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Research Article

Finger Printing Standard Colas Using Phosphate Oxygen Isotopes

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Abstract

Eutrophication is a globally significant challenge facing freshwater ecosystems and is closely associated with anthropogenic enrichment of phosphorus (P) from a number of sources in the aquatic environment. Phosphate oxygen isotopes ($\delta^{18}O$-PO_4) provide a possible tool for identifying P sources, although there are methodological challenges depending on associated dissolved organic matter. Cola drinks are commonly manufactured with high concentrations of phosphoric acid to make them a sharper flavour as well as slow the growth of bacteria. Although in themselves they are not likely to be a significant source of aquatic phosphate pollution globally, there is the potential for large spillages at the point of production in addition to the use of waste sludges from the manufacturing process being used as fertilisers in some countries. Here we present $\delta^{18}O$-PO_4 data for 8 different colas using a XAD and ion exchange column method previously published by the authors. Concentrations of SRP ranged from 77.6 to 192 mg P/L. Yields of $AgPO_4$ from 10 fold dilutions of the original cola solutions ranged from 60 to 87%. The range of $\delta^{18}O$-PO_4 was from 17.7-20.6 ‰ ± 0.3 with a mean of 19.3 which compares with a range of 19.3-20.0‰ ± 0.3 with a mean of 19.7 for commercially available phosphoric acids. Within brand there were significant differences in $\delta^{18}O$-PO_4 for the diet and regular colas. Diet colas also had lower SRPs than their regular counter parts. This work suggests that the method used is suitable for effluents with high very organic loads and that there are traceable differences between different colas.

INTRODUCTION

World food security and the growing production of biofuels rely on enhanced P inputs to ecosystems, largely through the application of inorganic fertilisers and feed supplements which have come from manufactured from mining phosphate deposits. China is the largest producer of phosphate and phosphate derivatives, which it uses mostly to cover its domestic needs whereas Morocco, has the largest phosphate reserves in the world and is the world leading phosphate exporter. In parallel with increased mining and processing of phosphate rock, widespread enrichment of aquatic ecosystems with P has occurred in many parts of the globe [1].

For use in industrial processes, phosphate is generally first converted to phosphoric acid (H_3PO_4) can be manufactured using either a thermal or a wet process. However, the majority of phosphoric acid is produced using the wet-process method. Wet-process phosphoric acid is used for fertilizer production. Thermal process phosphoric acid is commonly used in the manufacture of high-grade chemicals, which require a much higher purity, such as in food additives.

Phosphoric acid is added to soft drinks, and in particular cola's, to give them a sharper flavour. Additionally it slows the growth of bacteria, which would otherwise multiply rapidly in the sugary solution. Almost all of the acidity of cola drinks comes from the phosphoric acid and not from the carbonic acid from the dissolved CO_2. The colour of cola drinks comes from sulphite ammonia caramel. Waste products from the cola manufacturing process are commonly used as fertilisers because of their high residual phosphate content [2].

Over the past decade there has been increasing interest in understanding phosphate sources and environmental processing by using phosphate oxygen isotopes. This has included studies in the open ocean [3], in estuaries [4], in rivers [5] and even drinking water supplies [6]. Recently Tcaci et al. [7], developed a new method to r to analyse the phosphate oxygen composition of waters with very low PO_4 concentrations. This has required a number of methodological developments to encompass the wide range of matrices encountered in the natural environment [8].

In this study we took 8 Cola's from UK supermarkets, including 3 diet varieties, and extracted the phosphate before converting to Ag_3PO_4. The method used was first detailed in Gooddy et al (2015) on clean mains water in the distribution network, and subsequently applied in Gooddy et al. [9], for a multi-phosphate source impacted river, and again applied in Gooddy et al. [10], as tracer of phosphorus from waste water treatment works. In these studies the water matrix became increasingly challenging with lower concentrations of P but increasingly higher concentrations of DOC, which appears to have been problematic for previous methods (e.g. Young et al). The purpose of the present study is therefore to two fold; to assess how well the method used in Gooddy et al. [6,9,10], works with very high concentrations of DOC and to benchmark the $\delta^{18}O\text{-}PO_4$ composition of commercially available cola drinks.

METHODS

Sample selection

To examine the $\delta^{18}O\text{-}PO_4$ composition of Colas 5 different brands were selected. (Table 1). Within these brands, 3 had both diet and regular products. Diet Cola's all contain no sugar whereas regular cola's contain between 9.9 and 11 g/100ml of sugar. A small amount of additional carbohydrate is also present in three of the brands selected. All data comes from product packaging or the manufacturers' website.

Anion Exchange Resin

Dowex anion exchange resin was conditioned with 1M HCl (Aristar) in a large conical flask and placed on a shaker for 1 hour. The resin was then rinsed multiple times with UltrPure water until pH 6 was achieved.

Dax-8 resin was conditioned with methanol (analytical grade) in a large conical flask on a shaker for 2 hours. The resin was then rinsed multiple times with UltraPure water to leave a clean white resin slurry.

A column comprising two 60ml SPE cartridges was prepared for each sample. A frit was placed inside each SPE cartridge and a stopcock valve attached. The upper SPE cartridge was filled with 50ml Dax-8 resin topped with a 5 ml layer of UltraPure water, and the lower SPE cartridge with 50ml Dowex anion exchange resin with a 5ml layer of UltraPure water. The resin cartridges were closed at the top with SPE adapters and fitted together on a clamp stand.

The samples were pumped through the columns at 4ml/min with a Watson Marlow 205U peristaltic pump using purple/white pump tubes (internal dimeter of 2.79mm). Tygon flexible tubing (1/8 inch internal dimeter) was used to connect the samples and columns to the pump tubes. The columns were drained to a sink.

Elution

The phosphate was eluted by pumping 0.3M KCl through the anion exchange resin cartridges at 2ml/min. The first 110ml of eluant from each sample was collected in 125 ml Nalgene bottles.

Magic precipitation

5ml of 2M $MgCl_2 6H_2O$ and 5ml of 2M NaOH were added to each 100ml eluant sample. The samples were left for at least 2 hours to allow white $Mg(OH)_2$ precipitate to form.

The samples were transferred to 50ml centrifuge tubes and centrifuged for 10 minutes at 3000rpm. The supernatant was poured off leaving wet $Mg(OH)_2$ gel at the base of the centrifuge tube.

Samples were then processed according to McLaughlin et al. [11], for $\delta^{18}O$ analysis of DIP ($\delta^{18}Op$).

RESULTS

Data for the amount of silver phosphate (Ag_3PO_4) produced from the described method are show in Table 2. Yields range from 60.0% for Pepsi regular to 86.8% for Fentimans Curiosity with mean of 72.4±10.1%. Where there were a Diet and Regular pair for a given brand, Diet versions gave higher yields (2.9% higher for Waitrose Diet, 14.2% higher for Diet Cola, and 2.3% higher for Diet Pepsi) although with just three values and 2 of the 3 highest yields for both Fentimans Curiosity and Freeway this difference is not likely to be significant.

Concentrations for soluble reactive phosphorus and ammonium together with isotopic values for phosphate are presented in Table 3. Concentrations for SRP vary from 77.6 mg/L for Diet Coke to 191.6 mg/L for Waitrose Regular with a mean concentration of 152 ± 34.7 mg/L. Where there were a Diet and Regular pair for a given brand, Diet versions had lower SRP concentrations (22 mg/L lower for Waitrose Diet, 94 mg/L lower for Diet Cola, and 13.2 mg/L lower for Diet Pepsi).

Concentrations for NH_4 vary from 4.8 mg/L for Coca Cola Regular to 23.6 mg/L for Diet Coke with a mean concentration of 16.5 ± 6.1 mg/L. Where there were a Diet and Regular pair for a given brand, Diet versions had higher NH_4 concentrations (6 mg/L higher for Waitrose Diet, 18.8 mg/L higher for Diet Cola, and 5.8 mg/L higher for Diet Pepsi).

Table 1. Sugar and carbohydrate content of the 8 Cola's selected for this study.

Sample	Sugar	Carbohydrates
	g/100ml	g/100ml
Waitrose Diet	0	0
Waitrose Regular	9.88	9.96
Diet Coke	0	0
Coca Cola Regular	10.6	10.6
Diet Pepsi	0	0
Pepsi Regular	11	11
Fentimans Curiosity	10.5	11.4
Freeway (Lidl)	10.2	10.3

Table 2. Measured yields from the $\delta^{18}O\text{-}PO_4$ extraction method. Maximum Ag_3PO_4 Yield is the mass of silver phosphate that would be produced if all the phosphate in original solution was found in the final precipitate. Yield is simply calculated based on the ratio of the Actual to Maximum Yield and expressed as a percentage.

Sample	Maximum Ag_3PO_4 Yield	Actual Ag_3PO_4 Yield	Yield
	g	g	%
Waitrose Diet	0.229	0.1732	75.6
Waitrose Regular	0.259	0.1882	72.7
Diet Coke	0.105	0.1677	80.0
Coca Cola Regular	0.232	0.1526	65.8
Diet Pepsi	0.198	0.1235	62.3
Pepsi Regular	0.216	0.1298	60.0
Fentimans Curiosity	0.181	0.1568	86.8
Freeway (Lidl)	0.225	0.1789	79.4

Table 3. Soluble Reactive Phosphate (SRP) and ammonium (NH_4) concentrations along with phosphate oxygen isotopes ($\delta^{18}O\text{-}PO_4$) in different Cola's. Concentrations for SRP and NH_4 are prior to extraction.

Sample	SRP	NH_4	$\delta^{18}O\text{-}PO_4$	±
	mg/L	mg/L	‰	‰
Waitrose Diet	169.6	20.2	19.08	0.27
Waitrose Regular	191.6	14.2	17.65	0.21
Diet Coke	77.6	23.6	19.21	0.20
Coca Cola Regular	171.6	4.8	20.51	0.25
Diet Pepsi	146.8	19	20.00	0.11
Pepsi Regular	160	13.2	19.85	0.45
Fentimans Curiosity	133.6	22.2	18.66	0.35
Freeway (Lidl)	166.8	14.8	19.68	0.22

Values for $\delta^{18}O\text{-}PO_4$ vary from 17.65‰ for Waitrose Regular to 20.51‰ for Coca Cola regular with a mean of 19.33±0.89‰. Where there were a Diet and Regular pair for a given brand no trend was observed with Waitrose Diet and Diet Pepsi having higher ‰ values than the regular brand by 1.43‰ and 0.15‰ respectively, whereas Diet Coke had a lower ‰ value by 1.3‰. Given the measurement error there is no identifiable difference between the $\delta^{18}O\text{-}PO_4$ of Diet Pepsi and Pepsi Regular.

DISCUSSION

There are clear differences in the chemical composition of the various cola's just based on their SRP and NH_4 contents. For example, the diet products all contain lower concentrations of SRP since they contain no sugar to balance the acidity provided by the phosphoric acid. Sulphite ammonia caramel (E150d) is added to cola drinks to give colour, and is used specifically because it is stable in acid rich environments (Vollmuth, 2018). Figure 1 shows a cross plot of SRP and NH_4. In general, diet drinks contain more NH_4 than their non-diet counter-part and NH_4 concentration increases as SRP decreases which may reflect the need to balance acidity in the low sugar drinks. There is a weak linear correlation of 0.4. The composition of Fentimans Curiosity, with a relatively low SRP concentration and the second highest NH_4 concentration looks more similar to the diet drinks. Interestingly, this cola contains an extra 0.9g/100mL of carbohydrate additional to the sugar content [Table 1], which could possibly make it less sweet overall and might relate to the different balance of SRP and NH_4.

The relationship between $\delta^{18}O\text{-}PO_4$ and NH_4 is shown in Figure 2. Although the range is small, there appears to be an overall trend for lower $\delta^{18}O\text{-}PO_4$ with increasing NH_4 concentrations, with Waitrose regular cola as an obvious out-lier. With this point excluded there is a linear correlation of 0.72.

Figure 3 shows the full $\delta^{18}O\text{-}PO_4$ dataset for all samples and relates this to the previously measured range for phosphoric acid as determined by Gooddy et al., [16]. The study mean is at the lower end of the range for phsophoric acid and 5 out of 8 of the sampels fall within the range of the pure phsophoric acid. As mentioned previously there is no systematic relationship between diet and regular products, although both Waitrose and Coke have approximately a 1.5 ‰ difference between the diet and regualr brands this is inconsistent with the sugar content. For Pepsi both the diet and regular products have near identical $\delta^{18}O\text{-}PO_4$ compositions. It is interesting to note that the Waitrose and Coke diet products are furthest from the study mean.

Based on our understanding of how phosphate is processed in the environment, the shift in $\delta^{18}O\text{-}PO_4$ away from the pure phosphoric acid composition could have one of two explanations 1), there is some biological processing of the phosphate, or 2) there is some cleaving of the P-O bonds during the manufacturing process probably due to heating. Due to the high sugar content and the sterile production environment it would seem likely that the production process is the most likely cause. There may also be greater differences in the $\delta^{18}O_{H2O}$ of the water used in the different mansufacturing locations which could also contribute to this variation from the phosphoric acid composition.

Figure 4 shows how the $\delta^{18}O\text{-}PO_4$ values obtained from this study compare with other sources that were first summarised by Davies et al. [8], and then added to by Gooddy et al [6]. The figure demonstrates how the Cola's have a slightly wider range than 'Tap water – type B', which in turn reflected the isotopic composition of the phosphoric acid used in dosing mains water to prevent plumbo-solvency. Similarly, the cola's all sit with the range seen for chemical fertilisers, which suggests a common phosphoric

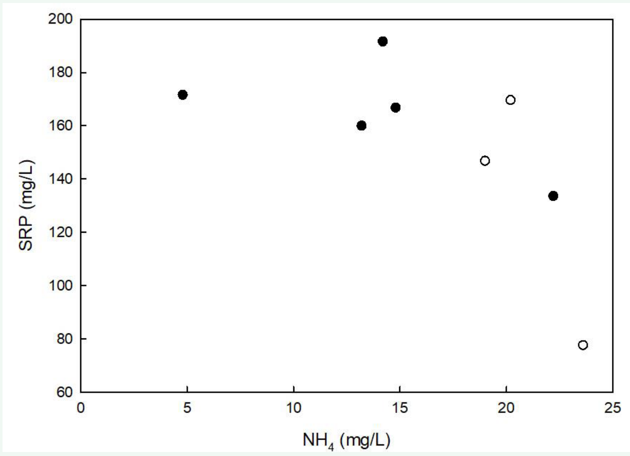

Figure 1 Relationship between NH4 and SRP for all 8 cola samples. Diet colas are shown as white circles.

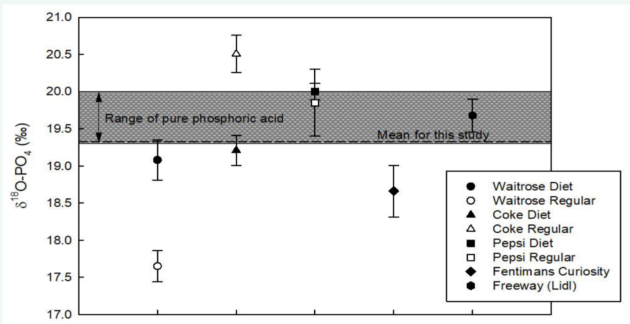

Figure 2 Relationship between δ18O-PO4 and NH4 for all 8 cola samples. Waitrose regular cola is shown as a white square.

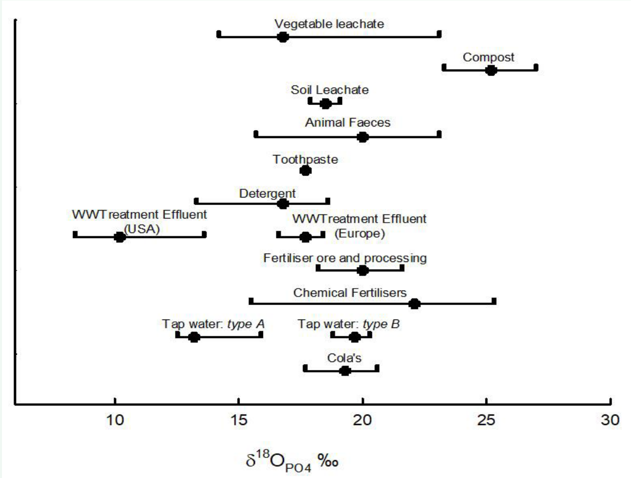

Figure 3 δ18O-PO4 composition shown for all samples and slip accoring to diet or regular products for each brand.

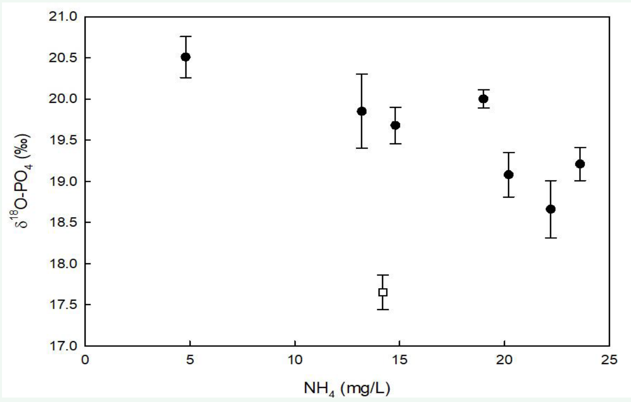

Figure 4 Range of colas relative to other δ18O-PO4 values.

acid source. Compared with many of the other source values from the literature, the range for cola's is relatively small all this may just reflect the relatively small numbers of samples analysed.

CONCLUSIONS

All of the cola's tested have different $\delta^{18}O\text{-}PO_4$ values, ranging from 17.7‰ to 20.5‰. This relatively wide range most likely reflects the different manufacturing processes (degree of heating) and may be a result of equilibrium reactions with the water used locally having different $\delta^{18}O_{H2O}$ compositions. This relatively wide range suggests that different cola sources may be traced based on their $\delta^{18}O\text{-}PO_4$ isotopic composition. Importantly, the data also suggest that method used is quite robust and able to deal with samples that contain high concentrations of organic matter.

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