We demonstrate how a combination of paper microfluidic devices and handheld mobile technology can be used by citizen scientists to carry out a sustained water monitoring campaign. We have developed a paper-based analysis device and a 3 minute sampling workflow that requires no more than a container, a test device and a smartphone app. The contaminant measured in these pilots are phosphates, detectable down to 3 mg L\(^{-1}\). Together these allow volunteers to successfully carry out cost-effective, high frequency, phosphate monitoring over an extended geographies and periods.
Citizen-led sampling to monitor phosphate levels in freshwater environments using a simple paper microfluidic device

Samantha Richardson,1 Alexander Iles,1 Jeanette M. Rotchell,2 Tim Charlson3, Annabel Hanson4, Mark Lorch,*1 and Nicole Pamme*1

1 Department of Chemistry and Biochemistry, University of Hull, UK, 2 Department of Biological and Marine Sciences University of Hull, UK, 3 Pocklington Canal Amenity Society, UK, 4 East Riding of Yorkshire Council, UK.

* Corresponding authors

M.Lorch@hull.ac.uk
N.Pamme@hull.ac.uk
Abstract

Contamination of waterways is of increasing concern, with recent studies demonstrating elevated levels of antibiotics, antidepressants, household, agricultural and industrial chemicals in freshwater systems. Thus, there is a growing demand for methods to rapidly and conveniently monitor contaminants in waterways. Here we demonstrate how a combination of paper microfluidic devices and handheld mobile technology can be used by citizen scientists to carry out a sustained water monitoring campaign. We have developed a paper-based analysis device and a 3 minute sampling workflow that requires no more than a container, a test device and a smartphone app. The contaminant measured in these pilots are phosphates, detectable down to 3 mg L⁻¹. Together these allow volunteers to successfully carry out cost-effective, high frequency, phosphate monitoring over an extended geographies and periods.
Introduction

The European Commission Water Framework Directive (WFD) sets out legislation to ensure that all waterbodies across Europe achieve a ‘good’ ecological status. (1, 2) In 2016, 86% of UK rivers failed to reach this status; of the assessed water bodies, 55% featured excess levels of phosphate, resulting in failure to reach the desired ‘good’ status. (3) Nutrient levels, however, are not constant, they vary widely spatially and temporally, and patterns are often missed due to infrequent measurement. (4) Therefore, to improve water quality and better understand whether nutrient levels meet the aims of the WFD, robust and frequent monitoring of water quality is vital to safeguard supplies and to manage the health of aquatic ecosystems. (5, 6)

Current routine water quality monitoring generally relies on established analytical methods such as high performance liquid chromatography (HPLC) or UV/vis spectroscopy, whereby a trained expert will go out into the field to collect samples which are then taken back to a laboratory, prepared and analysed. (7) These techniques are costly and time consuming and thus monitoring is carried out at low frequency and low spatial resolution. (5, 8) Routine monitoring is often only conducted on a monthly basis at best, with the sampling points along a river limited by time, resources and constraints in territory. (8, 9) A fuller understanding of water quality and contaminant dynamics, including sources and behaviour of contaminants are often lacking, hindering cost-effective and targeted environmental management. (9) A low-cost, easy to use method will facilitate better quantification of trends and pressures, underpin predictive modelling and provide the foundation for robust and cost-effective management of the aquatic environment.

High frequency sensing could be achieved with simple and low-cost devices operated by citizens or lightly trained agents. Data can then be uploaded to a cloud to build a picture of a larger area that is not easily obtainable by sending expert scientists into the field. According to the literature, some on-site systems have been developed as well as some autonomous systems for passive monitoring. (10, 11) However, currently such devices are relatively expensive, require several setup steps or require expertise to perform the manual steps in the workflow, such as calibration or sample preparation, making them unsuitable for regular low cost monitoring through volunteer-led sampling campaigns.
Dip tests or microfluidic Paper-based Analytical Devices, PADs, offer a promising alternative for on-site analysis with sampling methods simple enough to be completed by a non-expert. (12, 13) Devices can be readily produced by patterning commercially available hydrophilic cellulose filter paper with a hydrophobic material such as wax (14, 15) to create channels and reaction zones within the paper matrix. Reagents can be preloaded and stored in dry form on the devices. Fluid is transported into and through the paper by capillary forces. Meanwhile, via a smartphone app, users could conveniently photograph results from the PADs and upload these images along with records of the time and location of the measurements. Here, we set out to develop a PAD and app for truly simple on-site monitoring of contaminants by members of the general public. By taking such an approach we aim to allow regular and frequent on-site measurements by volunteer groups across a wide area not previously achieved.

The chosen analyte, phosphate, is an important example of a potential freshwater pollutant. (16) Phosphates are essential nutrients present in freshwater environments at low concentrations (0.005 to 0.05 mg L⁻¹). (17) However, it is well documented that aquatic levels are often artificially increased by run-off from agricultural and domestic activities. (17-19) Excessive amounts of phosphates, i.e. in the milligram per litre range, can lead to eutrophication; the rapid growth of algae. (20) In severe cases thick algal blooms reduce oxygen levels in water bodies and stop sunlight reaching beyond the surface of the water; in the most serious cases, the decomposition of the algae can lead to build-up of harmful toxins. (19-21) Problems associated with phosphate induced eutrophication include reduced fish populations, excessive death of fish during summer period, and changed composition of aquatic species in affected water bodies. (22)
Detection of orthophosphates, the main form of bioavailable phosphate linked to eutrophication, is typically performed using UV/vis spectroscopy via formation of the phosphomolybdenum blue (PMB) complex. Occasionally an ion exchange chromatography method is also employed. Both methods require laboratory equipment and expertise to operate. Phosphate test strips are commercially available from some providers, including Hach (UK); these rely on a colour change that can be compared to a colour chart. As the colour change is usually very subtle, it can be easily misinterpreted, leading to incorrect recording of phosphate levels. Other kits mostly require the mixing of reagents, typically nitric acid, making them less suitable for use by an untrained operator for on-site analysis.

Jayawardane et al. reported a paper-based microfluidic device for phosphate analysis from water samples. They created two reaction zones to separately store the reagents needed for the PMB reaction. Sulfuric acid at 6.6 M was employed, which required careful optimisation to avoid damage to the paper by hydrolysis of the cellulose. A Teflon sheet had to be placed between the two layers and sealed by lamination. Before use, the device had to be cut open and the Teflon sheet removed. A working range of 0.6 – 30 mg L\(^{-1}\) and limits of detection and quantitation of 0.15 and 0.48 mg L\(^{-1}\), respectively, were obtained. These are environmentally relevant levels. However, samples were added via a pipette and results were recorded via a flatbed scanner after 10 min incubation. The detection zones were 3 mm in diameter and hence rather small for visual inspection. The reported paper device was thus not usable by volunteers working in the field.

In contrast to the previously reported work, we set out to develop a simple to operate phosphate detection device with colorimetric readout that requires minimum input from the operator, yields a result within a few minutes that can be captured via a smartphone camera, thus avoiding the use of dedicated detection equipment. We show that such a system can be used by lightly trained volunteers to collect a significant data set, and this therefore is an appropriate tool to deploy across a wide area to elucidate patterns in spatial and temporal variations of waterway contamination pressures.
Experimental

Design and fabrication of the paper devices

Details of reagent preparation and stock solutions are given in SI1. Paper-based devices were wax printed on Whatman Grade 1 filter as outlined in SI2. The wax pattern was printed in two areas that were folded on top of each other to form an upper and lower reagent zone (Figure S2). The design used for field-based sampling, featured eight 10 mm circles enclosed with a rectangular wax box to reduce leaking in case of poor alignment between the upper and lower paper layers. These devices featured six detection zones (n = 6) as well as two negative control zones. At low analyte concentrations, any colour pigmentation in the water could potentially lead to a false positive result. Therefore, two of the eight circular zones were not loaded with any reagents, thus acting as blanks. This design also had a four-digit code so that individual devices could be identified.

The process of loading reagents and sealing the devices is shown in figure 1. Reagents were pipetted onto the respective circular zones and left to dry. The paper was backfolded and then sealed by lamination to maintain alignment and enclose the deposited reagents. More specifically, 5 µL of the ascorbic acid reagent and 5 µL of molybdate/antimony reagent were pipetted onto zone 1 and zone 2, respectively. The 5 µL volume was chosen as it was sufficient to cover the 10 mm zone effectively whilst leaving no excess liquid on the surface thus minimising drying time. The devices were allowed to air dry for about 30 min and then further dried overnight (-20 °C) to ensure complete evaporation of water, before being backfolded and laminated at 80 °C with matte finish 150 micron pouches (Lyreco, UK). Two slits in an x-pattern were cut with a scalpel into the back of the device to allow for water entry.
Fig 1: Production of PAD. (i) Circular reaction zones were wax printed onto filter paper. (ii) Ascorbic acid solution was added to each circle in zone 1, Mo/Sb reagent to each circle in zone 2. After drying, the paper was backfolded to align the sample zones. (iii) Devices were sealed by lamination to encase the reagents and prevent contamination. (iv) Slits were cut into the back of the device (zone 1) to allow for sample entry.

**Sampling and data collection**

Development of the analysis workflow was a key factor in the design of the device. The laboratory-based analysis workflow needed to be as similar as possible to the real-world analysis to achieve a device fit-for-purpose and to validate the sampling methodology. Thus, we decided to use a simple dip method (figure 2). In the laboratory, the device was placed in a dish containing approximately 20 mL of sample, with the front side facing upwards. Water soaks into the device until the cellulose in the reaction zone is saturated, the sample components are left to react with the pre-deposited reagents. Following optimisation, a short incubation time of 3 min was found to be sufficient to allow for a stable colour to form. After this time, the image was captured using either a flatbed scanner (CanoScan LiDE 220) or a smartphone camera (Huawei P smart). In the field, the same protocol was followed, the volunteers would dip the device on a sample of freshwater and the image of the device was collected using the volunteers’ own phones and the custom-developed RiverDIP app. Image analysis was carried out with ImageJ freeware as detailed in SI3. Benchmarking against the gold standard UV/vis spectrophotometry method was undertaken as described in SI4.
Fig 2: (a) The laboratory analysis workflow involved the paper device being placed into a dish with 20 mL aqueous sample. The sample entered through the slits in the back of the devices. After 3 min incubation, the formation of the blue colour on the upward facing side of the device was captured. (b) The workflow for volunteer sampling involved the collection of a water sample from a river and an aliquot being placed into a container. The paper device was dropped into the sample, the same as in the laboratory, and after 3 min, a photograph of the result was taken and uploaded via the RiverDIP app.

RiverDIP (fig 3) was developed in collaboration with Natural Apptitude Ltd (UK), a company specialising in app developing including for Citizen Science projects. The app is available via the Apple or Android Appstore. After logging on, the user is required to read safety information and confirm understanding. The user can then select to carry out a measurement, i.e. ‘start a new record’. The user will take a photo of the paper microfluidic device from within the app, following running down of a 3 min timer to ensure the test result is taken after the required incubation time. Furthermore, the user can upload photographs of the sampling location and water turbidity. In addition, the app records date, time and location of the sampling, with the user manually inputting the device code and name of the water body sampled. Users also give their own interpretation of the results and water turbidity using a colour sliding scale. They can then leave free-form comments if they wish. The data is uploaded onto a server and can be retrieved later for quantification of phosphate levels using image analysis software.
Fig 3: The RiverDIP app was custom-developed to record test results in the field, with GPS location and time alongside photographs of the water quality and the surroundings. (a) The ‘record navigation page’ shows the data that needs to be completed for each sampling. (b) ‘Capturing test PAD result’ will start a 3-min timer, after which an image can be captured and uploaded. Volunteers can compare the result to a colour intensity scale bar and select the colour intensity that matches their results the closest. (c) Screenshot showing a completed result ready for submission with date, time, record code and location and images of the PAD result, location and water quality.

Study areas
Volunteer sampling was carried out across the North Sea region in 2019 to 2021, with sampling across the UK, The Netherlands, Belgium and Germany. Alongside this wider sampling campaign some smaller sites were sampled more frequently in particular along a section of the Pocklington Canal (between GPS locations 53°54'57.5"N 0°47'01.5"W and 53°52'32.7"N 0°56'07.4"W) within the Humber catchment (UK). This is a typical example of a managed lowland waterway heavily influenced by rural agricultural activity, and, despite its status as a site of special scientific interest (SSSI), has a history of nutrient enrichment. The canal is approximately 15 km long, 8 km of this being navigable. Sampling was undertaken along a 7 km stretch of the canal including both navigable and non-navigable sections.
Comparison of PADs to the gold standard UV/vis analysis method was carried out using samples from the Pocklington Canal and River Aire. Both are located within the Humber catchment, however, the River Aire represented a water system heavily influenced by urban activity in comparison to the Pocklington Canal. This gave a range of water samples to study the effect of sample matrix on the colorimetric reaction chemistry.

**Results and Discussion**

**Optimisation of reagents and method**

The standard method for phosphate analysis via the phosphomolybdenum blue (PMB) reaction,\(^\text{(26)}\) as well as the previously reported paper-based method,\(^\text{(28)}\) rely on sulfuric acid to provide the acidity needed to minimise auto-reduction of the ammonium molybdate reagent. However, it is well documented that sulfuric acid readily hydrolyses cellulose,\(^\text{(29)}\) the main component of the filter paper material used in paper microfluidic devices. Therefore, instead of sulfuric acid we chose to use p-toluenesulfonic acid (TsOH), a non-oxidising solid acid with pKa -1.34 that allowed the required low pH to be achieved whilst avoiding paper hydrolysis.

A key factor when designing the sampling method was to have a short incubation period to avoid testing the volunteers’ patience when taking measurements in the field. The PMB reaction is time dependant, a heteropoly acid complex is reduced over time to form the brightly coloured product.\(^\text{(26, 31-33)}\) Many variations of this reaction have been reported with incubation times ranging from 90 s to several hours.\(^\text{(26)}\) As a starting point, we used concentrations of 0.01 M Mo reagent together with 0.01 M ascorbic acid as the reducing agent, similar to methods previously reported with incubation times of up to 10 min.\(^\text{(34-38)}\) Antimony tartrate is commonly added when using ascorbic acid as the reducing agent to improve the rate of reduction and to remove the need to heat the reaction.\(^\text{(25)}\) For the here reported work, 0.6 mM antimony tartrate was used.
The incubation time required to obtain a stable readout of the blue coloured PMB complex needed to be optimized. Ideally this process would take less than 5 min and be visible to the human eye. A series of experiments was performed with 5 µL of molybdate/antimony reagent (0.01 M Mo and 0.6 mM Sb in 2 M TsOH) and 5 µL of ascorbic acid (0.01 M) for phosphate concentrations of 0, 10, 100 and 1000 mg L\(^{-1}\). The reactions were carried out under laboratory conditions at room temperature (25 °C) and images captured using the flatbed scanner every minute and analysed with ImageJ. The results are plotted in figure 4. The colour was found to rapidly develop in the first minute of the reaction for all concentrations of phosphate solutions other than the 1 mg L\(^{-1}\) solution. The colour intensity then, more slowly, increased further up to 2 min at which point it began to plateau. At 3 min, the colour was found to be stable across the sample zone. Based on these findings, 3 min was deemed the ideal length for the volunteer-based dip tests; long enough for the colour to develop and become stable; yet still short enough for work with volunteers out in the field.

![Figure 4](image.png)

**Fig 4:** Formation of the blue PMB complex over time at different phosphate concentrations. The colour intensity was found to increase for up to 2 min and then plateau (n = 3).

To ensure maximum sensitivity from the device whilst minimising auto-reduction of the molybdate complex, a careful balance between [Mo(VI)] and [H+] must be achieved. Optimisation of these conditions is described in SI5.
Limits of detection and quantification

Following the optimisation experiments, calibration curves for to determine limits of detection (LOD) and limits of quantification (LOQ) were generated from devices prepared by pipetting of 5 µL of molybdate/antimony reagent ([Mo(VI)] = 0.01 M / [antimony] = 0.6 mM) in 2 M TsOH into zone 1 and 5 µL of 0.01 M ascorbic acid into zone 2 prior to lamination. This method was used to obtain calibration data (SI6) giving an LOD and LOQ of 3 mg L\(^{-1}\) and 8 mg L\(^{-1}\), respectively. Eutrophication can occur when phosphate levels exceed 0.1 mg L\(^{-1}\), however levels much higher were sometimes detected by the UK environment agency in recent years during routine water quality monitoring. Water quality records from 2018 show available phosphorous levels between 0.001 – 20 mg L\(^{-1}\). With unplanned sampling recording levels into the 100’s mg L\(^{-1}\). (40) The PAD devices we have developed here feature a relatively high limit of detection. Despite this the devices can still be used to screen large areas by taking a semi-quantitative approach. This can provide very useful information about a river system and highlight areas that may have excessive phosphate levels, which can then be further investigated.

Stability

To enable volunteer-based sampling campaigns, PADs need to be stable for a reasonable period of time to allow devices to be distributed and used in a realistic time frame. This was addressed to some extent by storing the two reagents separately on different sites on the paper devices on the two reaction zones which were back- folded to minimise contact (see figure 1). Whilst this approach increases the lifetime of the reagents, it does not prevent the auto-reduction of the molybdenum complex, which is typically controlled by low pH, however not eliminated completely.
To assess the viability of distributing the phosphate PADs to volunteer groups, we studied the performance of the devices following storage under different conditions over a 4 week period. Devices were prepared as stated in section 2.1 and then stored in the dark in a closed box, at room temperature with and without silica gel desiccant sachet, to elucidate how moisture affected long-term storage. Devices were also stored in a domestic fridge (4 °C) and freezer (-20 °C). Device performance was tested on day 0, day 7 and day 28 by performing the 3 min dip test with a 10 mg L\(^{-1}\) PO\(_4^{3-}\) solution. The results are shown in SI7. It was found that devices stored in the freezer were stable for the full length of the period investigated and this approach was therefore used for long term storage of the devices. The more important aspect of the stability test was that over a shorter period of time, i.e. 1 week, devices remained stable when stored in ambient conditions. After 1 week of storage, devices stored at room temperature yielded the same intensity compared to those used straight away when tested with 10 mg L\(^{-1}\) phosphate solution. Thus the devices are stable for long enough to allow for batch manufacturing, distribution via postal services, cold storage on arrival and subsequent use by volunteer groups.

**Inferences**

To ensure the PADs can be used to accurately analyse the levels of phosphate in river water, it was important to ensure cross-reaction with other species in a water sample matrix does not either interfere with the colour readout or give a false reading. Silicates (SiO\(_4^{4-}\)) in the water system are reported as the main interferent in the PMB reaction, they form heteropoly acid complexes with 12-MPA.(41) This is seen particularly at high pH(37, 42, 43) and temperatures.(42) To investigate the potential for silicate interference when using the PADs for environmental measurements, 10 mg L\(^{-1}\) phosphate solutions were doped with silicate at a range of concentrations (100 – 1000 mg L\(^{-1}\)) and the observed intensities were compared to a typical intensity value achieved when measuring a solution of 10 mg L\(^{-1}\) PO\(_4^{3-}\) only. The results as shown in figure S8.1 and S8.2 demonstrate that the silicate reaction occurs over significantly longer timescales. Therefore, over the 3 min incubation time period, silicate present in the water course does not interfere with the phosphate measurements. We also investigated whether silicates would produce a false positive result when no phosphate was present in the solution. At 1,000 mg L\(^{-1}\) silicate and an incubation period of 3 min the silicate readout was equivalent to a blank sample, thus demonstrating that silicates in the water system will not produce a false positive result.
Whilst no interference from silicates was found, many other species could be present in a water sample that may interfere with the PMB reaction. To investigate this, Milli-Q water and canal water samples were spiked with phosphate. Samples from the Pocklington Canal (Yorkshire, UK) were tested, the canal is a water source with high calcium carbonate (100’s mg L\(^{-1}\)) and chloride levels (up to 100 mg L\(^{-1}\)) as well as many other water soluble ions in sub-mg L\(^{-1}\) levels. (40) Both water samples yielded results with no significant difference to a blank sample \((t_{\text{stat}} = 5.127, t_{\text{crit}} = 5.849 \text{ at } \alpha = 0.05; n = 8 \text{ for a two-tailed } t\text{-test})\). Therefore, it was assumed both samples contained no detectable levels of phosphate. The canal samples were then spiked with phosphate, in the range of 0 – 10 mg L\(^{-1}\) and 0 – 1000 mg L\(^{-1}\), and compared to the same concentrations spiked into Milli-Q water (figure S8.3), demonstrating that there was no inference from the sample matrix.

**River water measurements**

Next, the paper PADs were tested with samples collected from the River Aire and Pocklington Canal (both within Yorkshire, UK). Water samples were stored at 4 °C prior to laboratory analysis. The results obtained from the PADs and the industry standard UV/vis spectroscopy method (44) are shown in figure 5. As can be seen, both methods gave comparable results. Whilst the traditional spectroscopy method gives more accurate results, both would yield the same phosphate level (to 1 s.f.). The PADs however can be used in the field and could be employed to rapidly gather large amounts of semi-quantitative data.
**Fig 5:** Comparison of PO$_4^{3-}$ levels in freshwater samples detected using UV/vis spectroscopy via PMB reaction (n = 3) and modified PMB method on the paper microfluidic device with flatbed scanner image capture (n = 6).

When carrying out field sampling it is important to account for changes in environmental conditions such as lighting, colouration or turbidity of the water samples and camera to camera differences. At low analyte concentrations, as would be expected, it is possible that any colour pigmentation in the water could lead to a false positive result. To address this, two negative control zones were introduced into devices used in the field. These zones were left blank so that the colour intensity read from them would relate only to the colour of the water sample (figure S9). Average relative intensity (ARI) for the real freshwater samples were calculated from the average pixel intensity (AI) of the reaction zones and the average pixel intensity of the blank zones (equation 1). Using this method, a calibration curve was constructed (figure 6) that would be suitable for the analysis of field sampling results.

\[
ARI = \frac{AI_{\text{reaction zone}} - AI_{\text{blank zone}}}{AI_{\text{ref. sq.}}}
\]

(eq. 1)
Fig 6: Typical calibration curve obtained with images captured from a smartphone camera. The obtained colour development in the sample reaction zones were standardised against both the internal standard (blue square) and the negative controls to account for interferences from varying light conditions and background colour from the water sample (n = 6).

To ensure comparability between results obtained using different image capture methods, we determined phosphate levels in freshwater samples when captured using a flatbed scanner (laboratory-based detector) and a smartphone (field-based detector) (table S9). We found that both image capture methods yielded the same phosphate concentration with all samples tested. Using a scanner to capture the image gave a more reproducible result as the lighting was constant, however, results captured using a smartphone, in a less controlled environment, still produced the same overall result. This shows the viability of these devices for volunteer-led sampling campaign to capture large amounts of data out in the field to reliably indicate levels of phosphate.
Following this optimisation, the devices were tested with volunteer groups from the local Canal and River Trust (CRT), who manage and maintain water courses such as the Pocklington Canal, UK. Group leaders were trained to carry out sampling, capture and upload the data to the RiverDIP app before leading sampling sessions across the Humber catchment area. This was further extended to volunteers across the North Sea Region specifically in Belgium, the Netherlands and Germany, who undertook sampling independently after initial training. During 2019 and 2021 over 350 samples were collected by volunteers across the North Sea Region.

By using volunteers to perform sampling across a large geographical area and with frequent measurement, large amounts of data could be gathered, showing trends and pressures not otherwise seen by traditional analysis. One way to represent field data is via a map demonstrating levels using coloured markers for each sampling point. Such data could be used to display results to a public audience. An example of this is shown in figure 7 representing all the successful tests carried out in this period, n = 332 Here, we display results gathered from volunteers across the North Sea region. In certain areas volunteers engaged in routine sampling across longer time periods. This allowed use to detect seasonal variations in phosphate levels along a 16 km stretch of the Pocklington Canal. Figure ESI 10. This demonstrates that the PADs, app and workflow are a cost-effective method to monitor waterways across large areas and the resulting data can be used to detect seasonal trends and contaminant pressures not seen with traditional analysis methods.

Fig 7: Data collected via citizen-led sampling sessions across the North Sea region presented for public dissemination via Google maps. Throughout 2019 and 2021 over 300 samples were collected by volunteer across the region. The location of the marker represents the GPS location of sampling, the colour of the marker represents the obtained phosphate level (none <1 mg L⁻¹, low 1-3 mg L⁻¹, medium 4-6 mg L⁻¹ and high ≥ 7 mg L⁻¹). Each marker links to other details captured on the RiverDIP app at the time of sampling; including the user’s own interpretation of the phosphate level, a photo to record water turbidity, and any notes made during the sampling.
Conclusions
We have demonstrated a simple to use paper microfluidic device suitable for on-site field analysis by lightly trained volunteers. The devices return semi-quantitative readings, with a limit of detection of 3 mg L^{-1} of phosphate levels in freshwater samples in just 3 minutes. Results obtained from using these methods were comparable to those obtained using the laboratory gold standard method of UV/vis analysis. The paper devices record a colour change which was captured using a smartphone camera and uploaded via the custom-designed RiverDIP app for further analysis. The image analysis method accounts for variations in environmental lighting conditions and turbidity in the sample. We also confirmed there was no interference from silicates nor other constituents of in a freshwater sample.

Devices can be manufactured and stored for at least 4 weeks, allowing distribution via mail. Field tests have confirmed that the PAD devices can be used by volunteers to gather the data with little input from experts. This approach has great potential for environmental data to be gathered across wide geographical areas, thus potentially providing freshwater quality readings with high spatial and temporal resolution to better monitor and respond to pressures.

Conflicts of interest
There are no conflicts to declare.

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Electronic Supplementary Information (ESI)

Citizen-led sampling to monitor phosphate levels in canal and river water using simple paper microfluidic device

Samantha Richardson,1 Alexander Iles,1 Jeanette M. Rotchell,2 Tim Charlson3, Annabel Hanson4, Mark Lorch,*1 and Nicole Pamme*1

1 Department of Chemistry and Biochemistry, University of Hull, UK, 2 Department of Biological and Marine Sciences University of Hull, UK, 3 Pocklington Canal Amenity Society, UK, 4 East Riding of Yorkshire Council, UK.

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1. Reagents
A stock solution of 1,000 mg L$^{-1}$ containing sodium dihydrogen orthophosphate (BDH, UK) was used to prepare working standards in the range from 0 to 1,000 mg L$^{-1}$. Molybdate/antimony reagent for the detection of orthophosphate was prepared from 0.01 M ammonium heptamolybdate tetrahydrate (Fisher Scientific, UK) and 0.6 mM potassium antimony(III) tartrate hydrate (99 %, Fisher Scientific, UK) dissolved in 2 M p-toluenesulfonic acid (TsOH) (Fisher Scientific, UK). The reducing reagent consisted of 0.01 M L-ascorbic acid (AA) (Fisher Scientific, UK). A stock solution of 1,000 mg L$^{-1}$ sodium orthosilicate pentahydrate (Fisher Scientific, UK) was used to prepare working standards to test for silicate interference. Reagents were prepared in plastic vessels to avoid potential silicate contamination from glassware. Stock solutions were stored in a refrigerator and kept for no longer than one week; ascorbic acid solutions were freshly prepared each day.

2. Paper Device preparation and Designs
Paper-based devices were designed using AutoCAD 2016 software and printed onto Whatman Grade 1 filter paper using a Xerox ColorQube 8570 wax printer with ColourQube wax inks as described by Carrilho et al.$^{1,2}$ Adapting a method from Peters et al.$^3$ the wax printed sheets were passed through a laminator (Fellows, model Callisto A4) three times at 125 °C. This allowed the wax to melt and flow through the porous paper to form a hydrophobic barrier throughout the entire paper thickness. The wax pattern was printed in two areas that were folded on top of each other to form an upper and lower reagent zone. **Design 1** (Figure S2a) featured eight pairs of circles, 10 mm diameter and 8 mm centre to centre distance, printed with 0.7 mm line width in orange wax. The orange colour allowed for a good contrast to the blue colour developed during the PMB reaction. As these devices were to be used by volunteers in the field, it was important to have a detection zone that can be easily visualised and to this end we settled on 10 mm diameter. Each device also included a set of six coloured squares as internal standard to account for changes in lighting conditions and camera to camera differences during image analysis. A further design change was introduced, **Design 2** (Figure S2b) whereby the eight circles were enclosed with a rectangular wax box to reduce leaking in case of poor alignment between the upper and lower paper layers. At low analyte concentrations, any colour pigmentation in the water could potentially lead to a false positive result. Therefore, two of the eight circular zones were not loaded with any reagents to act as blanks for in-the-field devices. Any colour in these zones would originate from the colour of the water sample. These devices thus featured six detection zones (n = 6) and two negative control zones, the latter allowing for correction due to water colouration or turbidity. A four digit code (marked XXXX in figure S2b) was also included so that individual devices could be identified. Design 2 was rolled out for use with volunteers.
Figure S2: (a) Design 1 with wax circle dimension before heating. (b) Design 2 with circular openings in a wax area to minimise leaking of reagents due to poor alignment of the reagent zones.
3. Image Analysis

Images, whether captured in the laboratory or in the field, were analysed with ImageJ freeware (National Institutes of Health, USA) (figure S3). Akin to a method published by Boehle et al.,\(^4\) the .jpg images were first inverted. The image was then split into the three separate colour channels (RED, GREEN and BLUE) and only the RED channel was used for further image analysis, as this was found to give the largest difference for measurements of varying sample concentrations. The area enclosed by the orange wax barrier was analysed by measuring the mean grey pixel intensity to give an average pixel intensity for each circle. Data was obtained from the six to eight circles on the paper device; outliers were removed using the Dixon Q-test\(^5\). To minimise effects from variations in environmental conditions, matte laminating pouches, instead of glossy pouches, were used; and an internal coloured square was included in the PAD design (see figure 1). Average relative intensity (ARI) was obtained by dividing the average pixel intensity (AI) for the individual circles by the average pixel intensity of the blue reference square (equation S1).

\[
ARI = \frac{AI_{\text{spot}}}{AI_{\text{ref. sq.}}}
\]  

\text{eq S1.}

Figure S3: ImageJ analysis process for individual paper device (a) The captured .jpg image was inverted and split into the RGB channels. (b) The first step in the image analysis process where the captured image was inverted. (c) The RED channel only was used for image analysis of each reaction circle and the blue printed square.
4. Benchmarking with UV/vis spectrophotometry

Results from the PADs were validated by comparison to the industry standard UV/vis spectrophotometry method. This was performed in triplicate. The molybdate/antimony reagent was prepared by adding 5 mL \( \text{H}_2\text{SO}_4 \) [5 M] to 0.5 mL potassium antimonyl tartrate [0.4 mM], 1.5 mL ammonium molybdate [0.3 M] and 3 mL ascorbic acid [0.1 M]. This solution could be stored for up to 4 h in a dark container. To perform the test, 1 mL of this reagent solution was added to 4 mL of phosphate sample or river water sample and vortexed for 30 s. After an incubation period of 20 min, absorbance was measured at 880 nm on a UV/vis spectrometer (Jenway 6705). A reagent blank was used as reference solution.

5. Optimisation of Molybdate and Toluene sulfonic acid concentrations

To ensure maximum sensitivity from the device whilst minimising auto-reduction of the molybdate complex, a careful balance between [Mo(VI)] and [H+] must be achieved. There are various forms of agents used to reduce the 12-MPA, the most common being ascorbic acid and tin chloride. Of these, ascorbic acid was the preferred choice due to the rapid formation of a stable product which is unaffected by chloride interference. The ammonium molybdate complex used in the PMB reaction can undergo direct reduction if the acidity is too low; typically reactions are performed between pH 0.5-1 to minimise this. However, increasing the concentration of H+ too far will inhibit the reaction, reducing the sensitivity of the device. A range of TsOH concentrations were screened (0-4 M, pH 5.3 - 1.4) whilst all other reagent concentrations and volumes remained consistent at 0.01 M Mo/Sb 0.6 mM and 0.01 M ascorbic acid, with 5 µL added to each reagent zone. Each device was dipped into a 10 mg L\(^{-1}\) phosphate solution for 3 min and an image captured using the flatbed scanner (figure S5a). The maximum reproducible sensitivity was achieved when using 2 M TsOH, therefore this was chosen as the concentration for further experiments. Removing TsOH from the system did not prevent the PMB reaction from occurring, however the results became erratic, seen by the high level of error produced from one reaction to the next. The lack of acidity allowed several Mo(VI) species to form, rather than just having the MoO\(_4\)\(^{2-}\) species present as needed for the 12-MPA complex to form efficiently. Increasing the acidity favours the formation of the 12-MPA species, thus better controlling the reaction, as seen at 1 M and 2 M TsOH. However, above 2 M TsOH concentration, the formation of the final colour product was suppressed, seen as a significant reduction in intensity when using 3 M or 4 M acid. Similar trends have been previously demonstrated by Nagul et al. when using excessively high acidities.\(^{(26)}\)
Figure S5a: Intensity of the blue colour formed from PMB complex after 3 min incubation in 10 mg L⁻¹ PO₄³⁻ solution with varying TsOH concentrations (n = 8).

With a fixed acid concentration of 2 M TsOH, the Mo(VI) concentration was then studied between 0.005 M and 0.02 M to maximise sensitivity without increasing the signal generated by a blank water sample. As can be seen from figure S5b, increasing the concentration of molybdenum in the system increased the amount of the PMB complex produced, thus increasing the colour intensity. A concentration of 0.01 M was shown to give the maximum response whilst still minimising background colour in the blank. Above this value, the intensity of the blank dramatically increased, showing that the direct reduction was no longer controlled. Although there was little difference between the intensity of 0.005 M and 0.010 M Mo(VI), lower concentrations would potentially lead to inconsistent results as shown in figure S5b, where the lower concentration produced a patchy response due to an insufficient quantity of Mo(VI) present in the reaction zone.
Figure S5b: Photographs of devices and blue colour intensity measurement when incubated for 3 min with varying [Mo (VI)] concentrations. Devices were tested in both 10 mg L\(^{-1}\) PO\(_4^{3-}\) solution and a blank of Milli-Q water (n = 8).
6. Laboratory-based calibration under optimal conditions

**Figure S.6** shows a typical calibration curve obtained under laboratory conditions, images were captured using a flatbed scanner (CanoScan LiDE 220) after 3 min incubation in sample solution. This was used when optimising and comparing laboratory-based methods to field results.

**Figure S6:** Linear relationship between concentration and intensity between 0 and 10 mg L\(^{-1}\) PO\(_4^{3-}\) using the optimised device preparation and 3 min incubation time. Images were captured using a flatbed scanner and analysed with ImageJ to compare the intensity of the internal standard to the reaction zone.

LOD (PO\(_4^{3-}\)) = 3 mg L\(^{-1}\) and LOQ (PO\(_4^{3-}\)) = 8 mg L\(^{-1}\) (n = 8).
7. Stability
Laminated devices with slits already cut into the back of the device were stored under different conditions to determine stability over a one month period.

| Storage method       | Intensity / a.u. | Day 0      | Day 7      | Day 28     |
|----------------------|------------------|------------|------------|------------|
| Freezer (-20 °C)     | 0.25 ± 0.004     | 0.25 ± 0.030 | 0.25 ± 0.010 |
| Fridge (4 °C)        | 0.25 ± 0.004     | 0.25 ± 0.020 | 0.32 ± 0.020 |
| Cupboard (20 °C)     | 0.25 ± 0.004     | 0.25 ± 0.020 | 0.30 ± 0.020 |
| Cupboard (desiccant, 20 °C) | 0.25 ± 0.004 | 0.41 ± 0.080 | 0.41 ± 0.040 |

8. Interferences
Silicates are the most common interferences in the PMB reaction and potentially present in water samples. The 3 min dip test was carried out with a range of silicate containing samples (100 – 1000 mg L\(^{-1}\)), as well as with samples containing only silicate and no phosphate. These were compared to a blank sample to study whether false positives may occur.

![Figure S8.1: Average intensity of blue colour formed after 3 min incubation of PAD in a range of sample solutions. A typical response for a 10 mg L\(^{-1}\) was compared directly with two samples containing silicate at 100 and 1000 mg L\(^{-1}\). This showed no significant difference between any samples investigated using ANOVA whereby \(F = 0.5589, F_{crit} = 3.467\) at \(\alpha = 0.05\); \(n = 8\). A comparison of a solution containing only 1000 mg L\(^{-1}\) silicate and a blank solution were also compared, again showing no significant differences. \(t_{stat} = 0.8584, t_{crit} = 2.145\) at \(\alpha = 0.05\); \(n = 8\) for two-tailed t-test.](image-url)
It is well established that the formation of the molybdenum silicate complex favours longer reaction times. This would suggest that over the required short incubation period, no interference should be seen, however, over longer periods secondary reactions may occur. To investigate this, PADs were monitored for 30 min after initial water entry with an image captured every minute.

**Figure S8.2:** Formation of the blue coloured molybdenum complex over time with solution of 10 mg L\(^{-1}\) phosphate with and without silicate present at 100 and 1000 mg L\(^{-1}\). This was also compared to the coloured complex formed when only silicates were present in solution at 1000 mg L\(^{-1}\), n = 6. (a) Reaction occurring over 30 min showing the formation of the molybdenum-silicate complex was far slower than the molybdenum phosphate complex. (b) Reaction over 5 min, showing clearly within the window of 3 min that silicate does not interfere or give a false positive result.
A river water sample has a complex matrix potentially containing many ions which could interfere with a readout. To explore this, aliquots of a real river water sample were spiked with phosphate were compared to Milli-Q water spiked with the same concentrations (Figure S4.3). The water samples were taken from the Pocklington Canal (Yorkshire, UK, GPS location 53° 54' 4.2588'' N 0° 48' 7.9164'' W), a water source with high calcium carbonate (100’s mg L⁻¹) and chloride levels (up to 100 mg L⁻¹) as well as many other water soluble ions at sub mg L⁻¹ levels.

**Figure S8.3:** (a) Comparison of colour intensity formed after 3 min incubation in a real water sample (Pocklington Canal, UK) and Milli-Q water, both were spiked with phosphate at 0 mg L⁻¹, 2.5 mg L⁻¹, 5 mg L⁻¹, 7.5 mg L⁻¹ and 10 mg L⁻¹ (n = 8). (b) Comparison of colour intensity formed after 3 min incubation in a real water sample (Pocklington Canal, UK) and Milli-Q water, both were spiked with phosphate at 0 mg L⁻¹, 1 mg L⁻¹, 10 mg L⁻¹, 100 mg L⁻¹ and 1,000 mg L⁻¹ (n = 8).
9. Field results
To account for water turbidity and false colour occurring from the water sample, negative control
zones were added to the PAD. These two zones contained no reagents therefore the only colour
produced here was due to the colour from the water sample (figure S9). A comparison of phosphate
levels ($\text{PO}_4^{3-}$) determined using 3 min dip test when images were captured using either a laboratory-
based method (flatbed scanner), or a field-based method (smartphone) (table S9).

![Figure S9](image)

**Figure S9**: (a) Design of a sampling device with two negative control zones, to account for any colour
from the water sample. (b) Examples of a result obtained from a volunteer showing the six reaction
zones and two negative controls. Images were captured whilst devices were still with the container to
ensure a simple procedure.

| Water sample         | Phosphate concentration / mg L$^{-1}$ | Scanner | Camera phone |
|----------------------|--------------------------------------|---------|--------------|
| River Aire (sample 1)| $2 \pm 0.060$                        | $2 \pm 0.200$ |
| River Aire (sample 2)| $2 \pm 0.060$                        | $2 \pm 0.100$ |
| Pocklington Canal   | $0 \pm 0.004$                        | $0 \pm 0.009$ |

During 2019 and 2020 a 16 km stretch of the Pocklington Canal was samples repeatedly, the data
gathered from this can be collated and potentially be used to observe seasonal trends. This was
achieved by plotting the results by season showing the percentage of results that were analysed
yielding phosphate levels in each category. In total 103 samples were undertaken during this period,
of which 97 yielded useful results.
Figure S10: Data collected via citizen-led sampling sessions across a 16 Km stretch of the Pocklington Canal during 2019 and 2020, plotted to show seasonal variation with continued sampling. The data is plotted to represent the percentage of devices categories at each phosphate level (none <1 mg L$^{-1}$, low 1-3 mg L$^{-1}$, medium 4-6 mg L$^{-1}$ and high ≥ 7 mg L$^{-1}$). $n = 97$.

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