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Determination of nitrate, phosphate and organically bound phosphorus in coral skeletons by ion chromatography

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Abstract

Nitrate, phosphate and sulphate incorporated in the aragonitic skeletons of corals (*Porites*) were analyzed by ion chromatography (IC). All anion analyses were performed using a Dionex 4500i IC system with Dionex columns, conductivity detector and computer interface. The anions were separated on an AS4A separator column behind an AG4 guard column, and the background conductivity of the eluent was suppressed using an AMMSII membrane suppressor. The suppressor was continually regenerated using an anion membrane suppressor regenerant cartridge and H_2SO_4. The eluent used was 1.8 m*M* Na_2CO_3–1.7 m*M* $NaHCO_3$. Bleached, oven-dried coral powder (200 mg) was dissolved in 0.5 ml of 30% HCl (Merck Suprapur) and diluted to 100 ml. Standards were also prepared in HCl, and the linearity was excellent for each anion ($r^2 > 0.999$) over the range 20–100 ng/g (ppb) for nitrate, 100–500 ng/g for phosphate and 0.5–12.0 μg/g (ppm) for sulphate. All samples and standards were passed through a chloride-removal cartridge (Dionex OnGuard AG) prior to injection. This cartridge consisted of silver and its sole purpose was to remove Cl^- which might otherwise overwhelm the separator column. Using a 250-μl injection loop, the detection limits were approximately 5 ng/g for nitrate and 10 ng/g for phosphate. The analytical procedure variability was 9% for nitrate, 2% for phosphate and 0.5% for sulphate. The concentrations of these species in the corals varied from 0.2 to 18 ppm, 8 to 118 ppm and 560 to 590 ppm, respectively. Coral samples taken from three coral heads at the Grande Rivière Noire Bay (slabs 1Ks, 5Ks, 6Ks) averaged 4.5 ± 0.5 ppm nitrate and 55 ± 1 ppm phosphate, compared with 2.2 ± 0.5 ppm nitrate and 16 ± 1 ppm phosphate for the samples taken further out in the lagoon (slabs 7Ks and 11Ks). Thus, the nitrate and phosphate concentrations clearly distinguish between corals taken from the bay, which are closer to anthropogenic N and P inputs, from samples collected from the lagoon, which are further from such N and P sources. To estimate the possible importance of organically bound phosphorus, samples were also treated with H_2O_2. Following dissolution in HCl, 1 ml of 30% H_2O_2 (Merck Suprapur) was added, and the samples were heated at 50°C for 3 h. The instrument was calibrated using standards prepared in HCl–H_2O_2. The phosphate concentrations measured in the samples dissolved in HCl–H_2O_2 were up to 100% higher than those dissolved in HCl alone, suggesting that organically bound phosphorus contributes significantly to the total phosphorus concentrations of the corals.

1. Introduction

The fringing reef ecosystem on the Island of Mauritius in the Indian Ocean is degenerating because of algal growth, coral diseases and degradation of lagoons. A reduction in coral vitality and a decrease in specimen variation is now well documented [1]. The ultimate cause of

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these problems may be the extensive eutrophication which arises from marine sewage disposal.

Anthropogenic inputs of sewage from towns, hotels and private houses on the beach could be responsible for the degradation of the corals either because of nutrients, toxic heavy metals or both [2,3]. These contaminants are transported directly into the lagoon by rivers, canals and groundwater, or may be washed out during the rainy season [4]. Once they have reached the reef ecosystem, there are a number of possible mechanisms by which the corals may be affected [5].

Phosphate is normally a trace constituent in seawater (of the order of 10 ng/g or less in uncontaminated waters), but elevated concentrations of this species can decrease calcification rates (i.e. coral growth rates) by as much as 50% [6]. Phosphate can also affect coral vitality indirectly by stimulating the growth of nuisance algae which reduce light intensity and promote sediment accumulation [7]. Bacterial infection of coral mucus may increase, causing localized tissue death and allowing algal growth to become established [8]. Thick algal mats have been known to form and may smother all underlying reef organisms [5].

To identify possible reasons for reef degradation, a geochemical study was undertaken using the widespread recent coral genus *Porites*. One goal of the project was to measure nitrate and phosphate concentrations in the coral skeletons and to use these as indicators of marine pollution. A second goal was to reconstruct the chronology of these types of marine pollution using the annual growth bands of the corals.

Previous investigations have used a colorimetric method (reaction with molybdenum blue) for measuring phosphate in acid digests of coral skeletons [9]. Because our interest included nitrate, we sought to measure nitrate and phosphate *simultaneously* in acid digests of coral skeletons using ion chromatography (IC). The main purpose of the present report is to summarize an evaluation of the IC method for measuring nitrate and phosphate in corals.

2. Experimental

2.1. Preparation of coral samples

Individual samples (approximately 5–7 g) of annual growth bands were removed from coral head slabs using a jig saw. The samples were placed in clean 100-ml polypropylene bottles, rinsed three times and left for two days in 18 MΩ deionized water. Following this, the samples were dried at 50°C. The bottles were then filled with 30% H_2O_2 (Merck Suprapur) and left for three days in order to remove any organic material from the sample surface. Next, the samples were rinsed and soaked in deionized water for another three days before drying at 50°C overnight. The dry samples were then powdered with an agate mortar and pestle and stored in small sample bottles.

2.2. Preparation of acid digests of coral powders

Dissolution in hydrochloric acid

About 200 mg of the powdered samples were weighed into 100-ml glass flasks. Samples were weighed out in duplicate in order to allow measurements both in HCl alone and in HCl–H_2O_2. Samples were dissolved in 0.5 ml of 30% HCl (Merck Suprapur). Dissolution was carried out at room temperature for 3 h. The digests were then diluted to 100 ml with deionized water.

Dissolution in hydrochloric acid and hydrogen peroxide

To evaluate the possible importance of organically bound phosphorus, the set of duplicate samples was dissolved in HCl as described above. In a second step, 1 ml of 30% H_2O_2 (Merck Suprapur) was added to destroy organic matter. The samples treated with H_2O_2 were warmed in a water bath at 50°C for 3 h in order to accelerate the oxidation process. Following this, the solutions were heated to 90°C to decompose any unreacted H_2O_2. This ensured that no further oxidation could take place. Trials

were made with H_2O_2 ranging from 1 to 5 ml, but 1 ml was found to be optimal. These digests were also diluted to 100 ml with deionized water.

2.3. Calibration of the ion chromatograph

Standards prepared in hydrochloric acid

Stock solutions were made up in 200 ml of deionized water from 1000 mg/l nitrate, phosphate and sulphate standards (Merck). From the stock solutions, the following working standards were prepared: 20, 40, 60, 80 and 100 ng/g (ppb) nitrate, 100, 200, 300, 400 and 500 ng/g phosphate and 0.5, 2.5, 5.0, 7.5 and 12.5 μg/g (ppm) sulphate. Suprapur HCl was used in the standards at the same concentration as in the samples to reduce the error of the analysis (i.e. matrix effect and/or blank values). The standards and samples were passed through a chloride-removal cartridge (Dionex OnGuard AG) to eliminate Cl^- prior to injection (Fig. 1). One cartridge was used per sample. Previous studies have shown that this cartridge has no significant effect on the measurement of HPO_4^{2-} even at concentrations of 20 ng/g [10]. The linearity of the standards was excellent in each case ($r^2 > 0.999$).

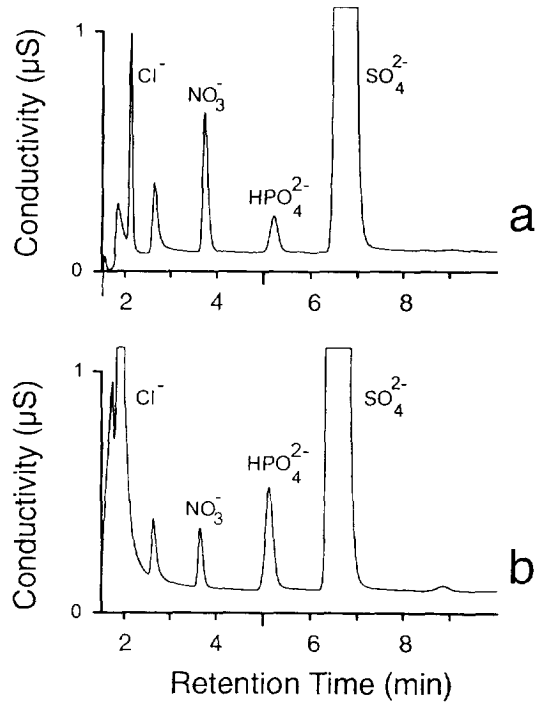

Fig. 1. (a) Chromatogram showing nitrate (150 ng/g), phosphate (150 ng/g) and sulphate (7.5 μg/g) in a working standard (HCl matrix), following injection through an AG chloride-removal cartridge. (b) Chromatogram showing nitrate (67 ng/g), phosphate (449 ng/g) and sulphate (10.9 μg/g) in an HCl digest of a coral skeleton, following injection through an AG chloride-removal cartridge.

Standards prepared in hydrochloric acid and hydrogen peroxide

The contribution of H_2O_2 to the blank values was evaluated by preparing three anion working standards in HCl and analyzing them with and without the addition of H_2O_2. After calibrating the IC system with standards made up only in HCl, the same standards were analyzed in duplicate after adding H_2O_2. Blanks containing only HCl and H_2O_2 were also measured. After correcting for blank values (phosphate in the blanks was below the limit of detection), nitrate and sulphate concentrations in the standards consisting of HCl plus H_2O_2 were 5–10% below the concentrations in the standards containing only HCl. At this time no explanation is given for this difference.

2.4. Accuracy, precision and quality control

There is no calcite or aragonite standard reference material certified for either nitrogen or phosphorus. Thus, a quantitative evaluation of the accuracy of the analyses was not possible. Using blind standards after every sixth sample injection, the analytical procedure variability was estimated to be 9% for nitrate, 2% for phosphate and 0.5% for sulphate. The precision of the analyses diminished in the order sulphate > phosphate > nitrate because the concentrations of the anions in the digests also decreased in that order. Relative to the detection limits, the concentration ranges for these anions in the sample digests were: nitrate, 1–2 × ; phosphate, 3–10 × ; sulphate, 100 × . The precision of the nitrate

analyses could probably be improved simply by analyzing more concentrated solutions (e.g. by diluting the digest to 25 ml instead of 100 ml).

3. Results

3.1. Nitrate

Dissolution in hydrochloric acid

Corals from the Grande Rivière Noire Bay (slabs 1Ks, 5Ks, 6Ks) average 4.5 ± 0.5 ppm nitrate, compared with 2.2 ± 0.5 ppm nitrate for the samples taken from the lagoon (7Ks and 11Ks). Thus, the nitrate concentrations clearly distinguish coral specimens taken in the bay, which are closer to anthropogenic N inputs (primarily from fertilizers used in the sugar cane industry and from livestock), from samples collected in the lagoon, which are further from such N sources.

Dissolution in hydrochloric acid and hydrogen peroxide

The nitrate concentrations in the samples dissolved in HCl versus those dissolved in HCl–H_2O_2 were not significantly different. It appears, therefore, that organically bound nitrogen is not a quantitatively significant N pool in the corals.

3.2. Phosphate

Dissolution in hydrochloric acid

Samples from the bay average 55 ± 1 ppm phosphate, compared with 16 ± 1 ppm phosphate for the samples taken from the lagoon. Again, therefore, the measured phosphate concentrations clearly distinguish coral specimens taken in the bay, which are closer to anthropogenic P inputs (primarily from municipal sewage inputs and livestock), from samples collected in the lagoon, which are further from such P sources.

Dissolution in hydrochloric acid and hydrogen peroxide

Samples treated with HCl–H_2O_2 yielded phosphate concentrations that were up to 100% higher than the phosphate concentrations measured in HCl alone. With respect to core 11Ks from the lagoon, however, in some cases there was no significant difference between the two treatments. In most cases, samples treated with HCl–H_2O_2 yielded phosphate concentrations that were 50% higher than the phosphate concentrations measured in HCl alone.

The difference between these two kinds of digests is attributed to organically bound phosphorus, with the following *caveat*. It has recently been shown that H_2O_2 does not completely destroy the organic components present in calcium carbonate minerals. Full-strength Clorox (5% NaOCl) is more effective, and more or less completely destroys all types of organic materials [11]. Until measurements of phosphate concentrations are also performed using samples treated with NaOCl, the phosphate concentrations measured in HCl–H_2O_2 should probably be interpreted as an estimate of the lower limit of organically bound phosphorus in these samples.

3.3. Sulphate

The sulphate concentrations in the corals ranged from 516 to 596 ppm, about twenty times higher than phosphate. The much higher concentrations of sulphate reflect the relatively high concentrations of sulphate in seawater (approximately 2700 mg/l). Despite the high background concentrations of sulphate in seawater, specimens collected from the bay show on average significantly higher sulphate values in the HCl digests (567 ± 3 ppm) than those from the lagoon (540 ± 3 ppm). This may be an indication of anthropogenic sulphur inputs to the Grande Rivière Noire Bay and warrants further study.

4. Conclusions

Nitrate, phosphate and sulphate in the skeletons of the scleractinian coral *Porites lutea* can be measured with sufficient sensitivity and precision using IC. The samples are simply dissolved in high-purity, concentrated HCl, diluted to volume, and passed through a chloride-removal cartridge prior to injection. Nitrate and

phosphate concentrations were found to be significantly higher in the samples collected from the Grande Rivière Noire Bay compared to the lagoon. Thus, both nitrate and phosphate concentrations in the coral skeletons are useful environmental indicators of reef eutrophication. Despite the high background concentration of sulphate in seawater, measured sulphate concentrations were also higher in the corals from the Grande Rivière Noire Bay than from the lagoon. Thus, even sulphate may also function as an indicator of coastal marine pollution.

Adding H_2O_2 to these digests significantly increased the measured phosphate concentrations in the majority of cases; in some samples, the difference was as large as a factor of 2. The difference in measured phosphate concentrations between the HCl versus HCl–H_2O_2 treatments was attributed to organically bound phosphorus which is incorporated within the skeletal fine structure of the coral specimens. Further studies are needed to determine how much of the total concentration of organically bound phosphorus was liberated by treatment with H_2O_2.

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References

[1] L. Hottinger, N. Muller, J. Muller and P. Vasseur, *Etude des Ecosystemes Littoraux de Maurice*, Rapport No. 1, Université d'Aix-Marseille I et III, Université de Maurice, 1990, 56 pp.
[2] F. Gendre, *Thèse Faculté de Sciences*, Institut de Géologie, Neuchâtel, 1992.
[3] J. Muller, *Etude des Ecosystemes Littoraux de Maurice*, Rapport No 5, Université d'Aix-Marseille I et III, Université de Maurice, 1991, pp. 255 and 257–294.
[4] I. Immenhauser-Potthast, *Ph.D. Thesis*, University of Berne, Berne, 1994.
[5] R.A. Pastorok and G.R. Bilyard, *Mar. Ecol. Prog. Ser.*, 21 (1985) 175.
[6] D.W. Kinsey and P.J. Davies, *Limnol. Oceanogr.*, 24 (1979) 935.
[7] D.I. Walker and R.F.G. Ormond, *Mar. Poll. Bull.*, 13 (1982) 21.
[8] R. Mitchell and I. Chet, *Microb. Ecol.*, 2 (1975) 227.
[9] R.E. Dodge, T.D. Jickells, A.H. Knap, S. Boyd and R.P.M. Bak, *Mar. Poll. Bull.*, 15 (1984) 178.
[10] W. Shotyk, *J. Chromatogr.*, 640 (1993) 309.
[11] S.J. Gaffey and C.E. Bronnimann, *J. Sed. Petrol.*, 63 (1993) 752.