APPENDIX 1 - WATER COLUMN AND SEDIMENTARY ENVIRONMENT

1.3 Project Execution Plan: Thiobacteria Survey MV *Snowgoose*

NAMIBIAN MARINE PHOSPHATE

VERIFICATION SURVEY

THIOBACTERIA SAMPLING

PROJECT EXECUTION PLAN

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1 INTRODUCTION

Namibian Marine Phosphate (NMP) is proposing to extract marine pelletal phosphate ore from deposits on the Namibian continental shelf. The phosphate will be extracted by dredging. The assessment of the environmental impacts associated with the project is therefore strongly reliant on a sound understanding of the sediment properties of the dredge area. The purpose of this verification survey is to confirm the sediment properties, water quality and local oceanographic processes of the seafloor and water column within the proposed dredge area. This information will then be used, in conjunction with previous scientific investigations of the region, to further inform the assessment of impacts on the marine environment as a result of the proposed dredging operations. This will aid in the final assessment of the impacts as detailed in the EIA / EMPR.

Following their review of the NMP Marine EIA for dredging of phosphate enriched sediments in ML 170, MFMR requested additions to the scope of work for the verification survey. One of the additions was to identify the presence or absence of thiobacteria within the mining licence area.

Thiobacteria, use hydrogen sulphide (H_2S) as fuel, effectively keeping the H_2S in the sediment and out of the water column. If the sediments are disrupted and the bacteria removed the H_2S may escape into the water column with (depending on scale and volumes) possibly significant impacts on the fauna and flora of the marine environment. Thiobacteria are ubiquitous on the Namibian continental shelf and generally in the world ocean. However, they have been shown to be temporally and spatially variable in the particulate organic matter (POM) enriched mud belt inshore of the proposed mining area (ML 170).

Although the presence of thiobacteria in ML 170 is suspected, given the sandy nature of the sediment (vertically and horizontally) compared to the H_2S-rich mud belt which is known to exist inshore of the mining area, the actual presence of the sulphur oxidising bacteria is questioned. Nonetheless, the detection of thiobacteria using the qPCR method, integrated with other Acid Volatile Source (AVS) assessments will provide a more robust indication of the potential presence and ecological importance of sulphur oxidizing bacteria in the survey area.

In order to determine the presence or absence of thiobacteria on the seafloor, the following survey is proposed:

Surface grab samples from 10 (ten) sample sites will be collected from the NMP licence area (ML 170). The samples will be recovered from the seabed with a van Veen grab deployed from the MV *Snowgoose*. 4 x 200ml sub-samples will be collected from each grab sample and these sub-samples (total 40) will then be stabilised and subsequently transferred to the University of the Western Cape, where they will be analysed for the presence of thiobacteria using the “real time Polymerase Chain Reaction” (qPCR) analysis procedure.

The samples are due to be collected during the last quarter of 2013.

2 FIELD SURVEY

2.1 LOCATION

The thiobacteria sampling sites are located within the NMP ML 170 Figure 2-1. These sites match those sampled during the NMP verification sampling programme, which focused on the northern enriched ore body called SP-1. 10 sample sites are targeted, these being the "T" [1]sites of the 26 verification sampling locations (SP-1). (Figure 2-2 and Table 2-1).

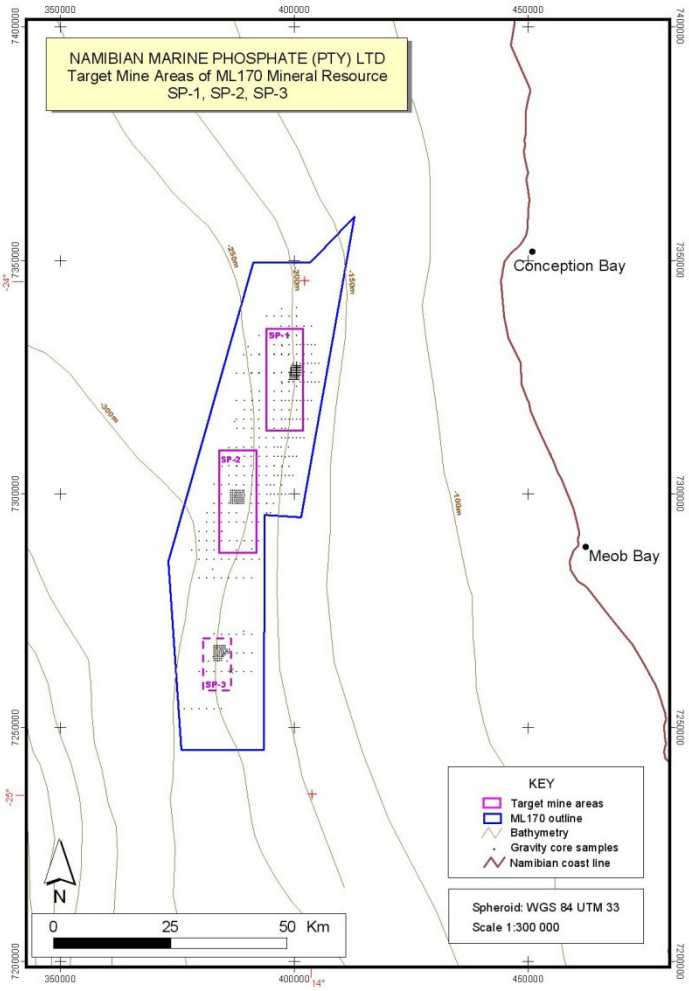


Figure 2-1: Regional location of the NMP Mining Licence 170.

[1] Provides for an east – west and north – south spatial assessment across the target mine site

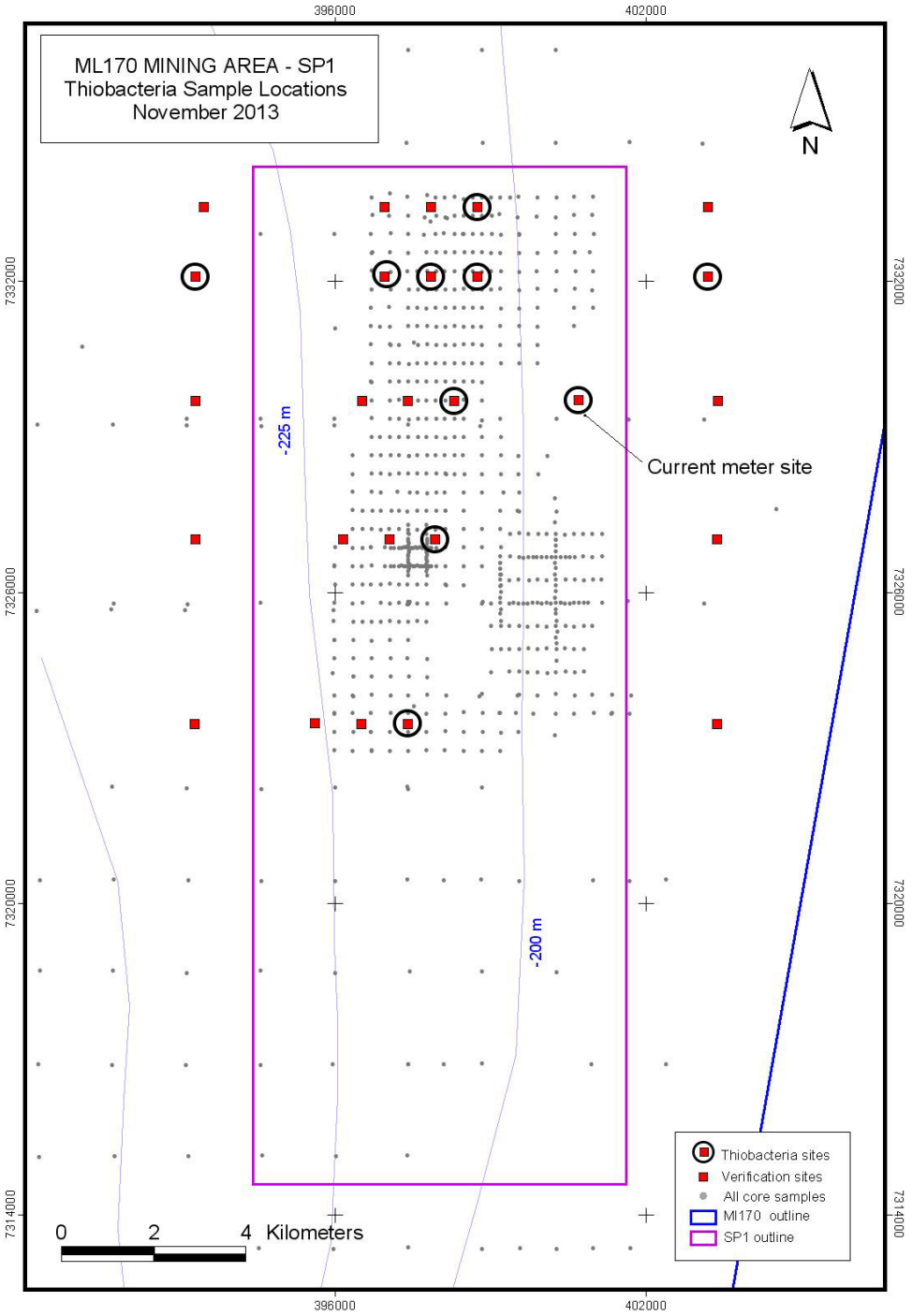


Figure 2-2: Planned thiobacteria sampling location in the SP-1 primary mine target area

Table 2-1: Co-ordinates of the planned thiobacteria sampling sites

Site	X	Y	Latitude	Longitude
1	397299	7323303	24°11.976'	13°59.324'
2	397888	7327305	24°09.810'	13°59.689'
3	398302	7330298	24°08.190'	13°59.946'
4	398802	7332998	24°06.729'	14°00.253'
5	397796	7332996	24°06.726'	13°59.659'
6	396803	7332998	24°06.721'	13°59.073'
7	392704	7332997	24°06.705'	13°56.653'
8	398800	7334498	24°05.916'	14°00.258'
9	403803	7332997	24°06.748'	14°03.205'
10	401016	7330084	24°08.316'	14°01.548'

2.2 TECHNICAL STAFF

The samples will be collected by the crew of the MV *Snowgoose*. Two of the crew (along with the NMP Representative) who normally collect the benthic samples will be familiarised with the thiobacteria sampling protocol.

2.3 SAMPLE COLLECTION EQUIPMENT

- Operational vessel (1) – MV *Snowgoose*
- Sampler (1 + spare) – van Veen Grab (of bite x m^2)
- Refrigeration (1) – chest deep freezer (-18 °C) with TidBits
- Bottles (50 units) – polypropylene (sterilised) (500 ml) – wide neck and cylindrical
- Sub sampler (4) – plastic scoop
- Hygiene (4 ℓ) – ethanol
- Fixing agent (1 ℓ) - RNAlater
- Gloves (box) – Nitrile
- Cloth (packet) – disposable cloth
- Temperature gauge – TidBit
- Photographic control – GoPro camera, with head strap mount and digital camera
- Waterproof marking paper and waterproof pens

3 SAMPLE PRESERVATION, CURATION AND QUALITY CONTROL

3.1 SAMPLING PROTOCOL

3.1.1 Prior to sailing

- Van Veen grab is to be tested and cleaned of any residual material (sea water wash).
- The freezer is to be operational for 48hrs prior to the collection of the samples.

- Temperate logging devices (TidBit) are to be placed in the freezer when it is first turned on.
- Sample collection equipment is to be verified against the list above (Section 2.3).

3.1.2 Sampling: Collecting a van Veen grab Sample

The standard van Veen grab sampling collection protocol is applied:

- One grab sample is collected at each of the stations.
- *Note*: For the van Veen grab sample to be acceptable for use the grab must be a minimum of 50 % full on recovery; otherwise another attempt on the site should be made before sub-samples are taken.

3.1.3 Sub sampling:

Please refer to:

1. The sampling protocol abbreviated list of sequential activities (This report, Annexure 1)
2. The Mock up bottle (This report, Annexure 2) and sample

3.1.3.1 Team

- The sub sampling team consists of two persons, Lead (LS) and Assistant (AS).

3.1.3.2 Preparation of the Equipment

- Technicians (AS & LS) are to be wearing a new pair or nitrile (disposable) gloves;
- The sub sampler (scoop) is to be cleaned with ethanol and disposable cloth before handling each sample;
- *Note*: the scoop may NOT come into contact with any item or person during the sampling process. Contact with human skin will contaminate the sample;
- 4 Nalgene bottles are required, inspect these to ensure that they have not been tampered with. Because they are sterile inside they should not be opened prior to use.
- 2 of the bottles are to have 50 ml of RNAlater poured in to them just before each grab sample is collected. The other 2 bottles remain dry.
- The bottles need to be labelled (Sample reference no.) with a permanent marker pen.

3.1.3.3 Collection of a Thiobacteria sub-sample

- Recover the grab to deck in a closed state.
- Open the inspection window and photograph the sediment in the recovered grab with station reference number label shown on the grab.
- Using the scoop the AS is responsible for collecting the 200 ml sample from the van Veen grab via the inspection window;
- LS is responsible for ensuring the that inspection window is open and that the sample bottles are prepared, this includes numbering and labelling;
- LS is responsible for sealing the bottles. Each bottle should be sealed immediately after the sample has been inserted;

- The 2 sample bottles containing the RNAlater (50 ml) and the sediment (200 ml), need to be shaken to distribute the liquid throughout the sediment sample;
- Upon a bottle being sealed it is to be placed in the freezer immediately;
- LS is responsible for ensuring that the bottles are sealed, correctly labelled and stored safely and securely (upright and do not roll around) in the freezer unit.

3.1.3.4 Photographing the Grab: Referencing the sample

- For each grab recovered from the sea bed, the sample number must be clearly written on waterproof paper, this number must be placed on the grab next to the inspection window and a photograph taken [DO NOT put the waterproof paper into the grab and on the sediment.]. Check the quality of the image to see if the sediment is recognisable as more than one attempt may be necessary to get a decent shot.

3.1.3.5 Bottle labelling & Marking

- Labels must be written on each bottle (and lid), these labels must correspond to the photograph and the datasheet entry (see example bottle in Annexure 2 of this report):
- e.g. for station No.1: T001-RNA-1 : T001-RNA-2
- e.g. for station No.1: T001-Non-1 : T001-Non-2
- The sample number, followed by RNA 1 – first bottle with RNAlater in it
- The sample number, followed by RNA 2 – second bottle with RNAlater in it
- The sample number, followed by Non 1 – first bottle with NO RNAlater in it
- The sample number, followed by Non 2 – second bottle with NO RNAlater in it

Bottles must be labelled by writing directly onto both the side and lid of the bottle with a permanent marker. The waterproof paper label (after a photograph has been taken of the grab sample) must be placed with the labelled bottle into a plastic bag (provided). Once the sample bottle has been filled, the plastic bag containing both the bottle and the waterproof paper label can then be placed into the freezer.

3.1.3.6 Filming

The collection of the thiobacteria samples is to be filmed by the party collecting the samples (GoPro camera is to be head mounted), General deck operations (GoPro camera to be mounted on the bridge). The camera must be activated as the grab comes out of the sea. The camera is to be deactivated once the thiobacteria subsamples have been packaged. Filming of at least 5 of the 10 stations is required.
The following are key aspects of the process to be filmed:

- Grab coming out of the sea and on to the deck;
- Nitrile gloves being put on;
- Sub sampling scoop being cleaned with ethanol;
- Sample bottles being prepared (50 ml RNAlater added, labelling, bottle marking);
- Sediment (200 ml) collected from the grab using the scoop. The sample is collected via the inspection window and subsequently put into the sample bottles;
- Sample bottles transferred to the freezer unit;

Two GoPros will be provided, one should cover the general activities on deck during the deployment of the grab. The other, as a head mount on the technical team leader / support technical person.

3.1.3.7 Position Control

The station sites are pre-selected to be aligned with the SP-1 verification sample site locations, with 10 of the 26 sites being targeted in this instance. Once the vessel comes on to station using the vessel positing system, the actual position of the vessel is recorded. This position is considered to be the location of the collected sample. There will be drift, however, this is not considered to be significant.

Positioning and depth is recorded by means of a Furuno Navigation system combining with 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 Sea Fisheries Jetty). A separate Garmin GPS linked up to the Maxsea Marine Software program is also used to confirm the accuracy of the main GPS on all samples and at the reference point.

3.1.4 Transporting Samples

The samples are to be transported to Cape Town in the same freezer unit as used on the MV *Snowgoose*. The freezer unit is to be kept in working condition at all times.

The freezer with the samples is to be delivered to:

Dr Bronwyn Kirby
Next Gen Sequencing Unit/Institute for Microbial Biotechnology and Metagenomics University of the Western Cape South Africa
Tel: + 27 (0) 21 9593033 Cell +27 0730498075

3.1.5 Quality Control

In order that quality controls are maintained, the following actions are to be adhered to:

- The technical team is to be familiarised with the sampling protocol;
- The NMP company representative is responsible for ensuring the quality management of the sampling procedure (Form 001- is to be completed for each sample site);
- Nitrile gloves are to be worn during the sub-sampling, A new pair of gloves is to be used per grab location;
- Only the provided nalgene 500 ml bottles are to be used;
- Do not use bottles that may have been contaminated: only use bottles where the seals are intact;
- The nalgene bottles are ONLY to be handled by persons wearing nitrile gloves;
- The sub-sampling scoop is to be cleaned with ethanol prior to use at each site, the scoop must NOT make contact with skin or clothing prior to use. If this should happen, re wash with ethanol;
- The freezer unit must be operating at full capacity for 48 hours prior to samples being stored within the unit;
- The collection of the thiobacteria samples is to be filmed by the party taking the sample, the GoPro camera is to be head mounted.

3.1.6 Analysis

This is to be carried out by Dr Bronwyn Kirby of the Next Generation Sequencing Facility/Institute for Microbial Biotechnology and Metagenomics, University of the Western Cape, South Africa.

ANNEXURE 1: SUMMARY OF SAMPLING PROTCOL

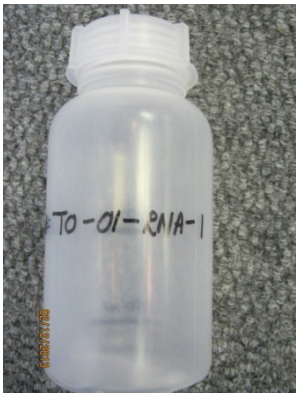
1. Sample labels are to be pre prepared on waterproof paper, and bottles are to be labelled as per Annexure 2 of this report.
2. Freezer unit to be activated 48 hrs before departure of vessel and temperature gauges (TidBit) to be inserted in the freezer
3. Van Veen grab to be checked and made ready for use at sea
4. Bottles to be kept in black storage containers prior to use
5. Both GoPro cameras to be activated (ensure they are charged prior to survey)
6. Van Veen grab to be deployed
7. Check that the recovered grab contains greater than 50% sediment
8. Recovered grab with station number to be photographed (referencing)
9. A new pair of nitrile gloves to be worn by the sampling team for each sample
10. 4 bottles to be prepared - labelled
11. Scoop to be cleaned with ethanol
12. Do not open the bottles before they are required for use
13. 50 ml RNAlater (fixing agent) to be added to each of 2 bottles
14. The other two bottles will only have sediment in them
15. 200 ml sediment to be put into each of the 4 sample bottles
16. Access the sediment through the inspection window, do not open the grab
17. The plastic scoops may need to be cut to fit the window of the grab.
18. The bottles with 50 ml RNAlater and sediment are to be shaken thoroughly
19. Ensure that all bottles are sealed and labeled correctly
20. Put each sediment filled bottle with its corresponding waterproof label into a transparent plastic bag
21. Put the plastic bag with the sample and its label immediately back in the freezer
22. All bottles (in their plastic bags) to be put into the black crates which must be in the freezer
23. Ensure that bottles are standing upright within the crates
24. Ensure that the freezer seals correctly
25. Dispose of nitrile gloves and any other waste products
26. Ensure that the sample record log is completed and signed off
27. Rinse out the grab prior to re-deployment
28. Turn off the GoPro cameras
29. Sail to the next station
30. Repeat the process.

NOTE: It is imperative to ensure that human skin does not come into contact with sample.

NOTE: It is imperative to ensure that the scoop is cleaned with ethanol between stations and that the scoop does not come into contact with human skin

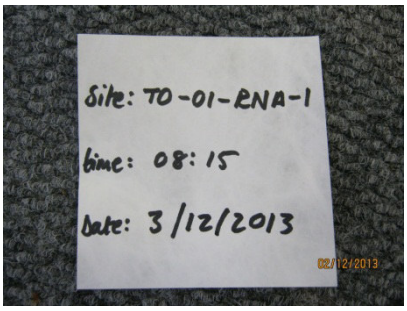
ANNEXURE 2: MOCK UP BOTTLE

Graphic – *a mock up bottle will be provided to the technical sampling team.*



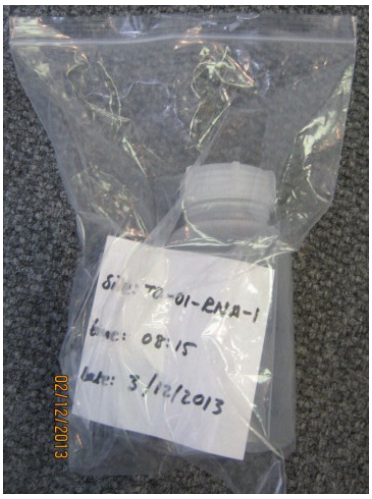
1. Bottle label (side): TO -01-RNA-1.

2. Bottle label (lid): TO -01-RNA-1



3. Waterproof Label: with site details (4 labels need to be written up per site, only 1 to be used in the photograph of the grab)

4. Sediment (250 ml) and RNA (50 ml) fill levels for each bottle.



5. Waterproof label and sample bottle placed in packet (once sample bottle is filled)

6. Bottle can now be placed in the freezer.