Department of Science, Information Technology and Innovation

RP128G: Sources of Bioavailable Particulate Nutrients-Phase 2

Protocols for sampling, processing and analysis of sediments for indicators of potentially bioavailable particulate nutrients.

Chemistry Centre, Landscape Sciences

30/06/2017

Queensland Government

Prepared by

Dr. Alexandra Garzon-Garcia, Dr. Joanne Burton, Dr. Phil Moody, Rob De Hayr
Landscape Sciences
Science Delivery Division
Department of Science, Information Technology and Innovation
PO Box 5078
Brisbane QLD 4001

Disclaimer

This document has been prepared with all due diligence and care, based on the best available information at the time of publication. The department holds no responsibility for any errors or omissions within this document. Any decisions made by other parties based on this document are solely the responsibility of those parties. Information contained in this document is from a number of sources and, as such, does not necessarily represent government or departmental policy.

If you need to access this document in a language other than English, please call the Translating and Interpreting Service (TIS National) on 131 450 and ask them to telephone Library Services on +61 7 3170 5725

Citation

Garzon-Garcia A, Burton J, Moody P, De Hayr R (2017). RP128G: Sources of Bioavailable Particulate Nutrients - Phase 2. Protocols for sampling, processing and analysis of samples for indicators of potentially bioavailable particulate nutrients. Department of Science, Information Technology and Innovation.

Acknowledgements

This report has been prepared by the Department of Science, Information Technology and Innovation (DSITI). DSITI acknowledges the funding and assistance from the Department of Environment and Heritage Protection, Office of the Great Barrier Reef, Reef Programs science program.

June 2017

Contents

Introduction

This document outlines the sample collection, processing and analysis methods for the measurement of the parameters identified in Project RP128G Phase 2 as components of indicators of potentially bioavailable particulate nutrients in sediments. The parameters were selected based on experiments that were designed to determine which pools of bioavailable nutrients currently measured in soils can be measured in sediments and best indicate algal growth in freshwater and marine conditions (Garzon-Garcia et al. 2017). The study found that a combination of bioavailable particulate nutrient parameters in multiple linear equations compose the best indicators of the potential bioavailability of particulate nutrients in fine sediment rather than a single parameter on its own.

Sediment sample collection methods

The indicators recommended for determining potentially bioavailable particulate nutrients in Phase 2 can be applied to sediment samples collected by a range of methods including: suspended sediment collected during flow events using time-integrated samplers (Phillips et al. 2000); river sediment deposit (lag) samples (e.g. Wilkinson et al. 2015); fine sediment generated from soil samples (e.g. Burton et al. 2015; Garzon-Garcia et al. 2017); or instream suspended sediment samples collected by automated refrigerated samplers (e.g. Garzon-Garcia et al. 2015, Wallace et al. 2015).

Sample processing

The following procedures describe the collection of sediment samples from the field during or after stream flow events.

Sediments may also be generated artificially from source soils for research and validation by following the proposed procedure in Appendix A.

Sediment samples from time-integrated samplers or river (lag) deposits

For sediment samples taken from time-integrated samplers or river (lag) deposits, remove any organic debris and then take to “air-dry” by drying in an air forced oven at 40°C to constant weight. Once air-dried, samples should be checked a second time to remove any remaining organic matter and then mixed well. Samples should then be fractionated to <63µm, preferably by dry-sieving, unless it is known from particle size analysis that the sampled sediment mainly consists of this fraction size (>80% of particles <63 µm).

Sediment samples from water (automated refrigerated samplers or grab samples)

Sediment can be extracted from water by centrifuging or filtering. If centrifuging the supernatant has to be completely discarded before drying the sediment to remove the carbon and nutrient fractions dissolved in the supernatant. Approximately 15-20 g of sample will be required to perform the required analyses. Once the sediment has been extracted from the water, it should

then be passed through a <63µm sieve, preferably by dry-sieving, unless it is known from particle size analysis that the sampled sediment mainly consists of this fraction size (i.e. >80% of particles <63 µm).

Alternately some analyses can be performed on the water directly and where appropriate are described in the Sample Analysis section.

Sample analysis

This section provides details on the laboratory analysis required to measure the indicators of potential bioavailable particulate nutrients outlined in Garzon-Garcia et al. (2017). It also provides detail on any associated calculations.

A number of the following methods are performed based on those described in Soil chemical methods – Australasia by Rayment & Lyons (2011) and the method code is referenced within the text where appropriate.

Particulate Nitrogen (PN) and Particulate Organic Carbon (POC)

Particulate nitrogen (PN) is defined here as the total nitrogen associated with sediments and particulate organic carbon (POC), the total organic carbon associated with sediments. They can be determined by catalysed, high temperature combustion methods in which the sample under analysis is combusted by heating it to 900 - 1300°C depending on the instrument (see manufacturers manual) by a resistance furnace in a stream of high purity oxygen. The organic carbon content of the sample is determined by the total amount of CO_2 produced by combustion (Rayment & Lyons method 6B2). Samples containing carbonate minerals should be pre-treated with dilute acid (Rayment & Lyons method 6B3). The nitrogen content of the sample is determined by the total amount of N_2 produced by furnace combustion and subsequent reduction in a Copper (Cu)-catalyst tube. Nitrogen is measured by a thermal conductivity detector while carbon is measured by an infrared radiation detector (Rayment & Lyons method 7A5).

These parameters can also be determined from water samples containing sediments by subtracting the analyte measured in the filtered sample (dissolved fraction) from that measured in the unfiltered sample (total fraction). For nitrogen this will be the Total Nitrogen (TN) measured on an unfiltered sample minus total dissolved nitrogen (TDN) determined on a filtered subsample (APHA/AWWA/WEF (2012) method 4500-N or 4500-NorgD). For carbon it will be total organic carbon (TOC) minus dissolved organic carbon (DOC) (APHA/AWWA/WEF (2012) method 5310).

PN = TN – TDN or TKN – DKN
POC = TOC – DOC

Mineral Nitrogen (Mineral N)

Sediments are extracted with 2 M potassium chloride (KCl), 1:10 soil to solution ratio for 1 hour at 25°C. Mineral N (i.e. ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N)) are determined by automated colorimetric procedures (Rayment & Lyons method 7C2).

Proposed method:
It is possible to extract the sediments present in water samples directly. The method proposed here is under development and is to be tested on automated refrigerated water samples with various concentrations of suspended sediments and particulate nutrients to guarantee that the extracted NH_4^+-N is detectable in the laboratory.

Adsorbed NH_4^+-N is extracted by the addition of 3M KCl solution to a measured volume of water in the ratio 1:5 such that the final concentration of KCl is 0.5 M(e.g., 200 ml of unfiltered water + 40 ml of 3M KCl solution). The solution should then be mixed in an end over end shaker for an hour before filtering through a 0.45 μm filter to quantify mineral N (i.e. NH_4^+-N (APHA/AWWA/WEF (2012) method 4500-NH_3G) and NO_3^--N (APHA/AWWA/WEF (2012) method 4500-NO_3^-F)). The adsorbed NH_4^+-N is then calculated by subtracting the dissolved NH_4^+-N previously determined in the water before the extract is carried out.

Particulate Organic Nitrogen (PON) is calculated as follows:

PON = PN – Mineral N

Dissolved Reactive Phosphorus (DRP)

Dissolved Reactive Phosphorus (DRP) is calculated as: *Colwell-P/Phosphorus Buffer Index (PBI)*

Colwell-P is determined using Rayment & Lyons method 9B by extracting air dried sample with 0.5M $NaHCO_3$ buffered to pH 8.5 with NaOH at a 1:100 soil/solution ratio for 16 h at 25°C . The sample extract phosphorus concentration is determined by an automated colorimetric method.

The determination of the Phosphorus Buffer Index (PBI) is derived from the Freundlich equation for describing the relationship between total P sorbed and final solution P concentration (i.e. the P sorption curve) based on Rayment & Lyons method 9I2b. The total amount of P sorbed by the soil is calculated as the amount of previously sorbed P, plus the amount of freshly sorbed P. The previously sorbed P is estimated as the Colwell–P status of the soil. Therefore, the 'total sorbed P' for use in calculating PBI is the addition of Colwell-P to the amount of freshly sorbed P. The amount of freshly sorbed P in the soil (mg P/kg) is calculated as the difference between the initial amount of P added (=1000 mg P/kg at the specified soil/solution ratio of 1:10) and the amount of P left in the equilibrating solution, expressed as mg P/kg air dry soil. Sample solution freshly sorbed P concentration is quantified by Inductively Coupled Plasma-Optical Emission Spectroscopy.

$$PBIadj = \frac{total\ P\ sorbed\ (mg/kg)}{residual\ P\ (mg/L)^{0.41}}$$

$$total\ sorbed\ P = Colwell\ P\ (mg/kg) + P\ added\ (mg/kg) - (residual\ P\ mg/L \times 10)$$

Water extractable Soluble Organic Carbon (SOC) and Soluble Organic Nitrogen (SON)

Sediments are extracted with deionised (DI) water using a 3:10 sediment to DI water ratio (for 1 h at 25°C in an end over end shaker). The suspension is centrifuged at 4500 rpm for 30 minutes, then filtered (0.45μm). The filtrate may need to be diluted to have enough volume to perform the following analytical procedures. The dilution factor needs to be recorded and the resulting concentrations from the analyses corrected for this dilution factor. This method was adapted from

the potential production of soluble organic carbon method, which in turn is an adaptation of the Potential Mineralisable Nitrogen in soils method (Bremner 1965).

Soluble Organic Carbon is determined on the filtrate by high temperature combustion or wet oxidation with persulfate (APHA/AWWA/WEF (2012) method 5310).

Soluble Organic Nitrogen is determined by subtracting the NH_4^+-N (APHA/AWWA/WEF (2012) method 4500-NH_3G) from the Dissolved Kjeldahl Nitrogen (DKN) ((APHA/AWWA/WEF (2012) method 4500-N org D).

Proposed method:
It is possible to extract the sediments present in water samples directly. The method proposed here is under development and is to be tried out in automated refrigerated water samples with various concentrations of suspended sediments and particulate nutrients to guarantee that the SOC and SON are detectable in the laboratory.

The water sample with a known volume is to be extracted with a 3M KCl solution. The KCl solution volume to be used should be calculated so that when mixed with the water, the minimum strength of the diluted solution is 0.5 M (maximum 6x dilution) (e.g., 200 ml of sediment suspension + 40 ml of 3M KCl solution). The solution should then be mixed in and end over end shaker for an hour before filtering through a 0.45 μm filter to quantify dissolved organic carbon (DOC) (APHA/AWWA/WEF (2012) method 5310) and total dissolved nitrogen (APHA/AWWA/WEF (2012) method 4500-N or 4500-NorgD). The DOC present in solution before the extract is carried out, should be subtracted from the DOC measured on the extract to obtain SOC. The DON (dissolved organic nitrogen) present in solution before the extract is carried out, should be subtracted from the DON measured on the extract to obtain SON.

Potentially bioavailable particulate nutrient indicators

Once individual parameters have been measured the data can be input into the equations developed in Garzon-Garcia et al (2017) to indicate the potentially bioavailable particulate nutrients. The equations were developed based on experiments designed to determine which pools of bioavailable nutrients measured regularly in soils best indicate algal growth. A combination of bioavailable particulate nutrient parameters compose the best indicators of the potential bioavailability of fine sediment rather than a single parameter alone.

The equations which can be used to indicate the potential bioavailability of fine sediment particulate N in marine conditions (PNB_m) are[1]:

(1) $PNB_{m1} = 0.198 + 0.457 \times PN + 0.004 \times \frac{POC}{PN}$

(2) $PNB_{m2} = 0.221 + 9.638 \times {NH_4}^+N + 0.402 \times PON + 0.001 \times \frac{SOC}{{NH_4}^+N}$

[1] Particulate organic carbon (POC); 2M KCl extractable ammonium (NH_4^+N); particulate organic nitrogen (PON); soluble organic carbon (SOC); soluble organic nitrogen (SON); dissolved reactive phosphorus (DRP). The units of all equation parameters are based on a w/w basis (e.g., mg POC kg^{-1} sediment).

The equations that can be used to indicate the potential bioavailability of fine sediment particulate N in freshwater conditions (PNB_{fw}) are[1]:

(1) $PNB_{fw1} = 0.467 + 0.015 \times POC - 0.001 \times \frac{POC}{PN}$

(2) $PNB_{fw2} = 0.438 + 3.387 \times SOC + 0.001 \times \frac{SOC}{SON}$

The equation that can be used to indicate the potential bioavailability of fine sediment particulate N and P in freshwater conditions ($PNPB_{fw}$) is[1]:

(3) $PNPB_{fw1} = 0.451 + 13.28 \times {NH_4}^+N + 451.7 \times DRP + 0.0005 \times \frac{SOC}{SON}$

References

APHA/AWWA/WEF (2012) - Standard Methods for the Examination of Water and Wastewater, 22nd Edition. Am. Public Health Assoc., Washington, USA.

Burton J, Moody P, De Hayr R, Chen C, Lewis S, Olley J 2015: Sources of bioavailable particulate nutrients: Phase 1 (RP128G), Department of Science, Information Technology and Innovation, Brisbane, Australia

Garzon-Garcia A, Wallace R, Huggins R, Turner RDR, Smith RA, Orr D, Ferguson B, Gardiner R, Thomson B, J. WMS 2015: Total suspended solids, nutrient and pesticide loads (2013–2014) for rivers that discharge to the Great Barrier Reef Great Barrier Reef Catchment Loads Monitoring Program, Water Quality and Investigations, Environmental Monitoring and Assessment Science, Science Division, Department of Science, Information Technology and Innovation

Garzon-Garcia A, Burton J, Moody P, De Hayr R, Franklin H, Burford M (2017). RP128G: Sources of Bioavailable Particulate Nutrients -Phase 2. Indicators of Potentially Bioavailable Particulate Nutrients to Algae in Freshwater and Marine Conditions. Department of Science, Information Technology and Innovation.

Olley J, Burton J, Smolders K, Pantus F, Pietsch T (2013b). The application of fallout radionuclides to determine the dominant erosion process in water supply catchments of subtropical South-East Queensland, Australia. Hydrol Process 27:885–895.

Phillips JM, Russell MA, Walling DE (2000) Time-integrated sampling of fluvial suspended sediment: a simple methodology for small catchments. Hydrol Process 14:2589–2602.

Rayment GE & Lyons DJ (2011). Soil Chemical Methods – Australasia. CSIRO Publishing.

Wilkinson SN, Olley JM, Furuichi T, Burton J, Kinsey-Henderson AE (2015). Sediment source tracing with stratified sampling and weightings based on spatial gradients in soil erosion. Journal of Soils and Sediments. DOI 10.1007/s11368-015-1134-2

Wallace R, Huggins R, Smith R, Turner R, Garzon-Garcia A, Warne MSJ 2015: Total suspended solids, nutrient and pesticide loads (2012-2013) for rivers that discharge to the Great Barrier Reef, Great Barrier Reef Catchment Loads Monitoring Program, Department of Science, Information Technology, Innovation and the Arts, Brisbane.

Appendix A

Procedure for generating sediment samples from bulk soils

The following steps should be taken to prepare the sediment samples from bulk soils:

- Organic matter (including litter, roots and charcoal) should be removed from whole soil samples. Soil lumps are then broken down by hand to as small as possible and samples are then air dried (oven-dry) at 40°C. Once air-dried, samples should be checked a second time to remove any remaining organic matter and then mixed well.
- The sample is then processed through a jaw crusher on setting 2. Any organic matter found should be removed. The sample then needs to be mixed thoroughly (e.g. using a cement mixer).

The sample then needs to be processed to separate out the fine fraction (<10 um) of sediment. This is done using the standard method for water-dispersible clay and the appropriate settling time based on Stoke's Law. Following separation, the fine fraction is dried at 40°C and then gently mixed and homogenised using a mortar and pestle.

The process for fractionation to <10 um specifically is as follows:

1. A 2 kg soil subsample is placed in a 20L clean bucket with tap and topped up with DI water to a calculated mark inside of the bucket (The height of the mark should be calculated based on Stokes law so that a 10 um diameter particle would take 46 minutes to settle at 20°C).
2. The sample and water should be sonicated for 2 minutes to break aggregates and mixed with a mixing stick for approximately 30 seconds.
3. The sample is then agitated with a plunger for 30 seconds, ensuring the sample is well mixed and dispersed.
4. The sample should be left undisturbed to settle for exactly 46 minutes.
5. The bucked tap is opened and the sample is allowed to drain into a clean bucket through a 63 um sieve to remove floating litter fragments.
6. The tap is closed and DI water is added to the bucket up to the mark again.
7. Steps 2 to 5 are repeated using a clean bucket to collect the second fraction of the sample.
8. The recovered supernatants (wet <10µm fraction) should be collected in 1L plastic containers washing any remaining sample from the bucket into the containers using a squirt bottle and dried in the oven at 40°C until constant weight of the recovered sediment was achieved. It is also possible to centrifuge (4800 rpm, 20 minutes) the supernatants to separate the <10um fraction from the water and then as much as possible clear liquid should be removed to speed the drying step.
9. Once the recovered sediment is dry, sediment coming from the same source soil sample should be combined into one sample and homogenised manually using mortar and pestle.