Physico-chemical factors controlling the speciation of phosphorus in English and Welsh rivers

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Phosphorus is a finite resource essential for global food production. However, excessive loss to river systems from diffuse sources (typically agricultural) and point sources (e.g. waste water treatment works and industrial effluent) can lead to negative environmental impacts, including changes to diatom and invertebrate community structure. Current environmental quality standards for phosphorus in the UK have been based on reactive phosphorus, which is poorly defined and comprises an unknown proportion of soluble reactive phosphorus and chemically extractable particulate phosphorus. This research assesses the influencing factors that may control soluble reactive phosphorus concentrations in rivers, including dissolved iron, as well as partitioning processes associated with the presence of total suspended solids, and questions the reliability of the assumptions used when setting environmental quality standards.

The extensive phosphorus speciation monitoring carried out across a wide geographic area of England and Wales shows that not all phosphorus as measured by the molybdenum blue method is either soluble or necessarily bioavailable, particularly at concentrations in the range in which the Environmental Quality Standard for 'Good' status (typically less than 100 µg P L^{-1}) has been set. Phosphorus speciation can change due to physico-chemical processes which vary spatially and/or temporally, including precipitation with iron and partitioning with suspended solids.

Keywords: phosphorus; speciation; eutrophication; water quality; standards

1. Introduction

A principal goal of the EU (see Electronic Supporting Information, S1 for abbreviations used) Water Framework Directive (WFD: 2000/60/EC) is "to protect, enhance and restore all surface waters and groundwaters with the aim of achieving Good Ecological Status (GES)" [1]. Ecological Status is a graded classification system which ranges from High to Good, Moderate, Poor and Bad for biological, physico-chemical and hydro-morphological elements; with overall status defined by the worst performing element [2].

A significant factor leading to the failure of a surface water habitat to achieve good status is the nutrient loading. Concern over the impact of nutrients, particularly phosphorus, on rivers has increased since the publication of the EU Urban Waste Water Directive and subsequently the EU Water Framework Directive [3]. Of the major nutrients controlling plant growth, phosphorus is widely accepted as limiting for riverine environments [4,5]. However, point and diffuse source discharges usually

result in concentrations of phosphorus that are not growth limiting but conversely lead to accelerated growth of undesirable phytoplankton and macrophytes [6].

The current UK guidance sets a site-specific phosphorus Environmental Quality Standard (EQS) expressed as reactive phosphorus (RP) using a combination of altitude and alkalinity (see Electronic Supporting Information, S2) to reflect different riverine typologies [7]. Regard should be given to the natural alkalinity and not an anthropogenically altered value [8].

Within surface waters, however, there exist numerous aquatic fractions of phosphorus [9], See ESI, Fig S1) not all of which are immediately bioavailable. RP is considered bioavailable and is therefore available for plant and algal growth within a watercourse. Historically, riverine RP concentrations have been determined by colorimetry, using the established phosphomolybdenum blue method [10-12] which is quick, simple, cheap, sensitive and not prone to interferences from typical riverine matrices [13]. Although the method is clear and reliable, sample pre-treatment and the nomenclature of the reported concentrations is often imprecise. Soluble reactive phosphorus (SRP) or dissolved reactive phosphorus (DRP) is a clear definition and has the pre-requisite of filtration of the sample prior to analysis, normally through a 0.45 µm membrane [11]. SRP is widely accepted as the most readily bioavailable fraction of phosphorus present in any given sample [14-16]. Several authors (e.g. Haygarth [17], Lapworth [18]) point out that colloidal inputs can impact on both SRP and TRP concentration, depending on the size of the colloids, i.e. <>0.45 µm, the accepted cut off between total and dissolved RP. However, much recent documentation expresses phosphorus concentrations using other terminology including molybdate reactive phosphorus (MRP), 'orthophosphate' [19], and RP, which fails to specify clearly how the sample should be treated prior to determination. The term MRP can give rise to ambiguity [20] as it may refer to filtered samples, where

MRP is equivalent to SRP measurements, or even unfiltered samples, where MRP is equivalent to SRP plus a fraction of particulate P which is reactive to the phosphomolybdenum blue method reagents. Jarvie *et al.* [20] note that MRP determined on <u>unfiltered</u> samples is routinely referred to as 'Orthophosphate as P' by the Environment Agency [16] in England and Wales.

It has been observed that measured concentrations of phosphorus in surface waters do not always correspond closely with indicators of ecological quality [21]. This is unsurprising when the impact of TRP, defined by Everall *et al.* [22] as the biologically available P contribution, is currently assessed using the response and community change of diatoms (The Trophic Diatom Index –TDI); yet the Trophic Diatom Index has been developed through measuring phosphorus as " P ≡ FRP (Filtered reactive phosphorus) ≡ 'orthophosphate' [23].

A further example of this confusion arises from more recent guidance regarding measuring phosphorus under the WFD. It advises that, where necessary, and to ensure accuracy of the method, samples be filtered using a filter not less than 0.45 µm pore size to remove gross particulate matter, [11]. However, there is no definition of specific mass or concentration of "gross particulate matter'. Furthermore, other recent documentation generated to support WFD environmental quality standard setting procedures states, for example: "*Most analyses by UK agencies are of molybdate reactive phosphorus in unfiltered samples **from which large particles have been allowed to settle** and referred to here as "reactive phosphorus". In practice, the difference between RP and SRP is usually minor*" [7, 11], cited in [24].

Most of the water quality data reported for phosphorus in the UK has used the assumption that the difference between RP and SRP is usually minor and therefore unfiltered samples have been analysed, potentially after settling for particularly turbid samples. A 2016 comparison between filtered and unfiltered samples [25] was undertaken, in which the relationship between unfiltered samples, allowed to settle and determined by the molybdenum blue method, henceforth denoted Total Reactive Phosphorus (TRP), and SRP (labelled as filtered P) appears to be 1:1 [25]. However, closer examination of the lower portion of the graph at the range of phosphorus concentrations of interest in UK rivers (and typically world-wide) which is ≤100 µg P L^{-1}; shows a significant amount of scatter in the data with SRP concentrations predominantly falling below the 1:1 line. Further examination of the premise that SRP dominates riverine phosphorus speciation shows that the conclusion was based on a constrained dataset, from rivers mainly sampled from the south and east of England, predominantly high alkalinity and low altitude catchments [25].

This raises the question as to what are the main factors controlling the form of phosphorus within the riverine environment? From a physico-chemical point of view, the difference between RP and SRP will be driven by ambient water quality factors that impact on (a) the reactivity of phosphorus species to the molybdate chemistry and (b) the formation of colloidal/particulate species which may or may not pass through a 0.45 µm membrane. Figure 1 shows the complexity of phosphorus biogeochemistry within flowing water. Within a freshwater system, SRP will undergo sorption processes in the presence of suspended solids, leading to adsorption onto particle surfaces or desorption depending on the ambient concentrations within each matrix. SRP can also be adsorbed or incorporated into colloids such as Fe and Al oxyhydroxides [26,13] (Figure 1), a process utilised within the wastewater treatment

works (WwTW) industry to reduce phosphorus concentrations within effluent to meet discharge permit conditions.
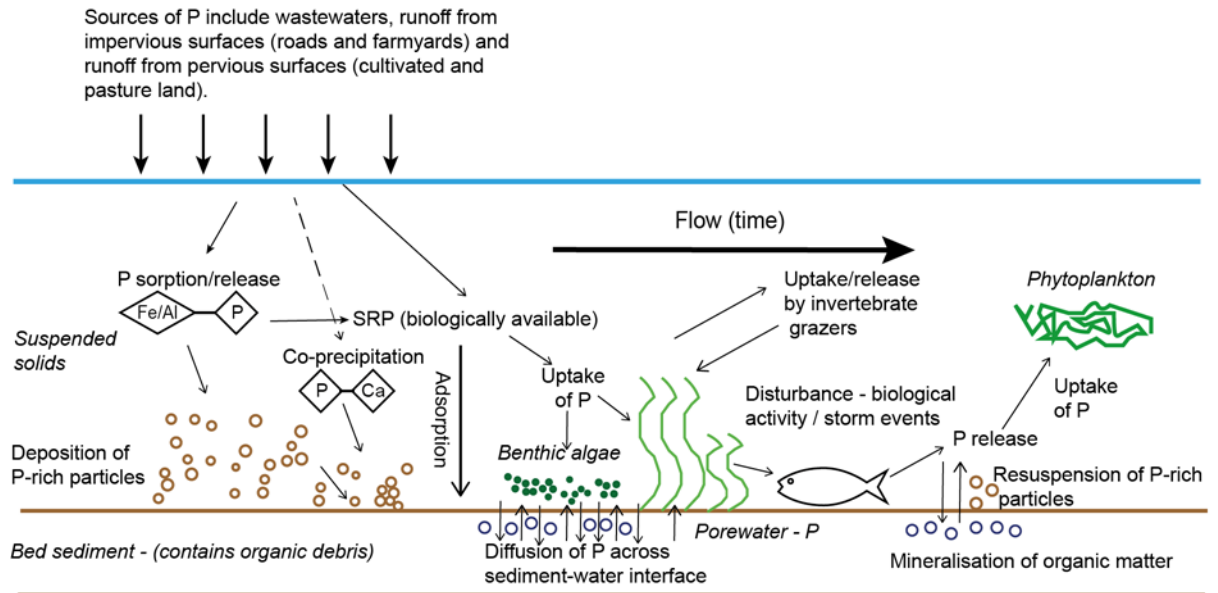

Figure 1: Conceptualised diagram of in-stream processes influencing P concentrations in flowing waters (reproduced with permission from Withers & Jarvie 2008).

Particulate phosphorus (PP) is defined as that fraction which is retained by filtration using a 0.45 µm membrane. PP may comprise biological material (animal, plant, bacterial), weathering products (mineral), inorganic precipitates and organic precipitates, as well as phosphorus associated with aggregates through metal binding or adsorbed to the surface of clay and mineral particles[14] Deposition of sewage-derived particulates enriched with P, particularly during an extended period of low summer baseflows, may provide localised bed-sediment hotspots and later act as a source of SRP . From an ecological standpoint, there is uncertainty over the degree of bioavailability associated with PP or molybdate 'unreactive' species which may pass through a 0.45 µm membrane. These phosphorus fractions within a water column may be utilized by algae and bacteria after hydrolysis by extracellular enzymes. These enzymes are usually only exuded under conditions of bioavailable P

deficiency [14]. Hence, some particulate phosphorus can become bioavailable either via natural partitioning or biological processes.

There has been, and will continue to be, substantial investment in reducing phosphorus loads entering waterbodies across Europe and beyond; via reduced agricultural loss from farms and fields and investment in new technology within WwTW. In order to ensure that the most scientifically robust guidance is provided, it is essential to fully examine the variety of phosphorus forms that occur throughout a range of geographical and physico-chemical conditions within riverine waterbodies, thereby allowing regulators to provide clear instructions on the pre-treatment of samples to ensure consistency and clarity of outcomes.

This study determines phosphorus speciation across a variety of water typologies, including alkalinity and altitude as well as physico-chemical conditions related to parameters that may influence speciation, such as dissolved iron and suspended solids and seasonality. Furthermore, a comparison between filtered and settled samples was undertaken to determine the impact of sample treatment on the quantification of RP.

2. Methodology

2.1 Site selection

The data were collected across seasons through 2016 – 2018 (except the separate Taw and Sedgemoor study, Jul-Sept 2015) from twelve catchments. All sites selected (Figure 2) were in England or Wales and the main criteria for river selection were:

- Variety of potential sources including WwTW, industry (e.g. dairies), septic tanks and agriculture (Table 1).

- Geographical spread across England and Wales.

- Typology including wide range of alkalinity and altitude.

- Contrasting water physico-chemical conditions (e.g. iron and suspended solids concentrations).

Within the selected river catchments, samples were collected from near source to the lower catchment.
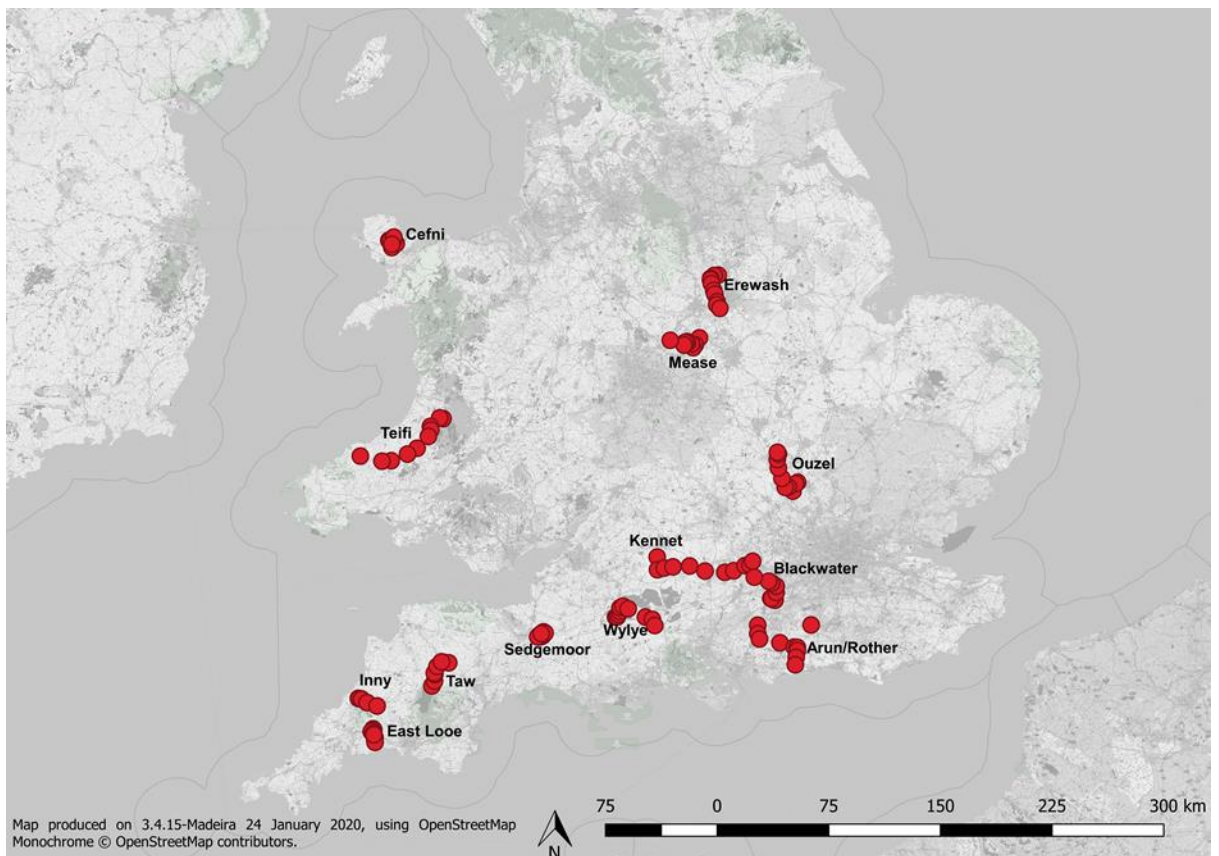

Figure 2: Geographical spread of catchments monitored in England and Wales from 2016-2018.

Table 1: Catchment sampling site characteristics.

Catchment	No. of sample sites and occasions sampled	Typology[1]	Dominant agriculture	Industry	WwTW mean flow (m^3/s)[2]	Total population equivalent (PE)[3]	% Effluent[4]
Erewash	10/2	Lowland, high alkalinity	Limited	50% urban some large industrial estates	0.90	238,769	64
Kennet	11/1	Lowland, high alkalinity	Arable/dairy	Limited, but increasingly urban towards Reading at end of catchment	4.52	402,260	57
Blackwater	10/2	Lowland, high alkalinity	Rural headwaters	Suburban with barracks and some industry	2.03	229,230	49
Ouzel	10/2	Lowland, high alkalinity	Mixed livestock/arable	20% urban, some industrial estates, Milton Keynes	0.42	106,113	27
Mease	11/2	Lowland, high alkalinity	Arable	10% urban, Biological Rotating Contactor present	0.13	32,556	21
Cefni	10/1	Lowland medium alkalinity	Arable	Mostly rural	0.13	26,883	20
Arun	10/2	Lowland, high alkalinity	Large improved pastureland	Limited	0.55	149,800	10
Looe	1/2	Lowland, low alkalinity	Mixed	Some urban (Liskeard - Al dosing)	0.04	12,768	6
Wylye	10/1	Lowland, high alkalinity	Arable	Only 1 major town (Warminster) with barracks	0.10	23,428	3
Taw	17/4	Upland, low alkalinity	Arable/dairy	Dairy, N. Tawton	0.03	7,346	2
Teifi	10/1	Upland, low alkalinity	Forestry, hill farming	Small WwTW	0.14	21,963	1
Upper Inny	8/2	Upland, low alkalinity	Mixed livestock	Dairy at Davidstow	0.02	58,407[5]	0.5
Sedgemoor	14/1	Lowland, medium alkalinity	Nature reserve and unimproved grazing for dairy cattle	Rural	<0.01	<1,000	<0.5

[1] Low alkalinity 15-55 mg $CaCO_3$ L^{-1}, High alkalinity 110-245 mg $CaCO_3$ L^{-1}.
[2] Based on DWF x 1.25
[3] PE is population including contribution of load from industrial discharges
[4] Based on total effluent flow as a proportion of mean river flow at outlet of catchment.
[5] Predominantly wastewater from the dairy.

These catchments fulfilled the objective of combining different potential sources of phosphorus, water typology and geography. The degree of urban influence was assessed using simple metrics associated with the population served by the WwTW, flow and catchment area (Table 1). The data show that catchments can be classified as follows: those heavily influenced by wastewater (Erewash, Blackwater and Kennet – at the mid to outlet of catchment), very rural catchments with greater rainfall and therefore dilution (Taw, Looe, Teifi and Sedgemoor), those with poor dilution from a combination of population density and lower rainfall (Inny, Blackwater, Ouzel and Mease) and those with a variety of population densities, dilution available and contribution of WwTW effluent (Arun /Rother, Cefni and Wylye).

To investigate the impact of settlement versus filtration on sample stability for a sub-set of Taw and Sedgemoor samples, aliquots for the determination of RP were shaken and allowed to settle for 24 h before the concentration of RP was determined in a decanted portion of the sample (as per the current recommendations). For the determination of SRP, water samples were filtered through a 0.45 µm pore diameter cellulose acetate filter membrane 24 h after collection. All water samples were sub-sampled in triplicate.

To take account of potential increases in particulate matter from any seasonal variation, five of the sites were sampled in both summer and winter.

2.2 Analytical methods

Water samples collected from the selected sites were measured for SRP, TRP, TSP and TP. Samples were collected mid-channel from the site in a clean bucket or measuring jug, agitated and sub-sampled for the separate tests. Samples were stored in sterile 15 mL centrifuge tubes. Tubes had been soaked in 10% HCl for at least 24 h before being rinsed 4 times in deionised water and once in high purity water.

Triplicate 12.5 mL samples for SRP were filtered into the tubes on site using 0.45 µm non-sterile hydrophilic Surfactant Free Cellulose Acetate (SFCA) membrane disposable filters (Cole-Parmer) and syringes, precleaned in 2% HCl (Primar Plus, Fisher Scientific) and rinsed twice with high purity water. Triplicate 12.5 mL samples for TRP were collected into the tubes on site using the clean syringe without filtration. Triplicate 9 mL samples for TSP were filtered (as above) into the tubes on site. Triplicate 9 mL samples for TP were collected into the tubes on site using the clean syringe. The 9 mL samples were all acidified in the field with 1 mL Primar-Plus Trace analysis grade nitric acid S.G. 1.42 (70%), supplied by Fisher Scientific. During summer sampling episodes, samples were kept in a cool box.

2.2.1 Reactive phosphorus (SRP and TRP)

Samples collected for SRP and TRP analysis were tested within 24 h, following the molybdenum blue method (ESI S3) (Blue Book Method A) [10] using EnviroMAT EP-L as a Certified Reference Material (CRM) (supplied by QMX laboratories).

Calibration standards were made up from 10 mg P L^{-1} stock solution. The range adopted was 0 to 1000 μg P L^{-1}.

To ensure analytical quality control 5 blanks were collected for each sample batch, including filter blanks. During each batch of analysis, EnviroMAT Drinking Water, Low (EP-L) external reference material was measured.

2.2.2 Total phosphorus (TP and TSP)

Samples collected for TP and TSP were refrigerated to 4 ^{0}C. Prior to analysis, the samples were placed in a water bath with the caps loosened and heated to 95 ^{0}C for 6 h to ensure complete acid digestion of the phosphorus species. Samples were analysed (ESI S4) for TP and TSP by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a Thermo Fisher Scientific X Series 11 or Thermo Fisher Scientific iCAP RQ instrument, depending on availability, within a laboratory managed under ISO 9001 certification. During the analysis, concentrations of iron and aluminium were also measured, using the same quality control steps as those described above.

2.2.3 Other measurements

Additional data were collected to assist with interpretation. Field measurements included conductivity and temperature (measured using Orion model 105 Conductivity meter), pH (Oakton Ion 6+ meter with Mettler Toledo pH probe) and Total Suspended Solids (HACH DR890 or HACH DR900). Within the laboratory, calcium was measured by ICP-Optical Emission Spectrometry (Thermo Scientific

ICAP 7400 Radial). PP (particulate phosphorus) and SUP (soluble unreactive phosphorus) were calculated by difference (PP = TP - TSP and SUP = TSP – SRP).

2.3 Statistical analysis

The combined datasets were subjected to a multiple linear regression (MLR) analysis which is a form of traditional linear regression but which can be used to explain the relationship between a dependent variable and two or more independent variables (standard linear regression has only a single independent variable). For example, standard linear regression takes the form shown in equation 1, where equation 2 shows the equation form for multiple linear regression. In both, 'y' is the dependent variable, and 'm' the independent variable(s). In MLR there are two or more dependent variables.

$$y = mx + b \qquad \qquad \text{Equation 1}$$

$$y = m_1x_1 + m_2x_2 + ... + b \qquad \qquad \text{Equation 2}$$

3.0 Results and Discussion

3.1 Relationship between TP, TRP and SRP

Descriptive catchment data for the river channels investigated can be found in ESI S5, together with sample data used within the paper ESI table S1. All data sets have been combined to show more comprehensively the relationship between SRP and TRP and SRP and TP.

Similar to the data produced in the study [25] discussed earlier, Figure 3 (a and b) shows that plotting the full range of observed values results in a trend line that approaches a 1:1 relationship, at least for SRP vs TRP. However, focussing on the concentration range that is of relevance regarding ecological standards and replotting only data <300 μg P L^{-1} accentuates the degree of scatter observed, with SRP concentrations significantly less than TRP or TP in many cases (Figure 3c and d).
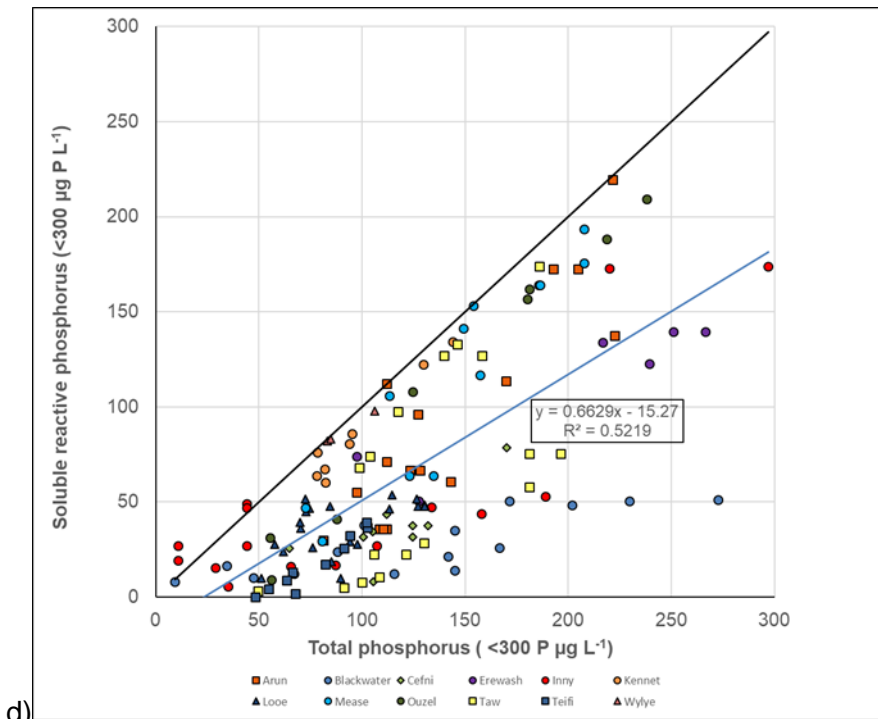

a)

y = 0.8874x - 18.221
R² = 0.9506
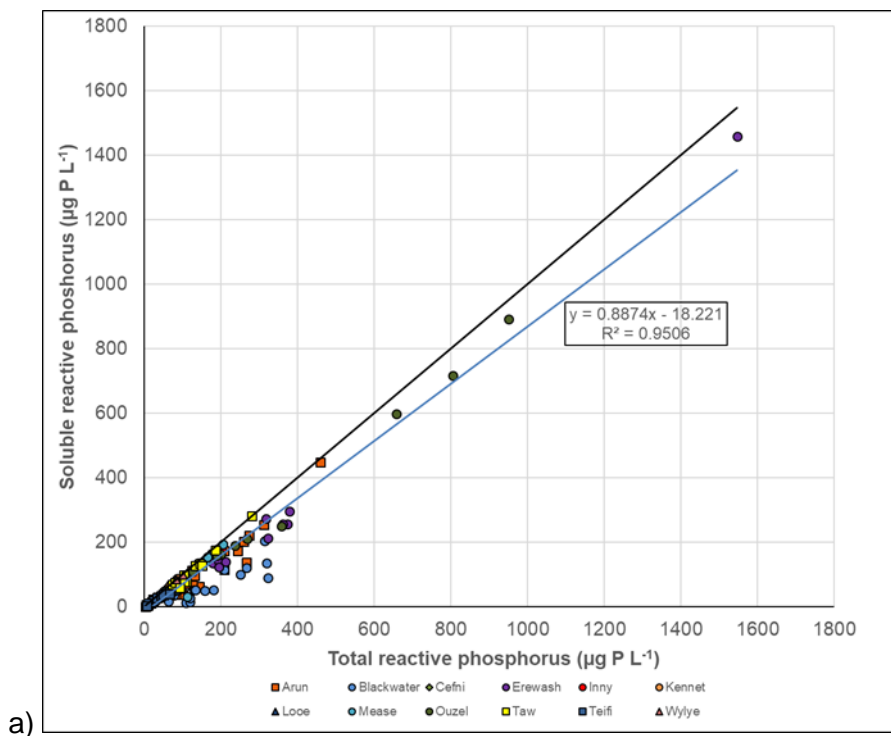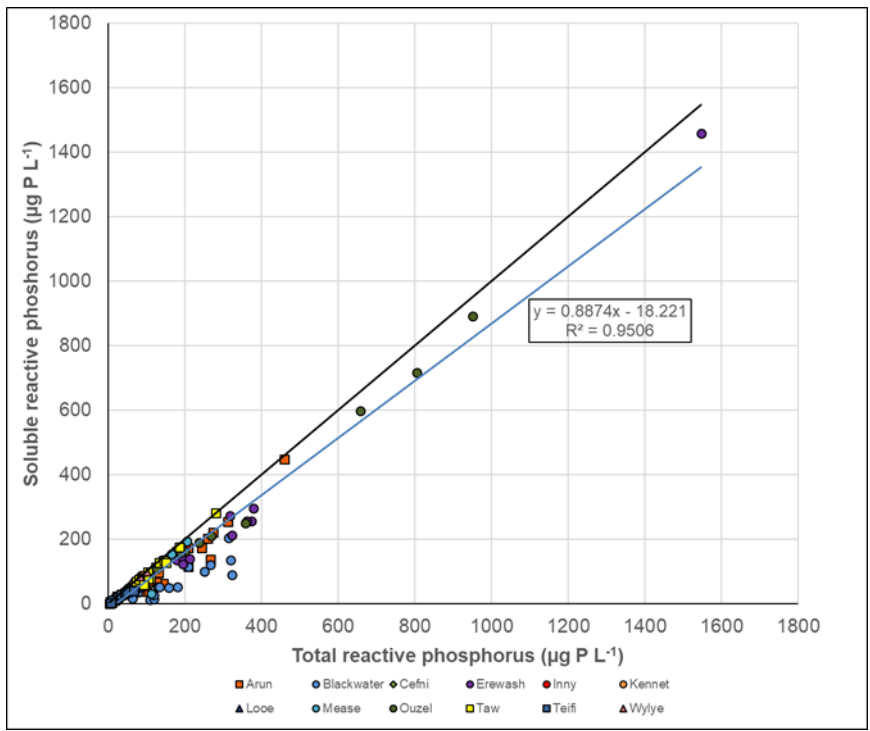
b)
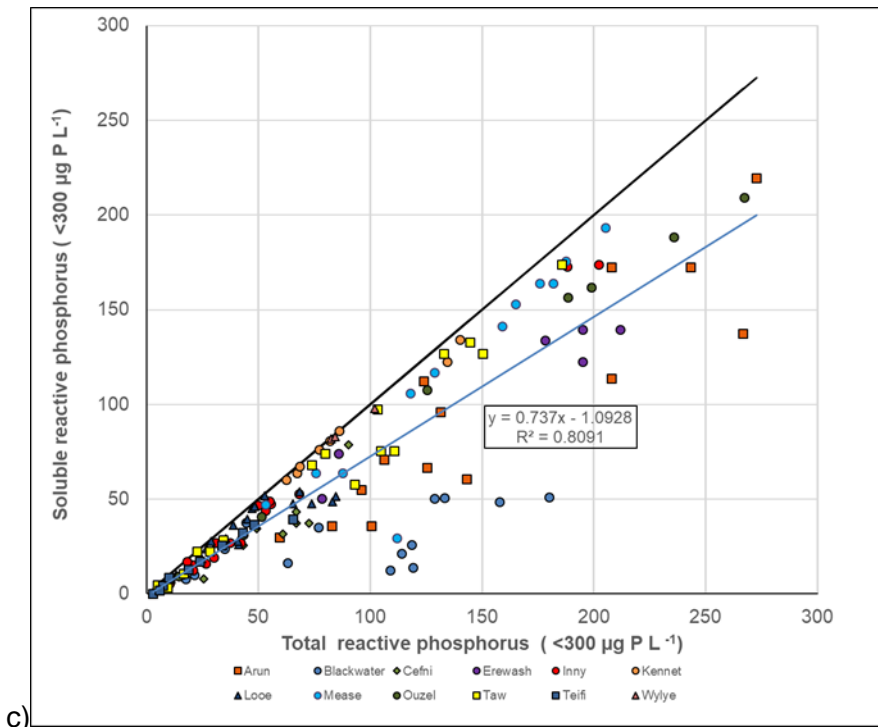
c)

d)

Figure 3: All SRP plotted against (a) TRP, (b) TP, (c) TRP <300 μg P L^{-1} and (d) TP <300 μg P L^{-1} for the 12 catchments sampled in England and Wales (2016-2018). Black line represents 1:1 relationship and blue line the trend.

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Examining the data in more detail using a cumulative frequency distribution for all of the data from the 12 catchments (Fig. S3 in ESI) shows that 70% of the SRP:TRP data has a ratio of <0.87 (i.e. SRP is 87% of TRP and approximately 30% of data have a ratio of <0.65, suggesting the presence of significant amounts of non-filterable reactive P. Regarding the SRP:TP ratio, 52% has a SRP:TP ratio <0.5, suggesting a significant portion of the TP present is non-reactive as well as non-filterable.

3.2 Predicting P speciation

Given the data available, it was possible to generate an algorithm by MLR to predict SRP from TRP (and vice versa), using dissolved Fe and TSS as additional explanatory variables. The ability to do this is useful in terms of being able to potentially predict SRP from Environment Agency phosphorus data which is predominantly reported as unfiltered orthophosphate (TRP). Using an MLR approach with TRP, dissolved Fe and TSS to predict SRP:

SRP (μg L^{-1}) = 0.904 x TRP (μg L^{-1}) + 0.0178 x Fediss (μg L^{-1}) - 0.00203 x TSS (μg L^{-1}) - 6.445

The MLR can be re-arranged algebraically to estimate TRP from SRP. The statistics generated for the MLR are given below (Table 2). A strong correlation was obtained between predicted and measured values (Figure 4). Approximately 50% of the predicted values are within 10% of the observed concentration, and 90% of values within 80% of the observed concentration. Furthermore, 50% of the predicted SRP values were within 6 μg P L^{-1} of the corresponding observed values, with 80% within 20 μg P L^{-1}. It should be noted that as with plotting TRP against SRP (Fig 3a), variation in the data is more pronounced at the lower concentrations than is

illustrated in the whole data set (Fig 4 inset). These lower concentrations are more relevant to regulatory bodies with regard to WFD requirements.

For a variable to be significant in terms of predicting the dependent variable (in this case SRP) from the independent variables (TRP, TSS and Fe) the critical value needs to be less than the 'T' statistic. The data in Table 2, shows TRP is statistically significant with a very high degree of confidence (better than 95% and $p<0.05$), whereas dissolved Fe and TSS were not (T statistic less than critical value and $p>0.5$).

Table 2: Statistical summary of multiple linear regression analysis

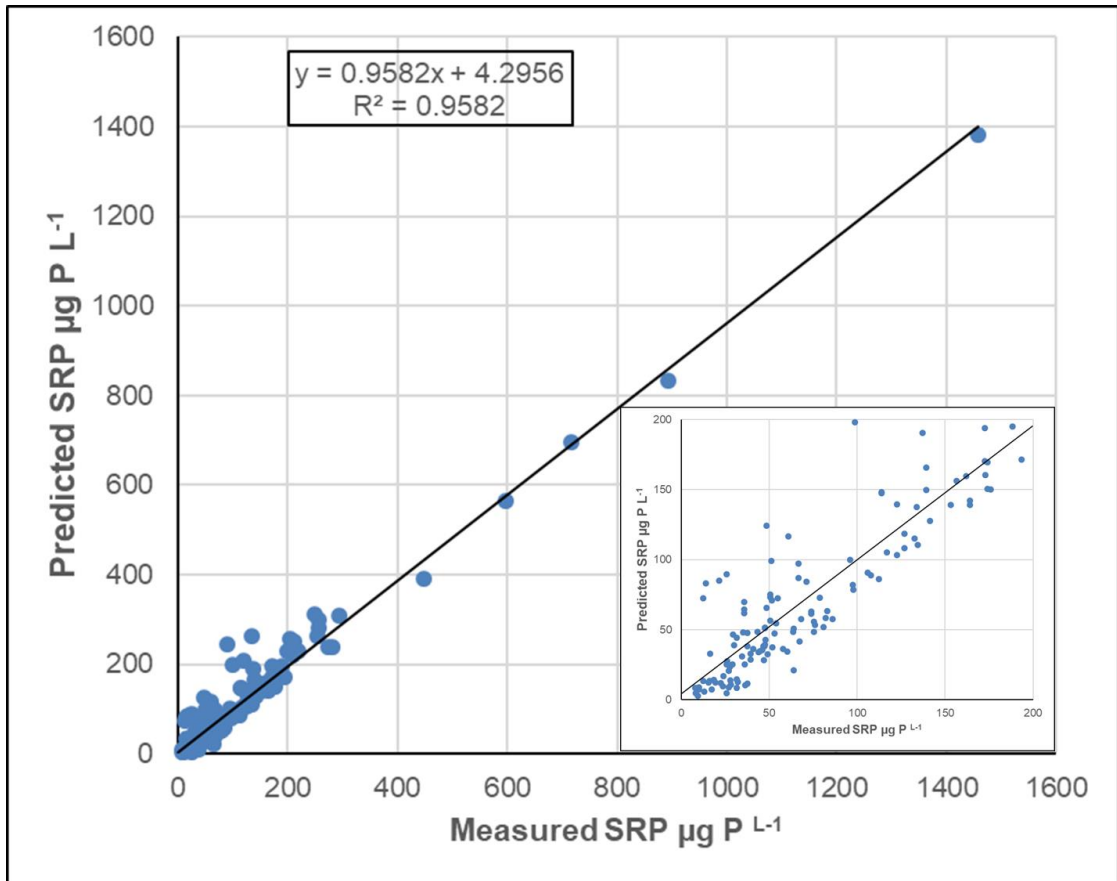|	TRP	Dissolved Fe	TSS	constant
T Stat	58.550	0.865	-5.277	-1.354
Critical	1.976	1.976	1.976	1.976
Statistically significant parameter	significant	not significant	not significant	not significant
P Value	3.5E-106	0.388331	4.44E-07	0.177454

Figure 4: Predicted vs measured SRP using the following algorithm: SRP = 0.904 x TRP + 0.0178 x Fediss - 0.00203 x TSS - 6.445, with line of best fit.

3.3   Sample preparation and impacts on P speciation

A comparison of phosphorus concentrations arising from the 24 h settled then decanted methodology and the filtration methodology was undertaken on a set of water samples from the Taw and Sedgemoor catchments (ESI Figs S2.9b and S5.13) A t-test was used to determine any significant differences ($p < 0.05$) in the mean values of each data pair. Thirteen of the 29 paired samples (45%) had mean concentrations of phosphorus that were generally low (43 µg P L^{-1}) small but significantly different for the two treatments. Higher concentrations (55 µg P L^{-1}) of phosphorus occurred most frequently in the settle/decant samples (11 of 13 sites, or 85%).
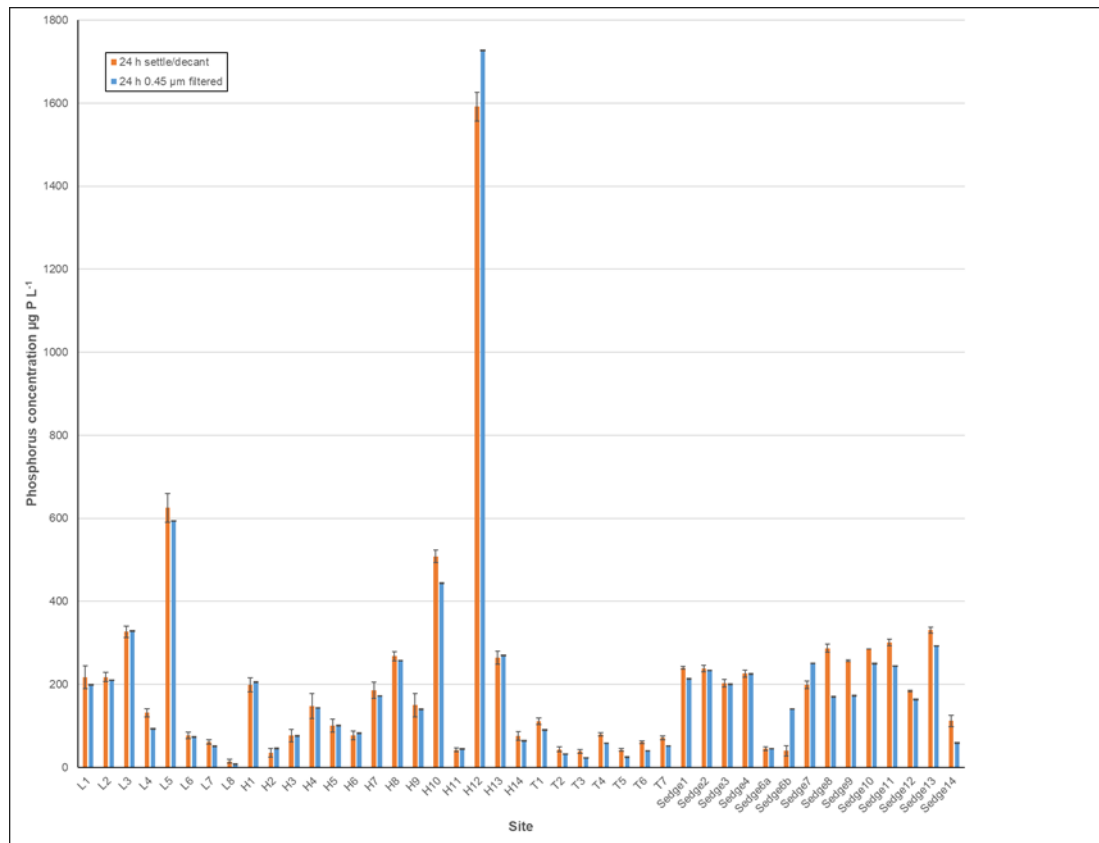
Figure 5: Phosphorus concentrations (µg P L-1) arising from river water samples settled/decanted or 0.45 µm filtered, both after 24 h. Error bars represent 2 standard deviation (n=3).

For the Sedgemoor samples (Figure 5), 10 of 14 paired samples (71%) had mean concentrations of phosphorus that were significantly different for the two treatments. Higher concentrations of phosphorus occurred most frequently in the settle/decant samples (7 of 10, or 70%). Samples for both surveys were collected during relative spate conditions in the Sedgemoor and Taw catchments and so the bias shown by the settle/decant samples can be ascribed to the presence of fine suspended solids and colloidal P that are reactive but not filterable. SRP concentrations will be lower than orthophosphate (meaning TRP) as particulate reactive forms will not have been included [16]. They cite a personal communication with Withers, which estimates that up to 20% of the particulate phosphorus may be reactive. The magnitude of the difference between SRP and orthophosphate concentrations will

depend upon the relative importance of soluble and particulate phosphorus losses in a catchment. Similar results have been shown previously for WwTW effluent [24]. The data therefore suggest a systematic difference in measured phosphorus concentrations occurring when samples are not filtered prior to analysis (i.e. defined as TRP). Although it may be argued that this is a conservative measurement when considering phosphorus potential bioavailability, the degree of bias between filtered and settled then decanted samples will not be consistent. This has the impact of making comparison of P concentrations between sites and between sampling occasions challenging owing to the presence of varying concentrations of suspended solids and colloidal P.

3.4   Impact of Suspended solids concentrations on SRP presence

Given the potential influence of suspended solids and colloidal material on the observed P concentrations, the datasets were further interrogated to seek any relationships or impacts of the presence and magnitude of suspended solids on the observed SRP concentrations. Higher river flows experienced during sample collection on some occasions when rivers were under spate conditions (e.g. >20 mg L^{-1}) would have led to enhanced concentrations of fine suspended solids and colloidal P in the water column (for example, from bed sediment resuspension and runoff from adjacent fields). Fine suspended solids and colloids (defined as particles ≤1μm in any one dimension) are slow to settle under gravity and would have been present in the collected samples. Owing to slow settling, they would have also been decanted with the sample prior to phosphorus determination for TRP but would have been filtered out to <0.45 μm for SRP. Under the acidic conditions of the colorimetric analytical procedure, a proportion of the fine suspended solids and colloidal phosphorus would have contributed to the measured phosphorus concentration.

Plotting the ratio of SRP to TRP/TP shows no clear trend, but suggests that where suspended solids are elevated, SRP tends to be low (Figure 6*: (a) % Soluble reactive phosphorus of total reactive phosphorus vs total suspended solids (b) Soluble reactive phosphorus of total phosphorus vs total suspended solids, based on samples collected from sites in England and Wales (2016-2018).*). The concentration of P distributed between the dissolved and particulate phase will ultimately be a function of partitioning and kinetics. Any P adsorbed to suspended solids has been suggested to be of lower reactivity and may therefore also impact on its immediate bioavailability [27]. The amount of suspended solids present in a catchment will be a function of catchment typology and land use (i.e. likelihood of soil being lost from land), seasonal variation in flow (i.e. typically low flows in summer and high flows in winter) or the occurrence of unseasonal weather patterns, e.g. heavy summer rainfall episodes. Overlaying these physico-chemical processes, it should also be noted that a decrease in SRP concentration would be expected in summer due to higher productivity and thus uptake by phytoplankton.
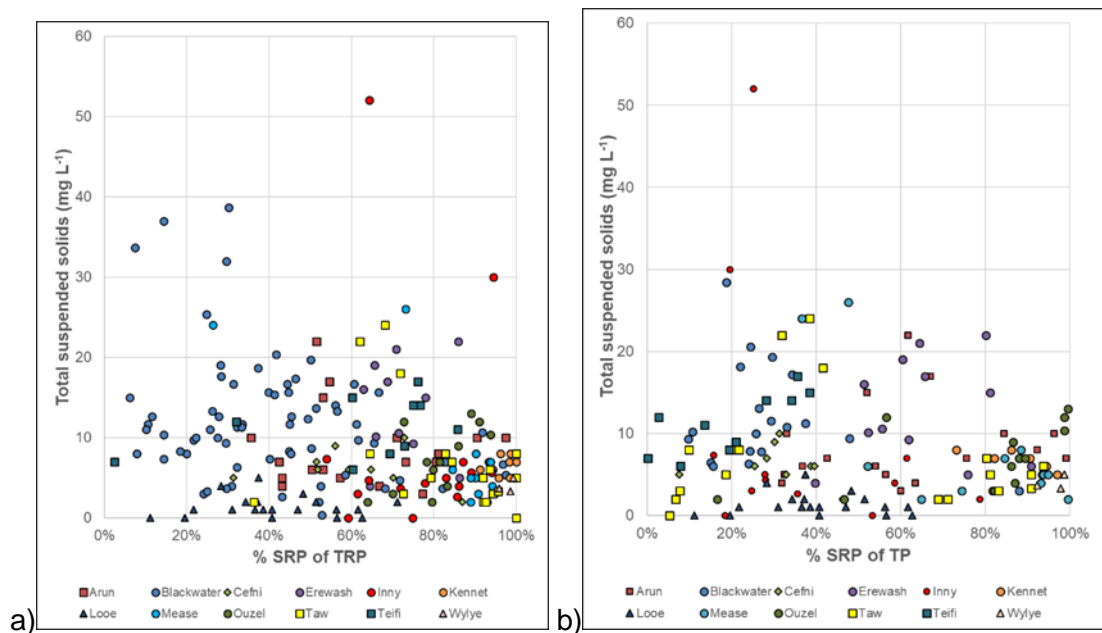
Figure 6: (a) % Soluble reactive phosphorus of total reactive phosphorus vs total suspended solids (b) Soluble reactive phosphorus of total phosphorus vs total suspended solids, based on samples collected from sites in England and Wales (2016-2018).

Jarvie *et al* [28] established that point sources (effluent) rather than diffuse sources (agricultural) of phosphorus provide the most significant risk for river eutrophication. They found that SRP was the dominant P fraction in all UK rivers monitored (n=7) (sample sites, n=54), averaging 67% of TP. Again, considerably less than the assumed 1:1 ratio. They noted that in times of low flow, this percentage increased. The time of this study should be noted as it occurred prior to many WwTW installing P stripping, following requirements of the EU Urban Waste Water Directive and WFD.

During higher winter flows, PP can form a significant proportion of phosphorus load to a river, but owing to the timing of such events – i.e. lower phyto-productivity, its relevance to eutrophication is questionable [28], which this paper assumes is a key driver for the existence of the EQS standard.

The significance of phosphorus cycling within the channel should not be understated. Withers *et al.* [26] cite sediment uptake rates of phosphorus of 0.16 g m^2 day^{-1} calculated by House and Casey (1989), i.e. twice the rate of phosphorus assimilation by algae. Thus, a higher concentration of total suspended solids within the channel would be associated with a lower concentration of SRP (Figure 6); that is unless suspended sediments or resuspended bed sediments resulted in changed redox conditions or were sufficiently contaminated to drive the partitioning of P from the particulate to the dissolved phase [29]. However, Jarvie *et al.* [28] have undertaken experiments to assess the significance of bed sediment as a source or sink of SRP using equilibrium phosphorus concentrations (EPC$_0$). They found that over 80% of the 84 river bed samples had potential for net SRP uptake from the water under low flow conditions, where SRP in the water column exceeded the EPC$_0$ of the bed sediment. Release of SRP from the sediment back to the water generally occurred where river water concentrations of SRP were low (<c.50 µg P L^{-1}). This is a further mechanism that will impact on the concentration of SRP within the river.

3.5 Iron as a controlling factor for observed SRP concentrations

Iron plays a key role in the biogeochemistry of phosphorus and as previously postulated [26] the presence of excess iron within the water column would be expected to form either non-filterable and/or non-reactive colloids. Plotting % SRP of TP and % SRP of TRP versus filtered (<0.45 µm filtered) iron concentrations (Figure 7*: (a) % Soluble reactive phosphorus of total reactive phosphorus vs filtered iron and (b) % soluble reactive phosphorus of total phosphorus vs filtered iron, based on samples collected from sites in England and Wales (2016-2018).*), shows no obvious trend, but as with suspended solids, high iron concentrations lead to suppressed SRP:TRP/TP ratios. Where % SRP of TP, or of TRP is 100%, all P is SRP. In a high Fe concentration situation, where a low % SRP of TP results, the difference between

SRP and TP is accounted for by unreactive phosphorus or particulate bound phosphorus being present. In a high Fe concentration situation where the % SRP of TRP is low, the difference between SRP and TRP is accounted for by particulate bound phosphorus. Where Fe concentrations are high SRP concentrations would be expected to be low, so the results shown in Figure 7a suggest an influence from other physico-chemical parameters.
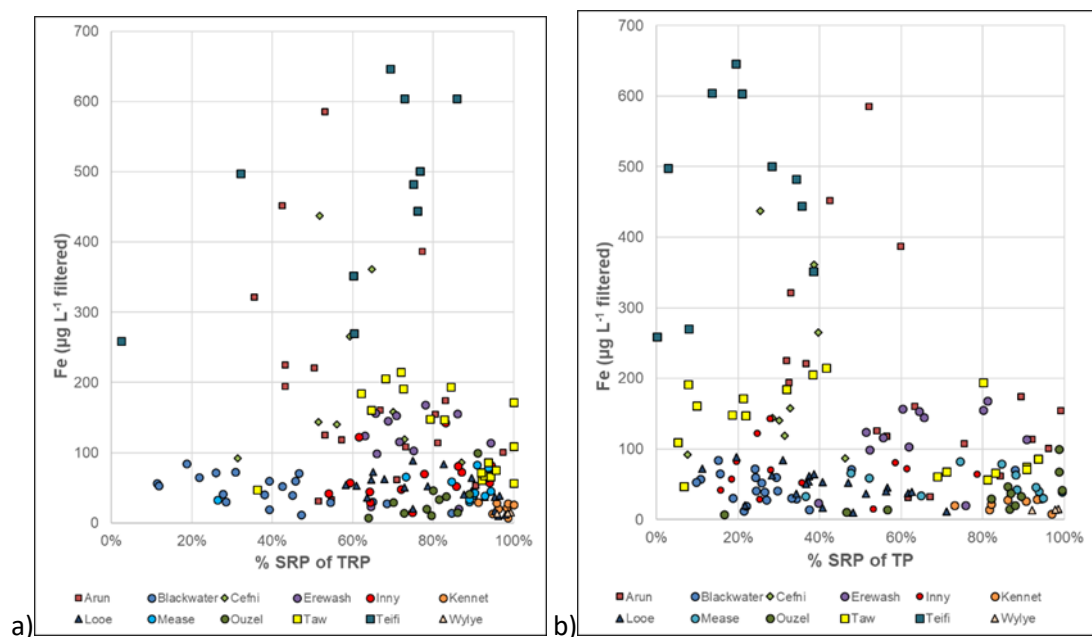

Figure 7: (a) % Soluble reactive phosphorus of total reactive phosphorus vs filtered iron and (b) % soluble reactive phosphorus of total phosphorus vs filtered iron, based on samples collected from sites in England and Wales (2016-2018).

Under oxic conditions, ferric iron (Fe (III)) forms insoluble oxyhydroxides that have a high affinity for phosphorus anions through sorption or precipitation reactions, thereby limiting phosphorus solubility [27,30]. Seasonal or spatial changes in iron speciation can change the phosphorus solubility [30]. Spatially, such changes can occur, for example, around industrial discharges (Taw, Inny), or seasonally, for example associated with iron-rich greensand geology in times of lower flow (Arun /Rother) [31]. Where sites (Cefni, Teifi, Taw in winter) exhibit higher concentrations of

iron (filtered), the proportion of SRP is lower and SUP higher (ESI S5.5, S5.6 and S5.9), suggesting that high concentrations of iron (mean 265 µg Fe L^{-1}) may reduce the chemical reactivity of colloidal iron phosphates to the molybdate reagents. The form of unreactive phosphorus (ESI, S3) cannot be determined by the methods used in this study.

Overall the presence of SRP in the water column is a product of a complex series of biogeochemical processes, not easily disentangled, nor easily predicted with a high degree of certainty. The data presented here, however, suggests that elevated iron and suspended solids can influence the proportion of SRP present in a sample.

This has significant implications regarding potentially explaining why there is still a significant mismatch between the derived phosphorus WFD standards and the ecological status classification [11]. For England for example, 68% out of the 221 sites have an ecological status that is either better or worse than predicted based on RP values alone. For the 29% of sites where the ecological status is better than the reported RP concentrations predict, it may be a case that the phosphorus present (measured as RP) is not necessarily 100% bioavailable. Under these conditions comparing the ecological status with SRP could result in a better agreement. That is not suggesting that all chemically non-reactive P is biologically unavailable, just that using a procedurally consistent method for pre-treating and reporting of phosphorus concentrations may provide a more scientifically robust approach.

4.0   Conclusions

Use of scientifically robust and consistent phosphorus speciation terminology in river systems is essential for scientists, regulators and industrial dischargers.

A recognised standardised approach needs to be set out using a robust methodology to ensure clear future regulation and that compliance monitoring is free from any ambiguity. The existing set of guidance and regulation prevents consistent determination of P in rivers in terms of trend analysis, seasonal cycling and compliance assessment; the ability to coherently replicate tests that are free from bias and subjectivity is essential for regulators and regulated alike.

Within a river, soluble reactive phosphorus concentration is not always equal to total reactive phosphorus. Biogeochemical processes including reactions between iron and SRP and total suspended solids and SRP can alter and change the speciation of phosphorus. This variation in physico-chemical state controlled by ambient conditions may go some way towards explaining the current mismatch observed between the ecology and chemistry within WFD waterbodies where RP is used for the determining the chemical status. Overall, the bioavailability of different phosphorus species, particularly the particulate and non-filterable reactive species, is not fully understood and further work in this area is needed.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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