabilising at around 200 individuals/10 cm² until the end of the experiment. During the same time, numbers colonising the CN material increased from zero to a slightly lower 'equilibrium' level of around 100–160 individuals/10 cm². In the TA samples, colonisation was slower, but reached similar numbers to the CP and CN samples at 97 days, and exceeded both control groups after 203 days. Colonisation was slower in TB than in TA, with very few organisms observed after 40 days but thereafter increased, and after 203 days, numbers in the TB mesocosm were higher than in all other samples. The average numbers of taxa colonising the mesocosms followed the same pattern as described for abundance, with numbers of taxa stabilising at 5–7 taxa.

Colonisation in the microcosms (Fig. 7) showed some similarities to the mesocosms. The total abundance in CP decreased from the initial numbers, while the numbers in CN increased and reached similar levels after day 65. However, there was comparatively higher colonisation in TA compared with all other samples throughout the experiment. There was no colonisation of TB after 40 days, but numbers were similar to those in CP and CN after 97 and 203 days. The abundance of taxa colonising the microcosms was consistently lower (1–6 taxa) than in the mesocosms.

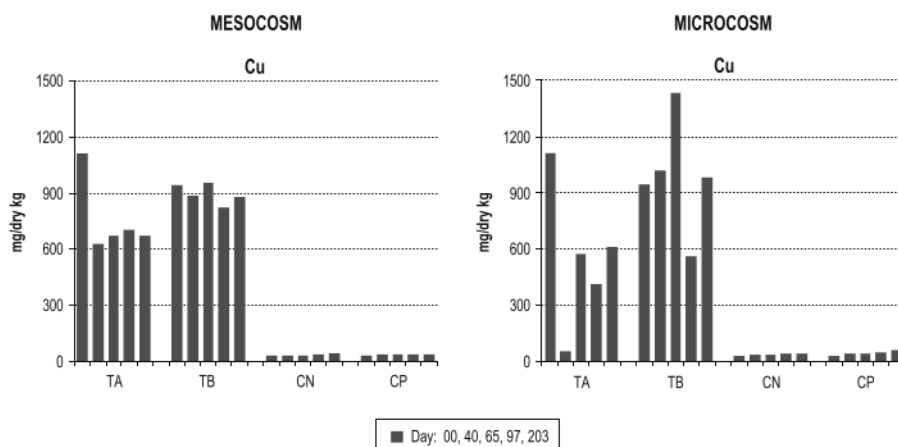


Fig. 4. Concentration of copper in test sediments throughout the experimental period.

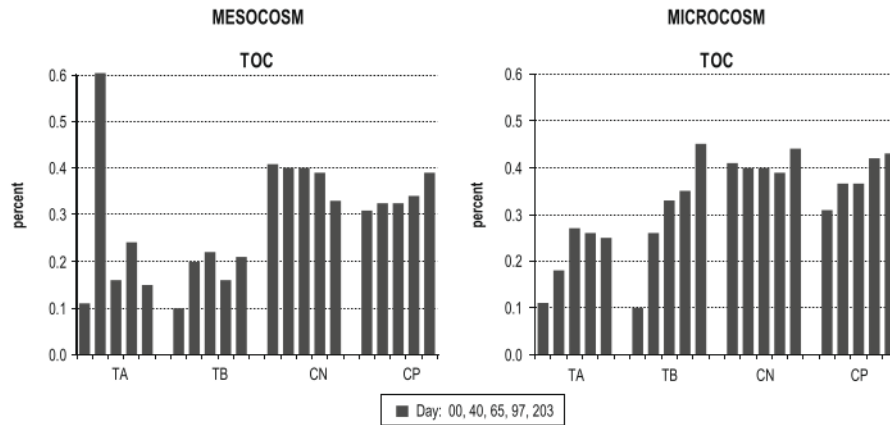


Fig. 5. Concentration of total organic carbon (TOC) in test sediments throughout the experimental period.

Table 3
Composition and abundance of meiofauna in sediment at the Benete Bay study site at the start of the experiment.

Taxa	Meiofauna in CP at start of experiment (individuals/10 cm ²)			Meiofauna in sediment settled after 40 days (individuals/10 cm ²)
	Sample 1	Sample 2	Sample 3	
Amphipoda	5.6	0	2.8	55.0
Harpacticoida	201.5	370.4	139.4	837.0
Kinorhyncha	1.4	0	0	0
Nematoda	314.1	456.3	1000.0	421.3
Oligochaeta	43.7	2.8	4.2	5.6
Ostracoda	1.4	0	0	22.4
Polychaeta	16.9	12.7	14.1	55.5
Turbellaria	15.5	25.4	23.9	23.9
Nauplii	22.5	62.0	5.4	95.2
Bivalve larvae	11.3	0	0	0
Acari	2.8	0	0	8.1
Tanaidacea	0	0	0	21.4
Total	621.2	919.7	1191.3	1545.4

Fig. 8 shows the composition of the main taxonomic colonising mesocosms and microcosms. Nematodes were the dominant group, particularly in the CP mesocosm and in all mesocosms after 203 days, but harpacticoid copepods were the dominant colonisers in CN and TA after 40 days, and in TB after 65 days. In the microcosms, nematodes were clearly dominant after 203 days.

3.6. Depth of colonisation

Fig. 9 shows the depth of colonisation in each 2 cm horizon from the surface to 10 cm depth. In the CN, TA and TB mesocosms, colonisation was highest at the surface (0–2 cm) after 40 days, but extended to include all horizons sequentially with time, with abundance generally inversely proportional to depth. The pattern was less distinct in the microcosms, as it was not always possible to obtain samples from all horizons in the microcosm tubes, because the material in the tubes was not always sufficiently consolidated to sample all layers effectively, particularly from the 40- and 65-day samples.

3.7. Statistical analyses

3.7.1. Univariate analyses

The data for mesocosms and microcosms were explored using SPSS (version 15), and abundance was square-root transformed

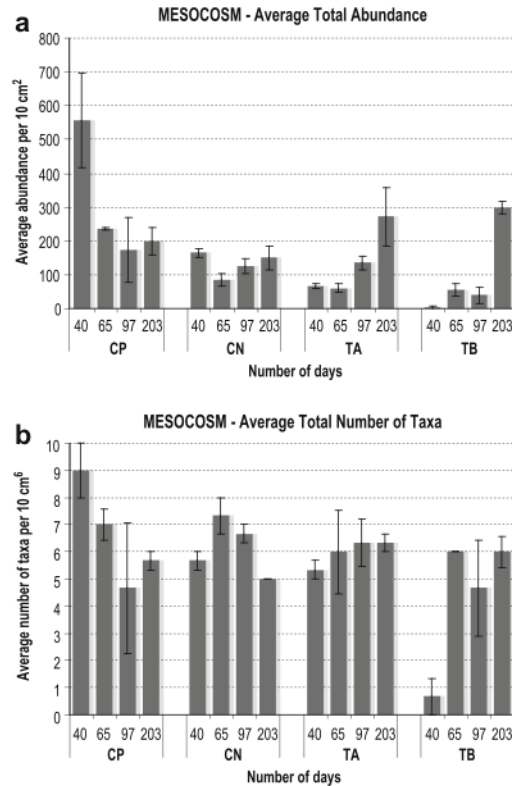


Fig. 6. Average abundance and numbers of taxa (±se) for all mesocosms.

in order to meet normality requirements. No transformation was necessary for numbers of taxa, and the variances of both dependent variables were homogeneous (Levene's test). Results of two-way ANOVA found significant interactions between time (days) and treatment (CP, CN, TA, TB; $P < 0.05$) for both the abundance and numbers of taxa in meso- and microcosms. The effect of the four treatments on the abundance and numbers of taxa in meso- and microcosms at each of the four time intervals was tested by one-way ANOVA.

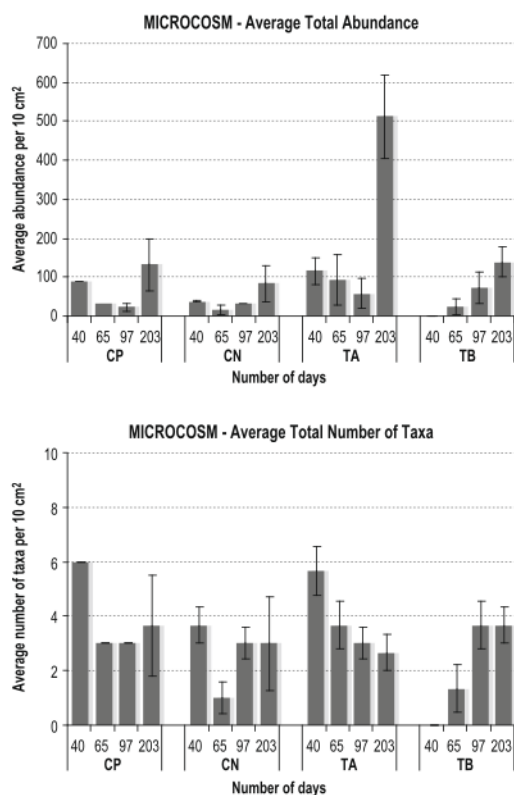


Fig. 7. Average abundance and numbers of taxa (\pm se) for all microcosms.

For the mesocosms, after 40 days there was a significant effect of treatment on abundance of meiofauna ($F = 34.336$, $P < 0.001$, df 3,8) and Tukey's post hoc tests showed CP significantly different from the other three treatments, CN significantly different from TB ($P < 0.05$ in both cases), and no difference between CN and TA. After 65 days, there was still a significant effect of treatment on abundance in the mesocosms ($F = 18.275$, $P = 0.001$, df 3,8), with post hoc tests at this time showing CP significantly different from the other three treatments, but with no significant difference between CN, TA or TB ($P > 0.05$). After 97 and 203 days, there was no significant effect of treatment on abundance in the mesocosms ($F = 0.863$, $P = 0.498$; and $F = 1.97$, $P = 0.197$ for 97 and 203 days, respectively). Similar results were found for numbers of taxa in the mesocosms. After 40 days, there was a significant effect of treatment ($F = 28.178$, $P < 0.001$, df 3, 8) with CP and TB significantly different from each other and also different from CN and TA; the latter two were not significantly different from each other. However, for numbers of taxa, there was no significant difference between the treatments after 65, 97 or 203 days.

In the microcosms, there was a significant effect of treatment on abundance ($F = 16.519$, $P = 0.005$, df 3, 8) after 40 days but insufficient cases for post hoc identification of treatment level. However, after 65, 97 and 203 days, there was no significant effect of treatment on abundance ($P > 0.05$ in the three cases). For numbers of taxa in the microcosms, there was a significant overall effect of treatment after 40 days ($F = 10.051$, $P = 0.015$, df 3, 8) but insufficient cases for post hoc identification of treatment source. After 65, 97 and 203 days, there was no significant effect of treatment on numbers of taxa ($P > 0.05$ in the three cases).

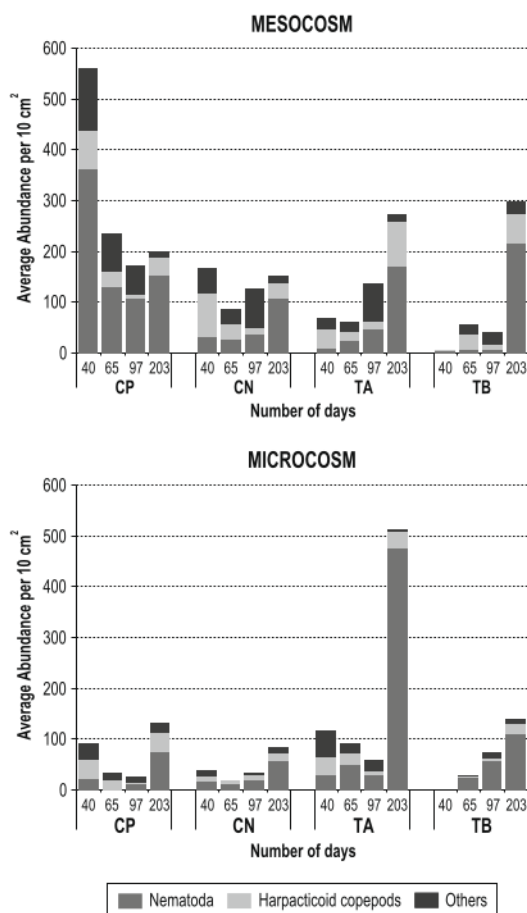


Fig. 8. Composition of the main meiofaunal groups colonising mesocosms and microcosms during the experiment.

These results are consistent with the trends apparent in Figs. 6 and 7, with clear differences in abundances in mesocosms detected between CP and all other treatments after 40 and 65 days, and numbers becoming similar in all treatments after 97 and 203 days.

3.7.2. Multivariate analyses

The two-dimensional MDS ordination plots based on assemblages of meiofaunal groups in the four different mesocosm test materials after 40, 65, 97 and 203 days (Fig. 10 upper) shows progressively closer clustering (and hence similarity) of the CP, CN, TA and TB samples over time. The 97-day and 203-day clusters are separated from each other, indicating some changes in assemblages common to all treatments between these two times. Results from the microcosm experiments (see Fig. 10 lower) are generally consistent with those of the mesocosms, with progressively greater similarity of assemblages in treatments over time. In both cases, the TB-40 day sample is not included in the MDS ordination plot (because of low and zero numbers in the mesocosms and microcosms respectively, and collapse in the ordination plots). Results of two-factor ANOSIM tests of similarity matrices showed no significant differences (at 5% level) in assemblages between all combinations of 40, 65, 97 and 203 days except 40 vs. 65 and 65 vs. 97. This result was the same for both mesocosms and microcosms. There were no overall differences between treatments CP, CN, TA and TB over all times combined.

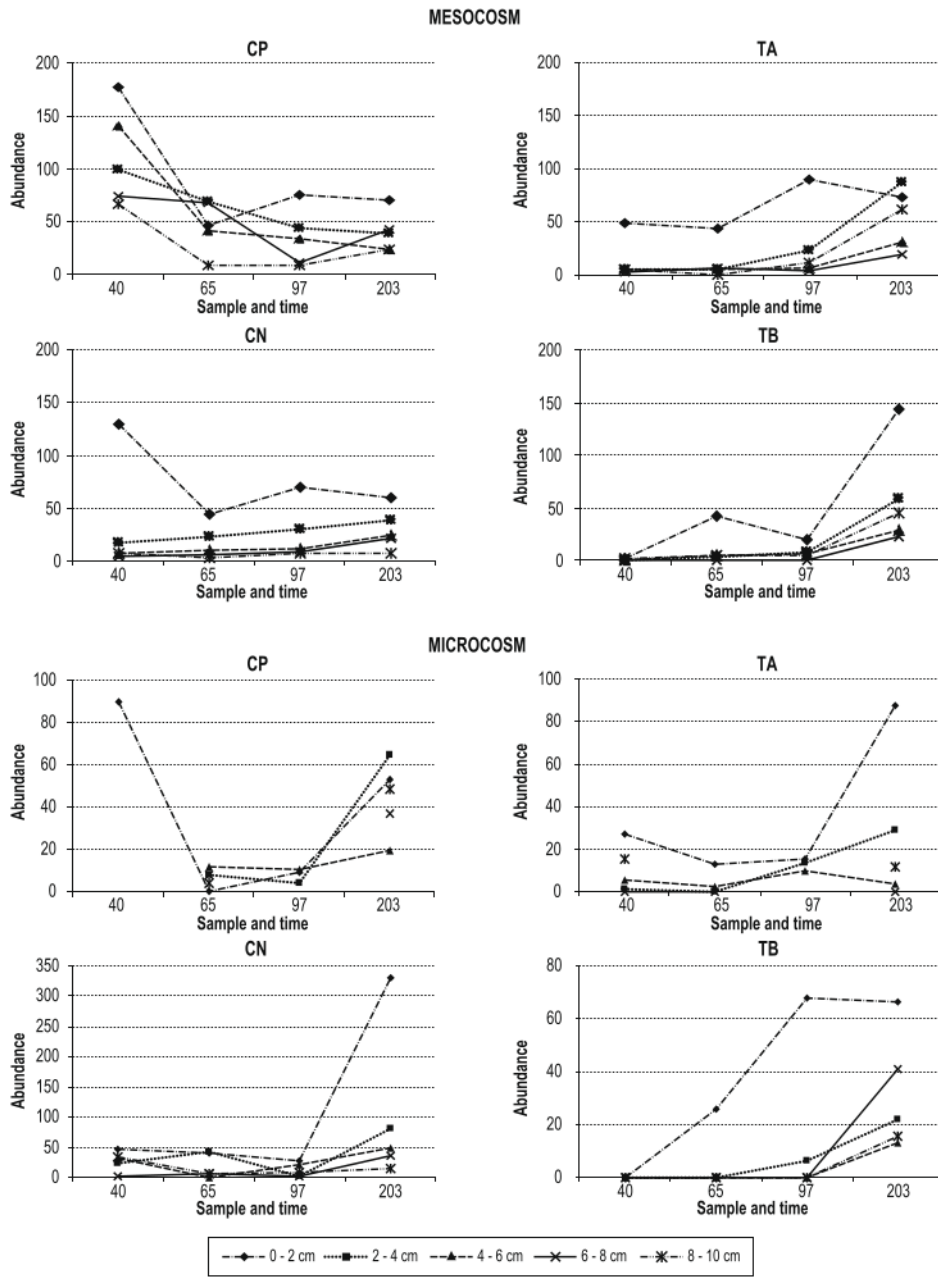


Fig. 9. Depth of colonisation in mesocosms and microcosms during the experiment.

4. Discussion

4.1. Physical aspects

The four test materials were similar in all physical and chemical measurements, except for the substantially higher concentration of total copper and detectable TCLP copper in the two tailing samples. While the total copper in the tailings is relatively high, studies by CSIRO (2005) using the Australia and New Zealand sediment qual-

ity guidelines methodology (ANZECC/ARMCANZ, 2000), found that only 4% of the total copper in run-of-mine tailing (i.e., TA) was in a potentially bioavailable form. The proportion of bioavailable copper in TB is not known, but may be higher than TA given its slightly higher copper TCLP measure. The initial inhibition of colonisation in TB may result from more readily leachable oxidized copper, as this tailing was derived from partially oxidized stockpile material. The effect decreased with time, probably through gradual leaching and geochemical stabilization of metals within the tailings sedi-

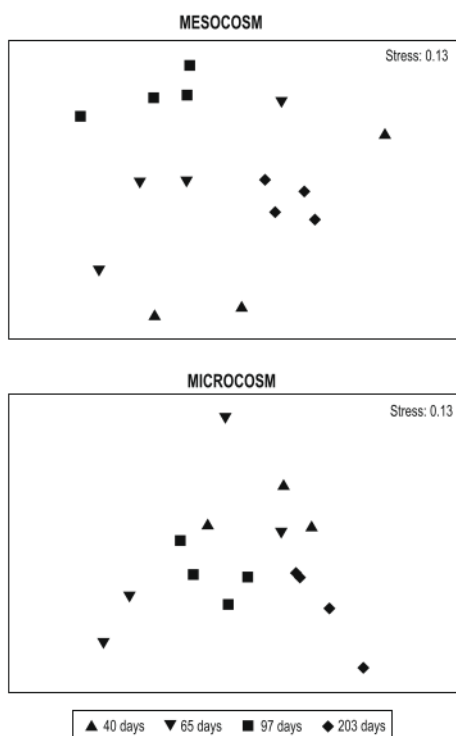


Fig. 10. MDS ordination plots for meiofauna communities in mesocosms and microcosms.

ment. Under actual field conditions the tailings would be leached and mixed with natural material during the turbulent descent of the tailing to the final deposition depth; a process that the present experiments did not simulate.

4.2. Recolonisation

As it was not practicable to conduct the experiments at the actual depths of tailing deposition (>3000 m) or on organisms obtained from those depths, the study was carried out at divisible depths, with the underlying assumption that the colonisation by meiofauna in the experiments would represent the same potential at the depths of tailing deposition, although at a faster rate. The regular observance of meiofauna in samples of tailing recovered from these depths during routine monitoring lends support for the assumption, and the main test was whether any inhibitory effect of the tailing on colonisation compared with natural sediment would be detectable. The overall finding was an increase in the numbers colonising the defaunated CN, TA and TB material from zero to levels statistically indistinguishable from those in the CP material after 97 days.

The decline from the initial levels in the CP material shows an effect of the experimental chambers, most likely a result of the reduced depth of oxygenation in the confined sediment, with this effect more marked in the smaller and narrower microcosms. The similar rate and levels of colonisation in the defaunated CN and barren TA material indicated no detectable inhibitory effect of the TA. In contrast, colonisation in TB was very low during the first 40 days, suggesting an effect of the higher proportion of bioavailable copper in TB.

Colonisation of defaunated sediment is reported in the literature to be relatively fast, and most microcosm experiments run

over periods of 30–50 days are sufficient to compare assemblages in defaunated and test material (Schratzberger et al., 2000a, b; Gyedu-Ababio and Baird, 2006). However, these microcosm experiments are typically inoculated with natural sediment containing the meiofauna and do not rely on passive input. Arroyo et al. (2006) found that colonisation of defaunated material by meiofauna attached to drift algae occurred in a matter of days, although partitioning between the settled algae and sediment differed between meiofaunal groups. In field studies, Schratzberger et al. (2004) found that nematode communities in defaunated control and high sand content (dredge-simulated material) became similar after 3 months, but return to ambient communities took much longer in organically enriched material.

In tailing deposited on the floor of a fjord at 160 m depth after 25 years of operations of the Island Copper Mine in Canada, Ellis (2000) found that in the areas where tailing deposition was less than 1 cm/year, or up to 25 cm during the mine's life, seabed biodiversity could not be distinguished from natural seabed benthos unaffected by tailing, and a deposition rate of 20 cm per year was found to be the threshold for significant impacts to benthic biodiversity.

The experiment also examined the extent to which recolonisation of settled tailing would be dependent on subsequent burial by natural sediment, as would occur after the closure of the mine. Over the 203-day duration of the experiment, the microcosm tubes acted as traps for drifting sediment, which accumulated to a depth of 8.3 cm. In contrast, no accumulation of natural sediment was observed in the wider mesocosm drums. In this way, the microcosms mimicked natural burial of deposited tailing, (potentially more rapidly than at 3000 m), while the mesocosms represented the un-buried tailing surface.

In the microcosms, most of the colonisation was observed in the overlying natural material, but analyses of meiofauna in 2 cm depth horizons showed that meiofauna also colonised the underlying TA and CN material, assuming the interface between tailing and settled material remained distinct. In the mesocosms, all colonisation was observed in the test material, as there was no measurable accumulation of natural sediment. This shows that the tailing is colonisable prior to any subsequent burial by natural deposition.

The potential sources of re-colonising meiofauna in the CN, TA and TB samples were from outside the chambers, on drifting particles or attached to other macrofauna finding their way in, while in the CP material, the assemblages were a combined result of survival of the original organisms and those introduced as for CN, TA and TB. Analysis of the meiofaunal groups in the settled drift particulate material showed some differences in the proportions of nematodes and harpacticoid copepods, with the latter being dominant in the settled drifting material. The harpacticoid copepods were dominant in many of the samples analysed after 40 and 65 days, as would be expected if they are dominant in the drifting material, whereas nematodes clearly resumed dominance after 203 days. Copepods are known to be mobile components of the meiofauna, dispersing on drifting algae (Gwyther and Fairweather, 2005; Armonies, 1998).

The present experiments were conducted over an extended period, in order to apply the results to seabed conditions in 3000 m depth, where changes would be expected to be slower, given the lower temperatures, food availability and faunal densities. Had the experiments been terminated after 40 or 65 days, (as with many other microcosm studies), the conclusions would have been different. The lack of any colonisation in TB after 40 days clearly indicates some initial inhibitory process. However, after 97 and 203 days, both the TA and TB were colonised by 9–10 meiofaunal groups and with assemblages and abundances that were not significantly different from the CP and CN controls. Similar processes

would be expected at the actual depth of tailing settlement, although potentially at a slower rate.

5. Summary

Colonisation by meiofauna was observed after 40 days in tailing from freshly mined ore (TA) and after 65 days in stockpiled, weathered ore (TB). Abundance in the defaunated controls (CN) and both tailing samples increased from zero to similar levels after 97–203 days, with no significant difference in communities, numbers of meiofauna or numbers of taxa between any of the treatments including CP. The present experiments have demonstrated that settled tailing was recolonised, with communities indistinguishable from controls after 97 days. Recolonisation was not dependent on accumulation or burial by natural particulate material.

Acknowledgements

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