APPENDIX 1 - WATER COLUMN AND SEDIMENTARY ENVIRONMENT

1.4 Cruise Report: Thiobacteria Survey MV Snowgoose

ENVIRONMENTAL VERIFICATION

CRUISE REPORT

THIOBACTERIA SAMPLING

Prepared for:

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24 February - 1 March 2014

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ML 170

THIOBACTERIA SAMPLING PROGRAMME CRUISE REPORT

for:

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DATES: 24th February to 1st March 2014

OBJECTIVE

To:

- Collect 10 grab samples from previously sampled sites in ML170, SP-1 area, intended for environmental lab analysis at the University of the Western Cape for thiobacteria.
- Collect 2 additional verification site cores intended for environmental laboratory analysis at the Council for Scientific and Industrial Research (CSIR), Stellenbosch. These fill earlier gaps.
- Obtain accurately positioned samples with QC /QA full integrity.

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1 INTRODUCTION

Sampling Cruise # 29 was undertaken to complete an environmental verification programme in ML170. A set of bottled thiobateria samples was taken from 10 grab samples sites. Two gravity core samples were also taken from ML170 filling two gaps in the sampling grid of the environmental verification assessment of August 2013. The thiobacteria samples were required to be collected in a contamination free manner which meant that handling protocols precluded any contact with skin. The thiobacteria samples and the two environmental cores were to be cooled and kept frozen to -18 deg °C from time of collection and during delivery to UWC and CSIR in Cape Town.

2 HISTORICAL BACKGROUND

Phosphatic nodules were first recovered off the Agulhas Bank, South Africa in 1891. In 1978 Dr M. Bremner, of the Marine Geoscience Unit at the University of Cape Town, completed his PhD thesis on "Sediments on the continental margin of South West Africa between Sylvia Hill and the Kunene River.", for which a regional set of grab and core samples was collected along the coastal waters of Namibia. Since then there has been a steady stream of research on the marine sediments on the Namibian continental shelf including the research cruises of the R V Benquela and the R V Professor Logachev. In 1992 and 1994 Gencor drilled 50 vibrocorer holes, mainly in EPL 3414 and 3415 and at waters depths of between 200 and 300 m though some holes did stray into EPL 3323. Though data only exist for 38 of these holes, the results were very encouraging with grades between 15 and 22% P_2O_5 and thicknesses in 8 holes in EPL 3414 lying between 2.62 and 5.05 m, Peripheral areas displayed thicknesses of between 1 and 2.0 m. However, bulk sampling in 2004 by the Ocean Phosphate Consortium in EPL 3415 demonstrated the local continuity of grade and thickness in an area where grades lay between 16.66 and 18.35% P₂O₅ and thicknesses between 3.1 and 6.36 m. The above results provided sufficient evidence to support the view that the Sandpiper Project Area (ML170) could host significant quantities of phosphate, a view that was later confirmed by the 2008 to 2013 grab and core sampling programmes (Cruise Reports 1 to 28).

3 SAMPLING VESSEL

The MV *Snowgoose*, an 18.83 m wooden vessel with a gross tonnage of 63.63 tonnes owned by Carpé Diem Namibia cc. based in Lüderitz, was again used as a platform for the sampling program. The crew consisted of 12 people including the skipper/owner Lukas Els.





Figure 1: Sampling vessel: MV Snowgoose.

4 SAMPLING EQUIPMENT

4.1 GRAVITY CORER

The third generation 600 kg gravity corer was taken on this cruise (No. 29). Additional weights were also carried to bring the corer weight up to 750 kg if required. The gravity corer comprises a spooling head section and attached to the head section is a 3m or 4m long steel pipe section that carries a 3 m or 4 m long 75 mm or 90 mm internal diameter plastic sample tube. On this cruise a 100 mm steel pipe and 90 mm core tube setup was used for the two environmental cores, while the 75 mm tubes were used for the 100 EPL cores. The 75 mm tube provides about 6.3 kg of material per metre of core while the 90 mm tube provides about 9.2 kg per metre. The 75 mm core tubes are fitted with one-shot aluminium can core catchers riveted and glued in. For the 90 mm tubes core catchers are made from clean oil cans which have slightly thicker sheet metal.





Figure 2: Third generation gravity corer in use.

4.2 GRAB SAMPLER

Samples were to be taken with a modified Van Veen grab sampler. The grab was redesigned to recover samples without flushing the fine sediment out of the grab during recovery. The grab had a scoop volume of 20 litres, with a 425 x 400 mm bite footprint. The grab can take a bite into the sediment of approximately 20 cm depth. The grab was deployed over the side of the vessel by means of a 7 tonne Atlas crane and a free fall winch. The winch cable was fed through a pulley attached to the crane, which allowed the handling of the grab over the side of the vessel before and after deploying the grab to the seabed. The grab was modified to effectively trigger only on impact with the seabed.

5 SAMPLING METHOD

5.1 GRAB DEPLOYMENT

The grab sampler is primed on the vessel gunwale and readied for deployment using the vessels lighter winch (10 mm cable). After lifting the grab over the side, the winch is released, allowing the grab to free-fall to the seabed where it triggers. The GPS position of each grab is recorded when it lands on the seabed on two independent GPS units.





Figure 3: Grab sampler illustrating seawater venting mesh (left). Gravity corer and pipes lying on deck (right).

5.2 GRAVITY CORER DEPLOYMENT

The gravity corer is deployed using the vessels main winch. This winch is not a free fall winch. The gravity corer works with a lever controlled mechanical braking system. A 50 kg weight on a 5 m cable keeps the brake in a locked position while the gravity corer is lowered towards the sea bed on the main winch cable (16 mm). When the 50 kg weigh reaches the sea bed the weight on the lever goes slack, releasing the lever, which allows the brakes to release. The gravity corer then takes a controlled drop of up to 5 m to the seabed on a built in 13 mm wire spool that sits at the top of the corer.

The GPS position of each gravity core is recorded when it lands on the seabed on two independent GPS units.

5.3 RECOVERED GRAB SAMPLES

For the specific purpose of this programme each of the 10 grab samples was collected and recovered onto the vessel deck. At this point the grab is not opened in the usual manner. Instead the sediment was accessed through the sea water venting mesh on the top of the grab. In this manner a series of four sub-samples per grab were recovered and bottled in 500 ml Nalgene bottles in a sterile manner and preserved with RNALater (a specialised compound for preserving the thiobacteria in the sediment). The bottles were then stored in a chest freezer and kept frozen all through the transportation to Dr Bronwyn Kirby of the University of the Western Cape, Next Gen Sequencing Unit and Institute for Microbial Biotechnology and Metagenomics. The sampling process was filmed and photographed for audit purposes.

5.4 RECOVERED GRAVITY CORE SAMPLES

The core tube is unbolted and pulled free from the steel core barrel. The depth of the core penetration into the sediment is determined from the mud line on the outer core barrel. A retaining cap is immediately put on the catcher end and the extent of the core fill is determined by inserting a wooden pole into the inner tube to tag the top of the sediment and the internal length of core recovered is recorded. A hacksaw is used to cut the inner tube to the length of core fill so that the other retaining cap can be put on. It may need two cuts to get to a snug fit with the top of the sediment.

The sequential sample number is marked on the end cap of the core with a permanent marker and a corresponding sample tag goes inside the core. Samples are labeled with a sequential 4-digit number, which is logged with the temporal, positional, penetration and licence information on the master logging sheet. The top and bottom of the core is marked.

6 SAMPLES

The two ML 170 cores (90 mm barrel) were frozen and taken to Lüderitz for onwards transport in two deep freezers to CSIR Stellenbosch for environmental analysis. The 10 thiobacteria samples were frozen and delivered to UWC, Belville, Cape Town.

Cores:

Licence	Numbers	Water depth min	Max	Cores / Sites
ML170	2982 - 2983	195	200	2 / 2

Grab Samples:

Licence	Numbers	Water depth min	Max	Grabs / Sites
ML170	T 01 to T 10	195	227	1 / 10

7 POSITIONING

Positioning and depth was recorded by means of a Furuno Navigation system combining a PS-8000 GPS, video echo-sounder and chart plotter. The positioning system is checked both before and after the cruise at a fixed reference point in Lüderitz harbour (the end of the Ministry of Fisheries and Marine Resources jetty). A separate Garmin GPS linked up to the Maxsea Marine Software program was also used to confirm the accuracy of the main GPS on all samples and at the reference point.

The latitude and longitude positions of all planned sample sites (see this report Annexure 1) were uploaded onto the Maxsea system and the vessel then occupied each site following the simplest cruise pattern. Once on the target position the gravity corer is lowered over the side and the actual position recorded when the corer hits the seabed. This is to account for any drifting of the vessel after the corer was deployed over the side. Each sample is recorded with a sequential number starting at 2901 and ending on 3002, along with coordinate information, sample size and other pertinent information. Data were hand recorded on pre-prepared work sheets and then entered into a database after the cruise. Failed core sites were also recorded. The same procedure was followed when taking the ten thiobacteria grab samples.

8 SAMPLE HANDLING AND STORAGE

The environmental cores are cut to length (halved or thirds), capped at either end of each marked up section, then stored flat in a deep freezer. Once in port the diamond inspector inspected the cores before being released for storage. They were then transported to the Carpe Diem facilities for temporary storage in the freezer before onward transport to the CSIR, Stellenbosch. In transit he thiobacteria samples were also kept in the freezer and these were delivered to UWC, Bellville. During road transport the samples are kept frozen in the freezer which was carried on a trailer and kept working using a generator on the tow vehicle. Temperature gauges are installed in the freezer unit, which allows for monitoring of the freezer temperatures during transportation.

9 CRUISE SUMMARY

The vessel left Lüderitz at 17:15 on Monday the 24^{th} February 2014 and set sail north to the northern end of ML170 with a strong SW wind (20 to 30 knots) and a 3 m swell. The vessel arrived at the concession area at 13:30 on Tuesday 25^{th} February with a 20 knot SW wind and 2.5 m swell. The vessel had to wait for an improvement in the weather for the next few hours while starting to get ready for sampling at 23:00 with a 5 knot SW wind and 2 m swell. Thiobacteria grab sampling started at 00:13 on the 26^{th} and was completed by 09:40 am. Problems were encountered with gloves (too small and easy to tear) and the GoPro cameras (various issues). Grabs filled reasonably well (20 kg plus) and consistently contained layer 1 shelly mud and phosphate.

Coring was completed at 02:45 on the 1st March and the vessel returned to Lüderitz arriving at 22:00 the same day. Cores and freezer samples were unloaded the following day.

10 QC PROCEDURES SUMMARY

The client representative verified;

- The effectiveness of the GPS positioning system (by comparison with an independent GPS and reference to a fixed position);
- The quality of sample collected (by following sampling procedures and observation);
- The reliability of the record keeping (records attached and each page signed off);
- Correct labeling of the samples;
- Good storage of samples (no leakage, and careful handling of samples).

Annexure 1: Thiobacteria Sample Locations

