BIOTIC INFLUENCES ON CHEMICAL FLUXES AND SEDIMENT-WATER EXCHANGES IN SEDIMENT DEPOSITS

by

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Synopsis

This is the first study undertaken in a controlled environment to understand the kinetics of the release of soluble reactive phosphorus and copper from sediments of natural systems with an associated biofilm, and to identify which of the size compartments affected those fluxes most. It was found that of the sediment size fractions in the system, the stones that had a substantial biofilm growth attached had the greatest influence

Differences in the responses were observed between the sediment size fractions and the two sites, where contaminant concentrations varied. The equilibrium phosphate concentration and a phosphorus transfer index were used to establish if there was a net uptake or release of phosphorus by the sediment size at the time of sampling. The sediment having a biofilm and associated particulate material resulted in a greater flux than fine sediment, which does not support a filamentous biomass. The kinetic results imply a different mechanism than diffusion being involved. It was demonstrated that both gravel and stone substrates can have an important control over the release of soluble reactive phosphorus due to their rôle as firm substrate for a biofilm growth.

Changes in the steady-state concentration of dissolved copper suggest that the bed sediment is responding to reduced river water concentration and setting a new steady-state. The kinetics of the reaction of the sediment to copper were of a similar order, and rate constants increased through the season, but were of a similar magnitude for both sites. The differences in the n and rate constant values indicate a difference in the mechanisms (*i.e.* the order of the kinetics) of uptake of copper through the seasons.

The kinetics are described by a rate law which yields a method of estimating the flux to the sediment for recovery time after a pollution incident and how far downstream the copper concentration would remain elevated after a pollution incident or sediment.

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Glossary

advective flux	movement of a mass of fluid resulting in solute transport - requires approximation of two vectors: the concentration gradient and the velocity	MWO
biofilm	defined here as benthic algæ and including filamentous macroalgæ	
bioturbation	disturbance of sediment layers by biological activity	
DBL	diffusion boundary layer	
diagenesis	recombination or rearrangement of constituents (as of a chemical or mineral) resulting in a new product	MWO
DO	dissolved oxygen	
EPC_0	equilibrium phosphate concentration	
epilithic	growing on stones	HYP
EPS	extracellular polymeric matrix	
flume	experiment channel for circulating a solution	
fluvarium	building containing flume channels	
ICP-MS	inductively-coupled plasma mass spectrometry	
macroinvertebrate	invertebrate animal large enough to be seen without magnification	HYP
NERC	Natural Environment Research Council	
OM	organic matter	
PTI	phosphorus transfer index	
rate constant	a mathematical proportionality constant for a given reaction if the concentration of the reactants is the only variable, *i.e.* varies, for example, with change the temperature of the reaction	GCO
rate law	(or rate equation) relates the rate of reaction of a particular reaction in terms of concentrations of chemical species, *i.e.* concentrations of reactants, catalysts, and inhibitors.	GCO
SRP	soluble reactive phosphorus	
stoichiometric	a : the quantitative relationship between constituents in a chemical substance b : the quantitative relationship between two or more substances especially in processes involving physical or chemical change	MWO
STW	sewage treatment works	
UFRP	unfiltered reactive phosphorus	
URGENT	NERC thematic programme for Urban Regeneration and the Environment	

Sources:
MWO – Merriam-Webster Online www.m-w.com/cgi-bin/dictionary
HYP - Hyperdictionary Online www.hyperdictionary.com/index.html
GCO - General Chemistry Online
http://antoine.frostburg.edu/chem/senese/101/index.shtml

Chapter 1
General Introduction

1.1: Preamble

This opening chapter reveals why the project was initiated and sets out the structure of the thesis. It also introduces the key aspects involved in the research and explains their importance and context.

1.2: Introduction

This project was an element of the third stage of the Natural Environment Research Council Thematic Programme for Urban Regeneration and the Environment (URGENT III). The overall aim of this programme was to develop a sustainable scheme to cleanse urban areas contaminated in the past, prevent the recurrence of contamination incidences, and allow the safe and cost-effective re-development of such areas in order to regenerate urban environments. The two main objectives were to establish the scope of the problems and risks, and to advance strategies for remediation and management. Generic models or solutions could then be developed and later applied elsewhere. It was anticipated that this would be achieved by gaining a thorough understanding of the interactions between the mechanisms of natural processes and those anthropogenically instigated. One specific area targeted for better understanding was surface water quality and river rehabilitation, and in particular the prediction of changes in chemical fluxes to and from contaminated sediments, and the understanding of the factors that control these fluxes.

1.2.1: River Tame

The catchment of the River Tame is located within the West Midlands, one of three conurbations that were designated as URGENT 'flagship' sites, and is typical of polluted systems in that type of area. The River Tame rises in the large conurbation to the north-west of Birmingham, and flows eastwards through the heart of the metropolitan area. Figure 1.1 is a map of the area showing the site locations, and Table I.I. gives details of the locations. It has been channelled, culverted, and otherwise diverted at

Table I Sampling site locations

Site location	Site code	National grid reference	Distance between (km)	Area of catchment (km^2)
Bentley Mill Way	BM	SO 990977		57
Sandwell Valley	SV	SP 029924	7	182
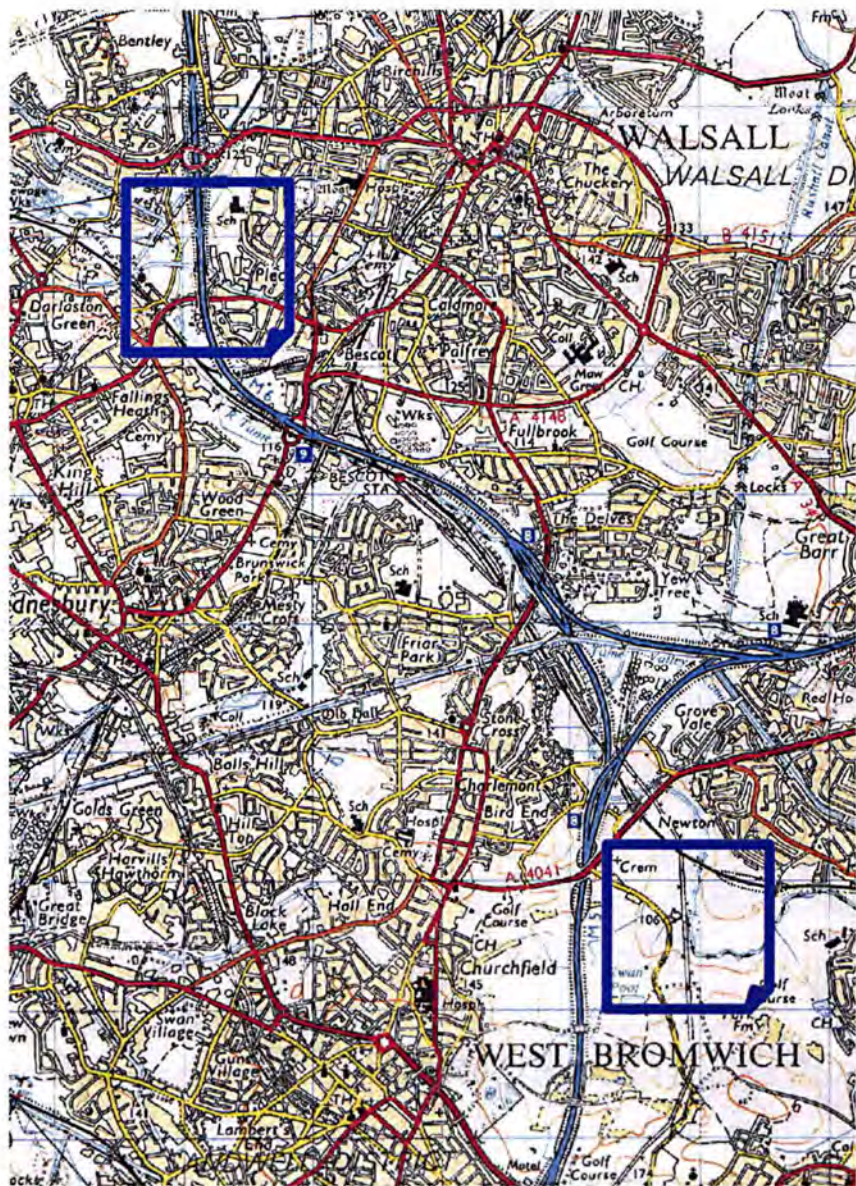
Figure 1.1: Map of the region to the north of Birmingham, UK, showing the path of the River Tame and the position of sampling sites. Upstream site: Bentley Mill Way (BM); downstream site: Sandwell Valley (SV). From Ordnance Survey Landranger Number 139, Birmingham.

various stages on its journey through the city. It is joined by several tributaries, including the Rea, Cole, and Blythe, before eventually turning northwards and feeding into the River Trent near Nottingham. It also contributes to water abstraction storage ponds for the region.

The industrial heritage of Birmingham means that certain areas of land, and consequently the run-off from these, are contaminated with trace metals, such as copper. In 1511 Birmingham was already known for its metalworking, the Royal Army placed an order for horseshoes and weaponry, and by 1700 corn mills were being converted to the production of metal rolling and ironwork. A quote from *Showell's Dictionary of Birmingham*, shows the importance played by brass manufacturing; 'In 1865 it was estimated that the quantity of metal used here in the manufacture of brass was 19,000 tons of copper' (Harman, 1885).

Sources of pollutants were old mine workings, smelting works, and other plants that no longer exist, but which also discharged effluent directly into the rivers. The River Tame has the additional problem, now common to many British rivers, of phosphorus-rich effluent, discharged from sewage treatment works without tertiary phosphate stripping facilities. The four treatment works currently discharging sewage effluent and settled storm sewage that impact the field sites are Willenhall (NGR SO9796098160) with a people equivalent (p.e.) of 45,000, Goscote (NGR SK02190192) p.e. 109,000 - discharging to Rough Brook, Walsall Wood (NGR SK03550388) p.e. 16,600 - discharging to Ford Brook, and Ray Hall (NGR SP0232094440) p.e. 162,000, (Figure 1.2) (pers. comm. Severn Trent Water). People, or population, equivalent (p.e.) is an estimate of the number of people served by a treatment plant. Sewage effluent is the major contributor of phosphorus, a nutrient that can contribute to eutrophication and the associated algal growth problems (Mainstone & Parr, 2002). Both phosphorus and copper, a trace metal that is toxic at high concentrations, may be stored in sediments for long periods by association with the particulate materials. These elements will remain sequestered in the sediment until changes in the physico-chemical properties of the water or the sediment cause their release. The algal biofilm, which develops on the sediment surface in the River Tame, and the benthic macroinvertebrates, epifaunal and infaunal which colonise the sediment, may play important rôles in the fluxes of copper and phosphorus. Macroinvertebrates can affect the properties of the sediment, the biofilm,
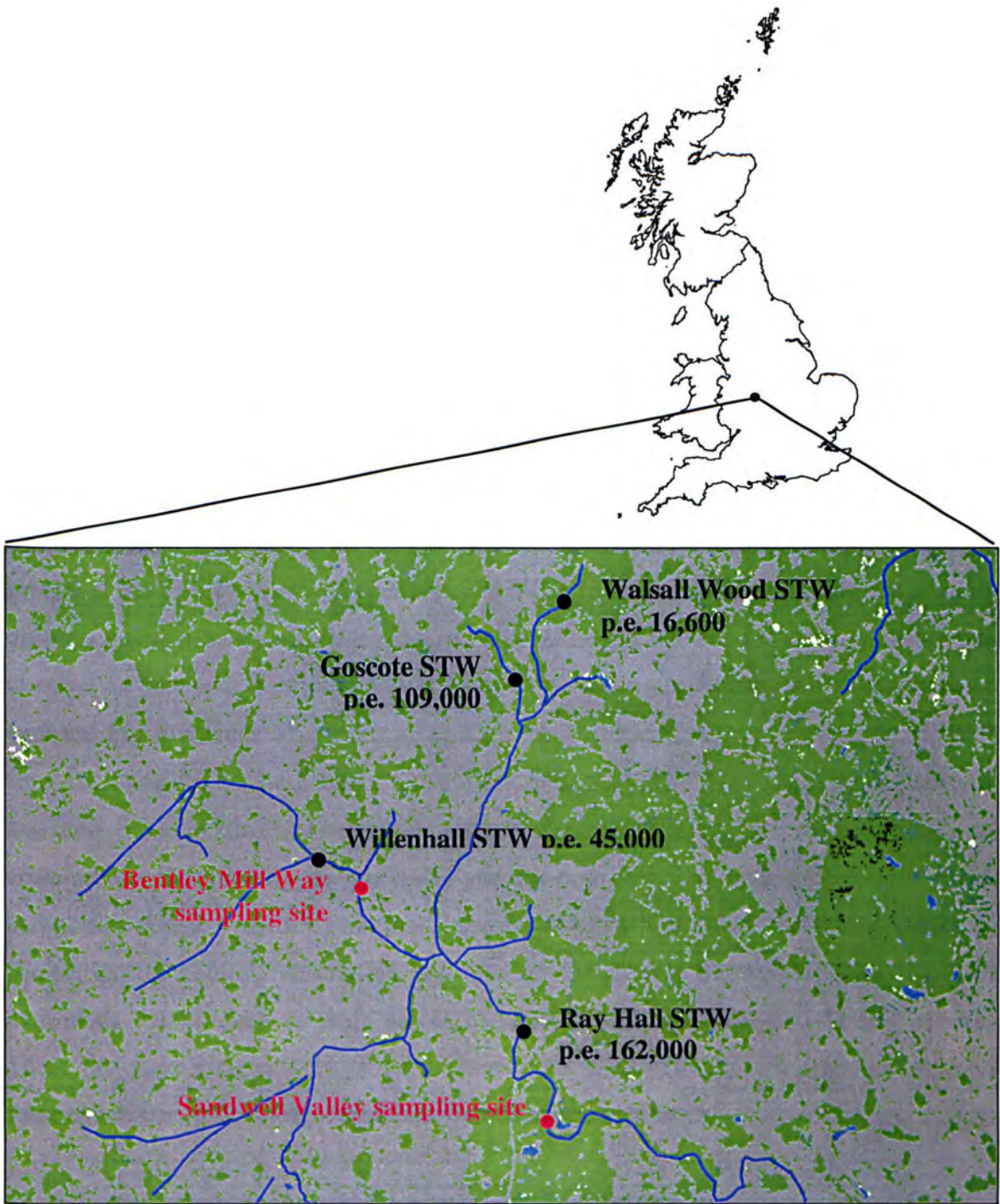
Figure 1.2. Outline of Great Britain with point indicating the position of the city of Birmingham and the mapped area shown enlarged. The positions of the two sampling sites (red points) and the four sewage treatment works impacting the sites (black points) are indicated on the schematic of the Upper River Tame catchment showing the river (blue line). Grey areas represent buildings or other constructs, the green areas represent any vegetated ground, and lakes or ponds are shown in blue. Map image based on digital spatial data licensed from the Centre for Ecology and Hydrology, © CEH. © Crown copyright.

and the water at the water-biofilm-sediment interfaces, and so may influence the mechanisms controlling chemical fluxes.

In order that these issues could be addressed within the section of river studied, and that a single catchment should be used so as not to complicate the water chemistry, two sites on the upper reaches of the river were selected. An upstream one situated at Bentley Mill Way (named for Thomas Bentley (1799-1848) who was a brass founder), recognised as being a fairly heavily polluted reach - the Environment Agency grading of the River Tame currently falls between *c* and *d* – and the second site downstream at Sandwell Valley, where the river is associated with a flood defence overflow, or 'balancing' lake and there is a nature reserve. Both these sites have Environment Agency gauging stations which provided river flow and water quality data. Figures 1.3 – 1.6 are photographs of the Bentley Mill Way and Sandwell Valley sampling sites, and the flood alleviation system at Sandwell Valley. These two sites differ in a number of ways, but the primary difference is that Sandwell Valley is a re-constructed channel created when the river was diverted as part of a flood alleviation scheme. It has a clay lining, which has become exposed in some areas where littoral erosion has occurred, and one bank is constructed from rock-filled wire-mesh cages. Upstream at Bentley Mill Way the river is narrower and more prone to bank and bed erosion than at Sandwell Valley, but both have a channel cross-section that is fairly straight-sided and flat-bottomed, and both sites include riffles and pools. There was little marginal macrophyte growth at either site during the study period, but the natural, non-mobile, bed load of the river at Bentley Mill Way includes man-made debris, such as large pieces of concrete drainage pipe and vehicle wheels. These provided a stable substrate for filamentous algal growth, and acted as traps for finer material in which submerged macrophyte species grew.

Much investigation has been done into the sediment-phosphorus interaction in lakes since eutrophication began to be seen as a problem (Likens, 1972; Moss, 1986; Golterman, 2001). In river systems the majority of research has been on the silt fraction of sediment as this was thought to be the key control of the phosphorus flux, and because these fine particles can be important contaminant carriers (Westrich, 1984; Droppo, 2001; Linge & Oldham, 2002). However, little information is yet available for lotic systems with a mixture of sediment sizes. An earlier work examined the effects of a diatom-dominated

Figure 1.3   The Bentley Mill Way sampling site on the River Tame, Birmingham, photographed in May, 2000. This site is situated immediately downstream of the James Bridge Aqueduct. The view is downstream from approximately the position of the first 10 m section of the measured 100 m experimental reach, and the entire length of the reach is visible.   Bank vegetation is still at a minimum, but in-stream the biofilm is fairly well developed.   Contaminant seeps can be seen on the left side as the orange leachate staining. The river is at low flow.

Figure 1.4 The Bentley Mill Way sampling site on the River Tame, Birmingham, viewed upstream towards the culverts under the James Bridge Aqueduct, photographed in March, 2000. Although the river is at low flow condition, the author demonstrates the depth of water at the position of one of the sediment sampling trays in position in the river bed. Man-made additions to the bed load can be seen mid-stream and under the bank on the right of the picture.

Figure 1.5 The Sandwell Valley sampling site on the River Tame, Birmingham, photographed in March, 2000. The view downstream, from the approximate position of the first 10 m section, shows the entire length of the 100 m experimental reach. This site is a constructed section of the river, made during the building of a flood alleviation scheme, the bed has a clay liner and the bank on the left of the picture is built of rock-filled wire cages. The riffles and pools included in the sampling reach are clearly visible because the river is at low flow conditions. The artificial berm constructed to prevent over-bank events during high flow can also been seen on the left.

Figure 1.6 A view upstream of the Sandwell Valley flood defence scheme on the River Tame, Birmingham. This section of the river has a concrete lining, and the bank on the right of the picture is lowered to allow overbank spillage into the overflow lake (far right). The return inlets of the flood sluices can be seen (arrows indicate) on the right bank leading from the overspill lake. The area around this site, and the overspill lake have been designated as a nature reserve, and there are large colonies of wildfowl present all year round.
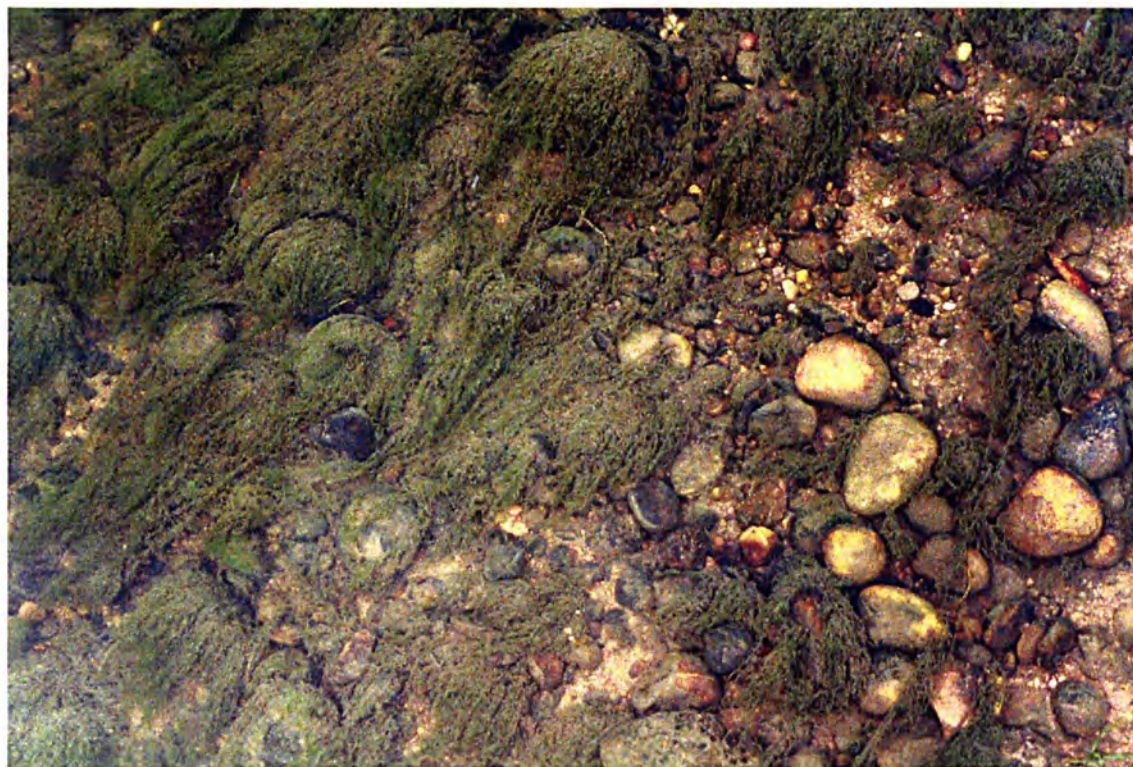
Figure 1.7 A typical section of mixed grain-size bed sediment in the 100 m experimental reach at the Sandwell Valley site on the River Tame, Birmingham. The photograph was taken in March, 2001, and shows that a small amount of filamentous algal growth had developed by this time. Flow direction is right to left. The average size of the larger cobbles which can be seen is approximately 6 – 10 cm diameter.

biofilm on the processes and chemical fluxes in the fine (< 2 mm) fraction of a river sediment (Woodruff *et al.*, 1999a, b).

Until this present study, there was no research into the rôle of larger size material – the gravel and stones - and the effects of benthic, filamentous macroalgæ, on the fluxes of contaminants across the sediment-water interface. Therefore, the River Tame, with its heterogeneous mix of different grain-size bed sediment (Figure 1.7 shows a typical section of bed sediment at Sandwell Valley), and seasonally fluctuating filamentous algal growth patterns, was found to contribute valuable understanding of these aspects of river systems.

1.3: Natural Waters

1.3.1: Introduction

Natural waters are a complex system comprising all phases – aqueous solution, solid, and gaseous – which, in a relatively small river such as the Tame, will be strongly affected by the growth of biota. In turn this growth is influenced by temperature and light intensity, which are controlled by channel characteristics. The River Tame, for example, generally has a high bed-area to water volume ratio, which means processes at the water/sediment interface have a greater effect on water quality than in deeper rivers. All these factors in concert determine when and to what extent reactions and processes occur, and the state of properties such as the pH (see section 1.3.2), conductivity (section 1.3.4), and dissolved oxygen (section 1.3.5) content.

The main chemical processes in natural waters have been identified (Stumm & Morgan, 1970) as acid-base, oxidation-reduction, gas-solution, and coordination reactions, and precipitation-dissolution, adsorption-desorption processes. Rivers are environments where water has a short residence time and chemical equilibrium is frequently not reached between the solution and the solid phases, whether in suspension or on the river bed. In riverine systems under low flow conditions, such as those frequently experienced by the River Tame after relatively short periods of low rainfall, the adsorption-desorption processes are dominated by those occurring at the water-sediment interface; unlike lakes where suspended particulate material plays a more significant rôle. However, because

approximately 90% of the contaminant load in river systems is transported by the particulate matter (Förstner, 1990), the interactions with the particulates may dominate when flow conditions lead to elevated suspended sediments, or when algal growth becomes a trap for suspended material, as has been observed in the River Tame.

In catchments not dominated by point discharges, concentrations of both dissolved and solid fractions of contaminants are strongly affected by surface run-off, and higher winter values are caused by increased weathering and transport in the catchment. Concentrations in solution seem to be highest during low river flow – this indicates that there may be some dilution effect of point sources in higher flow periods. In addition, there is a 'flushing effect', as brief, but significant, increases in concentration during the initial stage of storm run-off have been documented (Förstner, 1990). The significance of any sorption/desorption reactions occurring in a sediment need to be considered in the context of a particular water body and its hydrodynamic characteristics, *e.g.* mixing, dilution, and other factors influencing the concentrations of contaminants released from sediments. The sites on the River Tame for example, being in the upper part of an urban catchment where, typically, most rainfall enters the channel via overland flow, are prone to spates. Then, in dry spells water levels can fall quite low – down to $0.39 \text{ m}^3 \text{ s}^{-1}$ at Bentley Mill Way during the sampling period of spring 2000 to spring 2002, compared with an annual average of $1.0 \text{ m}^3 \text{ s}^{-1}$, and $1.9 \text{ m}^3 \text{ s}^{-1}$ minimum with a $3.2 \text{ m}^3 \text{ s}^{-1}$ average at Sandwell Valley for the same interval.

1.3.2: pH

The pH of water is determined by the anions present, *e.g.* in hard waters by HCO_3^- and CO_3^{2-}, and other weak acids balancing the base content – the acid-base reactions. Because most of the elements involved in these reactions are also involved in biological activity, *e.g.* uptake and release of CO_2 (a weak acid) during photosynthesis and respiration, these processes affect pH as well. Thus, in a neutral to slightly alkaline river such as the Tame, where extensive algal development can occur, photosynthesis will reduce the CO_2 present and raise the pH.

Variations in pH and reduction/oxidation (redox) conditions in the solution around particulates are important in the release and uptake of contaminants from sediments. Changes in the pH of a system are influenced by the buffering capacity of the system, and the buffering capacity of a sediment can affect contaminant mobilisation and immobilisation processes. The buffering capacity, *i.e.* the ability to resist a change in the hydrogen ion (H^+) concentration and thus a change in pH, of a freshwater system is dependent mainly upon the dissolved inorganic carbon (DIC, *i.e.* CO_2, H_2CO_2, HCO_3^- and CO_3^{2-}), equilibria as these supply the dominant ions (Stumm & Morgan, 1970). Low DIC content systems, such as upland streams like Mosedale Beck in the English Lake District – average pH 5.3 - (Lawlor *et al.*, 1998), have a low buffering capacity and therefore may experience larger fluctuations in pH compared with a well-buffered system.

Removal of trace metals from natural waters is affected by pH as this alters the metal speciation in solution, and the ionisation of particulate surfaces. Similarly, binding sites on algal surfaces are affected by pH, and thus the precipitation of metal compounds in biofilms (Liehr *et al.*, 1994). The thickness of a biofilm, the growth requirements of the constituent organisms, and the alkalinity (see section 1.3.3) of the overlying and sediment pore waters, will control the extent to which the pH is raised. Kuenen *et al.*, (1986) showed that pH in the interior of a biofilm may be as much as two pH units higher than water buffered by an alkalinity of approximately 50-100 mg L^{-1} as calcium carbonate alkalinity ($\approx 1 - 2$ milli equivalent per litre (meq L^{-1}) $CaCO_3$); their models predicted that pH variation could be even greater in poorly buffered waters. Levels of 1 meq L^{-1} $CaCO_3$ and 10 meq L^{-1} $CaCO_3$, represent moderate to very high alkalinities respectively. A hard water with values of 4 - 5 meq L^{-1} $CaCO_3$ gives very well buffered conditions, so with annual averages of ~ 5.4 meq L^{-1} at Bentley Mill Way and ~ 4.0 meq L^{-1} at Sandwell Valley (pers. comm.. Environment Agency, U.K.), pH in the River Tame would not be expected to alter much. However, changes of ~ 1 pH unit were noted at the sediment-water interface where a biofilm was present in a river with similar alkalinity to the River Tame (Woodruff *et al.*, 1999a).

1.3.3: Alkalinity

Total alkalinity is determined by the interaction of H^+ and anions, and for fresh water may be approximated by:

$$[Alk] = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+] \qquad (1)$$

i.e. alkalinity increases with increasing DIC. An increase in temperature will also cause a rise in alkalinity. A temperature gain leads to an increase in HCO_3^- and CO_3^{2-} because higher temperatures reduce the solubility of calcite and the concentration of dissolved CO_2 and H_2CO_3, which affects the dissolution/precipitation equilibrium of the carbon species. Within a biofilm, fluctuations in alkalinity, pH, and conductivity, may occur in diurnal as well as seasonal cycles (Heath *et al.*, 1993).

With reference to copper, it has been seen (Lee & Jones, 1987) that significantly greater concentrations need to be added to high alkalinity waters than low alkalinity water bodies to achieve an equivalent algal kill. This is because the copper carbonate ion pairs formed in the more alkaline waters, such as $CuCO_3^0$, are not available to the algæ.

1.3.4: Conductivity

The ability of an aqueous solution to conduct an electrical current is used to determine the dissolved mineral content, commonly called total dissolved solids, or dissolved salts. There are several factors that determine the degree to which water will carry an electrical current, which include: chemical speciation of the solution, the concentration and number of ions; the mobility of the ions; the oxidation state (valence); and the temperature of the water. The ionic content and composition of the water can have effects on the biofilm of a system; many diatom species have preferences for different conditions along the conductivity gradient, or for certain major ion proportions or concentrations. For example, many *Navicula* spp. would tolerate the high conductivity conditions of the River Tame, whereas most *Eunotia* spp. prefer lower concentrations (Kelly, 2000; Potapova & Charles, 2003). Total dissolved solids information is used to determine the ionic strength of a water and hence estimate the activity of individual ions. As well as sewage effluent, overland flow and urban runoff from roads - a common input in the River Tame catchment - can cause the concentration of dissolved solids such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) in a system to vary significantly.

In a study of rivers in the U.S.A. (Potapova & Charles, 2003) most had a moderate

conductivity of ~180 - 618 microSiemens per centimetre (μS cm^{-1}), and calcium and bicarbonate were the dominant ions. However, conductivity did range from 10 to 14,500 μS cm^{-1}. This can be compared with a conductivity > 9,000 μS cm^{-1} recorded in a tropical soda lake (Verschuren *et al.*, 1999), and an average conductivity recorded in the River Tame over the sampling period (March 2001 to April 2002) of ~1206 μS cm^{-1} corrected to 25°C – readings are referenced to 25°C to eliminate temperature differences associated with seasons and water depth.

1.3.5: Dissolved Oxygen

The percentage saturation of dissolved oxygen depends on the temperature of the water (as temperature increases the concentration of dissolved oxygen at 100% saturation decreases), and the elevation of the water testing site, or atmospheric pressure at the site. The dissolved oxygen (DO) concentration can indicate how much biological processes have affected the water recently.

Dissolved oxygen concentrations in natural waters are particularly linked to the photosynthetic reactions, and bacterial respiration in the bottom sediments, plus the physical mixing processes, and temperature changes occurring within the system. Ground waters that are low in oxygen, due to decay processes in the soil entering the river, can also reduce the average oxygen concentrations. Community respiration, mainly by bacteria, raises the CO_2 when the bacteria break down organic matter, and photosynthesis affects oxygen levels because there is a stoichiometric[1] relationship between CO_2 fixation and oxygen evolution. Lowering the oxygen concentration in the overlying water will reduce the flux of oxygen into a sediment, change the rate of the reactions which depend on oxygen, and consequently affect fluxes of trace metals and phosphate. In a study of the River Great Ouse (Heath *et al.*, 1993), which has a somewhat higher pH (~ 8.0 – 9.0) than the River Tame, photosynthetic activity appeared to have the greatest influence on the oxygen concentration in the water.

In addition to measuring the river water and flume channel overlying solution dissolved oxygen concentrations, a dissolved oxygen microelectrode was used to investigate

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[1] the quantitative relationship between two or more substances in chemical change processes

activity within the biofilm itself. Ion selective microelectrodes have been used since the late 1960s to investigate photosynthesis and respiration in microbial communities and biofilms (Revsbech & Jørgensen, 1986; Woodruff *et al.*, 1999a). They are able to do this because they give sufficient spatial resolution to study the internal changes through a biofilm, and because their size and structure cause little disruption to the biofilm, or sediment, when in use. Ion selective microelectrodes measure the activity of ions in solution, *i.e.* the effective concentration of the oxygen ions that are free to participate in chemical interactions.

1.3.6: Sediment

Sediments comprise a number of components. These are: minerals, polymeric organic acids, detrital matter, microorganisms, and benthic organisms (House *et al.*, 1995a).

The distribution and movement of sediment in a river is a function of both the sediment properties - primarily grain size and density, and of the flow characteristics - principally temperature and velocity (Painter, 1976). Grain size, sorting, and packing affect the porosity (the sum of all pores present in a unit volume of sediment) of a sediment, but although porosity determines the volume of water a particular sediment may hold, not all this water may be available for movement (Ward, 1967). The electromolecular characteristics of the sediment particles and the polar water molecules exert attractive forces, and with the smaller particles (clays and silts) these can have a significant effect on the transport of water (Polubarinove-Kochina, 1962). Porosity:

$$\text{Percent porosity } \alpha = 100w/V \qquad\qquad\qquad (2)$$

w is the volume of water required to fill all pore space, and V is the total volume of the sediment (Todd, 1967).

Pollutants generally have a greater affinity for the smaller grain-size fraction of the sediment, *i.e.* clay and silt particles (Förstner, 1990). The larger grain-size fractions tend to be relatively inert, but may acquire coatings such as iron oxides or organic substrates onto which the pollutants can adsorb. For example, an algal biofilm such as develops in the River Tame and other phosphate-rich streams during the spring bloom, will act as a

substrate. Sediments can also act as a reservoir for contaminant nutrients and trace metals, accumulating there until they are re-mobilised by changes in environmental conditions.

The ideal conditions for incorporation and fixing of trace metals, such as copper, in sediments include: the presence of suitable fine-grained mineral particles; non-turbulent environments, *e.g.* areas of low flow velocity in a river; steady deposition rates; and reducing (anoxic) conditions (Förstner, 1990). Sediments are complex environments where dissolved metals may be released not only from inorganic compounds, but also from organic matter; and where numerous adsorption sites may prevent the quantitative release of the produced dissolved metals. Metals precipitated in the sub-surface, anoxic sediment may be re-released to the porewater if the precipitate is transported back to the oxidising surface layer, for example by the burrowing of benthic organisms. This would be analogous to the recycling of redox sensitive metals between the oxidising surface layer and the reducing subsurface of the sediment, which is also accelerated by bioturbation. This recycling adds particulate metal to the metal derived from labile organic matter originating from the water column.

Nutrients are also stored in sediments, and the main factors established as regulating the release of nutrients from sediments are: the rates of deposition of organic detritus and decomposition – mainly bacterial; and the rates at which nutrients released to pore waters are transported to overlying water by diffusion and bioturbation (McCaffrey *et al.*, 1980). The factors controlling CO_2 and nutrients in pore waters, the magnitude of fluxes from advection[1] of water by organisms, and diffusion (ionic and molecular) across the sediment-water interface are other aspects known to contribute to nutrient re-cycling.

In calculating fluxes from porewater concentrations, McCaffrey *et al.* (1980) assumed metabolites were to be transported across the sediment-water interface in either of two ways. The first, a random model by biodiffusion, includes simple diffusion but is dominated by animal-driven advection of water; and the second, an ordered process called biopumping, where the organisms are assumed to pump the water from various

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[1] Advective flux as described here denotes transport of dissolved chemical species due to transport of water and sediment by organisms.

depths in the sediment to the overlying water. No account was taken of the effects of a
biofilm on the sediment surface. The biodiffusion model was not thought to be very
accurate as the calculated results were linear not exponential as would have been
expected from a random process (McCaffrey *et al.*, 1980). Also, where there were no
positive concentration gradients, random processes would not have caused diffusion to
occur. Random processes would only have produced fluxes towards the interface along
positive concentration gradients.

In laboratory experiments biofilm growth has been shown (Woodruff *et al.*, 1999a) to
have a significant effect on the development of porewater solute concentration gradients,
and the composition of overlying water in a recirculating fluvarium channel. Salomons
(1985) suggested that it is vital to establish whether the concentrations of pollutants in
sediment porewaters are controlled by adsorption/desorption processes or by
precipitation/dissolution, because these different processes may have an important effect
on the way the contaminants impact the environment.

Although equilibrium may not always be achieved between the solid and aqueous phases
of a river because of the flow speed (short residence time), Pavlou (1987) asserts that
transport between the sediment and water does occur rapidly via molecular exchange. In
the water column molecular exchange occurs between suspended particulates and water,
and in sediments between consolidated sediment particles and interstitial water. And
because this exchange is continuous, it maintains the contaminants in the system at
chemical equilibrium. Pavlou states "The instantaneous concentration of the contaminant
in either of the two components (sediment or water) can be expressed as a function of its
concentration in the other component and an equilibrium constant specific to that
contaminant." Pavlou refers to these constants as *partition* coefficients, but the term
'partition' implies some knowledge of the mechanism involved, therefore it is preferable
to use 'distribution' instead. These two terms are often used interchangeably in the
literature, sometimes mistakenly. The distribution coefficient

$$K_D = \frac{C_s^x}{C_w^x} \qquad\qquad (3)$$

where K_D is the distribution coefficient, C_s^x and C_w^x are the concentrations of

contaminant x in sediment s and surrounding water w.

Pavlou (1987) found a statistically significant relationship between the organic content of a sediment and K_D for copper, and so states that K_D needs to be adjusted, *i.e.* normalised, to account for the organic carbon (OC) present. As organic substances (and Fe) control the trace metal sorptive properties of sediment particles, sediments with high OC generally show a greater affinity for trace metals, and therefore have a higher K_D.

According to Pavlou (1987) the 'safe' level for a contaminant in sediment can be defined as its concentration in the sediment which ensures that the concentration in interstitial water does not exceed established water quality criteria. However, where interstitial waters are high in colloids the metals, or other toxic substances such as pesticides (Warren *et al.*, 2003), can adsorb to the colloids and the toxicity is reduced by making the substance less bioavailable.

1.3.7: Phosphorus

1.3.7.1: *Introduction*

This Group V non-metal has three allotropes – white, black and red. The white allotrope exists as P_4 molecules, which are very reactive because of the bond strain due to the molecular shape and the low (298 kJ mol^{-1}) bond energy.

- *Compounds of Phosphorus.* Phosphorus forms two important oxides, phosphorus$_{(III)}$ oxide (P_4O_6) and phosphorus$_{(V)}$ oxide (P_4O_{10}). When phosphorus$_{(V)}$ oxide reacts with water, phosphoric acid (H_3PO_4) is produced. This acid is commonly used in the manufacture of fertilizers, along with nitrogen as both are essential for plant growth, but it generally needs to be present in the simple inorganic form for it to be taken up by the plants. Other phosphorus compounds are important ingredients in detergents, water softeners and pharmaceutical products.

Phosphate minerals comprise principally hydroxyapatite $Ca_5(PO_4)_3OH$ (HAP), the most stable form at normal temperature and pressure. However, there are a number of other more soluble calcium phosphate minerals that may form before HAP. Phosphorus may occur in non-occluded and occluded forms. The occluded species are those found within
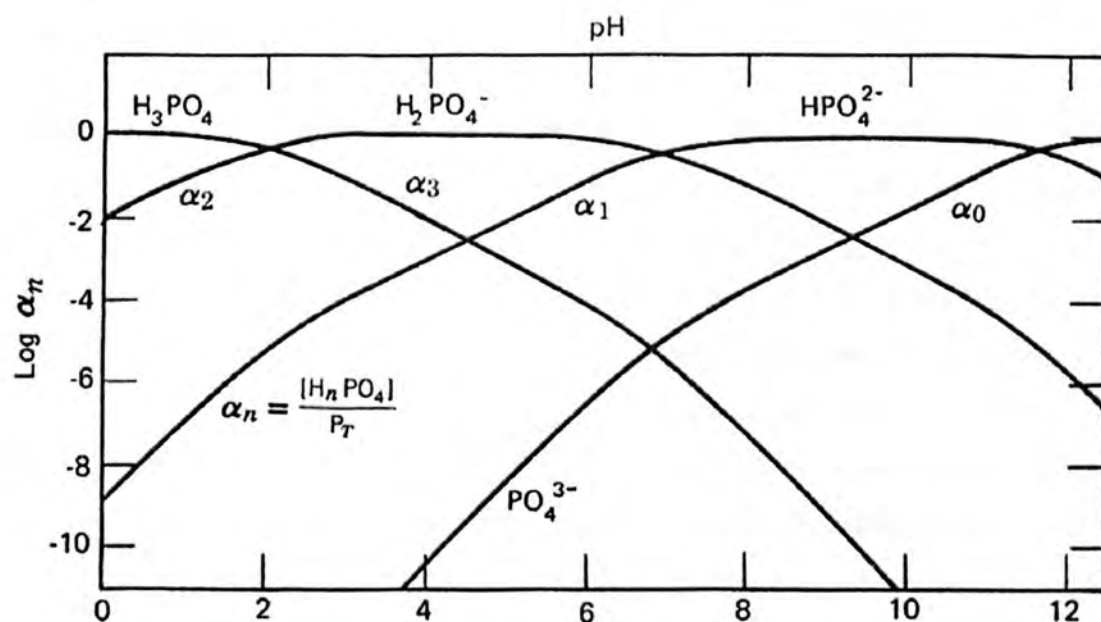
Figure 1.8   A diagram showing the distribution of the various species of phosphorus ion in solution. The x-axis shows the pH of the the solution. The y-axis is the log of the fraction (α) of that species (expressed in terms of their activities) at that pH, P_T being the total phosphorus. Diagram adapted from Stumm and Morgan (1970).

the matrices of amorphous hydrated aluminium ($AlPO_4.2H_2O$), iron oxides ($FePO_4.2H_2O$) and alumino-silicates, and are not readily available for uptake. Non-occluded species are more soluble and therefore more labile; these include the orthophosphate ion bound to the surface of calcium carbonate ($CaCO_3$) or silicon dioxide (SiO_2).

Combined within aquatic biomass, usually bacteria or algæ, phosphorus occurs in an organic form. Soluble reactive phosphorus (SRP) is the most abundant and bioavailable form of this element. It is variously known in the literature as dissolved reactive phosphorus (DRP); Murphy and Riley, or molybdate reactive phosphorus (MRP); filterable reactive phosphorus (FRP); and commonly (but often incorrectly) as orthophosphate. Strictly, orthophosphate ions (PO_4^{3-}) are negligible in natural waters unless they are very alkaline, *e.g.* pH 12 (Figure 1.7). These definitions of the labile phosphorus are operational rather than chemical, *i.e.* they depend on the methods used for assay. In these definitions are included all simple dissolved inorganic phosphorus forms, but it may also include phosphorus adsorbed to the surfaces of colloids in suspension if they are smaller than the pores of the filters used in a particular method, *e.g.* < 0.45 μm. However, the definition would not include organic phosphorus compounds, or inorganic polymeric phosphorus, unless they were hydrolysed during the analysis.

1.3.7.2: *Speciation in natural waters*

The PO_4^{3-} ion is a strong base[1] and is hydrolysed by water to give the orthophosphate form HPO_4^{2-}. This species and $H_2PO_4^-$ are the predominant phosphates found in waters and sediments with a normal pH range (Figure 1.8). Hydrogen phosphate is most available at near neutral pH values, but in alkaline conditions may react with calcium ions to form calcium phosphate minerals such as octacalcium phosphate, or calcium hydroxyapatite (HAP) which is relatively insoluble.
e.g. for HAP:

$$3HPO_4^{2-} + 5Ca^{2+} + H_2O \rightarrow Ca_5(PO_4)_3(OH)_{(s)} + 4H^+ \qquad (4)$$

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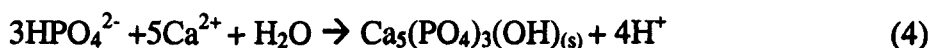
[1] In the Brønsted-Lowry definition - a proton acceptor.

Phosphorus is an important element in aquatic chemistry (Makareth, 1953; Stumm & Morgan, 1970; Jarvie, H.P. *et al.*, 1998) and can be limiting to algal growth in certain conditions. The residence time of phosphates in aqueous and sediment porewaters can vary from minutes to tens of hours.

1.3.7.3: *Processes involving Phosphorus*

Phosphorus interacts with suspended and bed sediments by: sorption, precipitation, and through uptake and release by the biota of the river.

* Adsorption and desorption. Phosphorus has a strong affinity for fine particulate material in sediments, such as iron and aluminium oxides that occur either as independent particles or as coatings on other grains, due to the charge on the surfaces. It is understood that phosphate anions are exchanged with hydroxyl anions attached to other elements (Morgan, 1997). Desorption is brought about by changes in the pH of a system and whether ærobic or anærobic conditions dominate.

* Mineral precipitation. Redox conditions are a significant factor in the reactions of phosphorus (Mortimer, 1941; Golterman, 2001; House, 2003a) with other compounds, for example with iron to form Fe-P complexes. However, in calcium-rich sediment (high alkalinity conditions where iron is less soluble), redox conditions were thought to be less important to phosphorus release (Granéli, 1979). This was because where ærobic conditions formed a barrier of precipitates at the sediment-water interface, the burrowing action of macroinvertebrates released phosphorus from the sub-sediment by bringing interstitial water to the surface. Bound phosphorus may be associated with calcite (as a co-precipitate), or may form siderite ($FeCO_3$), vivianite (Fe_2PO_4) and other calcium phosphate minerals. Co-precipitation of phosphate with $CaCO_3$ may occur in hardwater regions in the summer, particularly during algal blooms (Heath *et al.*, 1993) when photosynthesis reduces the concentration of CO_2 in the water and thus changes the balance of carbonate ions. Phosphorus may also precipitate iron phosphate, the Fe(II) oxidation state in anoxic conditions and Fe(III), usually as $Fe(OH)_3$, in oxic conditions.

* Biological processes. The uptake and release of phosphorus by filamentous algæ, in particular *Cladophora* spp., and diatoms can have a significant impact where very large algal mats and skeins develop, and these can move phosphorus within a river

system when they are detached from their substrate and transported downstream during spate events. Also, concentrations of dissolved gases and nutrients may be different from those of the overlying water due to the microenvironment created (Woodruff *et al.*, 1999b). Macrophytes are also recognised as influential in the transport of phosphorus (Casey & Downing, 1976), through uptake from the sediment and release to the water during autumn die-back and decomposition (Moss, 1986), and also by their physical impact on fine material (Sand-Jensen, 1998) by slowing the water flow and allowing fine material to be deposited. Bioturbation by macroinvertebrates also causes phosphorus within a sediment to be brought into contact with water, and thus be released into solution, either by re-suspension or irrigation of sub-surface layers (House *et al.*, 1995b). It is also thought that those macroinvertebrates which use the deeper sediment as food may cause additional mineralization of phosphorus via digestion of the sediment particles (Granéli, 1979).

There can be a distinct phosphorus concentration gradient in sediment and exchange depends upon the movement of phosphorus across a boundary layer, the thickness of which is controlled by the rate of transport of the phosphorus through the sediment. This transport rate is determined by several factors including: molecular diffusion; physical mixing; bioturbation; and uptake or release by microorganisms (House *et al.*, 1995a). This transport also affects the concentration of SRP in river water. In a polluted system, such as the River Tame, concentrations of SRP up to $\sim$ 123.4 μM were recorded, and this compares with concentrations as low as < 0.03 μM that have been found in relatively unpolluted systems (Lawlor *et al.*, 1998).

1.3.8: Copper

1.3.8.1: *Introduction*

A soft, reddish metal, copper is sometimes found naturally in its elemental form and its compounds are extensively distributed in the Earth's crust. Two common copper ores are *chalcocite*, and *chalcopyrite*, with typical compositions of Cu_2S and $CuFeS_2$ respectively. Copper is noted for its high thermal and electrical conductivity - the pure metal is widely utilized in electrical equipment, and has been used in alloys since the Bronze Age. Last

of the first transition element series, it has a configuration of $3d^{10} 4s^1$ and this leads to a large range of +1 oxidation state entities. Complexes are formed where the numbers of atoms of a ligand ion, or molecule, attached to the central copper atom are greater than its normal oxidation state, or covalency, permits. At relatively low temperatures copper is attacked by the halogens and sulphur, dissolves in oxidising acids, *e.g.* HNO_3, H_2SO_4, in the presence of air, but is resistant to reducing acids, *e.g.* dilute HCl (Stumm & Morgan, 1970; Nicholls, 1974). Electrochemically:

$$Cu^{2+} + 2e > Cu \qquad\qquad (E^o = +0.34V) \qquad\qquad (5)$$
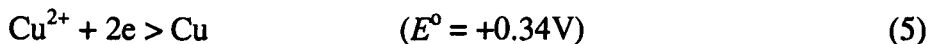
- *Compounds of Copper(II) (d^9).* In aqueous solution copper(II) is the most stable state, and when copper(II) salts are dissolved in an excess of water the hexaquo-ion $[Cu(H_2O)_6]^{2+}$ is formed. Many different coordination compounds may result from the substitution reactions of $[Cu(H_2O)_6]^{2+}$ with ligands[1], *e.g.* with ammonia $[Cu(NH_3)_4(H_2O)_2]^{2+}$. If an alkaline solution of copper(II) is treated with a mild reducing agent it will produce a fine orange precipitate form (Nicholls, 1974). The hydrate of copper(II) nitrate, $Cu(NO_3)_2.3H_2O$, is highly soluble in water.

- *Compounds of Copper(I) (d^{10}).* It is only the insoluble salts of copper(I) that are stable in water. Soluble ones undergo disproportionation[2], *e.g.* Cu_2SO_4:

$$Cu_2SO_4 + 6H_2O \rightarrow Cu + [Cu(H_2O)_6]^{2+} + SO_4^{2-} \qquad\qquad (6)$$
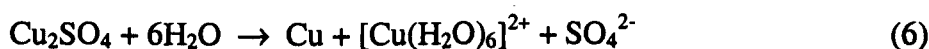
The red copper(I) oxide occurs naturally in *cuprite* (Nicholls, 1974).

1.3.8.2: *Speciation in natural waters*

Copper in urban waterways often originates from industrial waste streams from metallurgy, paint and dye, the electrical and electronics industry, cleaning, duplicating, electroplating/finishing, chemical manufacturing, explosives, and textile manufacture

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[1] Ligands may be defined as donor molecules or Lewis bases. They are said to donate a pair of electrons to the central atom of a complex by co-ordinate bond formation (Nicholls, 1974). Water being a ligand means that complexes are always formed when a transition-metal ion is introduced to aqueous solution.
[2] Disproportionation occurs when, in the same reaction, some atoms of the same element are oxidized and some reduced (Lister and Renshaw, 1993).

(Barnhart, 1978). After entering a river any metal dust particles, *e.g.* from smelters, or effluents containing metals associated with inorganic or organic matter undergo little or no change. The majority of this metal load is transported by particulate matter in an unpolluted river, but in polluted systems the dissolved fraction is significantly higher especially for copper and some other metals (Förstner, 1990). Variations in the trace metal content of stream sediments are a function of a number of potential controlling factors. These factors have been grouped into the following six categories by Dahlberg (1968): the influence of lithological units; hydrological effects; geological features; cultural, *i.e.* man-made, influences; type of vegetation cover; and the effects of mineralised zones.

Although an essential trace element copper is toxic at high concentrations and may have adverse effects on aquatic biota far below drinking water limits, *i.e.* 2 mg L^{-1} in the U.K. (DWI, 2000), and the 'free' or aquo-metal ion form has been suggested as the most available to organisms compared to the particulate, complexed or chelated forms (Förstner, 1990). However, adsorption, filtration, sedimentation, complexation, precipitation, and redox reactions can act as barriers to the movement of metals. Organic substrates play a major rôle in the binding of metals such as copper because they are involved in metabolic processes, and so, according to Förstner (1990), they may be the chief agents of the transfer of metals in the food chain.

1.3.8.3: *Dissolution-precipitation*

The ratio of dissolved to solid fractions of an element can reveal the mobility of that element. These ratios are influenced first by the inputs, and then by the interactions, occurring in different environmental compartments, especially in rivers. Three main factors affect the distribution of trace metals between solution and particulates: 1) the chemical form of the dissolved metals originating from both natural and anthropogenic sources; 2) the type of interactive processes (sorption/desorption- or precipitation-controlled mechanisms), and; 3) the concentration and composition of particulate matter, mainly with reference to surface active phases (Förstner, 1990).

1.3.8.4: *Adsorption-desorption*

Metals use organic surfaces on particles for adsorption and these surfaces could be formed in different ways (Hart, 1990): by bacteria and algæ; by the breakdown of organic matter or the aggregation of lower molecular weight organics; or, by low molecular weight organic material being adsorbed onto clay or metal oxide substrates (Davis & Gloor, 1981). The difference between those three surface types with respect to metal uptake is not yet well understood, but carboxylic and phenolic functional groups on part of the organic matter adsorbed on the particulates in natural waters are available for binding with trace metals. The capacity of organic matter to adsorb trace metals usually falls between the levels for metal oxides, such as metastable iron and manganese oxides, and clays. The oxides are particularly efficient at adsorbing inorganic components because they have a high degree of isomorphic substitution (Jenne & Zachara, 1987). Reactions occurring between pollutant-loaded sediment particles and the aqueous phase can be seen most clearly in the composition of interstitial waters in the sediments, which can also give information of the types and the extent of those reactions (Förstner, 1990).

In aquatic environments algæ have been observed (Liehr *et al.*, 1994) to remove metals, and some algæ have a significant number of reactive sites with a high affinity for metals such as Cu and Zn. The mechanisms, though difficult to distinguish, may be: extracellular – by incorporation in an extracellular polymeric matrix (EPS[1]), possibly by attachment to specific sites, or trapping of particles of precipitates; cell surface – by adsorption, ion exchange, or complexation to sites on cell surface; and intracellular – by active uptake and accumulation inside the cells. Boult *et al.* (1997) found that a biofilm colonising an acid mine drainage contaminated stream was affecting the flux of metal (Fe) in the stream. This has been postulated to be by scavenging metal from the water to reactive groups within the film bacterial EPS matrix, which has a large surface area for cation exchange. It is thought possible that metals fixed in this way may remain associated with the organic matter because EPS is also an efficient complexing agent, and that in less contaminated streams this may have a more significant affect on metal fluxes.

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[1] Extracellular polymeric substances - a polysaccharide mucus produced by bacteria and diatoms

1.3.8.5: *Toxicity*

It has been suggested (Cairns *et al.*, 1978) that because certain algal groups, *e.g.* diatoms, may have a much lower tolerance to chemical toxicity than others, *e.g. Chlorococcales*; and some groups, *e.g. Cyanophyta* and *Chlorophyta* (see section 1.3.9), are more tolerant to warmer waters than others, such as diatoms and flagellates, contaminated and heated waste water discharges could cause significant alterations in algal community structure. However, Cairns (1978) also showed that certain species of algæ grew optimally at copper concentrations just below the lethal level – much higher than the levels found in most natural waters – but that toxicity appeared to increase as water temperature increased (between 5° and 25°C). Blue-green algæ, especially filamentous, bloom-forming species, are generally more sensitive to copper toxicity than chrysophytes, the golden-brown algæ, chlorophytes, or diatoms (McKnight & Morel, 1980). Though (Agrawal & Misra, 2002) state that the formation of zoosporangia, their viability, and the germination of zoospores in a variety of algæ, including *C. glomerata,* was affected by the presence copper (and other trace metals) at concentrations of 0.5-10 mg L^{-1}.

When compared with concentrations of acid-available copper in the relatively unpolluted Dun-Kennet canal system, between October, 2000 and September, 2001, of between < 1 μg L^{-1} and ~ 25 μg L^{-1} (pers. comm. W.A.House), and the maximum concentration in some small rivers in a Swiss agricultural catchment of < 5 μg L^{-1} (Xue, H. *et al.*, 2000), those measured in the River Tame, between 2001 -2002 for this study, of between ~ 7.0 and 150 μg L^{-1} clearly show the river to be impacted. However, Environment Agency data does not confirm this, with recorded concentrations reaching maxima of only ~ 13 μg L^{-1} (pers. comm., Environment Agency), but assay method may account for this apparent difference.

1.3.9: Biofilms

Algæ can form communities in any illuminated, aquatic environment, even on a damp surface or in a moist atmosphere (Leadbeater & Callow, 1992). However, those which live either on or in an aquatic substrate are known as benthic algæ, and in freshwater habitats they commonly include: *Bacillariophyta* (diatoms), *Chlorophyta* (green algæ),

Cyanophyta (blue-green algæ), and *Rhodophyta* (red algæ) (Stevenson, 1996; Stevenson *et al.*, 1996). Other divisions may also occasionally be found in benthic habitats, but to a much lesser extent and usually in different physiological forms, for example as resting cells. The algal mats formed by these colonies are known as biofilms, and are a microbial aggregation that may frequently include bacteria and fungi (Cooksey, 1992) as well as the unicellular and filamentous algæ; there may also be macroinvertebrates and inorganic components such as silt and minerals present. Diatoms are generally the primary colonisers of benthic substrates and from settlement the durability of their attachment to a firm substrate increases with time. In addition, diatom species that have a flat-lying habit resist water flow shear stress better than sessile species or bacterial communities (Blenkinsopp & Lock, 1994), although in a cultured bacterial monopopulation study by Peyton (1996) shear stress did not appear to affect film thickness. The colonisation of a substrate is also influenced by external factors including: the nature of the substrate, the supply of nutrients, competition from other organisms, and predation by grazers, which alter the growth, and hence, the composition of a biofilm (Cox, 1988). Because organisms in biofilms are closely attached to each other they resist the mass transfer of products out of the biofilm, and this leads to the environment in the biofilm being different to the environment of the bulk water (Liehr *et al.*, 1994; Battin, 2000). Biofilms are also sometimes referred to as periphyton, but according to Stevenson (Stevenson, 1996) this description does not include macroalgæ. Macroalgæ mostly comprise the *Chlorophyceæ* (green algæ) such as *Cladophora* species.

It is known that the biofilm is encased in a highly hydrated, fibrous, species-specific, exopolysaccharide, also known as extracellular polymeric substance (EPS), a matrix produced by diatoms (Hoagland *et al.*, 1993) and bacteria; though the diatom mucilage has a slightly different composition from the bacterial EPS, having smaller amounts of neutral polysaccharides (Leadbeater & Callow, 1992). By using confocal scanning laser (CSL) microscopy, it has been seen that populations within the biofilm grow in microcolonies separated by less-dense zones of the matrix that are highly permeable water channels (Costerton *et al.*, 1994). These channels give good permeability access from bulk water to the colonized surface and have convective flows within them. However, quantifying porosity of a biofilm has problems associated with the degree of heterogeneity within a film, and differentiating between water in the pores and that in the EPS matrix (Lewandowski, 2000). Boult *et al.* (1997) assert that diffusion affects the

growth and activity of the biofilm, along with any sloughing-off that might be induced autogenically (Stevenson, 1996), or by the hydrodynamics of a stream. This microenvironment, created by the diffusion properties, and reactive groups of anions in the EPS matrix, may also enable the biofilm to survive in otherwise adverse conditions, such as acid mine drainage (Boult *et al.*, 1997).

Above the biofilm surface lies a thin layer of water known as the diffusion boundary layer (House, 2003). A velocity gradient exists in this layer (Borchardt, 1994) where laminar flow precludes mixing with the overlying bulk water and so nutrient movement is governed by molecular diffusion. However, there appears (Lau, 1990) to be some resistance to diffusion through this layer, and the resistance is proportional to the thickness, which can range from micrometres to millimetres (Borchardt, 1994).

As a photosynthesising entity the biofilm uses carbon dioxide and produces oxygen during daylight (if irradiance level is sufficient) and the converse in darkness. It utilizes dissolved inorganic carbon (DIC) (from atmospheric CO_2) to form the organic compounds it requires, and the highest production levels normally occur during the spring bloom. As the thickness of a biofilm increases, self-shading may cause a cessation of photosynthesis and lead to anærobic conditions in the underlying layers (Leadbeater & Callow, 1992). However, it is thought possible that cells residing in different light microhabitats within a biofilm may become photoadapted and develop different photosynthetic responses (Hill, 1996). In a study by Costerton *et al.* (1994) where dissolved oxygen (DO) distribution through the biofilm was measured using DO microelectrodes, they found that if the electrode passed directly through an area of the biofilm composed of a microcolony, and the associated dense EPS, DO levels decreased with depth. However, when the electrode passed through an area that constituted the less dense water channels they describe, significant DO levels were detected even through to the colonized surface.

1.3.9.1: *Cladophora*

This generally attached filamentous macroalga is widely distributed, and found in both freshwater and marine habitats (Fritsch, 1935; Dodds & Gudder, 1992). In rivers it is prevalent in those of < 10 m width (Haslam, 1978) where there is a flow > 20 cm s^{-1}

(Whitton, 1970), and it is commonly part of a stream community, *i.e.* not especially associated with eutrophication. The most commonly described species in eutrophic conditions are *C. glomerata*, *C. rivularis* and *C. fracta* (Whitton, 1970). Normally small-celled, and multi-branched, it forms clumps that can range in size from a few centimetres to skeins as long as 30 metres (HMSO, 1984). It is phosphate rich conditions which can promote the growth of long, less branched filaments with relatively large cells (Marks & Power, 2001), that form the dense algal mats and skeins. Figure 1.8 illustrates such growth as it occurs in the River Tame. Such excessive growth can lead to exaggerated diurnal oxygen fluctuation, and, when die-back occurs in autumn, could cause de-oxygenation in the water column and the surface sediment in areas of low flow velocity where the organic matter accumulates.

In simple terms the growth cycle of *Cladophora*, *e.g. C. glomerata* (Whitton, 1970), begins in spring with vegetative reproduction. Starting with branching from the thick-walled basal fragments of filaments that have over-wintered attached to rocks, the maximum biomass is reached by early summer when vegetative growth then slows. Some cells can develop zoospores during summer (Dodds & Gudder, 1992), from which new filaments develop when they settle on a suitable substrate. When temperatures and light levels fall in late autumn, the long skeins of filaments become separated from the basal rhizoids, which remain attached to the substrate quiescent until conditions become suitable for growth again.

Propagation in *Cladophora* spp. can take place in both the intercalary[1] (internode) and apical (end) cells of the branches (Whitton, 1970), and can occur as (Fritsch, 1935):

Vegetative

In this mode of propagation the separation of cells happens with no apparent changes in the protoplasts, *e.g.* fragmentation - the separation of short strands of the filaments. This type can lead to abundant growth. In conditions unfavourable for reproduction *Cladophora* can form the akinetes - cells with a thickened membrane and build-up of fuel reserves within the protoplast. From this dormant phase new growth develops;

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[1] describes a meristem (actively dividing plant tissue) that grows in the internode of a stem

Asexual

Zoospores (bare flagellate protoplasts) are formed by division of cell contents. *Cladophora* can produce hundreds of zoospores either by division of the protoplast after nuclear division, or repeated division of the nuclei and then segmentation of the protoplasm into an equal number of parts (known as swarmers);

Sexual (not observed in all species)

In isogamy the fusion of morphologically identical gametes[1] occurs. In oogamy a large non-motile female cell (or ovum) is fertilised by a smaller and active male cell (spermatozoid). This occurs mainly in multi-cellular species. The sexual cells are unicellular and usually different from vegetative cells. Both types of cells can be formed on a single individual plant. Sexual cells are normally produced in high nutrient conditions and after vegetative reproduction has peaked. Light, temperature and hydrogen-ion concentration also affect sexual reproduction and it is commonly the last stage of active growth in a seasonal cycle. In oogamous forms the thickened membrane formed after fusion results in a resting-cell, or spore, which is able to withstand period of dessication.

1.3.10: Macroinvertebrates

The chemical diagenesis of surficial sediments is complicated by macrobenthos because sediment and interstitial water are vertically and laterally advected by feeding, defecation, and respiratory pumping. Burrows may act as conduits for exchange via fluid advection, solute diffusion, or sediment slumping, and the secretion of mucus and excretion of metabolites may lead to 'hot spots' of bacterial activity (Matisoff *et al.*, 1985). Also, infaunal macrobenthos which construct semi-permanent burrows, such as the chironomid larvæ that were found to be plentiful in the River Tame, can significantly alter the redox conditions in sediments (Granéli, 1979). *Chironomidæ* numbers recorded in the River Tame in the macroinvertebrate study (Jill Patten – unpublished) were comparable to those found in White Clay Creek (Borchardt & Bott, 1995), which is a stream with a similar bed composition. Oligochætes, another pollution-resistant family of subsurface deposit

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[1] A mature male or female germ cell capable of initiating formation of a new individual by fusion with a gamete of the opposite sex.

feeders found at both Bentley Mill Way and Sandwell Valley, mix sediment within their life zones (McCall & Fisher, 1980), but numbers were in the lower range of those documented in the Borchardt and Bott (1995) study.

Diffusion is the main mediator of the vertical transport of dissolved substances through sediment in the absence of macroinvertebrates, but this is a much less efficient process than biopumping, *e.g.* the respiratory pumping of water. Precipitation may form a chemical barrier to the movement of phosphorus (and iron) in sediments overlain by ærobic water, but macroinvertebrates can burrow through the barrier and thus promote transport across the sediment-water interface (Granéli, 1979). Another option is that tubificid oligochætes, which use deeper sediment as food, digest sediment particles in their intestines and so increase the rate of mineralization. The effect of the worms on the exchange of phosphate between the sediment and the water seems to depend on the proportion of redox-sensitive to redox-insensitive phosphorus, and the worms will only affect the insensitive portion by their effect of increasing the physical contact between the sediment and the water (Davis *et al.*, 1975).

1.4: Project Aims and Objectives

The main aim of this study has been to study the internal chemical, biological and hydrological processes of a river system that cause the deposition or remobilization of contaminants, and how these processes control the fluxes from sediments heavily polluted with nutrients (P) and trace metals (Cu), into the overlying waters.

1.4.1: Aims

The specific aims of this project were to assess the influences an algal biofilm has on chemical fluxes at the sediment-water interface and the kinetics of these exchanges.

◊   To use experimental flume channels to measure fluxes of phosphorus and copper, and certain physico-chemical parameters (pH, DO, temperature and conductivity) in natural, heterogeneous sediments from two selected sites on the River Tame, and to study the kinetics of these movements.

◊ Observe seasonal changes in biofilm development in the field and assess the effects of biofilm development on the fluxes, with a focus on filamentous algæ, in particular *Cladophora*, using the experimental channels.

1.4.2: Objectives

In order to achieve these aims, several core objectives were identified. These were divided into two aspects. Firstly, the field-based component, observing and sampling on the River Tame, and secondly, the flume channels that could simulate the natural system, but under controlled conditions.

1.4.2.1: *Field*:

◊ To observe the development of the biofilm over a complete growth (and die-back) cycle, noting any alterations in appearance that occur due to changes in hydrological conditions or specific events.

◊ To record differences in the physico-chemical characteristics of the river water and relate these to the flume experiments.

1.4.2.2: *Laboratory*:

◊ To use the flume channels to measure the flux of ions across the sediment-water interface from sediment to the overlying solution over the growth cycle of the natural biofilm.

◊ To use the flume channels to explore the influence on the fluxes of the different individual size fractions composing the bed sediment separately, *i.e.* stones, gravel, and silt fractions defined by specific sizes.

1.5: Thesis Plan

The material, methods, and modelling theory used in this study are described in Chapter 2. Chapter 3 investigates the effects of the sediment size fraction on the kinetics of

phosphorus release, especially with respect to the consequences of a biofilm being present. In Chapter 4 the efflux of phosphorus from a natural sediment of mixed grain-size over an annual cycle of development and decline of an algal biofilm associated with the sediment, and models of the kinetics of those exchanges, are examined. The results of copper flux measurements are presented in Chapter 5. Finally, Chapter 6 summarises the results of the experimental work with reference to the definitions of the URGENT III programme, and discusses the implications of the findings. It also draws conclusions from the results of the study, and suggests further work that would improve our understanding of the system and processes examined.

Chapter 2

Methods

2.1: Introduction

All materials and analytical techniques used in this study are described in the following chapter. Also, details of the methods used by Jill Patten in the macroinvertebrate study carried out for her undergraduate work experience project, and executed at the Centre for Ecology and Hydrology Dorset.

2.2: Materials

2.2.1: Field Surveying and Sampling

A one hundred metre reach was demarcated at each of the two sites (Bentley Mill Way and Sandwell Valley) and divided into 10 m sections with marker pegs on the bankside. Rectangular cross-sectioned polypropylene sampling trays, 10 x 40 cm and 5 cm deep, were installed in the bed-sediment longitudinally in-line with the direction of water flow and at a depth such that their top edges were flush to the bed-surface. Ten trays were installed randomly in the measured reach at each site to ensure that potential variability in the bed characteristics were represented. Their positions within the grid system, as described below, were determined by Excel random number generation; should a position be unsuitable due to the depth of the water, the next number in the sequence was selected. Trays were 'seeded' with material (stones, gravel and smaller sediment particles) taken from the hole in the riverbed in which they were placed. Trays were marked using a 'tell-tale' marker rope, however this proved inadequate to find some trays when the biofilm was fully developed. An example of samples tray *in situ* is shown in Figure 2.1. All site visits, Table II.I, were made during the period December 1999 to April 2002, and samples were taken regularly throughout biofilm development and decline phases.

During site visits a set of field observations was made, where possible recording the changes in the general appearance of each measured reach and its margins. Quadrats, delineated using the pegs at 10 m intervals along the bank downstream and visually estimated at 0.5 m intervals across the stream, were examined and the dominant feature, *e.g.* type of bed sediment, presence of algal growth, mapped on a plan of each quadrat.
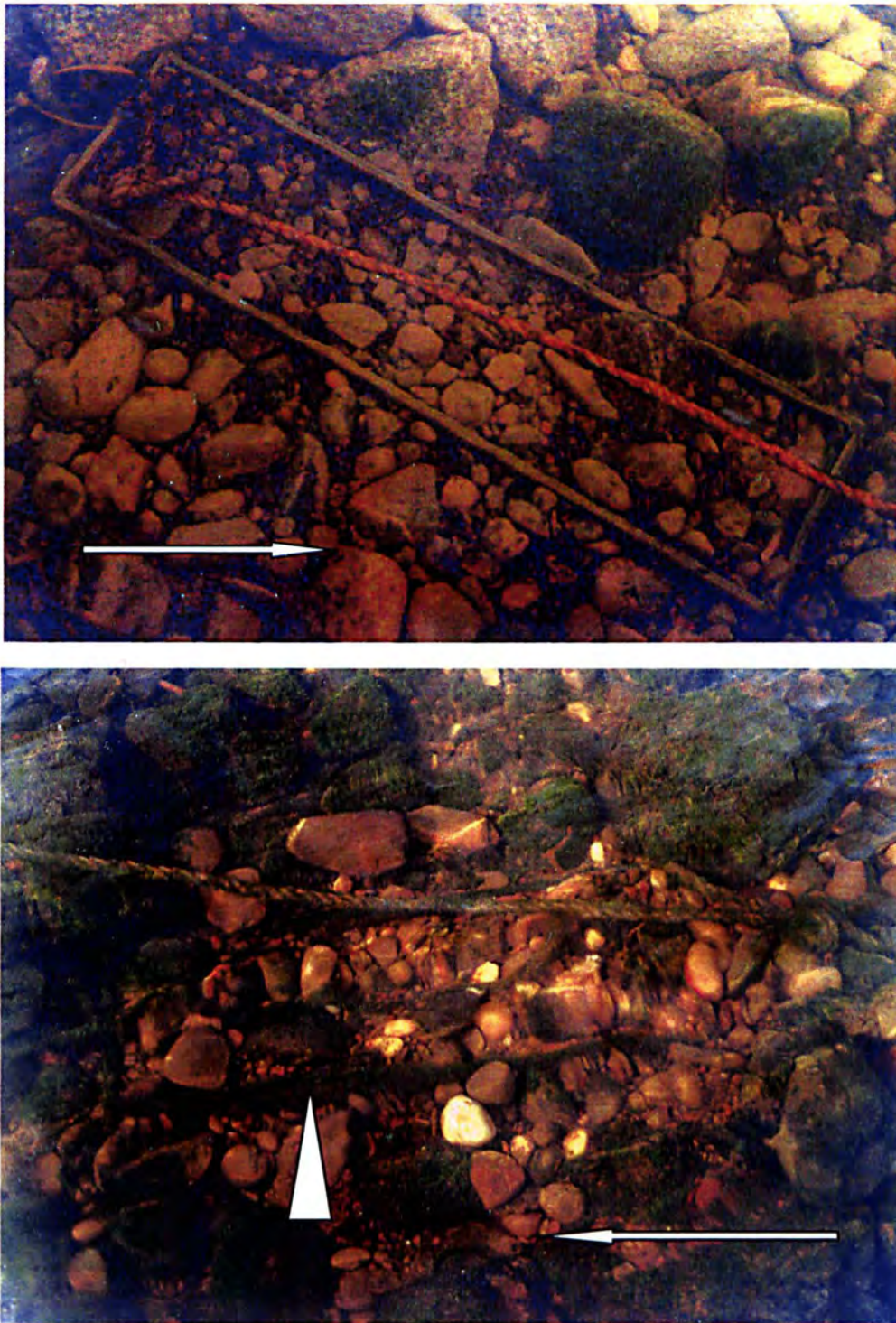
Figure 2.1: Sediment sampling trays in position in the River Tame in April, 2001, after three months in situ. The sediment composition is the typically heterogeneous mix of grain sizes found in the Tame. The rope 'tell-tails' used for locating the trays can be seen in the centre of the pictures. Top – at Bentley Mill Way. Direction of flow is left to right. Bottom – at Sandwell Valley. Direction of flow is right to left. Both images are the same scale. Note: The directions of flow and edge of the tray outline is indicated by the arrows.

Table II.I: Date of site visits to Bentley Mill Way (BM) and Sandwell Valley (SV) with visit function or Experiment number where appropriate.

BM		SV	
Date	*Expt.No.*	*Date*	*Expt.No.*
01-Dec-1999	Pilot study	02-Dec-1999	Pilot study
07-Mar-2000	Pilot study	07-Mar-2000	Pilot study
18-Aug-2000	Pilot study	18-Aug-2000	Pilot study
17-Jan- 2001	Install trays	16-Jan- 2001	Install trays
16-Feb-2001	Check trays	16-Feb-2001	Check trays
-	No visit	29-Jan-2001	Check trays
19-Mar-2001	Experiment BM 1	19-Apr-2001	Experiment SV 1
2-May-2001	Experiment BM 2	2-May-2001	Experiment SV 2
13-Jun-2001	Experiment BM 3	13-Jun-2001	Experiment SV 3
02-Aug-2001	Experiment BM 4	02-Aug-2001	Experiment SV 4
11-Sep-2001	Experiment BM 5	10-Sep-2001	Experiment SV 5
31-Oct-2001	Experiment BM 6	31-Oct-2001	Experiment SV 6
10-Apr-2002	Experiment BM 7	09-Apr-2002	Experiment SV 7

Other details included: noting weather conditions, evidence of any recent high-flow events, macrophyte growth, physical changes in the channel such as bank collapse, water turbidity, and any unusual features such as obvious contamination events. An example of the field record sheet is given in Appendix 2.1. Field measurements of pH, dissolved oxygen (DO), and conductivity were made using Ciba-Corning M90 Sensors, from river water freshly collected in a polypropylene jar rinsed in river water. The sensors were calibrated in the field using the methods given in Appendices 2.2 -2.4. Three readings were taken in order to get an average value. Water temperature was recorded from a 100 Ohm (Digitron 3204 Pt) platinum resistance thermometer (PRT) sensor placed directly in the flowing surface water. Bulk water was sampled into 1 L polypropylene bottles rinsed twice with river water, ensuring no bottom sediments were disturbed during sampling. Filtered water was obtained by filtering river water through a syringe fitted with a 0.45 μm cellulose nitrate membrane placed in a small filter body. A fresh membrane was put in the filter body for each sample and was wetted using distilled water to ensure the filter settled in the body securely. Water samples were collected for the determination of SRP, trace metals, and major-ion analysis. The filtered water sample bottles, 100 ml polypropylene, were acid washed (5% Aristar HNO_3 solution) for 24 h prior to use. Immediately upon return to the laboratory the water samples for trace metals analysis were acidified to give a 1% v/v concentrated nitric acid using Ultrapur grade.

Sample collection trays were inspected at each visit and their suitability for removal and transport to the fluvarium recorded. Suitability was judged by the progress of development of settled, mixed size, sediment giving as close an approximation to the appearance of the actual river bed as was feasible given the dimensions of the trays. Trays where no 'development' had occurred, usually due to flow conditions preventing accumulation, were relocated to a new random position. Additional sediment samples were collected into glass pots by digging out to the same depth as the trays using a trowel. These were obtained from adjacent to each tray location to supply porewater and to characterise the sediment. All water and sediment samples were transported to the laboratory within 12 hours of collection and, if not used immediately for experiments or analyses, stored in the dark at 5°C. Flow velocity at each tray was measured using a Geopacks Stream Flowmeter positioned ~ 5 cm above the tray *in situ*. The fins of the flowmeter were 8 cm in diameter and in very shallow positions, or where the biofilm was very dense, it was not always possible to obtain a reading. In this case the nearest position where it was possible to measure the flow was used. Sediment porewater was extracted for SRP and trace metal analysis by centrifugation at a centrifugal force of ~9500 g for 20 minutes in polypropylene centrifuge bottles with reinforced aluminium collars, and filtered (cellulose nitrate, 0.45 μm).

In experiments where selected sediment size fractions were used rather than the sediment trays, the samples were collected separately, but in their storage and transport, were treated in the same manner as the material taken from the river in the sampling trays:

I. Fines – defined here as < 2 mm size fraction

These were taken from areas within the measured reach where flows were sufficiently low for accumulation to occur. This material was transported back to the laboratory and sieved to remove any > 2 mm particles prior to being put in the sampling trays for containment, before placement in the flume channels.

II. Gravel – for this study defined as the 2 mm – 20 mm particle size fraction

Gravel samples were collected using two separate techniques in order to give two sub-samples of the same fraction. The first sub-sample obtained was prepared by sieving on site, using 2 mm and 20 mm mesh-size sieves, and washing with river water to remove any fine particulates present on the gravel or in any algal growth attached to the gravel. The second sub-sample was carefully sieved on site, using the 20 mm sieve size, but without using any washing water, in order to minimise the

disturbance to the fine attached matter. At the laboratory this sub-sample was then very gently rinsed using river water collected at the same time as the sample, through a 2 mm sieve to wash out the finer particles with as little disturbance to the biofilm as could be achieved. The gravel sub-samples, designated 'washed' for those sieved and rinsed at site and 'unwashed' for those fine sieved in the laboratory, were placed in the flume channels in trays in the same way as the fines.

III.   Stones – here defined as any material > 20 mm

These were removed from the measured reaches individually, without any treatment *in situ*, until sufficient were obtained to fill the pair of flume channels completely. These were again separated into 'washed' and 'unwashed sub-samples. The 'unwashed' ones were put into the flume channels without any further handling, but the 'washed' selection were carefully rinsed in river water in the same way as the 'unwashed' gravel sub-sample. The stones were stored prior to use in a tank with circulating, ærated river water that was collected at the same time as the stones.

2.2.1.1: Environment Agency water quality data

Environment Agency (EA) water quality data were obtained using samples taken at the EA Sampling Points: 59022250 River Tame (Wolverhampton) Bescott NGR SP0040096200, and 59014850 River Tame Sandwell Valley NGR SP0290092800, between 1 March, 2001, and, 30 April, 2002. The Bescott sampling point is 2.3 km downstream of the upper sampling point of this study at Bentley Mill Way.

2.2.2: Flume Channels

2.2.2.1: Description of fluvarium

The flume channels are situated within the fluvarium building illustrated in Figure 2.2. This building is set over a mill stream in the vicinity of the River Frome (NGR SY861877) and has a water supply from the millstream in addition to mains water. The roof is glass to allow the interior to receive ambient daylight, and there are large extractor ventilation fans to circulate fresh air through the building.

Figure 2.2 The fluvarium in which the flume channels are situated. Top image shows the entrance which is situated on the downstream end of the building, and the glass roof. Lower picture shows the rear of the building with ventilation fans (green boxes) and the Mill Stream running under the building. All sluices were open so there was little water visible in the channel at the time.
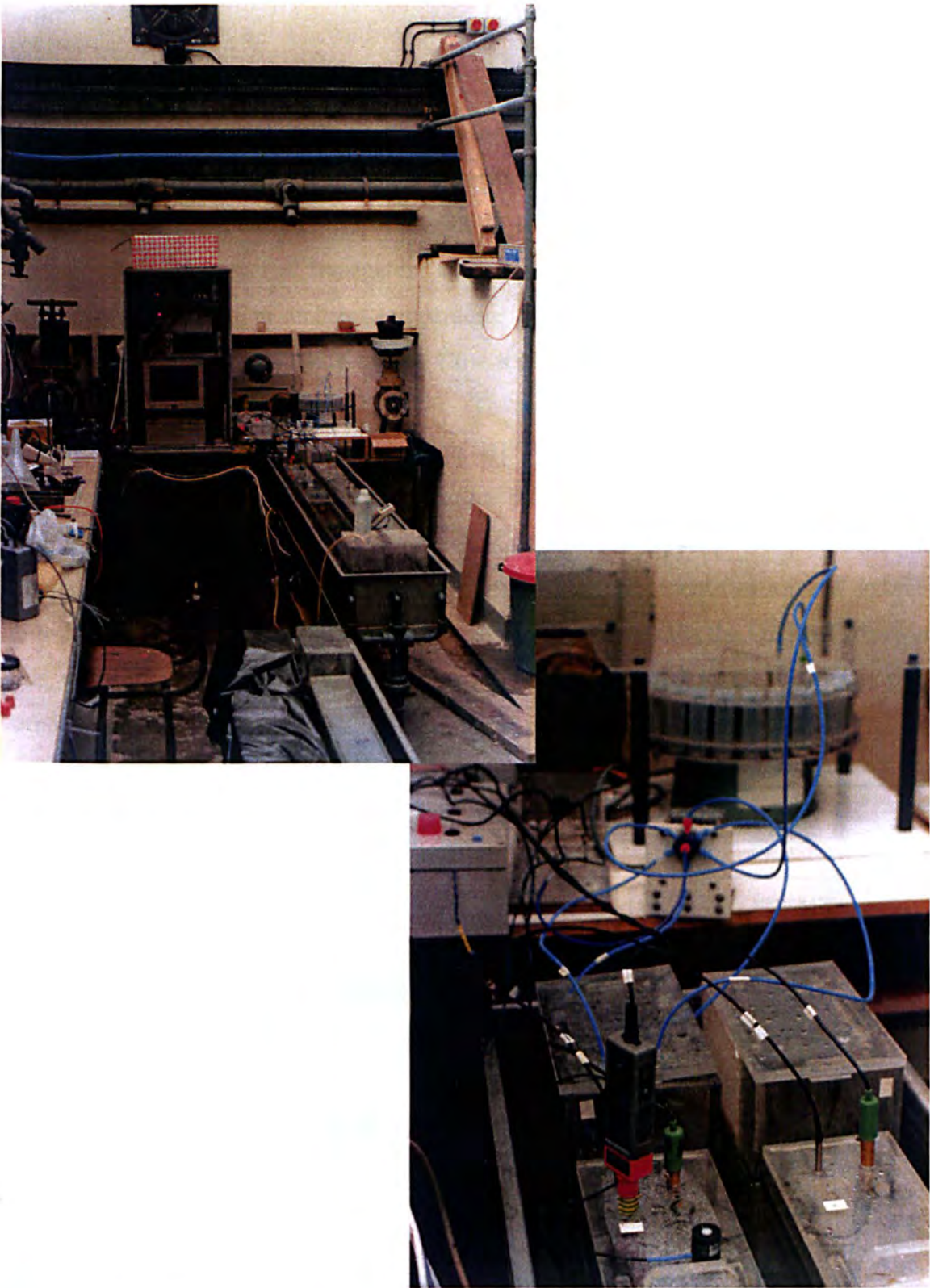
Figure 2.3: Flume channels in the temperature regulating bath, with associated sampling equipment, in the fluvarium (top left). Detail of sensor probes in position in the flume channels, and automated sampler valve and carousel in the background (top right).

2.2.2.2: Operation of flume channels

Upon completion of the construction of the flume channels in the fluvarium, the sampling equipment and computer with a new QBASIC control program, Appendix 2.5., were installed. Figures 2.3 and 2.4 are photographs and a schematic of the flume channels respectively, These channels were developed from an earlier design (House *et al.*, 1995b) and now comprise a pair of transparent acrylic re-circulating channels with controllable water flow, immersed in ambient temperature river water that flows through an outer bath. Flow was measured using turbine flow transducers (RS 257-062) downline of the water pumps (Little Giant Type U62B1). To measure pH, temperature, and dissolved oxygen, paired probes (Hanna Instruments HI 1911 glass pH electrode, Jenway 9010 polarographic DO electrode, and temperature probes) were fitted in the channels, and light level was monitored by a sensor (Li-Cor LI-185A) positioned just above the channels. The data from these probes was stored, along with identification and sediment information from each experiment, in the accessory computer via the QBASIC program. This was retrieved at the end of each experiment and stored in the main laboratory computer network.
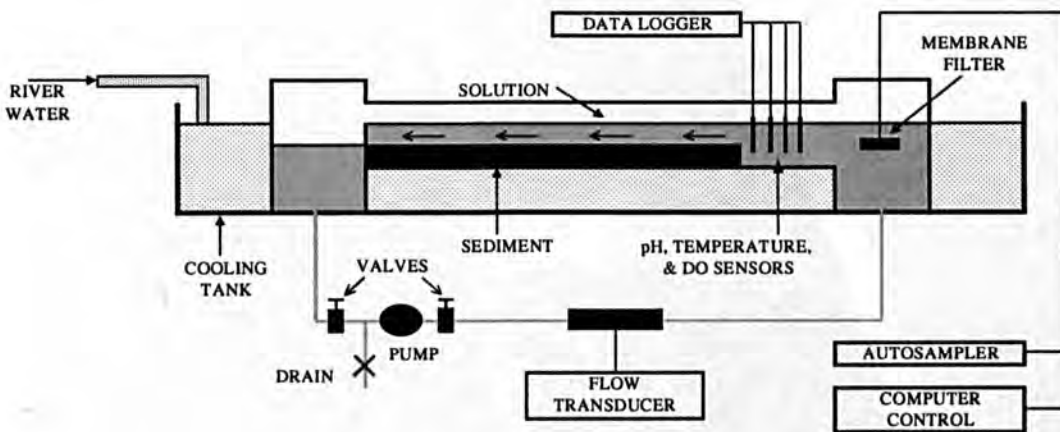
Figure 2.4: Schematic of flume channel systems as used in flux experiments. These channels are situated inside a fluvarium building that ambient light via a glass roof and water supplied from a natural watercourse.
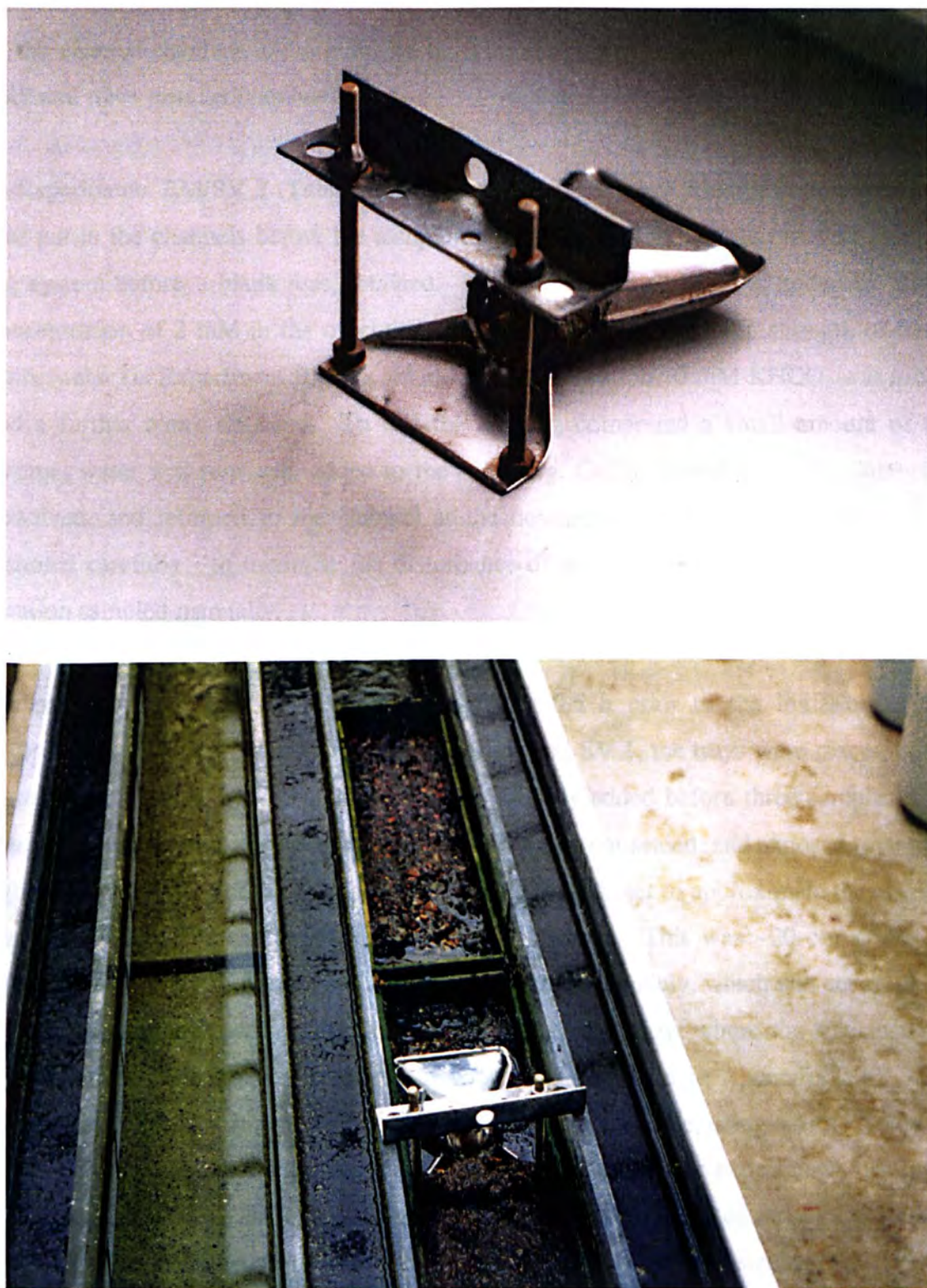
Figure 2.5: Top – Details of tool used in the sectioning of sediments in the sampling trays showing top plate used for section depth adjustment. Can be variably adjusted by 1 mm increments. Bottom – sectioning tool in use in tray in position in a flume channel. This photograph is reproduced with the kind permission of W.A. House.

Before any flux experiments were undertaken a control experiment was performed without sediment present in the channels. Twenty litres of de-ionised water were placed in the cleaned channels and left for 48 h. This water was then sampled and tested for SRP and trace metal contamination.

In Experiments BM/SV 2 (Table II.I) and onwards the twenty litres of de-ionised water was put in the channels before the sample trays. This was allowed to circulate through the system before a blank was obtained. Then, sufficient $CaCl_2$ was added to give a concentration of 2 mM in the overlying solution to simulate the ionic strength of River Tame water (in Experiment BM 1 a solution of concentration 10 mM $KHCO_3$ was used), and a further blank obtained. To add the ionising compound a small amount of the channel water was removed, added to the solid, *e.g.* $CaCl_2$, stirred until all solids were dissolved, and returned to the channel at the downstream end. Then the trays were installed carefully - to minimise the disturbance of any fine sediment present - and the solution sampled manually.

Six pairs of samples were taken manually over 0.5 h prior to the initiation of the autosampler. In the first two experiments, BM 1 and SV 1, the trays were placed in the channels first and the water and $CaCl_2$ were carefully added before three samples were taken manually over 1 h. The system was covered, but not sealed, and exposed to natural light through the roof of the fluvarium. Flow velocity was set to approximate the average flows recorded when the trays were located in the river. This was ~20 cm s^{-1} for all experiments except the one which used fine sediment exclusively, which was set at ~9 cm s^{-1} to simulate the velocity measured in the areas of low-flow where the fine material accumulated. Photographs, examples of which can be found in Appendix 2.13, were taken of the surface sediment and biofilm, and a traced plan made, using acetate sheets, to record the appearance of each tray – no plans were drawn for the experiments using the three individual size fractions. The plans identified the position of stones (> 20 mm diameter), the composition of areas between the stones by particle size, gravel (2 – 20 mm), and fines (< 2 mm), and any areas covered by dense algal growth. These plans were used to calculate the ratio of the areal coverage by stones to the smaller size fractions. An example of a traced plan can be seen in Appendix 2.14.

Two sampling runs were undertaken – the first to observe the release of SRP and copper

to the overlying solution, and the second, after standard additions of SRP and copper to the water, to record uptake into the sediment (Appendix 4.1). Concentrations of the standard additions of SRP, as KH_2PO_4 solution, used in each experiment are shown in Table II.II. The concentration of the copper standard addition, as $CuCl_2$ solution, was 4.46 μM in all experiments. Each run lasted for a period of approximately 48 h. Samples were taken manually prior to the automated sampling run starting, and then, using the automated sampling system and computer control program, on start-up and then at pre-set intervals of usually 120 minutes. The automated sampling system comprised: an uptake tube to each flume channel with a filter body, holding a 0.45 μm cellulose nitrate membrane, submerged in the downstream end of the channels; a valve system to control in- and outlet of sample powered by a stepping motor (Philips ID35); a syringe for drawing the sample; a pump for powering suction; and a carousel for the sample bottles. The syringe was flushed and purged between each sample collection to avoid inter-channel contamination of samples. Calibration of the automated pH, DO, and temperature probes was performed using standard buffers (pH 7 and pH 10), Na_2SO_3 solution and air, and mercury thermometer accurate to 0.5°C. Conductivity, plus supplementary pH and DO, measurements were made manually, using the field probes (Ciba-Corning M90) at intervals during an experimental run, and these probes were calibrated before each use. The QBASIC control program (Appendix 2.5) was developed from a FORTH program used in previous work (Taylor & Kunishi, 1971), extended to include a calibration sub-routine, and modified to accommodate the extra sampling and monitoring of the second channel.

At the end of each experiment, 1 litre of overlying solution was collected, for suspended solids analysis, and the remainder drained off to allow the trays to be sectioned horizontally through depth for sediment characterisation. Any surface water remaining was carefully removed manually, using a pipette. Sectioning was performed using the specially constructed tool, Figure 2.5. This tool was constructed of a flat cutting edge connected to a collection box; supported on adjustable-height runners that allowed the whole tool to be moved along the channel edges smoothly and remove a section of sediment of defined depth. The presence of macroinvertebrates was noted, but due to the destructive nature of the sediment characterisation techniques, no quantitative analysis was undertaken. Where possible each tray was split into 0-5 mm, 5-20 mm, 20-20 mm,

20-30 mm, and 30+ mm depth longitudinal sections; large stones were removed prior to sectioning and stored separately. These sections were then stored in sealed containers, in the dark, at ~4°C for analysis of chlorophyll a and size (see sections 2.3.3 and 2.3.4). For future reference, a ~50 g sub-sample of wet sediment (*e.g.* for particle-size analysis) from each tray was collected and stored in the dark at ~4°C, and the remainder air dried and stored.

Table II.II: Concentrations (μM) of standard additions of SRP as KH_2PO_4 used in each of the flume channel uptake experiments, including those using separate sediment size fractions (numbered a or b). Key: BM – Bentley Mill Way; SV – Sandwell Valley; SRP – soluble reactive phosphorus.

		1	2	3	3a	4	5a	5b	6	7	7a
BM	SRP	2.94	5.88	5.88	-	5.88	2.94	-	5.88	90.0	90.0
SV	SRP	5.88	5.88	5.88	5.88	11.76	-	96.8	5.88	180.0	-

A small (~30g) sub-sample of sediment was obtained from each tray and characterised by porosity, density and organic matter (OM) (see section 2.3.4). Suspended solids present in the overlying solution were measured by volumetric filtration (glass microfibre GF/F) and drying overnight at 105°C.

In the experiments using the stones size fraction, the pair of flume channels were marked into ten equal sections of 20 cm length. The 'washed' sub-sample placed in one of the channels and 'unwashed' in the second. Ten randomly selected stones were closely packed into each 20 cm section. At the end of the flux experiments two stones were removed from each section of both channels for chlorophyll a analysis, and the remaining eight used for the macroinvertebrate study.

2.3: Analytical Techniques

2.3.1: Soluble Reactive Phosphorus

Solutions were prepared using ultra-pure water of conductivity <0.1 μS cm^{-1} at 20°C (Purite, Analyst HP). For each batch of samples analysed for SRP, a calibration was performed, using seven standards over a range of 0 – 12 μmol dm^{-3} and a control blank,

and regression line applied. The river water and flume channel samples were then analysed for SRP spectrophotometrically using a method developed from a standard colorimetric technique (Murphy & Riley, 1962; Stephens, 1963), Appendix 2.6, and Beckman DU 520 UV/Vis spectrophotometer with a 4 cm path length cell, at 880 nm. The limit of determination for SRP using this method was 0.3 μM.

2.3.2: Copper

The copper and phosphorus experiments were carried out simultaneously on the same sediment. Thus the field surveying and sampling equipment installed in the river, and techniques used in the field, are the same and have been described fully in Section 2.2.1. Section 2.2.2 includes a complete description of the flume channel equipment, including a schematic, and an explanation of the treatment of the sediment in the collection trays at the end of the experiments.

For the dissolved fraction of the copper the aqueous samples were analysed using inductively-coupled plasma mass spectrometry (ThermoElemental PQ Eclipse ICPMS with calibration range: 0 - 10 μg L^{-1} (0, 0.5, 5, 10 standards, and detection limit: 0.2 μg L^{-1} (2 sigma blank)). On the day of sampling, the samples were filtered using a 0.45 μm cellulose nitrate membrane and the filtrate acidified. Acidification to 1% was achieved using concentrated nitric acid (Aristar or Ultrapur grade) to help prevent precipitation/adsorption of the trace metals during transport and storage. For the acid available, or acid recoverable fraction, *i.e.* total copper, unfiltered samples were acidified to 1% with concentrated nitric acid (Aristar or Ultrapur grade), and then agitated for 24 h at room temperature before being filtered using a 0.45 μm membrane filter. The dissolved copper concentration was then determined by ICP-MS as discussed above.

2.3.3: Chlorophyll *a*

For the measurement of chlorophyll *a*, to give an estimate of the biomass expressed as mg m^{-2}, surface sediment (stones >10 mm and the 0-5 mm section) from each tray was prepared and extracted in methanol (Marker, 1972, 1976; Revsbech & Jørgensen, 1986) (Appendix 2.7). For the 0-5 mm depth section which comprised any material of size < 10

mm diameter, as much water as possible was drained off and this water filtered (cellulose nitrate, 0.45 μm). The filter and sediment samples were combined, vacuum-dried in a Buchner flask using another cellulose nitrate membrane (0.45 μm). All sediment and filters were then extracted in methanol in wide-necked vessels with air-tight lids. Methanol extraction was carried out as described in HMSO publication (1983). The extract was then re-filtered, and the filter washed with solvent. The filtrate and washings were combined, and adjusted to a known volume; a small error could be introduced here by the unknown water content of the extract. Absorbance measurements of the samples obtained, plus a methanol blank, were made spectrophotometrically (Beckman DU 520 UV/Vis spectrophotometer) at 630, 645, 665, and 750 nm (Marker, 1972). The rocks and small stones (> 10 mm) were treated as the 0-5 mm section, but any stones too large to fit in an extraction vessel were treated separately. In a vessel large enough to accommodate them, the stones were shaken in water to remove any loosely attached material. The resulting wash was filtered and the filter pad combined with the smaller stones and other filters in the extraction vessel. Any large skeins of filamentous alga that were collected separately were frozen prior to treatment to ensure complete extraction of chlorophyll a (Marker, 1972; 1976).

2.3.4: Sediment Characterisation

For characterisation, the sediment was dried at 105°C overnight in a vial of known volume ($\pm$ 0.317 ml), and the porosity and density calculated (Appendices 2.8 – 2.10), then the organic matter (OM) content was measured by ashing the sediment in a furnace at 550°C overnight (Appendix 2.11). Suspended solids present in both stream-water at the site, and the overlying solution in flume experiments, were measured by volumetric filtration (glass microfibre, GF/F) and drying overnight 105°C (Appendix 2.12). The details of the methods for the determination of the volume of a vial, and the porosity, density, OM content of a sediment, and suspended solids are given in Appendices 2.7 – 2.11.

To establish the mass ratio of fine to coarse material in the sediments sampled from the River Tame, a known mass of the dried sediment representing each experiment was sieved through a 20 mm mesh size and then 2 mm. The separate fractions were weighed

and the ratio calculated. It had also been planned to calculate the percentage areal cover by each size fraction from the acetate maps, however because of the difficulty of distinguishing the proportions of the areal coverage for the two smaller size fractions, gravel and fines, it was not possible to determine the percentage cover by this method. However, the results of the gravimetric measurements for the stones fraction and the percentage areal cover for the stones from the acetate maps (Appendix 2.15) were compared and a reasonable correlation ($R^2 = 0.6$) was obtained. Therefore, because the trays were only 5 cm in depth, the percentage mass ratios were considered a sufficiently close approximation of the surface area represented by each size fraction for use in flux calculations.

2.3.5: Major ions

The major ions present in the river water at time of collection, and in the overlying solution of the flume channels during the experiments (Appendix 2.16), were determined using liquid chromatography analyses – Dionex DX-100 Ion Chromatograph with automated sample injection, and standard AS14 column set for anions and CS12A column set for cations. The limits of detection for the inorganic ions in this study are: cations – 0.20 mg L^{-1} Na^+, 0.20 mg L^{-1} K^+, 0.30 mg L^{-1} Mg^{2+}, 0.8 mg L^{-1} Ca^{2+}; and anions - 0.14 mg L^{-1} F, 0.02 mg L^{-1} NO_3^- and 0.03 mg L^{-1} SO_4^{2-}.

2.3.6: Dissolved oxygen

In addition to measuring the dissolved oxygen (DO) of the river water and of the overlying solution in the flume channels using the standard probes discussed in section 2.2.2.2, DO measurements were carried out in a surface sediment and in a biofilm attached to a stone *in situ* in the flume channel. This was achieved using an ion-selective microelectrode similar to the one which can be seen in use in the flume channels in Figure 2.6. The Clark-type microelectrode employed in this study included a guard cathode (Model 737-GC) and a Keithley 485 Autoranging Picoammeter. Adjustment of both guard cathode and sensor currents was controlled by an electronic interface. The rate and distance of descent of the microelectrode, in increments of 13 μm, was controlled by a micromanipulator linked to a computer. Amperage and depth readings

from the picoammeter and stepper-motor control were recorded as the microelectrode penetrated the biofilm, or sediment. Precise positioning of the microelectrode tip in relation to the sediment or biofilm surface was pinpointed using a swan-necked cold light source and magnifying glass. This was aided in the case of the sediment by vibrating the tip of the electrode slightly, which showed the slight movements of the sediment particles. Measurements in the biofilm were made in both light and dark conditions. The electrode was calibrated using a fine silt sediment that had been allowed to stand for 1 h for a DO horizon to develop, first in the overlying water, which was saturated with respect to atmospheric oxygen, and then in the anoxic zone of the sediment. The equilibrium concentration of DO in the water when atmospheric pressure was not unity[1], was calculated using the atmospheric pressure method after Benson and Krause (1980), using the Equation

$$C^P_0 = C^*_0 \, P \, [\, ((1 - P_{wv} / P) \, (1 - \theta_0 P)) / ((1 - P_{wv}) \, (1 - \theta_0)) \,] \qquad (1)$$

where C^*_0 is the unit standard atmospheric concentration by volume, which indicates that it is a concentration under standard conditions, *i.e.* in equilibrium with a standard atomosphere at unit pressure (see definition for unit standard oxygen concentration below); P is total pressure of 1 atmosphere (atm); P_{wv} is the saturated vapour pressure of water in atmospheres at the temperature of equilibrium; θ represents the negative of the second pressure coefficient, *i.e.*

$$\theta_0 = 0.000957 - (1.426 \times 10^{-5} t) + (6.436 \times 10^{-8} t^2) \qquad (2)$$

Benson and Krause calculated values for the quantity in square brackets (Eqn 1) for pressures of 1.1 – 0.5 atm and temperatures of 0° – 40^oC. This calculation was derived from the Bunsen coefficient for oxygen and the unit standard oxygen concentration, *i.e.* the concentration of DO per unit volume of solution, measured at equilibrium temperature, when in equilibrium with an atmosphere of standard composition and saturated with water vapour at a total pressure, including that of the water vapour, of 1 atm. This results in a value in units of μg-atoms dm^{-3}, but which can be converted to μmol by the simple expedient of dividing the μg-atoms by 2.

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[1] Atmospheric pressure at sealevel = 1 atm (76 cm-Hg)
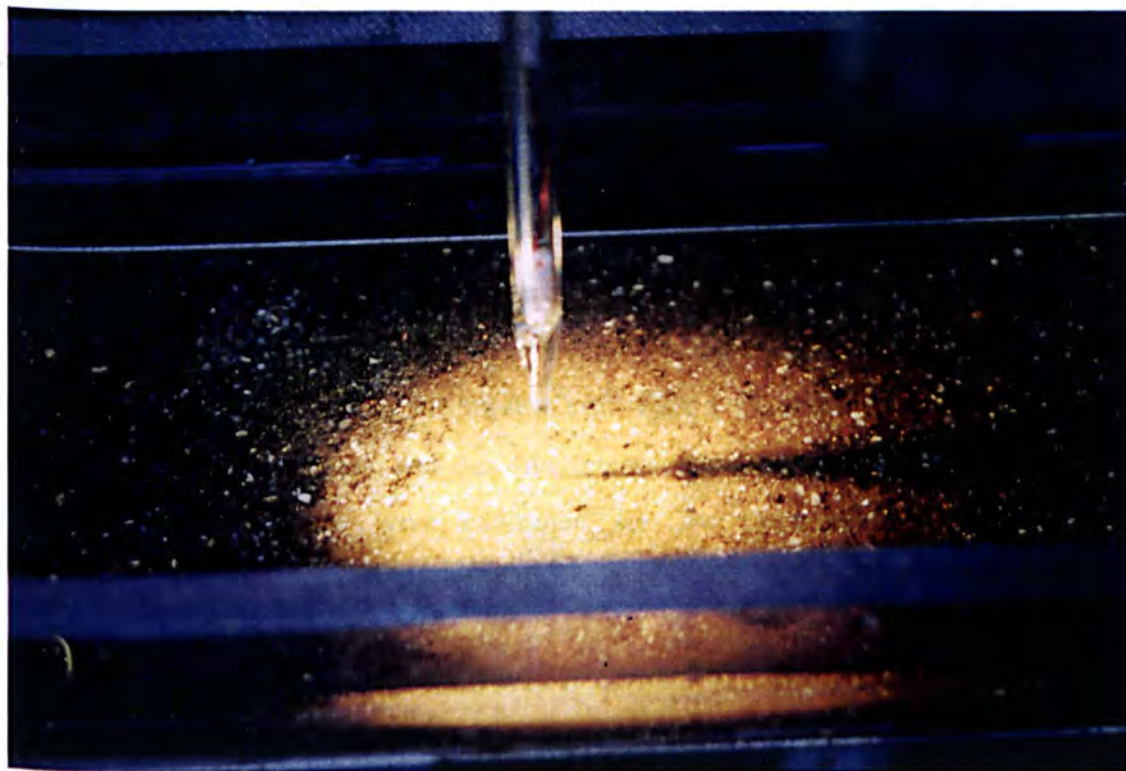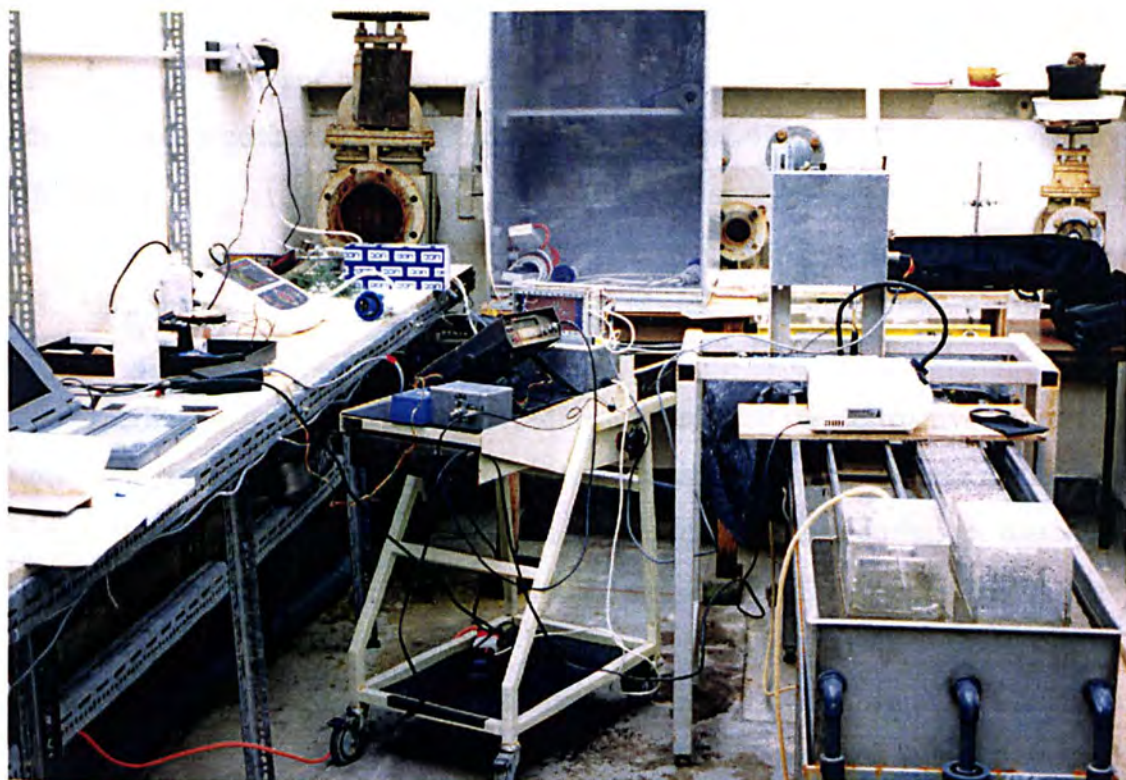
Figure 2.6: Top - Dissolved oxygen profiling in the flume channels at the fluvarium using a Clark-type oxygen microelectrode, with a guard cathode (Model 737-GC) and a Keithley 485 Autoranging Picoammeter. Bottom – Detail of a DO microelctrode at the surface of a sandy sediment. These photographs are reproduced by the kind permission of W.A. House.

Atmospheric pressure and temperature at the time DO measurements were made for this study were recorded from a barometer (Casella, London. 4792) located in the vicinity of the fluvarium.

2.3.7: Macroinvertebrates

In the experiments where trays of river sediment were used the destructive nature of the sediment characterisation techniques meant that it was not possible to make a quantitative analysis of invertebrates. However, individual invertebrates, seen during the dissembling of the trays at the end of experiment, were noted and described at family level.

After the flux experiments on the stones were completed a macroinvertebrate census was performed using methods based on those developed by Marker and Casey (1982). Samples were obtained from each stone in each section of the flume channel in the following way. Firstly, the filamentous algæ and loosely attached periphyton (Stevenson *et al.*, 1996) with its associated macroinvertebrate community were removed using a toothbrush, then more firmly attached epilithic species were detached using a very stiff nylon brush, after this encrusted algæ were scraped off with a scalpel, and finally the stone was abraded vigorously with a wire brush. These extracts were scraped into a minimal amount of distilled water and this sample then divided into coarse and fine fractions by sieving with a 600 µm mesh sieve. Five 1 ml aliquot sub-samples of these two fractions were then taken and the invertebrates counted and identified. The scalpel and wire brush fractions were examined for the presence/absence of diatoms. Identification of macroinvertebrates was made to family using a microscope at x40 magnification and relevant keys.

The exposed area of the stones was calculated using a tin foil method (Calow, 1972), and the area of the stones from which the chlorophyll *a* extracted used to estimate the biomass associated with the stones in the channels. This biomass estimate was made using a correction for the presence of chlorophylls *b* and *c*, and the equation given in Appendix 2.7, resulting in a weight per area value.

2.3.8: Modelling

The equilibrium phosphate concentration (Taylor & Kunishi, 1971) and two transport models, the Parabolic Equation (House *et al.*, 1995a; House & Denison, 1997), and the Diffusion Boundary Layer model (House & Denison, 2002; House, 2003a, b), were used to examine the kinetics of phosphorus movement across the sediment-water interface. The Phosphorus Transfer Index (PTI) (House *et al.*, 1998) was used to indicate the direction and concentration gradient for the movement of the SRP.

2.3.8.1: *Equilibrium Phosphate Concentration*

The uptake and release of SRP from particles that controls the concentration of SRP in river waters is sometimes referred to as the 'buffer mechanism', and maintains almost constant values because the sediment acts as a reservoir of phosphorus. In order to determine the availability of phosphate adsorbed onto the particulate matter of a sediment, it is helpful to establish the equilibrium phosphate concentration (EPC_0) (Taylor & Kunishi, 1971; Froelich, 1988; House *et al.*, 1995a; House & Denison, 2000). This equilibrium concentration is an empirical reference point on a sorption curve, which indicates the capacity of the sediment to take up or release phosphate if solution concentrations, or other factors, alter. If the SRP concentration in solution is greater than the EPC_0 of the sediment it will take up phosphorus, or if the concentration is lower than EPC_0, SRP will be released from sediment into solution (House & Denison, 1997, 2000). The sorption curve is also known as the sorption isotherm (Froelich, 1988) and is illustrated in Figure 2.7. The slope (K) of this sorption curve is the adsorption coefficient, otherwise known as the Henry's law constant, distribution coefficient, or partition coefficient, and an indication of the affinity of a sediment for phosphorus, and is measured in litres per gram ($L\ g^{-1}$). When near EPC_0, K has been defined as the number of moles of phosphorus that need to be added to, or subtracted from, a system to change the phosphate concentration in solution by $1\ mol\ l^{-1}$. Differences in the chemical and physical composition of sediment may lead to differences in K values for sediments that have an equivalent EPC_0. This is related to the amount of adsorbed phosphate that is available for instantaneous exchange at a particular concentration (Taylor & Kunishi, 1971). Flow velocity and the effects of water depth, laminar and turbulent flow, on the
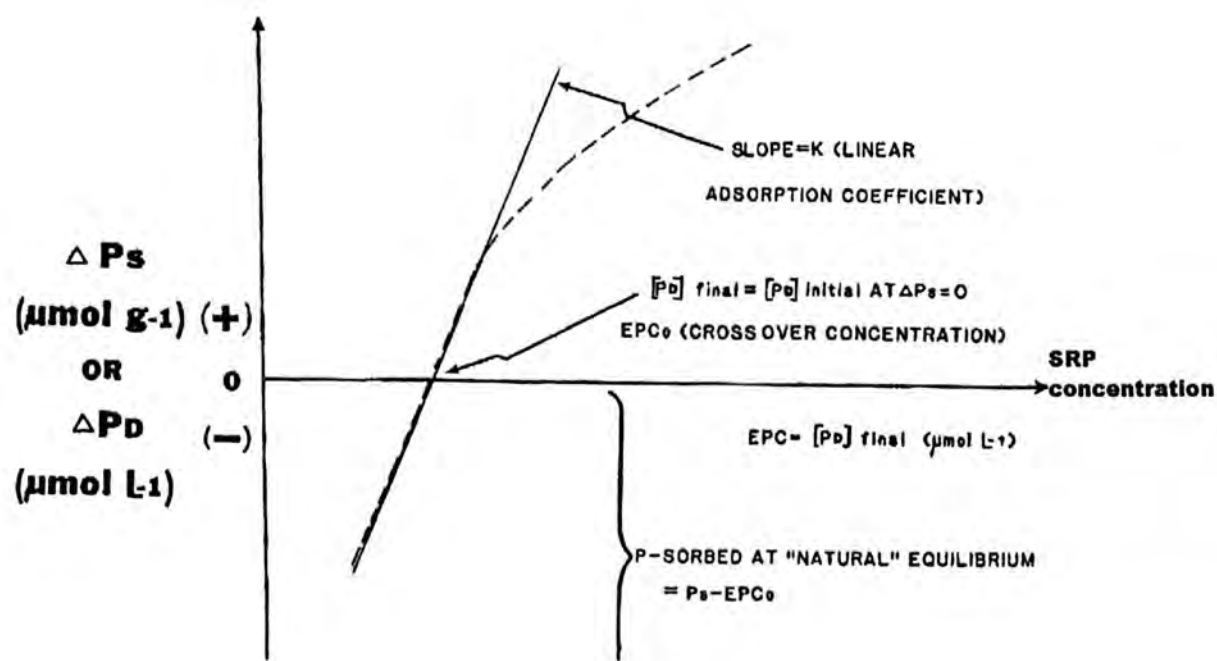
Figure 2.7 A schematic depicting a typical sorption isotherm. On the y-axis, ΔP_S is the phosphorus sorbed onto the surface of, or into, a solid, such as a sediment particle, ΔP_D represents the phosphorus dissolved in the bulk solution, *e.g.* river water, and the x-axis represents the concentration of soluble reactive phosphorus. The slope K is the linear adsorption coefficient, also referred to as k_p, and EPC_0 (also sometimes known as the crossover concentration) is the concentration of dissolved phosphorus when the final concentration of SRP, $[P_D]$ final, is equal to the initial concentration of SRP, $[P_D]$ initial, when the change in adsorbed phosphorus is zero. P-sorbed at 'natural' equilibrium denotes the 'native' (House *et al.*, 1995) phosphorus extant in the natural system before any changes occur. Diagram adapted from Froelich (1988).

mixing of the water are contributing factors as to whether equilibrium between the water and sediment in a river will be reached. At equilibrium the rates of adsorption and desorption reactions are the same, but if the concentration of SRP increases or decreases phosphorus would adsorb to, or be released from, sediment particles in order to re-establish equilibrium. The magnitude of the movement away from the EPC_0 indicates how much phosphate would be adsorbed, or desorbed, if a sediment re-equilibrated at a new EPC_0.

The amount of labile phosphorus can be defined as the amount that needs to be removed from a sediment for it to be in equilibrium with a given water concentration (Taylor & Kunishi, 1971), such as those set in water quality strategies, and this can be estimated from the sorption curve. However, at high or very low SRP concentrations the 'buffer' capacity of a sediment is reduced and in these cases the curve is not usually a straight line. Therefore any estimate made that significantly differs from the EPC will be larger than that obtained by simply extrapolating the K at EPC_0 – see Figure 2.7 (Froelich, 1988). Sediments that are able to maintain steady SRP concentrations in the overlying water tend to have high K values, *i.e.* a steep slope near EPC_0.

Experimentally, the EPC_0 is the concentration of inorganic phosphorus in a solution such that no change in the concentration occurs when it is in contact with a natural sediment for 24 h (House, 2003b), and has been utilized in many investigations to predict the behaviour of a sediment, or soil, when exposed to phosphate solutions (Klotz, 1991; House *et al.*, 1995b; House & Denison, 1997; Gardner *et al.*, 2002; Pan *et al.*, 2002).

In this study the EPC_0 was determined by measuring the approach to the steady-state concentration of SRP reached in the overlying solution, over the period of the experiment, and modelled using the Parabolic Equation or the Diffusion Boundary Layer transport models. Phosphate transferred to and from a solid is usually normalised to the mass of the solid, but in this case the surface area of the sediment was used.

2.3.8.2: *Parabolic Equation*

The Parabolic equation (House *et al.*, 1995a; House & Denison, 1997) *i.e.*,

$$\mathrm{d}n(t)/\mathrm{d}t = K_p [c(t) - \mathrm{EPC_0}]^2 \qquad\qquad\qquad\qquad\qquad (3)$$

where $n(t)$ is the amount of SRP flux per unit area of sediment at time t; $c(t)$ the SRP concentration; K_p the parabolic rate constant. Integrating this gives

$$n(t) = K_p \int_0^t (c - \mathrm{EPC_0})^2 \, \mathrm{d}t \qquad\qquad\qquad\qquad (4)$$

The parabolic rate constant, K_p, was computed from a linear regression analysis, *i.e.* the parabolic rate constant (K_p) is the slope resulting from a plot of $n(t)$ (the flux of SRP) against the integral in Eq. (2).

2.3.8.3: *Diffusion Boundary Layer Model*

A diffusion boundary layer (DBL) model (House & Denison, 2002), assumes that the flux of SRP across the sediment-water interface, $\mathrm{d}n(t)/\mathrm{d}t$, where $n(t)$ is the amount of SRP passing per unit area of sediment at time t, is regulated by diffusion across the boundary layer

$$\mathrm{d}n(t)/\mathrm{d}t = D_\mathrm{m}[c(t) - c_i(t)]/\delta \qquad\qquad\qquad\qquad (5)$$

where $c(t)$ is the SRP concentration at the top of the boundary layer, $c_i(t)$ is the SRP concentration in the interstitial water at the sediment surface, and D_m is the molecular diffusion coefficient of the dominant phosphorus ion ($\mathrm{HPO_4^{2-}}$) at the solution pH (see Figure 1.6), with a boundary layer of thickness (δ) (House & Denison, 2002), which can be estimated by

$$\delta = 2500/v \qquad\qquad\qquad\qquad\qquad\qquad\qquad (6)$$

where δ is in μm and v is the water velocity in cm^{-1}. If V_t is the total volume of the circulating water, A_s is the surface area of the interface, in this case the total area of the trays used for the fines and the gravel, and the total channel area (0.2 m^2) for the stones. An effective depth of water for the channel (d) may be written as $d = V_t / A_s$, and with the

approximation that interstitial water concentration, c_i is constant and equal to EPC_0 over the length of the experiment (House, 2003b), then for the release of SRP from a sediment. Equation (1) has the solution

$$c(t) = EPC_0[1 - \exp(-kt)] + c_0 \exp(-kt) \qquad\qquad (7)$$

where the rate constant $k = D_m/(\delta d)$ and c_0 is the concentration of SRP at the start of the monitoring – designated as $t = 0$, and allows the EPC_0 to be calculated from the kinetic data.

When the concentration of SRP at the top of the boundary layer $c(t)$, is much less than the EPC_0 of the bulk sediment ($c(t) << EPC_0$) and the concentration of SRP in the solution is considered to be zero, the limiting diffusion flux, *.eg.* the maximum efflux from the sediment, is controlled by the temperature, and thus viscosity, of the water, the molecular diffusion coefficient of the dominant phosphate ion, HPO_4^{2-}, the thickness of the boundary layer across which the ions must pass, and the velocity of the overlying water. It is also equivalent to the adsorption coefficient (rate constant k_p used in the Parabolic model - see section 2.3.8.2), which indicates the affinity of the sediment for phosphorus, *i.e.* how much phosphorus a sediment would be able to take up.

The boundary layer model (Eq. 3) was applied with the diffusion coefficient of the dominant ion, *e.g.* HPO_4^{2-} at the pH of the River Tame, at 25^0C ($=7.34 \times 10^{-6}$ cm^2 s^{-1}) (Li, Y. -H & Gregory, 1974) extrapolated to values for other temperatures, *e.g.* 4.7×10^{-6} cm^2 s^{-2} at 10^0C, by the expedient of correcting D_m through the relationship between the molecular diffusion coefficient of HPO_4^{2-} and the viscosity of water at a given temperature; which is a constant between $5 - 25^o$C (House *et al.*, 1995b; House & Denison, 2002). Root mean square (r.m.s.) deviation was used to optimise the agreement between the measured SRP concentration and the model prediction (Eq.3) by varying the EPC_0.

2.3.8.4: *Phosphorus Transfer Index*

In a natural sediment of complex composition, such as is found in the River Tame,

prediction of how that sediment will respond to a given concentration of SRP in the water is more complex than standard methods, for example sequential chemical extraction, can describe. The Phosphorus Transfer Index (PTI) (House *et al.*, 1998), was therefore developed in conjunction with the EPC_0 method of predicting the behaviour of dissolved phosphorus in contact with a sediment. This Index can thus predict whether there will be influx or efflux of SRP, as the resultant sign associated with the calculation indicates the direction of movement of the SRP. The kinetics of the interaction are related to the magnitude of the resulting value.

$$PTI = (SRP/EPC_0 - 1) \tag{8}$$

When the PTI is zero the sediment is in equilibrium with SRP in the overlying water. If the result gives a positive value, there is a net flux of SRP from the water to the sediment, and if negative, the net flux of SRP is from the sediment to the water.

Chapter 3
The Effects of Sediment Size Fraction and Associated Algal Biofilms on the Kinetics of Phosphorus Release.

3.1: Introduction

This chapter reports on the investigation of the effects on the kinetics of phosphorus release of three distinct sediment size fractions. For the purposes of the study these were defined as: stones, having a diameter of greater than 20 mm; gravel with diameters of between 2 and 20 mm; and fines being less than 2 mm in size. The consequences of a biofilm being present on the larger sized material was also considered.

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The Effects of Sediment Size Fraction and Associated Algal Biofilms on the Kinetics of Phosphorus Release.

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Abstract

Experiments using flumes containing sediment of three different size fractions, from two sites on the R. Tame, investigated the influences of sediment particle-size, and an associated biofilm, on sediment-water exchanges in heterogeneous sediment deposits.

This is the first study undertaken to understand the kinetics of the release of soluble reactive phosphorus from sediments of natural systems to identify which of the size compartments affected those fluxes most. Samples of fine material (< 2 mm), gravel (2 – 20 mm), and stones (> 20 mm) were collected over a period of several weeks and brought to a fluvarium where they were placed in artificial, controlled flow, flume channels. Synthetic solutions of similar ionic strength to the river were prepared using calcium chloride. Temperature, pH, and dissolved oxygen of the solution overlying the sediment were monitored automatically whilst filtered samples were obtained at 2 h intervals over 48 h. The biomass, expressed as mg m^{-2} chlorophyll a, of the algal component of the biofilm from the surface of the sediment was estimated using methanol extraction.

Differences in the responses were observed between the sediment size fractions and the two sites, where contaminant concentrations varied. The equilibrium phosphate concentration and a phosphorus transfer index were used to establish that there was a net uptake of phosphorus by all three sediment size fractions, from both sites, at the time of sampling. The kinetic results showed very fast initial reactions of phosphorus release from the larger size fractions with a well-developed filamentous algal growth present implying a different mechanism than diffusion being involved. The stones and associated biofilms also released more phosphorus than the fine fraction, $e.g.$ final release concentrations for the most contaminated site were: fines ~ 2.5 μM, gravel ~ 6.5 μM, and stones ~ 65.0 μM (expressed as soluble reactive phosphorus). Phosphorus fluxes, calculated assuming the concentration of phosphorus in the sediment was less than the equilibrium concentration, were a maximum at the most contaminated site, $e.g.$: fines 6.4 nmol m^{-2} s^{-1}, gravel 27 nmol m^{-2} s^{-1}, and stones 109 nmol m^{-2} s^{-1} (normalised with respect to the river bed area). These results confirm that sediment having a biofilm and associated particulate material results in a greater flux than fine sediment, which does not support a filamentous biomass. Removal of the fine particulates trapped in the algal

growth reduced soluble phosphorus release. These factors demonstrate that both gravel and stone substrates have an important control over the release of soluble reactive phosphorus.

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Key words: soluble reactive phosphorus; equilibrium phosphate concentration; algal biofilm; sediment.

Introduction

Many urban areas are heavily contaminated - a legacy of industrialisation and rapid expansion. To facilitate safe and cost-effective regeneration of these urban centres an effort is being made to identify, through an understanding of the processes involved, any potential ecological risks posed by contaminated river sediments.

Dissolved phosphorus is an important element in aquatic chemistry (Makareth, 1953; Stumm & Morgan, 1970; Jarvie *et al.*, 1998) and by association with particulate materials and the formation of phosphate minerals, may be stored in sediments for long periods. It will remain sequestered in a sediment until changes in the physico-chemical properties of the water or the sediment, for example the development of anoxic conditions (Golterman, 2001), cause its release. One of the factors that can influence such change is the growth of an algal biofilm at the sediment surface (Woodruff *et al.*, 1999a, b).

The exchange flux of soluble inorganic phosphorus between sediments and natural waters makes it available to algæ. Sediment-phosphorus interaction in lakes has been studied in detail since eutrophication began to be seen as a problem (Likens, 1972; Moss, 1986). However, little information is yet available for lotic systems with a mixture of sediment sizes. Some previous work has examined the effects of biofilm development on chemical fluxes and processes in the fine (< 2 mm) fraction of a sediment (Woodruff *et al.*, 1999a, b). The majority of research has been on the silt fraction of sediment as this was thought to be the key control of the phosphorus flux, and because these fine particles can be important contaminant carriers (Westrich, 1984). There is no research into the rôle of gravel, stones, and the effects of benthic biota – particularly filamentous algæ – which can comprise part of the biofilm on a sediment surface (Cooksey, 1992; Leadbeater & Callow, 1992). Therefore, in order to assess the contribution of the various compartments that can comprise a natural river sediment, six experiments were carried out in artificial flumes separating the material into < 2 mm, 2 – 20 mm, and > 20 mm size fractions. The main aim of this study was to measure the flux of phosphorus (as the bioavailable soluble reactive phosphorus (SRP)) out of natural sediment components, and to study the release kinetics. This was undertaken with the ultimate objective to assess what influence these compartments in a natural system will have on the sediment-water exchange.

Methods

Field Sites

Flowing through a large conurbation to the north of Birmingham, U.K., the River Tame eventually joins the River Trent between Lichfield and Burton-on-Trent, where it supplies water to abstraction storage ponds. It has a history of contamination from heavy industry and is still subjected to effluents from numerous sewage treatment works; the largest of which impacting the field sites is Ray Hall (NGR SP0232094440) with a people equivalent (p.e. an estimate of the number of people served by a treatment plant) of 162,000 (pers. comm. Severn Trent Water). After a preliminary pilot study of sites at various locations on the upper reaches of the Tame, two sites were selected – Bentley Mill Way (BM) and further downstream at Sandwell Valley (SV) (Table I). Both these sites are within a single catchment above the confluence with the Rivers Rea and Cole. They were selected to provide the broadest possible representation of bed-sediment types, although they are dominated by coarser material. Higher and lower flow regimes in this upper catchment were also represented, and the sites were sufficiently distant from each other to detect differences in water and sediment chemistry, yet avoid the complications caused by inputs from additional catchments. All site visits were made over the period November 2000 to April 2003.

Field Surveying and Sampling

During site visits when flow conditions allowed, a set of field observations was made recording the changes in the general appearance of the bed of each measured reach and its margins showing the dominant feature, *e.g.* silt, gravel, or *Cladophora*, mapped on a plan. Other details included: the weather conditions, evidence of any recent high-flow events, macrophyte growth, physical changes in the channel such as bank collapse, water turbidity, and any other features such as obvious contamination events. Field measurements of water temperature, pH, dissolved oxygen (DO), and conductivity were made using Mettler-Toledo Checkmate 90 Sensors. Bulk and filtered (cellulose nitrate membrane, 0.45 μm) water samples were taken for the determination of SRP, trace metals, and major-ion analysis.

Additional entire sediment was obtained from each location at removal of a sample, to characterise the sediment for organic matter, porosity, density. Flow velocity at each sampling position was measured using a Geopacks Stream Flowmeter. Any large clumps or skeins of filamentous algæ on the surface of the stones were removed either before or after removal from the stream-bed, and prior to any other treatments. These additional samples, used later for chlorophyll *a* analysis, were then placed in numbered plastic bags, sealed and stored in the dark until they could be frozen upon return to the laboratory.

Size Fractions

Six SRP flux measurement experiments were carried out in a flume (House *et al.*, 1995b) to assess the contribution from the various sediment compartments. Samples were collected from both field sites. These separated the material into < 2 mm, 2 – 20 mm, and > 20 mm size fractions; subsequently referred to here as fines, gravel, and stones respectively, although it is noted that the size range for the classification of gravel as defined on the Udden-Wentworth grain-size scale is 2 – 256 mm (Appendix 3.1).

The fines, collected in the autumn of 2001, represented sediment accumulating in very low flow areas (typically < 5 cm s^{-1}) within the river. They were sieved (2 mm size mesh) at the laboratory and placed in rectangular cross-sectioned, 10 x 40 cm and 5 cm deep, polypropylene sampling trays to contain them within the flume channels. This size fraction did not support a filamentous algal biofilm growth, but did develop a diatomaceous coating that was disrupted by collection and sieving. However, this biofilm has been observed to redevelop rapidly, *ca* 6 days (Woodruff *et al.*, 1999a, b).

The gravels, collected when biofilm was present in late summer 2001 and spring 2002, were divided into a 'washed' fraction by sieving and rinsing one portion in the river at the time of collection, and an 'unwashed', control fraction by sieving the second portion at the laboratory. Care was taken to remove as little biofilm and associated particulates as possible during the sieving of the 'unwashed' fraction. These gravel samples were also put into trays.

The stones were also collected during the summer of 2001, when biofilm development had occurred. SRP release was measured on stones with an active biofilm and associated fine particulates intact, and also on stones that had been rinsed gently to remove any loosely associated fine material. At the end of the experiment the stones in each channel were characterised by OM, porosity, density, and size, biomass established by chlorophyll measurement, and a macroinvertebrate census performed (see section below).

Experimental Flumes

The flumes were developed from an earlier design (House *et al.*, 1995b). They comprised a pair of transparent acrylic re-circulating channels with controllable water flow, immersed in ambient temperature river-water that flowed through an outer bath (River Frome, NGR SY861877). Flow was measured using turbine flow transducers downline of the water pumps. To measure pH, temperature, and conductivity, paired probes were fitted in the channels, and the light level monitored by a Li-Cor sensor. Both channels in the pair were treated in an identical manner. DO concentrations and conductivity were measured manually at regular intervals using the field meters.

The ionic strength of R. Tame water was simulated using twenty litres of de-ionised water and sufficient $CaCl_2$ to give a 2 mM solution. The channels had transparent acrylic covers, but were not sealed, and exposed to natural light. Next the trays, or stones, were placed in the channels, and the solution circulation started. The trays were carefully introduced to the channels after the solution to reduce disturbance of any fine material trapped in the biofilm. The flow rate for the stones and gravel experiments was set to give an average velocity of ~20 cm s^{-1}, which approximated to the average of the flows recorded *in situ* at the time of field sampling. For the experiment using the fine sediment, the flow rate was set at ~9 cm s^{-1} to represent the low flows recorded in the areas in the river where fine sediment accumulated. Photographs were taken of the surface sediment and biofilm, and a traced plan made to record the appearance of each tray or channel. The experiments were undertaken over a period of 48 h to observe the release of SRP to the overlying solution. Samples of the overlying solution (filtered through 0.45 μm cellulose nitrate membrane) were taken manually prior to the start of the experiment and then, using an automated sampling and filtering system at pre-set intervals of 120 minutes.

At the end of each experiment the overlying solution was drained to allow the trays to be sectioned longitudinally, through depth, for sediment characterisation. In the gravel and fines experiments the presence of macroinvertebrates was noted, but due to the destructive nature of the sediment characterisation techniques, no quantitative analysis was undertaken. A small (~30 g) sub-sample was obtained from each of the fines trays for sediment porosity, density, and organic matter content determination. The trays containing gravel and fines were longitudinally sectioned, and the 0-5 mm, and where necessary due to surface collapse the 5-10 mm, depth sections analysed for chlorophyll *a*. In the experiments with stones, two stones from the ten in each 20 cm long sub-section of the channels were chosen at random (the two nearest the centre of each sub-section) for chlorophyll *a* analysis. The chlorophyll *a* extracted from the surfaces of the samples was measured to assess biomass (Marker, 1976). The remaining eight stones from each of the channel sections were carefully removed from the channels and stored in the dark at ~4°C, prior to further analysis, and any loose material that had become detached from the stones during the experiments was also collected and stored for subsequent study.

Analytical Techniques

Solutions were prepared using ultra-pure water of conductivity < 0.1 μS cm^{-1} at 20°C (Purite, Analyst HP). For each batch of samples analysed for SRP, a calibration was performed, using seven standards over a range of 0 –12 μM. Samples were analysed for SRP spectrophotometrically using a colorimetric method (Murphy & Riley, 1962; Stephens, 1963) and Beckman DU 520 UV/Vis spectrophotometer with a 4.0 cm path length cell, at 880 nm.

For measurement of chlorophyll *a* to estimate biomass, surface sediment (the surface section from each tray from the fines and gravel, or the two stones randomly selected from each section in the experiments with stones), was extracted in methanol (Marker, 1972, 1976; HMSO, 1983). *Cladophora*, and other filamentous algæ samples were frozen overnight prior to extraction to ensure more complete removal of pigment (Marker, 1972). Samples and extracts were stored in darkness, in air-tight containers and, at ~4°C. For the fines and gravels as much water as possible was drained off the sectioned material, and this water filtered through 0.45 μm cellulose nitrate membrane. The filter and sediment samples were combined and extracted in methanol in a wide-

necked vessel with air-tight lid. The extract was then re-filtered (cellulose nitrate 0.45 µm), and the filter washed with the solvent. The filtrate and washings were combined, and adjusted to a known volume. Absorbance measurements at 630, 645, 665, and 750 nm (Beckman DU 520 UV/Vis spectrophotometer) were used to measure chlorophyll *a* (Marker, 1972). The stones were extracted in methanol in the same way as the 0–5 mm depth section, except that prior to extraction excess water was allowed to drain off without suction. For the fines and gravel the contact surface area of the water-sediment interface was taken as the total area of the trays containing the material, whereas for the stones experiments the exposed surface area was calculated from measurements made using a tin foil method (Calow, 1972; McCreadie & Colbo, 1991). The foil used was weighed so that the chlorophyll *a* measurements made from the two stones sub-sampled from each section could be normalised with respect to the surface area of the stones. With reference to the precision of chlorophyll *a* sample collection and analysis, overall variance of sampling and analysis was established by using a nested analysis of variance technique (HMSO, 1983, 1984). Organic matter for each section was determined from material removed from the stones using a nylon brush treatment (Marker & Casey, 1982), and overall values for each channel were calculated from these results.

For characterisation, the sediment was dried at 105°C overnight in a vial of known volume (± 0.317 ml), porosity and density were calculated, and the organic matter (OM) content obtained by ashing at 550°C overnight. Suspended solids present in both stream-water at the site, and flume experiments, were measured by volumetric filtration (glass microfibre GF/F) and drying overnight 105°C.

Theory

The equilibrium phosphate concentration (EPC_0) has been utilised in many investigations to predict the behaviour of a sediment, or soil when exposed to phosphate solutions (Klotz, 1991; House *et al.*, 1995b; House & Denison, 1997; Gardner *et al.*, 2002; Pan *et al.*, 2002). The EPC_0 is the concentration of SRP in a solution such that no change in the concentration occurs when it is in contact with a natural sediment for 24 h (House, 2003b). If the SRP concentration in the solution is greater than the EPC_0 then the sediment will take up phosphorus, but desorption from sediment will take place if the concentration is lower than EPC_0 (House & Denison, 1997, 2000).

In this study the EPC_0 was determined by measuring the approach to the steady-state concentration of SRP reached in the overlying solution, over the period of the experiment, and modelled using a diffusion boundary layer (DBL) model (House & Denison, 2002), which assumes that the flux of SRP across the sediment-water interface, $dn(t)/dt$, where $n(t)$ is the amount of SRP passing per unit area of sediment at time t, is regulated by diffusion across the boundary layer

$$dn(t)/dt = D_m[c(t) - c_i(t)]/\delta \qquad (1)$$

where $c(t)$ is the SRP concentration at the top of the boundary layer, $c_i(t)$ is the SRP concentration in the interstitial water at the sediment surface, and D_m is the molecular diffusion coefficient of the dominant phosphorus ion (HPO_4^{2-}) at the solution pH (Table III), with a boundary layer of thickness (δ) (House & Denison, 2002), which can be estimated by

$$\delta = 2500/v \qquad (2)$$

where δ is in μm and v is the water velocity in cm $^{-1}$. If V_t is the total volume of the circulating water, A_s is the surface area of the interface, in this case the total area of the trays used for the fines and the gravel, and the channel area for the stones. An effective depth of water for the channel (d) may be written as $d = V_t / A_s$, and with the approximation that interstitial water concentration, c_i is constant and equal to EPC_0 over the length of the experiment (House, 2003b), then for the release of SRP from a sediment, this equation has the solution

$$c(t) = EPC_0[1 - \exp(-kt)] + c_0 \exp(-kt) \qquad (3)$$

where the rate constant $k = D_m/(\delta d)$ and c_0 is the concentration of SRP at the start of the monitoring – designated as $t = 0$, and allows the EPC_0 to be calculated from the kinetic data. The limiting flux when $c(t) << EPC_0$ is $D_m c(t)/\delta$ and is the same as the rate constant k_p used in a previous model (House & Denison, 2002). The boundary layer model (Eq. 3) was applied with the diffusion coefficient of HPO_4^{2-} at 25^0C (=7.34 x 10^{-6}

$cm^2 s^{-1}$) extrapolated to 4.7 x 10^{-6} $cm^2 s^{-1}$ at 10^0C (Li & Gregory, 1974; House *et al.*, 1995b). Root mean square (r.m.s.) deviation was used to optimise the agreement between the measured SRP concentration and the model prediction (Eq.3) by varying the EPC_0.

To indicate the direction and concentration gradient for the movement of the SRP, a Phosphorus Transfer Index (PTI) was used (House *et al.*, 1998).

$$PTI = (SRP/EPC_0 - 1) \qquad\qquad\qquad (4)$$

When the PTI is zero the sediment is in equilibrium with SRP in the overlying water, if a positive value, there is a net flux of SRP from the water to the sediment, and if negative, the net flux of SRP is from the sediment to the water.

Results and Discussion

The results of six flume experiments are discussed. The sediments used for these were collected from both Bentley Mill Way (BM) and Sandwell Valley (SV) at intervals over the sampling period (Table II). These two sites differ in a number of ways, and the sketch surveys that were performed at sample collection times summarise the main distinctions. The primary difference is that SV is a constructed channel created when the river was diverted as part of a flood alleviation scheme. It has a clay lining, which has become exposed in some areas where bed erosion has occurred, and one bank is built from rock-filled wire-mesh cages. The river at BM is approximately half the width it is at SV, but both have a rather straight-sided and flat-bottomed channel cross-section. Within the measured reaches both had only one small stand of marginal plants at the time of sampling, which covered no more than 1 % of the bed area, and BM had much more frequent bank and bed erosion events. Both reaches include riffles and pools, but the natural, non-mobile, bed load of BM is augmented by man-made detritus, such as large pieces of concrete drainage pipe and vehicle wheels. These provided a stable substrate for filamentous algal growth, *e.g. Cladophora*, and acted as traps for finer material in which deeper-rooted, more spate-tolerant species of submerged angiosperm grew, *i.e. Sparganium emersum,* and *Potamogeton crispus* (Haslam, 1978). Overall, both reaches had few areas where fine silt and sand could accumulate, with the bed area occupied by

this fraction estimated to be ≈ 5 %. BM had more larger-sized stones at the top end of the measured reach, but more gravel present further down stream mostly incorporated with the hard-packed bed sediment. SV had an approximately equal areal coverage of gravel and stones. The invertebrate populations at both sites comprised mainly pollution-tolerant, detritus feeders such as *Nematoda, Oligochæta,* and *Chironomidae,* and also *Hirudinea.* Results from the invertebrate census taken from the experiments with stones indicated BM to have lower species diversity than SV, and the *Oligochæta* and *Chironomidae* were shown to be correlated to OM.

Solution Chemistry

The physico-chemical constitution of the river water at the sampling times is shown in Table II. A greater river flow was recorded for SV than at BM. These flow data were obtained from the Environment Agency, UK, recorded by their gauging stations located in the vicinity of the two sites. The larger volume of flow is expected at SV because of diffuse inputs, the contribution from two brooks, and four sewage treatment works (STW) outfalls between the sites. The water temperatures recorded accord with the times of day the measurements were made and seasonal averages, with April, 2002, the lowest temperature at which samples were obtained, and September, 2001, the highest, but with no substantial difference between the sites over the sampling period. The SRP measurements made from the river water spot sampled show SV to have consistently higher concentrations than BM. There was considerable variability between the water velocities measured at the sediment sampling positions – from < 5 cm s^{-1} in the areas where fine material accumulated up to ~ 20 cm s^{-1} where the stones were collected.

In the experimental flumes the solution pH, temperature, and the ambient light showed diurnal fluctuations as expected. The solution in the flume was not artificially ærated, but was found to remain well oxygenated. In the experiments with more biofilm, the increase in light lead to increased photosynthetic activity. This activity would reduce CO_2 concentration, altering the balance of anions in the carbonate system and hence the pH (Stumm & Morgan, 1970). Both anions and cations were found in higher concentrations in the solution at the end of the experiments with the SV fractions, and overall the dominant major ion was sulphate (Appendix 2.16). The constitution of the overlying

solution used in the fluvarium experiments is described in Table III, which also includes data for the associated sediment. These physico-chemical characteristics comprise averages and minima/maxima extracted from all manual or automated readings taken during each experiment. The temperature measurements for April and June were higher than those recorded at the time of sampling in the river, but all others are comparable with the field measurements. This may have been due to either a weather induced temperature differential over the country – the field site and laboratory being ~250 km apart – or the hour of sampling. The mean solution pH in the experiments with fines and gravel were neutral, but in the experiments with stones the solutions were slightly alkaline. The oxygenation of the solution through mixing in the re-circulating system ensured the DO concentration of the overlying solution remained high throughout all the experiments. However, the slower flow in the experiments with fines, combined with a higher sediment oxygen demand, resulted in lower DO concentration in solution. The molar concentration of DO in the overlying solution is dependant on atmospheric pressure and solution temperature (Benson & Krause, 1980), *i.e.* a lower temperature would result in 100% saturation occurring at a higher molar concentration. Steady levels of conductivity were lower than spot measurements in the field.

Although the overlying water comprised a 2 mM calcium chloride solution, most of the measured major ions were registered as initially present because the process of filling the channels containing the sampled sediment released ions into the water column before the first sample could be obtained.

Sediment Characteristics

The sediment characteristics, *i.e.* organic matter, porosity and density, found for the sediment collected at the same time and position in the riverbed as the relevant size fractions used in the flume experiments were taken from, are shown in Table II. Measurements of the chlorophyll *a* for the three sediment size fractions used in the flume experiments, along with the calculated surface area of the stones can be found in Table III.

Organic matter (OM) can be a very important constituent of a sediment, especially in one that has a substantial proportion of large grain-size material. This is because gravel and

stones can acquire coatings of organic substrates onto which pollutants adsorb (Förstner, 1990). The OM content of the fine material was higher than for the other two fractions at both sites, and also exhibited comparable and higher porosity. The OM content in the areas where the stones were taken from was comparable, but the SV gravel sample had a smaller amount than the BM gravel.

The densities of the sediments were consistent with a high quartz content, except that taken from the vicinity of the SV fines fraction, which was rather low and is in concert with the organic matter (OM) figures, because high OM implies low particle density.

Porosity values generally correlated well with values for typical sediment interstices given by Todd (1967) of between 30 and 50% for gravel and sand of mixed grain size; except for the sample for the fine material. These are high, yielding an equivalent of >60% porosity, when compared with Todd's values of ~45% for medium to coarse mixed sand. Grain size affects the water available for movement, and small particles may have a significant effect on the transport of water through a sediment due to their electro-molecular characteristics (Polubarinove-Kochina, 1962; Ward, 1967).

Algal Biofilm Development

Chlorophyll *a* analyses are often used to estimate the biomass of algæ in aquatic environments. However, pigment content may vary depending on the algal species present, *e.g.* chlorophyll *a* can range between 0.4 – 4.0 % as dry weight (HMSO, 1983) and complete extraction is sometimes difficult to achieve (Marker, 1972; Marker & Casey, 1982). Therefore pigment determinations should not be regarded as a definitive technique for the measurement of primary production, but used in conjunction with other biomass assessment methods. However, this method provided sufficient information on growth rates, in this case, to relate the growth rate to other factors and events. Chlorophyll *a* was found to increase with increasing fraction size due to the attached filamentous algæ, *i.e. Cladophora*, but the SV stones had a greater mass associated with them than the BM stones. Chlorophyll *a* estimates calculated from absorbances measured for the surfaces of the stones and gravel, and in the 0-5 mm surface section of the fines (normalised to the geometric bed area), illustrate the order of benthic biomass development to be found in the Tame at a particular time (Table II). These are low

compared with values found by Marker (1976) of between 23-37 mg m^{-2} in a chalk stream in Dorset, U.K. in January, and between 255-290 mg m^{-2} in April. Values found for a large, impacted, lowland, gravel-bed river in south-west France were between 112 - 254 mg m^{-2} (Ameziane *et al.*, 2002). The low biomass may be attributed to the hydrological and climatic conditions that prevailed in the Tame early in the year, *i.e.* streambed scouring by high-flow events, and cold weather slowing the onset of the spring bloom.

Cladophora is an attached filamentous alga, it is widely distributed, prevalent in streams of < 10 m width (Haslam, 1978), and commonly part of a stream community, *i.e.* it is not necessarily associated with eutrophication. Normally small-celled, and multi-branched, it forms clumps that can range in size from a few centimetres to skeins as long as 30 metres. It is phosphate rich conditions, such as those found in the Tame, which can promote the growth of long, less branched filaments with relatively large cells, that form the dense algal mats and skeins. Such excessive growth can lead to exaggerated diurnal oxygen fluctuation, and, when die-back occurs in autumn, can cause de-oxygenation in the water column and the surface sediment (HMSO, 1984).

Soluble Reactive Phosphorus Dynamics

Residence time of phosphates in aqueous and sediment systems can vary from minutes to tens of hours. Soluble phosphorus interacts with suspended and bed sediments by: sorption, mineral precipitation, coprecipitation, and through uptake and release by algæ, diatoms and invertebrates (House *et al.*, 1995b). The rate of phosphorus transport through the sediment is affected by the thickness of the 'active surface layer'. Diffusion of phosphorus across the sediment-water interface in silt deposits had been thought to dominate flux responses (Froelich, 1988; House *et al.*, 1995a; Haggard *et al.*, 1999), and so the removal of the fine material by scouring of the stream-bed during high flow conditions would lead to a reduced capacity for the bed-sediment to adsorb or release phosphorus. However, there has been no other comparable research on phosphorus transport in coarser gravel and stone sediments.

SRP released in each of the experiments is shown in Figure 2, (a) fines, (b) gravel, and (c) stones. Sub-samples from the gravel and the stones were carefully rinsed to remove fine material trapped in the filamentous algæ present, and the paired control samples were

unwashed. The fine material was sieved to remove particles greater than 2 mm diameter. This sieving disrupted any diatomaceous coating the sediment had had *in-situ*, and as no filamentous biofilm growth was present, no unwashed paired sub-sample was made for the two sites.

Fines

After a brief initial period of fast release and uptake activity, the SRP concentrations from the experiment with the SV fines (Figure 2a) demonstrated a gradual continuing release over ~ 12 – 20 hours until they reached a plateau. This pattern is echoed by the BM fines but without the rapid reaction in the first few minutes. However, the concentrations of the SRP are low, with BM achieving a steady-state at ~ 1.0 μM and SV at ~ 2.3 μM. These concentrations are similar to those found for a silt-dominated, natural sediment by House and Denison (1997) using the experimental flumes in an earlier study, and are unexpectedly lower than the concentrations observed in the experiments with the larger two size fractions discussed below.

The diffusion model failed to predict the initial fast activity of SRP for the experiment with SV fines. However, agreement with the longer-term release kinetics is evident (Figure 3a). The DBL model produced an excellent agreement with the data for the BM fines as shown by the low r.m.s. in Table IV. The SRP fluxes when $c(t) \ll EPC_0$ (Eq.1) were calculated as 2.2 and 6.4 nmol m^{-2} s^{-1} for BM and SV respectively, and are comparable to the flux measured by House and Denison (2002) for the silt sediment from the R. Blackwater in southern U.K. This is also a river contaminated by treated sewage treatment works effluent (no tertiary phosphorus removal), and produced evasion fluxes over two annual cycles of between 1.6 and 25.4 nmol m^{-2} s^{-1}. The silt sediments had a similar OM content, porosity and density, and the main factor contributing to the difference in the EPC_0 values and fluxes is the greater concentration of SRP in the overlying water at SV compared with BM (Table IV). The EPC_0 values are low and below the river water SRP concentration at the time of sampling leading to positive PTI values and hence a phosphorus flux from the water to the sediment, *i.e.* net uptake of SRP at the time of sampling, if sorption capacity is available.

The antecedent flow conditions indicate a storm event (Figure 4a) with high flow volumes (Table II) at both sites ~ 6 days prior to sampling and a smaller rise in flow

volume 2 days before. Deposits of fine material would have been considerably reduced by the storm event, as scouring was observed to occur at both sites, but particularly at BM. However, there was little time, < 3 days, for the re-accumulation of fines before the next, smaller event, and therefore this would have caused less disruption to the depleted fines deposits. The SRP concentrations (Table II) from the field spot samples show good agreement with corresponding data from the Environment Agency (Table II and Figure 4) even though the latter are measured as unfiltered reactive phosphorus (UFRP), and they are consistently higher than the EPC_0s confirming the net uptake of SRP by the sediment.

Gravel

The results for the gravel fraction (Figure 2b) are more complex, with rapid initial activity in two phases – a very quick release, re-adsorption, and re-release, followed by the gradual evasion. The earliest phase occurred before the first sample was taken, leading to a relatively large c_0 of 4.76 μM (Table IV). The gradual release occurred over ~ 15 hours, to a final concentration higher than in the experiment with fine sediment. The results then diverge from the pattern seen in both experiments with the fines and the stones – the final concentration for the BM gravel unwashed sub-sample is close to that of the SV unwashed fraction, and there is only ~ 1.0 μM difference in the magnitude of the final concentrations between the SV washed and unwashed sub-samples, but a difference of ~ 4.0 μM for the BM washed and unwashed ones. It was noted that the initial concentrations were not zero in either the gravel or stones experiments (see Stones section).

The DBL model does not describe the initial rapid releases observed in the experiments, as indicated by the intersection of the y-axis in Figure 3b. These rapid effluxes were either caused by the release of SRP from particulates disturbed during the immersion of the material in the flume channels, or a kinetic effect associated with material trapped within the filamentous biofilm. However, the model does predict the second phase of release (Figure 3b). The presence of the biofilm on both gravel fractions is indicated by the chlorophyll *a* values (Table III). The washed gravel samples exhibited similar initial activity but over a much smaller concentration range, and therefore the model prediction gave a better fit. As expected, the rate constants for these experiments are similar and the EPC_0 for the BM unwashed sample is higher than the washed, whereas for SV the values are comparable. Although the EPC_0 values are higher than those obtained from the

experiments with the fines, this may be caused by the greater river water concentration of SRP at the time of sampling. The order of the EPC_0 values is consistent with the more stable flow at the SV site compared with BM (Figure 4b) enabling more fine sediment to be trapped in the epilithon. The SRP fluxes (when $c(t) \ll EPC_0$) are 29 and 27 nmol m^{-2} s^{-1} for BM and SV gravels (unwashed), higher than for the fine fraction from the R. Tame, but at the upper limit for the silt sediment from the R. Blackwater. Thus, unexpectedly, the gravel sediment and associated fine material trapped in the biofilm, gives rise to a greater flux of SRP than the fine fraction alone. There is evidence from the results of the BM gravel experiment that reducing the trapped sediment has a direct effect on the reactivity of the gravel, although this is not confirmed for the SV gravel, in which there was little difference between the kinetics (Figure 2b) or EPC_0 values (Table IV). This is corroborated by a comparison of the final SRP concentration in the experiment with SV fines, which was similar to BM washed gravel. The BM unwashed gravel fraction, and both washed and unwashed SV gravel samples, reached similar final concentrations to the BM stones. The results show that the initial fast release of SRP (not described by the diffusion control model) is crucial to the determination of the comparatively high EPC_0 values relative to the fine sediment.

Stones

In the experiment with stones (Figure 2c) the concentration of the SRP in the solution for BM reached a final concentration of ~6.5 μM after ~24 hours; occurring at a concentration comparable to that achieved at the end of the experiments with gravels, with a difference between washed and unwashed fractions of ~ 2 μM. The release curves for the experiments with SV stones show much the same characteristics, $i.e.$ gradual release, but over a much greater concentration range, $i.e.$ ~70 μM, and without reaching a plateau. There is also a difference in the behaviour of the two sub-samples especially evident in those from the SV site, with the unwashed sample resulting in a higher concentration of SRP released to solution, but echoing the release pattern of the washed material. This difference indicates a link between the presence of a developed biofilm, entrapped sediment, and the amount of SRP released to the overlying solution. The hydrograph for the month preceding sampling indicates that after high volume flows, the river had a relatively stable flow for ~ 20 days allowing fine sediment to accumulate again. Both SV and BM samples had comparatively high chlorophyll a concentrations indicative of substantial algal biofilm growth on this, more physically stable, fraction

compared to the gravel. More growth was present on SV sample than BM, and hence more fine material was likely to be trapped and so contribute to SRP release. The EPC_0 values calculated for the SV site samples are an order of magnitude higher than the other sediments, although the SRP concentration in the river at the time of sampling was similar to that when the gravel from SV was sampled. The PTI values are also the lowest of all the sediments indicating their relative closeness to equilibrium, *i.e.* when PTI = 0. The agreement with the DBL model for the SV samples is relatively poor compared with that obtained in the fines experiment (Figure 3c, Table IV). This is because the release of SRP in the period *ca* < 10 h is greater than expected by diffusion alone. This, combined with the effects of washing, indicates a mechanism involving sediment, entrapped by the epilithic filamentous growth, that has intimate contact with the flowing water so that the exchange reactions become rate controlling rather than diffusion across the boundary layer.

The SRP fluxes (when $c(t) \ll EPC_0$) are 36 and 346 nmol m^{-2} s^{-1} for BM and SV respectively, both much greater than for the gravel and fines fractions, and when compared with results from studies with silt sediments (House & Denison, 2002).

Implications for the River Tame

Overall, the SV sediments released higher concentrations of SRP to the overlying solution, which corresponds with the EPC_0s calculated (Table IV) and the spot samples of SRP concentration made in the field (Table II). These concentrations are the same order of magnitude as those obtained by the Environment Agency (Table II and Figure 4), taking into account the difference in the method of analysis. These observations are supported by the volume of discharge from the four STW, which can be as much as, *e.g.* Goscote 60 ML d^{-1} (pers. comm. Severn Trent Water), at concentrations of up to almost 10 mg L^{-1} unfiltered reactive phosphorus UFRP (pers. comm. Environment Agency). The EPC_0 values for both sites generally increased with the concentration of SRP in the river (Figure 5) with large deviations from equilibrium when the PTI is zero. The results obtained for the SV unwashed stones gave the closest approach to equilibrium with the water, *i.e.* PTI = 0.41. The same sample gave the largest amount of SRP released over 48

h, which is consistent with the amount available from exposure in the river, and accumulation of silt in the biofilm.

The relative importance of the three substrates in terms of their SRP release can be estimated using Eq.1 and compared with the river load when point inputs are reduced and a target SRP concentration is set at, for example 3.2 μM (0.1 mg l^{-1}; a proposed target for lowland rivers on clay and alluvium in the Environment Agency's national strategy for eutrophication control.) (Mainstone & Parr, 2002). For the comparison it is assumed that each substrate comprises the entire river bed for the 100 m reach at each site, and the river water discharge is taken as that at the time of sampling. In this case, all the sediment apart from the fine sediment from BM would release SRP to the overlying water. Considering a 100 m reach at each site, the percent interaction (expressed in terms of the instantaneous load calculated on the sampling days) is 0.01 % for the SV fines, increasing to 0.37 % and 0.11 % for the BM and SV gravel, and 0.66 % and 3.76 % for the BM and SV stones. These percentages are linearly related to the length of the river section and so, in the absence of other processes and inputs, will determine the SRP concentration over the 7 km distance between sites, for a bed completely composed of gravel or stones. If the target SRP concentration were 6.5 μM (the 0.2 mg L^{-1} interim target suggested for heavily enriched rivers in the EA strategy), then only the bed composed entirely of stones and associated biofilm would release SRP to the river water; with percent interactions of 0.1 % for BM and 1.75 % for SV. As both sites were dominated by the gravel and stones fraction, which produce the largest fluxes of SRP, these will have the greatest influence on the river water concentration.

After measuring the SRP release and calculating the EPC_0s, the lowest releases (expressed on the basis of the geometric area of the bed) were found for the fine material, with the release from the stones the highest. In fact, the EPC_0 value for the stones from SV was an order of magnitude greater than the EPC_0 for the stones from BM. This indicates a more important rôle for the larger fraction of the sediment by supporting the filamentous algal growth which acts as the trapping mechanism for the fine particulates which control the release of SRP. The chlorophyll a values (Table 3) support this - SV having the highest concentration, as does the application of the model (Figure 3), which works well for the silt as the release will be diffusion controlled across the sediment-

water interface, but does not describe the kinetics for the gravel as effectively. This implies that diffusive transport is not as important a mechanism in the gravel fraction because of the ease of suspension of the fine material and also the relatively rapid exchange of SRP with the suspended material. However, this mechanism does not work in quite the same way for the stones because of their much greater capacity to support a large biofilm mass, shown by the higher chlorophyll *a* concentration. This binds the fine material within it more strongly and subsequently diffusive transport better describes the kinetics seen in Figure 2.

In all the sediments sampled from the field sites (Table II) there was a higher percentage of gravel than fines, with stones contributing the greatest percentage. BM had a larger percentage contribution from the gravel and stones fractions than SV. This larger-sized material determines the surface roughness of the stream-bed, and so increases the turbulence of the flow. Where filamentous growth is present, although this tends to reduce turbulence, the more turbulent flow created by the stones would lead to less fine particulate material accumulating within the biofilm, but if a large volume of growth is present even residual particulate material would contribute significantly as a source of SRP.

Comparing both the BM and SV river water concentrations of SRP, at the time of removal of the sediment, with the final concentrations achieved by the end of the release experiments for both sites (Table II and Figure 2), it can be seen that there was a higher concentration in the river than was reached in the overlying solution in the flume channels. This indicates a net movement of SRP into the sediment at that time. Flow conditions at the times of sampling were comparable for both sites, though the storm events evident from the hydrographs for the month prior to sampling (Figure 4) show when fine material would have been depleted by scouring.

Conclusions

This is the first study to be made of the differences in behaviour, with respect to their effects on the flux of SRP, of the size fractions which comprise most natural, heterogeneous river sediments.

Removal of fine material trapped in filamentous algal growth on stones reduces SRP release into solution, and from this evidence it is concluded that turbulent flow, created by stream-bed surface roughness, and stream velocity contribute to the flux and magnitude through the re-suspension of the fine particulates.

The release kinetics indicate that the larger size sediment fractions, those in the 2 - 20 mm and > 20 mm range, with a well-developed biofilm present exhibit more release than the fine sand and silt, < 2 mm, fraction, but SV fine sediment releases more SRP than the fine material from BM, as a result of SV being exposed to higher SRP concentrations. The kinetics of these reactions confirms that another mechanism is involved than simple diffusion. Sediment from the two sites (Bentley Mill Way and Sandwell Valley) reacts in similar ways, though over a different concentration range.

Higher EPC_0 values for SV than BM indicates a greater amount of SRP adsorbed at SV. Comparing the equilibrium concentration values of the SRP in the overlying solution in the fluvarium channels with river water values at the time of sampling can establish whether there would be a net flux of P into or out of the sediment at these times. For example, where the river water values are much higher than the equilibrium concentrations observed in the solution in the channels, there would be a strong flux of P into the sediment at that time.

Differences in behaviour were observed both between the sites and the sediment size fractions. The results indicate that both the gravel and stone substrates have an important control over the release of SRP when the overlying water concentration is reduced below the EPC_0 value.

Table I Sampling site locations

Site location	Site code	National grid reference	Distance between (km)	Area of catchment* (km²)
Bentley Mill Way	BM	SO 995994		57
Sandwell Valley	SV	SP 929028	7	182

*From Environment agency, U.K.

Table II. Physico-chemical constitution of the river water at sampling time including temperature with temperature deviation from seasons average (dev), dissolved oxygen (DO) and soluble reactive phosphorus (SRP). Also, data for the associated river sediment (†) as sampled for the three sediment size fractions used in the flume experiments, fines (< 2 mm), gravel (2 - 20 mm), and stones (> 20 mm).

	Bentley Mill Way (BM)			Sandwell Valley (SV)		
	fines	**gravel**	**stones**	**fines**	**gravel**	**stones**
Experiment code	6	7a	5b	6	5a	3a
Date sampled	31-Oct-01	10-Apr-02	11-Sep-01	31-Oct-01	10-Sep-01	13-Jun-01
River discharge (m^3 s^{-1})*	0.8	0.5	0.5	2.8	3.2	2.3
Flow velocity min/max (cm s^{-1})	<5/5	<5/35	30/30	<5/5	12/31	15/60
Temperature (^{o}C) (dev)	12.2 (0.5)	10.3 (-0.3)	17.2 (-2.1)	12.2 (0.0)	17.8 (-0.8)	15.3 (1.7)
pH	7.2	7.0	7.2	7.6	8.0	7.3
DO (% saturation)	67.0	78.0	-	63.0	-	111.0
Conductivity (μS cm^{-1} at 25°C)	841.0	1380.0	1094.0	817.0	1241.0	1437.0
SRP (μM)	16.6	45.4	25.7	57.8	102.4	109.2
†Organic matter (%)	8.3	7.5	5.9	9.1	2.6	6.5
†Porosity (% average by site)	65.6	53.0	48.9	63.5	34.2	44.2
†Density (g cm^{-3})	2.5	2.5	2.6	2.2	2.7	2.9
†% by mass Fines	26.3	10.7	9.0	25.4	22.8	20.7
†% by mass Gravel	35.9	22.6	21.8	35.3	34.9	34.5
†% by mass Stones	37.8	66.7	69.2	39.3	42.3	44.8

*Mean daily discharge data from the Environment Agency, U.K.

Table III. Physico-chemical constitution of the overlying solution used in the fluvarium experiments, and associated sediment data (†) of the three sediment size fractions, fines (< 2 mm), gravel (2 - 20 mm), and stones (> 20 mm) of unwashed material. Means and minima/maxima for temperature, pH, dissolved oxygen (DO), conductivity, and major ions, and mean/maximum for light intensity.

	Bentley Mill Way (BM)						Sandwell Valley (SV)					
	fines		gravel		stones		fines		gravel		stones	
Date sampled	31-Oct-01		10-Apr-02		11-Sep-01		31-Oct-01		10-Sep-01		13-Jun-01	
Flow rate (cm s^{-1})	9		20		20		9		20		20	
Mean Temp ($^{\circ}$C)	12.4		15.6		17.4		12.5		17.5		21.1	
Temp ($^{\circ}$C)	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
	11.0	13.1	13.4	16.4	16.5	18.3	11.45	13.4	16.3	18.7	17.6	25.2
Mean pH	6.5		7.3		7.2		6.4		7.1		7.5	
pH	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
	5.7	6.9	7.1	7.4	7.1	7.4	5.6	6.9	6.1	7.7	7.2	7.9
Mean DO	86		94		96		84		92		93	
DO	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
(% saturation)	83	86	87	98	84	104	80	88	87	96	62	138
Mean Conductivity	516		538		500		485		514		532	
Conductivity	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
(μS cm^{-1} at 25°C)	476	528	519	564	485	506	461	500	471	518	529	547
Light Intensity	av.	max.	av.	max.	av.	max.	av.	max.	av.	max.	av.	max.
(μE m^{-2} s^{-1})	14.4	67.3	43.3	253.5	49.9	285.6	16.1	78.4	68.2	399.3	156.9	817.7
† Chlorophyll a (mg m^{-2})	3.0		6.4		22.8		2.3		8.6		62.7	
†Measured surface area (cm^2)	-		-		1784.4		-		-		1695.4	

Table IV. Rate constant (k) values, equilibrium phosphate concentrations (EPC$_0$) with associated r^2 and r.m.s, for sieved fine sediment, and washed and unwashed gravel and stones. Also, shown are river water SRP concentrations at time of sampling, from Bentley Mill Way (BM) and Sandwell Valley (SV) and the Phosphorus Transfer Index (PTI) values.

Sample	c_0 (μM)	k ($10^{-5} s^{-1}$)	EPC_0 (μM)	R^2	r.m.s.	River SRP (μM)	PTI
BM sieved fines	0.1	2.3	1.2	0.84	0.21	16.6	12.8
SV sieved fines	0.88	2.2	3.5	0.79	1.2	57.8	15.5
BM washed gravel	1.1	1.3	2	0.56	0.86	45.4	21.7
BM unwash gravel	2.72	1.5	6.3	0.85	2.47		6.21
SV washed gravel	1.35	1.7	5.8	0.92	3.11	102.4	16.7
SV unwash gravel	4.76	1.7	5.6	0.74	1.88		17.3
BM washed stones	2.67	1.5	6.5	0.94	1.46	25.7	2.95
BM unwash stones	4.48	2.3	7.7	0.82	2.44		2.34
SV washed stones	10.8	1.9	50.2	0.99	9.3	109.2	1.04
SV unwash stones	15	1.4	65.5	0.99	11.8		0.41
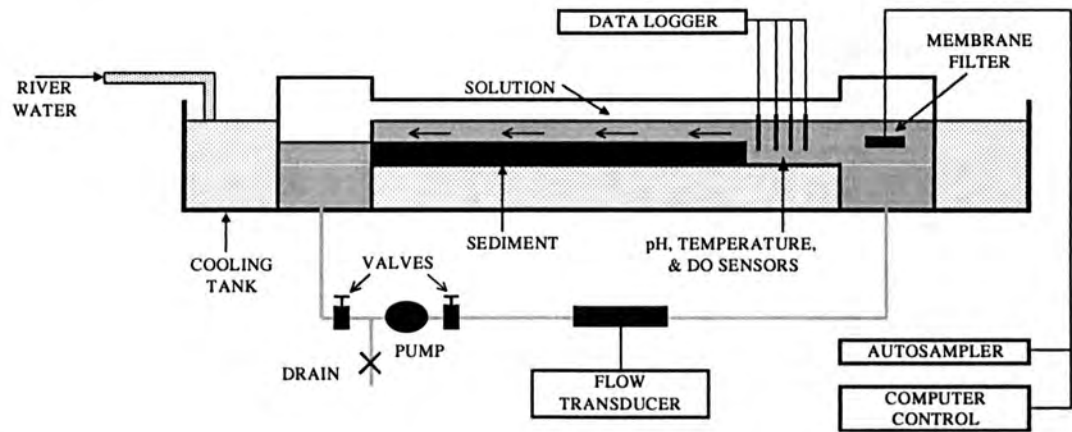
Figure 1. Schematic of flume channel system (House *et al.*, 1995b) used to measure the release of soluble reactive phosphorus by sediment. The fines and gravels fractions were placed in trays before insertion into the channels, the stones were placed directly into the channels and the channels marked into measured sections for sampling purposes.
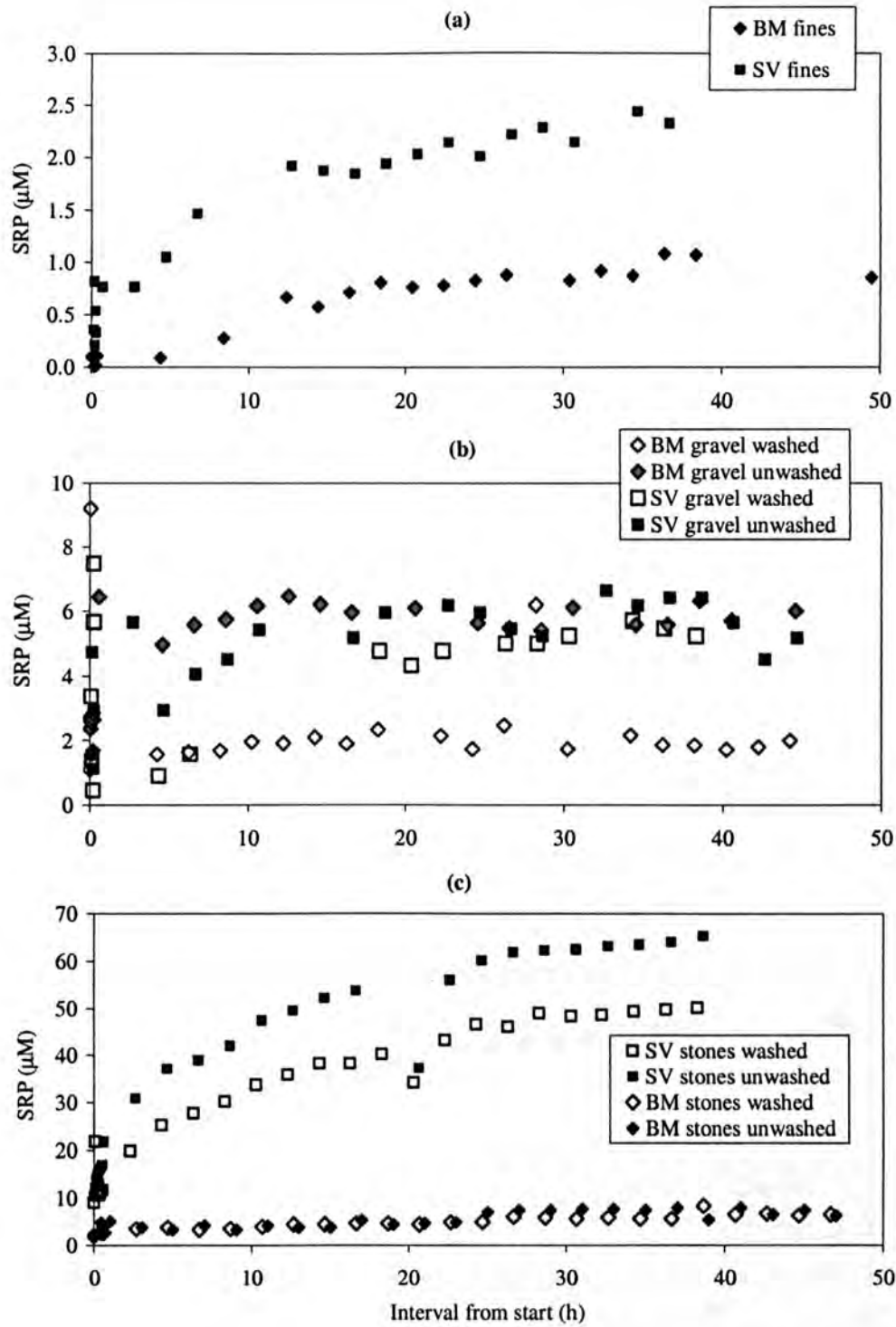
Figure 2. Measured concentrations of SRP released to the overlying solution in the experiments using a) < 2 mm fraction (fines) , b) 2 - 20 mm fraction (gravel), and c) > 20 mm fraction (stones) from Bentley Mill Way (BM) and Sandwell Valley (SV). The fines material was sieved to remove and particles greater than 2 mm diameter. Sub-samples from the gravel and the stones were carefully rinsed to remove fine material trapped in any filamentous algæ present (open points), and control samples were unwashed (closed points). It should be noted that the scale on the y axis differs between these plots.
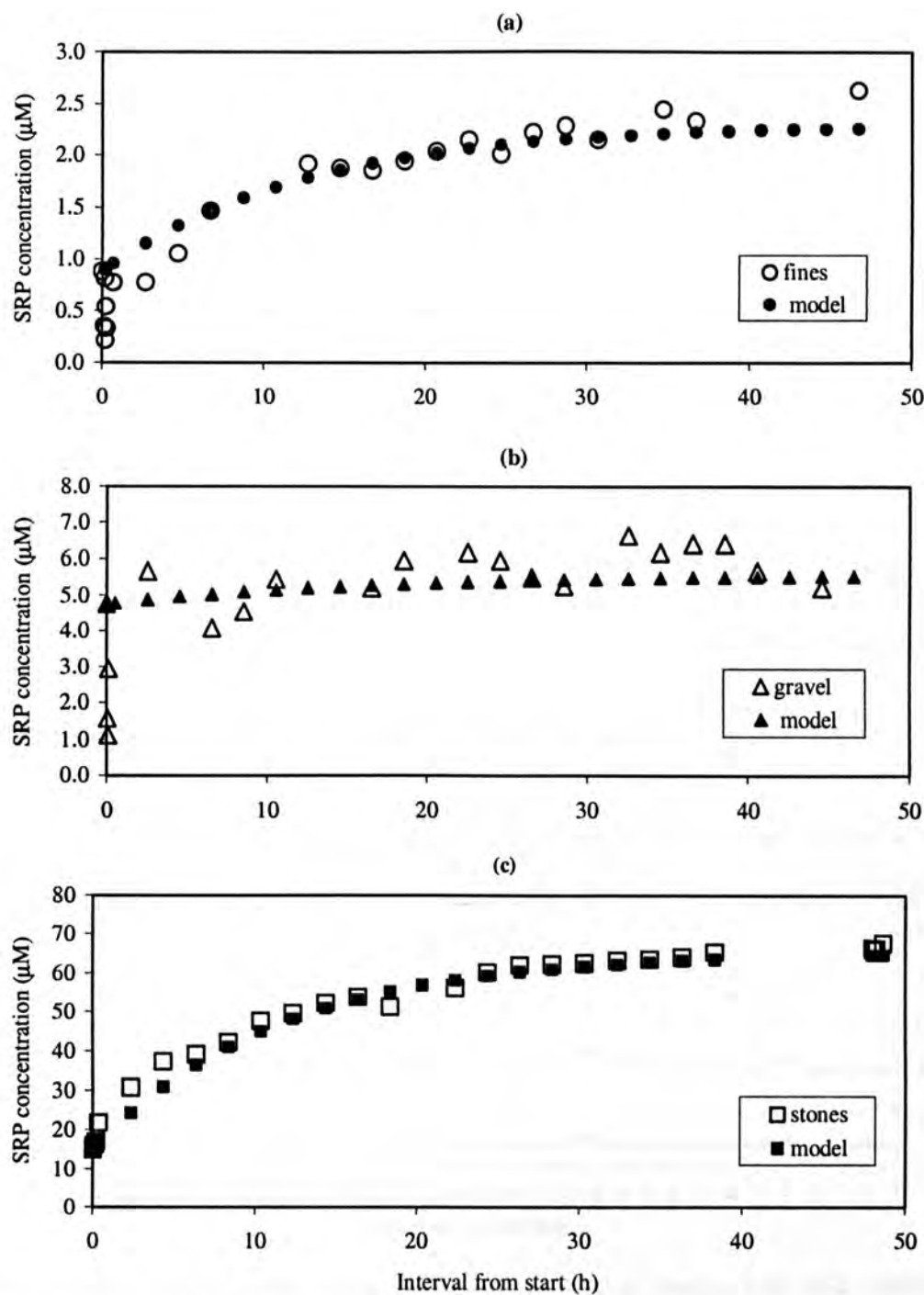
Figure 3. Release of SRP to the overlying solution in the flume channels from the unwashed samples of the three size fractions, (a) fines < 2mm, gravel 2 - 20 mm, and (b) stones > 20 mm, from Sandwell Valley. Comparison of experimental results (open points) and the release modelled (closed points) using the diffusion boundary layer model (House and Denison, 2002). It should be noted that the scale on the y axis of these plots differs.
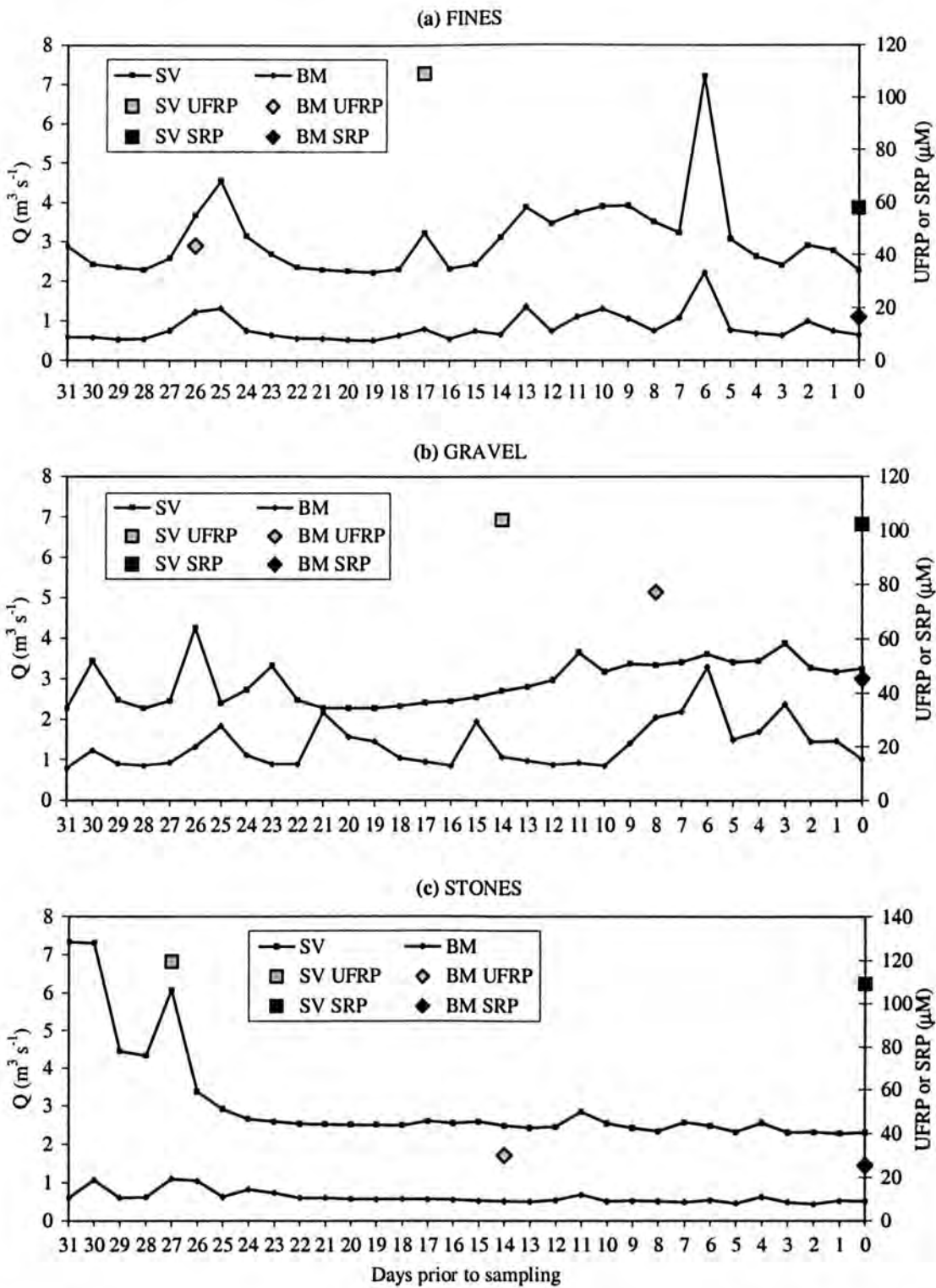
Figure 4. Hydrographs of flow conditions, in m^3 s^{-1} (Q), at Bentley Mill Way (BM*) and downstream Sandwell Valley (SV) for the month prior to the sampling of the three size fractions: (a) fines < 2 mm, (b) gravel 2 - 20 mm, and (c) stones > 20 mm. River water concentrations of SRP (closed points) on sampling dates and unfiltered reactive phosphorus (UFRP) as measured by the EA on the day closest prior to the sediment sampling dates (shaded symbols) are shown on the second axis. Daily mean Q and spot UFRP concentration data from the Environment Agency, U.K.
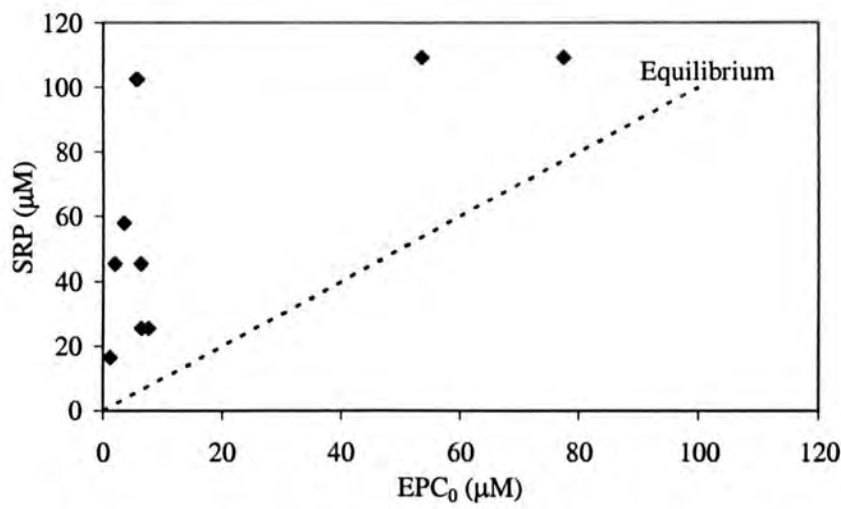
Figure 5. River water concentrations of SRP at sampling time against EPC_0s for all three size fractions (fines < 2 mm, gravel 2 - 20 mm, and stones > 20 mm), including washed and unwashed sub-samples of the gravel and stones, from both Bentley Mill Way and Sandwell Valley.

Chapter 4

Kinetics of Phosphorus Release from a Natural Mixed Grain-Size Sediment with Associated Algal Biofilms

4.1: Introduction

In this chapter the release of phosphorus from a natural sediment of mixed grain-size over an annual cycle of development and decline of an algal biofilm associated with the sediment, and models of the kinetics of those exchanges, are examined.

It should be noted that although this part of the study is presented after the size fractions experiments these experiments using the natural sediment were performed in sequence through the biofilm growth cycle.

A second phase of each experiment, to examine the uptake of phosphorus by the sediments after the addition of a standard solution, was also undertaken. However, as the amount of phosphorus released from the sediment during the first phase of the experiments was high, it was found that the standard additions were insufficient to show an uptake response clearly in the majority of cases. Therefore the seasonal sequence of experiments, and consequently the effects of the biofilm growth on the phosphorus flux over that cycle, could not be evaluated. Where no uptake was evident, *i.e.* no increase of SRP concentration was detected after the standard addition, the data from this phase of the experiments was amalgamated with the first phase and the whole considered as release. The data from the phosphorus uptake experiments are given in Appendix 4.1.

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Kinetics of Phosphorus Release from a Natural Mixed Grain-Size Sediment with Associated Algal Biofilms

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Abstract

Experiments using flumes containing mixed grain-size sediment with an associated algal biofilm, from two sites on the R. Tame, investigated the sediment-water exchanges in heterogeneous sediment deposits. These results were considered in the light of findings of a previous study (Gainswin *et al.*, (submitted)) by considering this natural system in relation to the effects of the different sizes of material comprising the sediment.

Sediment samples were collected in trays installed in the river over a period of one growth cycle (March, 2001 – April, 2002) and placed in flume channels with controlled water flow. The temperature, pH, and dissolved oxygen of the solution overlying the sediment were monitored automatically whilst filtered samples were obtained at 2 h intervals over 48 h. The biomass, expressed as chlorophyll *a*, of the algal component of the biofilm from the surface of the sediment was estimated using methanol extraction. The composition of the sediment *viz* size fractions, organic matter and porosity, were determined at the end of the experiments.

The equilibrium phosphate concentration and a phosphorus transfer index were used to establish that a net uptake of phosphorus by some of the samples occurred at the time of sampling. The results were modelled using a Diffusion Boundary Layer model and the maximum flux from the sediment (or limiting diffusion flux) compared for each of the samples. The limiting diffusion flux was highest at the most contaminated site – reaching ~180 nmol m^{-2} s^{-1} (normalised with respect to the river bed area). The limiting diffusion flux calculated for the composite samples was in agreement with the flux estimated from the contributions expected from the individual size fractions (Gainswin *et al.*, (submitted)). The dominance of the flux contribution from the stones size fraction (> 20 mm) confirms that sediment having a filamentous biofilm, and associated particulate material, results in a greater flux than a silt sediment without such a biomass.

Key words: soluble reactive phosphorus; equilibrium phosphate concentration; algal biofilm; river sediment.

Introduction

There is now a strong focus on the regeneration of urban areas, and it is important to find the most effective and efficient techniques. Industrialisation has often left these urban heartlands severely contaminated, and it is essential to recognize any potential ecological risks from contaminated river sediments by understanding their complex internal processes.

One of the most common contaminants in rivers is dissolved phosphorus (Mainstone & Parr, 2002), an important nutrient (Bowes & House, 2001), which when associated with particulate materials may remain sequestered in sediments for long periods. However, physico-chemical changes in the system can cause it to be released (Golterman, 2001; House, 2003b), and the development of an algal biofilm on the river bed (Woodruff *et al.*, 1999a, b) is one of the factors that can influence such changes.

Large quantities of soluble inorganic phosphorus in waterways are the most common cause of eutrophication (Likens, 1972; Moss, 1986). In lentic systems the sediment-phosphorus interaction has been studied in detail (Mortimer, 1941; Moss, 1986), but the bulk of research has been on silt sediments as this size fraction was thought to be the primary responsible for fluxes of phosphorus. Although some earlier work concentrated on the effects of biofilm development on processes in the fine (< 2 mm) fraction of a river sediment (Woodruff *et al.*, 1999a, b), little information is yet available for riverine systems with a mixed grain-size sediment. A previous paper reported research into the effects different grain-size material (Gainswin *et al.*, (submitted)) and an associated filamentous algal biofilm had on chemical fluxes at the sediment surface. The aims of the work reported here were to measure the flux of phosphorus, as the bioavailable soluble reactive phosphorus (SRP), out of a natural, heterogeneous sediment comprising various grain sizes, and to study the kinetics of these movements. Also, to build on the findings of the previous study (Gainswin *et al.*, (submitted)) by considering this natural system in relation to the effects of the different sizes of bed sediment material on the exchanges. This was carried out over an entire biofilm growth cycle with the objective of assessing what effects the seasonal changes in a natural system would have on the sediment-water exchange.

Methods

Field Sites

The River Tame rises in the large conurbation to the north-west of Birmingham, U.K., and flows eastwards through the metropolitan area, eventually joining the River Trent, and contributing to water supplies for the region. Seriously contaminated by heavy industry in the past, water quality has improved over the last twenty years (EnvironmentAgency, 2000). However, it is still subjected to effluent from a number of major sewage treatment works with no tertiary phosphorus removal mechanism. The four works discharging secondary treated sewage effluent impacting the field sites are Willenhall (NGR SO9796098160), Goscote (NGR SK02190192), Walsall Wood (NGR SK03550388), and Ray Hall (NGR SP0232094440) (pers. comm. Severn Trent Water). The two study sites considered to be the most suitable for sampling on the upper reaches of the Tame were: Bentley Mill Way (BM) (NGR: SO 995994) and, approximately 7 km downstream, Sandwell Valley (SV) (NGR: SP 929028). Both these sites are within a single catchment area, but had relative sub-catchment sizes of 57 and 182 km^2 respectively, and were chosen to provide representation of the different bed-sediment types and flow conditions in the upper reaches of the Tame. The sites were sufficiently distant to detect differences in water and sediment chemistry, yet close enough together to avoid the complications caused by inputs from additional catchments. All site visits were made over the period November, 2000, to April, 2003, and samples were taken regularly throughout biofilm development and decline phases.

Field Surveying and Sampling

A one hundred metre reach was marked out at each site, and divided into 10 m sections with marker pegs on the bankside. Rectangular cross-sectioned, polypropylene sampling trays, 10 cm x 40 cm and 5 cm deep, were installed in the bed-sediment longitudinally in-line with the direction of flow, and at a depth such that their top edges were flush with the bed-surface. Ten trays were installed randomly in the measured reach at each site to ensure potential variability in the bed characteristics was accounted for. Their positions within a grid system, which is described below, were determined by Excel random number generation, but should a position be unsuitable due to water depth, the next number in the sequence was selected. Trays were seeded with the material taken from

the hole in the riverbed in which they were placed.

Observations were made during site visits, where possible recording the appearance of each measured reach. Quadrats, delineated using the pegs at 10 metre intervals along the bank downstream and visually estimated at 0.5 m intervals across the stream, were examined and the dominant feature, *e.g.* silt, gravel, or filamentous algæ, mapped. Other details noted were: current and previous weather, evidence of any recent high flow events, macrophyte growth, physical changes in the channel such as bank collapse, water turbidity, and any unusual features such as obvious contamination events. Water temperature was recorded from a 100 Ω platinum resistance thermometer (PRT), and pH, dissolved oxygen (DO), and conductivity were measured using Mettler-Toledo Checkmate 90 Sensors. Bulk and filtered (cellulose nitrate membrane, 0.45 μm) water samples were taken for the determination of SRP, trace metals, and major-ion analysis.

On removal of a sample tray for an experiment, additional entire sediment was obtained from each location to characterise the sediment for organic matter, porosity, density, and size fraction. Flow velocity at each sampling position was measured using a Geopacks Stream Flowmeter. Any large clumps or skeins of filamentous algæ on the surface of the stones were removed either before or after removal from stream-bed, and prior to any other treatments. These additional samples, used later for chlorophyll *a* analysis, were then placed in numbered plastic bags, sealed and stored in the dark until they could be frozen upon return to the laboratory.

Experimental Flumes

The flumes, developed from an earlier design (House *et al.*, 1995b), comprised a pair of re-circulating channels with controllable water flow (Gainswin *et al.*, (submitted)). Flow was measured using turbine flow transducers downline of the water pumps. Paired probes, fitted in the channels, measured pH, temperature, and conductivity, and a Li-Cor sensor monitored light levels. Both channels in the pair were treated in an identical manner. DO concentrations and conductivity were measured manually at regular intervals using the field meters.

A 2 mM CaCl₂ solution was used to simulate the ionic strength of R. Tame water. Twenty litres of this solution was put in the channels and they were covered, but not

sealed, and exposed to natural light. Next the sample trays were carefully introduced to the channels, to reduce disturbance of any fine material trapped in the biofilm, and the circulation started. The flow rate was set to give an average velocity of ~20 cm s^{-1}, which approximated to the average of the flows recorded *in situ* at the time of field sampling. Photographs and traced plan recorded the appearance of the surface sediment and biofilm in the trays. Samples of the overlying solution (filtered through 0.45 μm cellulose nitrate membrane) were taken manually prior to the start of the experiment and then, using an automated sampling and filtering system, at pre-set intervals of 120 minutes. An experimental period of 48 h was used to observe the release of SRP to the overlying solution.

At the end of each experiment photographs were taken of the surface sediment and biofilm, and a traced plan made, using acetate sheets, to record the appearance of each tray (Appendices 2.13 and 2.14). The plans identified the position of any visible stones > 20 mm diameter, the composition of areas between the stones by particle size, gravel (2 – 20 mm), and fines (< 2 mm), and any areas covered by dense algal growth. The overlying solution was then drained to the level of the sediment surface to allow the trays to be longitudinally sectioned for sediment characterisation. A small (~30 g) sub-sample was obtained from each of the trays for sediment porosity, density, and organic matter content determination. The trays were sectioned longitudinally through depth, and the 0-5 mm, and where necessary due to surface collapse the 5-10 mm, depth sections were analysed for chlorophyll *a*. Any stones greater than 10 mm size, which would impede the sectioning due to the design of the sectioning tool, were removed prior to this and stored separately before extraction. The chlorophyll *a* extracted from the surfaces of the samples and stones was measured to assess biomass (Marker, 1976).

Analytical Techniques.

Solutions were prepared using ultra-pure water of conductivity < 0.1 μS cm^{-1} at 20°C (Purite, Analyst HP). SRP analysis was carried out as described previously (Marker, 1972; Gainswin *et al.*, (submitted)) and using a colorimetric method (Murphy & Riley, 1962; Stephens, 1963).

For measurement of chlorophyll *a* to estimate biomass, the surface sediment layer (0-5

mm) of the trays and associated filamentous algæ were extracted in methanol (Marker, 1972, 1976; HMSO, 1983), and absorbance measured at 630, 645, 665, and 750 nm, using a spectrophotometer. The surface area of the water-sediment interface was taken as the total area of the trays containing the material. This was to reduce variabililty introduced by any potential differences in particle sizes. The precision of chlorophyll *a* sample collection and analysis, was established by using a nested analysis of variance technique (HMSO, 1983, 1984).

After drying the sediment at $105^{\circ}C$ overnight in a vial of known volume ($\pm$ 0.317 ml) porosity and density were calculated, and the organic matter (OM) content was obtained by ashing at $550^{\circ}C$ overnight. Suspended solids present in both stream-water at the site, and flume experiments, were measured by volumetric filtration (glass microfibre GF/F) and drying overnight $105^{\circ}C$. Where possible, sediment porewater was extracted for SRP analysis by centrifugation at a centrifugal force of ~9500 *g* for 20 minutes, and filtered (cellulose nitrate, 0.45 μm).

Theory

When the concentration of SRP in a solution in contact with a sediment does not change over a period of 24 h, the system is said to be in equilibrium. This has become known as the equilibrium phosphate concentration (EPC$_0$ (Taylor & Kunishi, 1971; Froelich, 1988; House *et al.*, 1995a; House *et al.*, 1995b; House & Denison, 2002), and has been used to predict the behaviour of a sediment when exposed to a phosphate solution. The EPC$_0$ (Klotz, 1991; Gardner *et al.*, 2002; Pan *et al.*, 2002) may be defined as the concentration of SRP in the interstitial water of a sediment at which no change in the concentration in an overlying solution occurs after a 24 h exposure to that sediment (House, 2003b). Furthermore, when the concentration of SRP in the overlying solution is greater than the EPC$_0$ the sediment will take up phosphorus, but SRP will be released from the sediment if the concentration is lower than the EPC$_0$ (House & Denison, 1997, 2000).

The EPC$_0$ of the sediments used in these experiments was established from measurements of the concentration of SRP in the overlying solution, during the period of an experiment, as it approached a steady-state. These EPC$_0$ values were obtained by calculation using a diffusion boundary layer (DBL) model (House & Denison, 2002). This model assumes

that the flux of SRP across the sediment-water interface, $dn(t)/dt$, where $n(t)$ is the amount of SRP passing per unit area of sediment at time t, is regulated by diffusion across the boundary layer

$$dn(t)/dt = D_m[c(t) - EPC_0]/\delta \qquad (1)$$

where $c(t)$ is the concentration of SRP at the top of the boundary layer, D_m is the molecular diffusion coefficient of the dominant phosphorus ion (HPO_4^{2-}) at the solution pH, with a boundary layer of thickness (δ) (House & Denison, 2002), which can be estimated by

$$\delta = 2500/v \qquad (2)$$

where δ is in μm and v is the water velocity in cm s^{-1}. If V_t is the total volume of the circulating water, and A_s is the surface area of the interface (in this case the total surface area of the trays used to contain the sediment, or the channel area for the stones), an effective depth of water for the channel (d) may be written as $d = V_t / A_s$. With the approximation that EPC_0 is constant over the length of the experiment (House, 2003b), then for the release of SRP from a sediment, equation (1) has the solution

$$c(t) = EPC_0[1 - \exp(-kt)] + c_0 \exp(-kt) \qquad (3)$$

where the rate constant $k = D_m/(\delta d)$ and c_0 is the concentration of SRP at the start of the monitoring – designated as $t = 0$, and allows the EPC_0 to be calculated from the kinetic data. By varying the EPC_0, and using root mean square (r.m.s.) deviation, agreement between the measured SRP concentration and the prediction (Eqn 3) was optimised.

In order that the heterogeneous composition of the trays could be described in terms of the size fractions and any associated biofilm, and a maximum flux be calculated when considering the natural mixed system in relation to the three different size components, the DBL model was adapted to express the limiting diffusion flux, f_L, for the release of SRP from the sediment when the concentration in the overlying solution is zero

$$f_{L} = -D_{m} EPC_0 / \delta \qquad (4)$$

The limiting diffusion flux when $c(t) \ll EPC_0$ is the same as the rate constant k_p used in a parabolic model that has also been used to describe the release kinetics (House & Denison, 2002). When c=0 each experiment with a dominant size fraction, *i.e.* stones, gravel or fines, yielded a limiting flux (Gainswin *et al.*, (submitted)). These fluxes, f_{Ln}, were used in conjunction with the fractional coverage of a particular size fraction, A_n, occupied within the total area, A_s, of the natural sediment. The resultant calculated flux, f_{Lc}, from the composite system could then be compared with the flux measured from the natural sediment and associated biofilm used in the channels

$$f_{Lc} = [(A_1 \ f_{L1}) + (A_2 \ f_{L2}) + (A_3 \ f_{L3})] / A_s \qquad (5)$$

where subscripts 1, 2 and 3 indicate the values associated with the stones, gravel and fines fractions respectively. These have been determined and discussed in a previous study (Gainswin *et al.*, (submitted)). The calculated values (Eqn 4) for the individual size fractions for stones, gravel, and silt respectively were 36, 29, and 2.2 nmol m^{-2} s^{-1} for BM, and 346, 27, and 6.4 nmol m^{-2} s^{-1} for SV.

It had been planned to calculate the area of the size fractions from the acetate maps but, because of the difficulty of distinguishing the proportions of the areal coverage for the two smaller size fractions, it was not possible to determine the percentage cover by this method. However, using a gravimetric method, the relative proportions of each size fraction in a sample could be estimated. The results of the gravimetric measurements for the stones' fraction and the percentage areal cover for the stones from the acetate maps were compared and a reasonable correlation, r = 0.77 (n = 10, p = 0.01) obtained. The mass ratios were thus considered a sufficiently close approximation of the surface area represented by each size fraction.

To be able to indicate the direction of the flux of SRP and perturbation from equilibrium for a sediment a Phosphorus Transfer Index (PTI) was used (House *et al.*, 1998).

$$PTI = (SRP/EPC_0 - 1) \qquad (6)$$

When the PTI is zero, the sediment is in equilibrium with SRP concentration in the overlying water, if a positive value, there is a net flux of SRP from the water to the sediment, and if negative, the net flux of SRP is from the sediment to the water.

Results and Discussion

The sediments used for these experiments were collected from both sites at intervals over the biofilm growth cycle between March, 2001 – April, 2002 (Table I), but excluded winter sampling because of the adverse river conditions. The two sites differ in a number of ways, primarily that BM is on the original river whereas SV is in a re-directed reach. At the SV site the river is roughly twice the width (~ 10 m) of the BM reach (~5 m), but both have a straight-sided and flat-bottomed channel cross-section. Both are dominated by coarser sediments, but the natural, non-mobile, bed-load of BM includes man-made detritus such as concrete and metal items, which provides additional substrate for filamentous algal growth, *e.g. Cladophora*, and traps finer sediments. Both sites were visually estimated to have bed area occupied by fine particulate material (< 2 mm in size) of less than 25 %, with the rest being approximately equally divided between gravel and stones. The gravel at BM was mostly within hard-packed bed sediment.

The antecedent flow conditions in the river prior to the time of sampling, and the number of days since a rainfall induced high flow event, are described in Table II, listed by experiment.

Solution Chemistry

Table I describes the physico-chemical constitution of the river water, and sediment characteristics, at the sampling times. The flow volumes recorded were greater at SV than at BM. This increased flow at SV is due to the contributions between the sites from four sewage treatment works (STW), two brooks, and diffuse inputs. These flow data were obtained from the Environment Agency, UK, recorded by their gauging stations located in the vicinity of the two sites. The water velocities measured at the sediment sampling positions varied substantially – from > 20 cm s^{-1} in riffle areas to < 5 cm s^{-1} in the areas where fine material accumulated. The lowest temperature at which samples

were obtained was recorded in March, 2001, at BM, and the highest temperature at SV in September, 2001. However, there was no major difference between the sites overall, and these temperatures are consistent with the times of day at which the measurements were made and seasonal averages. The pH, DO and conductivity were generally found to be higher at SV. The SRP measured from water samples taken at the time of sediment collection show SV to have consistently higher concentrations than BM, which is related to the effluent inputs. SRP concentrations were observed to peak during the summer months, and this was particularly evident at SV, though an unexpectedly low concentration was recorded for BM in June 2001. The summer peaks are indicative of the lower dilution of sewage effluent during the low flow periods. The sediment organic matter, porosity and density were comparable for both sites.

The character of the simulated river water used in the flume experiments is described in Tables III a, and III b, which also include data for the associated sediments. These physico-chemical characteristics include averages and minima/maxima extracted from all manual or automated readings taken during each experiment. Flow velocities were set to approximate the average flows recorded during sampling at the positions within the measured reach from which the samples were taken. The temperature measurements were slightly elevated compared with those recorded at the time of sampling in the river. This may have been due either to the hour of sampling, or a weather induced temperature differential over the country – the field site and laboratory being ~250 km apart. In the flume channels the mean solution pH was neutral except for SV solution in Experiment 1, which was slightly more alkaline, and in Experiment 6, which was mildly acidic. Mixing of the overlying solution by the re-circulating system lead to oxygenation remaining high throughout all the experiments, but with diurnal fluctuations. Levels of conductivity were generally higher at BM than at SV with the measurements made in spring having the highest values. In the experiments with more biofilm growth, increases in light levels during an experiment lead to increased photosynthesis, and the consequent reduction in CO_2 concentration resulted in the observed raised pH (Stumm & Morgan, 1970).

Sediment Characteristics

The additional samples of sediment collected simultaneously and contiguously with the samples used in the flume experiments yielded the organic matter, porosity and density

values given in Table I. Measurements of the chlorophyll *a* from the sediment used in the flume experiments can be found in Tables III a and III b.

In a sediment comprising mainly large grain-size material the organic matter can be an important constituent because gravel and stones are often coated by organic substrates which adsorb pollutants (Förstner, 1990). The organic content of the sediment varied considerably both between sites and experiments, but with the larger percentages generally found in the sediment with lower density.

The densities of the sediments were consistent with a high quartz content, except that taken from the vicinity of the sample used in Experiment 3 for SV, which was slightly higher than would have been expected from the organic content. The low density of the SV sample used in Experiment 6 is in accord with the high organic matter content. Typical sediment porosities of between ~30 and ~50% for mixed gravel and sand sediment (Todd, 1967) were exhibited by all samples except Experiment 6, which contained chiefly fine material, and that of Experiment 1 of BM. Porosities greater than 60 % indicate very loosely packed, silt material.

Algal Biofilm Development

The biomass of algæ in aquatic environments is commonly estimated using chlorophyll *a*, but complete extraction can be difficult to achieve (Marker, 1972; Marker & Casey, 1982), and therefore pigment determinations should not be regarded as a definitive technique. In this case however the method provided sufficient information to relate the growth rate to other factors and events, and a seasonal growth trend was evident (Figure 1). As expected biomass was found to increase during the spring bloom, especially with the development of filamentous algæ, including *Cladophora* spp., attached to the larger material. *Cladophora* is endemic in smaller rivers and streams (Haslam, 1978) and is commonly part of a stream community. In phosphate rich conditions, such as those found in the Tame, dense skeins of *Cladophora* are produced as the growing season progresses. This growth can lead to large diurnal oxygen fluctuations (HMSO, 1984). The values found (Table III) are a little lower than those of between 255-290 mg m^{-2} (25.5-29.0 μg cm^{-2}) in April in a chalk stream in Dorset, U.K, (Marker, 1976), and also of another study

in a similar stream where the averages from measurements extending over two years were 450 mg m^{-2} and 573 mg m^{-2} (Hartley, 1997). The biomass recorded for SV in June was particularly low considering the time of year and long period of low flow prior to sampling (see Figure 2 b). The hydrological and climatic conditions experienced by the Tame early in the year, *i.e.* streambed scouring by high-flow events (Figure 2), and cold weather slowing the onset of the spring bloom, may account for this lower biomass.

Soluble Reactive Phosphorus Dynamics

Diffusion across the sediment-water interface in fine silt deposits has been thought to govern phosphorus fluxes (Froelich, 1988; House *et al.*, 1995a; Haggard *et al.*, 1999), with the rate of this transport through the sediment being affected by the thickness of the 'active surface layer' (House *et al.*, 1995b). Thus, if silt sediments were removed by turbulence and stream-bed scouring during high flow events, the bed-sediment would have a diminished potential to remove phosphorus from, or release to, the overlying water. However, there has been no other comparable research on the rôle of coarser gravel and stone sediments in phosphorus exchange.

The SRP released into the overlying solution during each of the experiments using natural bed-sediment is shown in Figure 3. The concentrations measured for the sediment from BM are all below 10 μM (Figure 3 a); concentrations for the SV sediment (Figure 3 b) were all in excess of this with values ranging from ~ 11 – 30 μM. In Experiment 4 (in which sediment was collected from the river after there had been a prolonged period of low discharge - Figure 2) both sites resulted in relatively high concentrations.

The release of SRP into the overlying solution during the flume experiments (Figure 3) was compared with a release calculated using the diffusion boundary layer model (DBL) (House & Denison, 2002), (Figure 4, BM a – e and SV f – j). The diffusion model generally produced an excellent agreement with the data for the latter phase of the experiments, as shown by the low root mean square (r.m.s.) values in Table IV, when the longer-term release kinetics dominate the exchange. However, the diffusion model could not predict the initial fast activity of SRP, and this is most clearly shown by the intersection of the y-axis in Figure 4 j. The rapid initial exchanges, particularly evident in

Figure 4 c, were either caused by material trapped within the filamentous biofilm, or the release of SRP from particulates disturbed during the immersion of the material in the flume channels. This is supported by the relatively high proportion of fine material and biomass associated with this sediment sample ~27 % and ~92 mg m^{-2} respectively (Table V).

The SRP fluxes (f_M) calculated for SV samples were greater, up to ~180 nmol m^{-2} s^{-1}, than those from BM, and are high (Table VI) when compared with the flux measured by House and Denison (2002) for a silt sediment from the R. Blackwater, U.K.; another river impacted by treated sewage effluent, but which resulted in effluxes of up to only *ca.* 25 nmol m^{-2} s^{-1} during the sampling period. As a result, and surprisingly, a mixed grain-size sediment and attached biofilm, with entrapped fine material, produced a larger net release of SRP than a silt sediment with a flux similar to that for fine material alone (Experiments BM/SV 6). The lower values, particularly for Experiment 2 with the BM sample, occur when the river water concentrations of SRP are also low. A flux 5.6 nmol m^{-2} s^{-1} at BM occurred when the concentration of SRP in the river water was unusually low - 5.9 μM. All the sediments had comparable OM content, porosity and density, except those in Experiment 4, so the greater concentration of SRP in the overlying water at SV compared with BM is indicated as the main factor contributing to the difference in the EPC$_0$s and fluxes (Table VI). The EPC$_0$ values obtained for the SV sediments are higher than those from the experiments with the material from BM, but this is due to exposure to higher river water SRP concentrations at SV. Figure 5 (a and b) illustrates the correlation between the river water concentration of SRP at the time of sampling and both the EPC$_0$ (r = 0.93, n =10, p = 0.001) and measured fluxes for the natural sediment (r = 0.95, n = 10, p = 0.001), plus in Figure 5 c that of the measured flux (f_M) for the natural sediment and the limiting flux calculated (Eqn 5) for the composite sediment (f_{Lc}), which has an r = 0.89 (n = 10, p =0.001). The agreement between the EPC$_0$ and limiting flux correlations with SRP reflects the close link between f_L and EPC$_0$ expressed in Eqn (4).

Where stones are the dominant fraction of the natural sediment, the measured fluxes are greater than in the samples where there is a more even proportionality between different size fractions. This observation is confirmed by the results of the investigation using the individual size fractions. In those experiments, despite there being a difference in the

resultant flux between the sites of an order of magnitude, the stones and associated biofilm from both BM and particularly SV resulted in greater measured fluxes than either of the gravel or fines fractions. It has been observed in a previous study (House & Denison, 2002) that rapid exchange reactions become the rate controlling mechanism, rather than diffusion across the sediment-water interface, where fine particulate material is suspended in the water. Similar fast kinetics are expected for particulates trapped within the growth of filamentous algæ (and probably submerged macrophytes) that is in close contact with the flowing water (Gainswin *et al.*, (submitted)). The limiting diffusion fluxes calculated for the composite sediments are distinctly different for the two sites (Table VI), resulting in values an order of magnitude higher for SV, and when broken down into the component parts it can be seen that stones and the associated biofilm dominate the flux. This is particularly evident at SV where the stones to gravel/fines ratio was greatest (Table V).

A clear seasonal pattern is evident in the average chlorophyll *a* concentrations (Figure 1) measured over the period April 2001 - April 2002, with the spring bloom clearly indicated at both the beginning and end of the sampling period. The values for April 2002 were higher than the preceding April, but this may be explained by the higher temperatures experienced (Table I), or higher light levels (Jarvie *et al.*, 2002). The concentrations of SRP in the river water at the time the samples were removed from the river are superimposed and there is a pattern of higher concentrations during summer when flows are lowest and lower in winter when river discharge is greater. The data from the Environment Agency seems generally to confirm this observation (Figure 2).

Implications for the River Tame

The mean daily flow volumes presented in the hydrographs (Figure 2) for the year February, 2001 to February, 2002, show how the flow conditions experienced at both sites are highly variable, with spate volumes in excess of 4 m^3 s^{-1} above base flow at BM, and 10 m^3 s^{-1} above base flow at SV. During periods of high rainfall the contribution of treated sewage effluent from Ray Hall STW, which is situated between the BM and SV sampling sites, can be as much as 68 ML d^{-1} (pers. comm. Severn Trent Water) – resulting in a considerable input of SRP.

The concentration of SRP in river water and the EPC_0 are not the same, and hence the system is not in a steady-state, but reacting to fluctuations in the SRP concentration. However, the good correlation between these factors and the fluxes measured for the natural sediment (Figure 5 a and b) indicates that by governing the EPC_0, the SRP concentration is the main factor determining the limiting diffusion flux.

As the main factor determining limiting flux has been found to be the SRP concentration in the river, evidence is not clearly seen of other characteristics that are expected to be important influences. These factors include: the amount of biofilm present, which could be influenced by season and shading (growth rate), grazing by macroinvertebrates, and disturbance by high flow events (stripping of fine particulates from within the biofilm, or erosion of the biofilm itself); and antecedent flow conditions – causing accumulation or dispersal of fine material, and dilution or concentration of SRP in the water (Bowes & House, 2001; House, 2003b; Bowes *et al.*, (submitted)). The present data are insufficient to examine these in depth as limiting fluxes at comparable SRP concentrations are needed.

The concentration of SRP in the river water prior to sampling would affect the EPC_0 of the sediment and thus the flux from trapped particulate materials (greater exposure to SRP results in higher EPC_0 values). The higher EPC_0 values for SV indicates the site has been consistently exposed to higher river water concentrations of SRP and this has resulted in a greater amount of SRP being adsorbed onto the sediment there. All EPC_0 values were below the concentration of SRP in the river water at the time of sampling, and this resulted in positive PTI values (Table IV) and hence net uptake of SRP by the sediment at the time of sampling. The SV sediments released higher concentrations of SRP to the overlying solution, which corresponds with the EPC_0 values calculated (Table VI) and the spot samples of SRP concentration made in the field (Table I). These are consistent with those obtained by the Environment Agency (Figure 2) when the difference in the method of analysis is taken into account. The volume of discharge from the four sewage treatment works, which can be as much as 68 ML d^{-1} from an individual works, in this case Ray Hall (pers. comm. Severn Trent Water), at concentrations of up to almost 10 mg L^{-1} unfiltered reactive phosphorus UFRP (pers. comm. Environment Agency) supports these results. In most cases the EPC_0 values for both sites increased with the concentration of SRP in the river (Table IV), with large deviations from

equilibrium when the PTI is zero. Experiment BM 1 gave the closest approach to equilibrium with the water, *i.e.* PTI = 0.65, and at SV Experiment 7 was the closest to equilibrium, *i.e.* PTI = 1.75, and the same sample gave the largest amount of SRP released over 48 h. This is consistent with a relatively high concentration of SRP in the river, and a long period since a storm event allowing accumulation of silt in the biofilm. Overall, the PTI values for SV indicate that the longer the time elapsed since a storm event, which would reduce fine material present by re-suspension (Wood, 1977), the greater the capacity of the sediment to exchange phosphorus with the overlying water.

An example of when biomass estimates may be affected by the position of the trays at SV due to shading, and therefore affecting the algal growth, occurred in Experiment 4 where two of the three sample trays collected were from positions where trees on the bank created shade over the river. This effect would not be as strong at BM as there are no trees on either bank of the measured reach.

In a previous study (Gainswin *et al.*, (submitted)) the relative importance of the different size fractions with respect to the percent interaction of the SRP with the bed sediment was considered. In this previous study it was assumed that each substrate comprised the entire river bed for the 100 m reach at each site, the river discharge was taken as that at the time of sampling and a target SRP concentration was set at 3.2 μM. This concentration is the 0.1 mg l^{-1} proposed target (for lowland rivers on clay and alluvium) in the national eutrophication control strategy planned by the Environment Agency (Mainstone & Parr, 2002). The results of these experiments (Table IV) are consistent with the results of the previous work. The importance of the larger size fraction material is particularly evident in experiments using sediments from SV where the stones dominated the mix. The relative importance of the three substrates in terms of their SRP release is apparent from the breakdown of calculated limiting diffusion flux (Tables V and VI).

In these cases, all the sediment apart from that sampled for two of the BM experiments (in May and June, 2001) would release SRP to the overlying water. This was when the biomass was at the maximum recorded for BM and the fine fraction was greater than in the other experiments. These percentage interactions are linearly related to the length of

the measured river reach and so, in the absence of other processes and inputs, will determine the SRP concentration over the 7 km distance between sites, for a bed dominated by the larger size fraction material. Therefore, because these fractions produce the largest fluxes, they will have the most effect on the river water concentration of SRP because the bed sediment at both sites was predominantly composed of gravel and stones.

Conclusions

This is the first study to be made of the effects of a mixed grain-size sediment on the flux of SRP with respect to differences in behaviour caused by the size fractions which comprise most natural, heterogeneous river sediments. The main conclusions are summarised:

- The larger sized material (stones with a greater than ~20 mm diameter with an associated biofilm) in the study reach is a major control of the limiting flux. The measured and calculated fluxes resulting from these experiments indicate that larger size components in a sediment give rise to a greater release than a sediment mainly composed of fine sand and silt. This was observed at both sites, and especially at SV where the contribution from the gravel was less in relation to the stones.

- The SRP flux from the stones was predicted from the results of a previous series of experiments (Gainswin *et al.*, (submitted)) in which the behaviour of the three size fractions individually was investigated. The response is consistent with the results of these experiments using a natural mixed grain-size sediment from both BM and SV, as can be seen in Figure 5 c of the predicted versus measured flux. The limiting flux from the natural sediment, for SV in particular, is dominated by the effects of the flux from the stones and associated biofilm. Evidence from previous experiments (Gainswin *et al.*, (submitted)) indicates that reducing the sediment trapped in the biofilm has a direct effect on the reactivity of the sediment.

- Although the SRP concentrations at the two sites (Bentley Mill Way and Sandwell Valley) are very different, they react with the sediment in a similar way when the sediment composition is comparable. Unlike the flume experiments, where SRP in

the overlying solution was approaching a steady-state, the river system was not able to equilibrate and was continuously responding to variations in the SRP concentration.

- The concentration of SRP in the river water is a dominant factor in the mechanism controlling the limiting diffusion flux of phosphorus from a sediment because exposure to high concentrations of SRP will raise the EPC_0 and thus the limiting flux. A comparison of the equilibrium concentration value for a sediment and river water values can establish whether there would be a net influx or efflux of SRP to/from a sediment at that time.

- The results indicate a possible faster exchange response mechanism to be in operation, caused by trapped particulates very rapid interaction with SRP changes, compared with the diffusion mechanism in the settled bed sediment. The kinetics of these reactions confirms that another mechanism is involved than simple diffusion.

Table I. River discharge (*) and the physico-chemical constitution of the river water at Bentley Mill Way (BM) and Sandwell Valley (SV) on the River Tame, U.K., during the sampling periods including the soluble reactive phosphorus (SRP) concentration, and data for the associated river sediment (†) sampled concurrently.

	Bentley Mill Way (BM)				
Experiment	**1**	**2**	**3**	**4**	**7**
Date sampled	19-Mar-01	02-May-01	13-Jun-01	02-Aug-01	10-Apr-02
River discharge (m^3 s^{-1})*	0.9	1.0	0.6	1.6	0.5
Temperature (°C)	8.6	11.8	12.6	15.8	10.3
pH	5.6	7.0	7.2	7.0	7.0
DO (% saturation)	-	84.0	56.0	51.0	78.0
Conductivity (μS cm^{-1} at 25°C)	1199.0	1225.0	1368.0	-	1380.0
SRP (μM)	15.7	32.5	5.9	57.3	45.4
†Organic matter (%)	-	-	6.6	11.1	7.5
†Porosity (% average by site)	-	-	59.2	58.5	53.0
†Density (g cm^{-3})	-	-	-	2.5	2.5
	Sandwell Valley (SV)				
Experiment	**1**	**2**	**3**	**4**	**7**
Date sampled	18-Apr-01	02-May-01	13-Jun-01	02-Aug-01	09-Apr-02
River discharge (m^3 s^{-1})*	3.6	3.4	2.2	5.1	2.1
Temperature (°C)	9.8	10.8	15.3	17.6	12.0
pH	7.1	7.3	7.3	7.4	8.2
DO (% saturation)	-	102.0	111.0	85.0	85.0
Conductivity (μS cm^{-1} at 25°C)	-	1305.0	1437.0	-	1365.0
SRP (μM)	62.8	68.1	109.2	123.4	92.0
†Organic matter (%)	-	-	6.5	3.4	4.7
†Porosity (% average by site)	-	-	44.2	35.1	47.7
†Density (g cm^{-3})	-	-	-	2.9	2.7

*Data from the Environment Agency, U.K.

Table II: The antecedent flow conditions in the River Tame, U.K., prior to each experimental sample collection for the period March, 2001, to April, 2002, with the number of days elapsed since a high flow or storm event.

	Bentley Mill Way			Sandwell Valley	
Expt	Days	Conditions		Days	Conditions
1	2	Small event 2 days prior, part of a 2 week series after 1 month of low flow		8	3 weeks of high flow including a large event of 10 day duration 8 days prior
2	4	Small event 4 days prior, after ~1 month of high flow events		7	Small elevated flow event of 10 day duration 7 days prior
3	30	Large event 30 days prior with ~ 25 days of falling flow volume		26	Steady low flow for 26 days
4	15	Large but short duration event 15 days prior, but overall steady low flow volume		14	Large event 14 days prior during period of steady low flow
5	23	Series of small events 23 days prior		21	Rising base flow with events
6	1	~ 30 day series of small to medium events		5	17 days of elevated base flow with high volume event
7	21	flow gradually falling to low		21	Flow gradually falling to low

Hydrometric data provided by the Environment Agency, U.K.

Table III a. Physico-chemical constitution of the overlying solution and associated sediment (†) from Bentley Mill Way (BM) used in the flume experiments. Means and minima/maxima for temperature, pH, dissolved oxygen (DO), conductivity, and mean/maximum for light intensity.

	Bentley Mill Way (BM)									
	1		**2**		**3**		**4**		**7**	
Date sampled	19-Mar-01		02-May-01		13-Jun-01		02-Aug-01		10-Apr-02	
Flow rate (cm s^{-1})	20		20		20		20		20	
Mean Temp (°C)	11.3		14.5		18.5		19.6		13.5	
Temp (°C)	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
	9.8	13.3	13.0	15.9	17.1	20.9	17.6	21	12.5	14.8
Mean pH	8.3		7.9		7.6		7.5		7.3	
pH	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
	7.9	8.7	7.3	8.6	7.3	8.1	6.6	8.3	6.7	7.9
Mean DO	-		94.5		63.9		94.8		89.1	
DO	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
(% saturation)	-	-	90.8	97.7	55	79	82	105.9	79.6	96.7
Mean Conductivity	864		566		530		481		567	
Conductivity	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
(µS cm^{-1} at 25°C)	757	1030	509	598	510	549	454	519	521	607
Light Intensity	av.	max.	av.	max.	av.	max.	av.	max.	av.	max.
(µE m^{-2} s^{-1})	89.8	345.2	82.5	612.3	91.8	816.3	56.3	382.3	40.7	262.5
† Chlorophyll *a* (mg m^{-2})	-		83.6		92.2		20.8		68.9	
†Organic matter (%)	-		3.0		9.1		9.9		2.4	
†Porosity (% site averages)	70.3		46.6		54.6		52.6		52.9	
†Density (g cm^{-3})	2.6		2.7		2.6		2.4		2.6	

Table III b. Physico-chemical constitution of the overlying solution and associated sediment (†) from Sandwell Valley (SV) used in the flume experiments. Means and minima/maxima for temperature, pH, dissolved oxygen (DO), conductivity, and mean/maximum for light intensity.

| | Sandwell Valley (SV) | | | | | | | | | |
	1		**2**		**3**		**4**		**7**	
Date sampled	18-Apr-01		02-May-01		13-Jun-01		02-Aug-01		09-Apr-02	
Flow rate (cm s^{-1})	20		20		20		20		20	
Mean Temp (^{o}C)	13.0		14.8		19.2		20.0		13.7	
Temp (^{o}C)	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
	11.6	14.6	12.7	16.4	17.8	21.9	17.9	22.1	12.4	15.3
Mean pH	7.4		7.9		7.7		7.5		7.6	
pH	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
	6.5	8.2	7.3	8.8	7.4	8.0	6.7	8.4	6.9	8.6
Mean DO	69		92		88.2		85.8		-	
DO	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
(% saturation)	63.8	76.3	70.8	103.7	83.0	93.3	28.5	113.5	-	-
Mean Conductivity	480		530		560		461		553	
Conductivity	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
(μS cm^{-1} at 25°C)	441	501	489	577	560	560	436	481	509	580
Light Intensity	av.	max.	av.	max.	av.	max.	av.	max.	av.	max.
(μE m^{-2} s^{-1})	-	-	82.6	702	92.7	802	85.1	622	46.3	480
† Chlorophyll a (mg m^{-2})	22.1		100.9		56.7		14.1		35.4	
†Organic matter (%)	2.7		6.8		5.8		4.2		9.7	
†Porosity (% site average)	39.4		40.0		42.6		33.3		44.6	
†Density (g cm^{-3})	2.8		2.8		3.0		2.6		2.7	

Table IV. Initial concentrations (c_0) of SRP in the flume experiments, rate constant (k) values, equilibrium phosphate concentrations (EPC$_0$) with associated R^2 and r.m.s, for Experiments 1 to 4 and 7 from Bentley Mill Way (BM) and Sandwell Valley (SV). Also, river water SRP concentrations at time of sampling, the Phosphorus Transfer Index (PTI) values, and the percent interaction of P with the bed sediment at a river water SRP concentration of 3.2 µM.

Experiment		c_0 (μM)	k (10^{-5} s^{-1})	EPC$_0$ (μM)	R^2	r.m.s.	River SRP (μM)	PTI	% P Interaction
BM	1	1.76	9.1	9.51	0.83	0.72	15.7	0.65	0.40
	2	1.79	1.2	2.76	0.9	0.77	32.5	10.78	-
	3	0.35	2.2	1.16	0.46	0.6	5.9	4.09	-
	4	4.47	2.0	9.07	0.68	3.11	57.3	5.32	0.26
	7	2.19	1.2	6.3	0.85	2.47	45.4	6.21	0.37
SV	1	6.23	1.4	21.0	0.83	2.38	62.8	1.99	0.59
	2	1.7	1.1	17.4	0.69	5.21	68.1	2.91	0.51
	3	2.3	1.8	34.5	0.91	4.77	109.2	2.17	1.97
	4	6.85	1.8	35.3	0.76	7.06	123.4	2.50	0.95
	7	2.64	1.0	33.5	0.84	6.77	92	1.75	1.83

Table V. The calculated SRP flux (f_M) and percentage interaction of phosphorus with the bed sediment, calculated from experimental measurements at Bentley Mill Way (BM) and Sandwell Valley (SV) on the River Tame, U.K. The average percentage by mass between fine material < 2 mm, gravels of 2 - 20 mm, and stones > 20 mm, at the time the time of sampling, and biomass (as chlorophyll a), from the content of the experimental sampling trays. Also, river discharge at time of sampling.

				Bentley Mill Way			
Exp't	Date sampled	f_M (nmol m^{-2}s^{-1})	% by mass Fines	% by mass Gravel	% by mass Stones	Biomass (mg m^{-2})	Discharge (m^3 s^{-1})*
1	19-Mar-01	37.32	33.6	25.5	40.9		0.9
2	02-May-01	11.91	23.1	29.6	47.3	83.6	1.0
3	13-Jun-01	5.63	26.7	34.9	38.4	92.2	0.6
4	02-Aug-01	45.34	11.9	29.8	58.3	20.8	1.6
7	10-Apr-02	26.35	11.0	26.5	62.4	10.3	0.5
				Sandwell Valley			
Exp't	Date sampled	f_M (nmol m^{-2}s^{-1})	% by mass Fines	% by mass Gravel	% by mass Stones	Biomass (mg m^{-2})	Discharge (m^3 s^{-1})*
1	18-Apr-01	87.67	21.9	25.0	53.1	22.1	3.6
2	02-May-01	75.16	10.6	22.6	66.8	100.9	3.4
3	13-Jun-01	172.22	9.4	17.7	73.0	56.7	2.2
4	02-Aug-01	181.11	3.6	19.2	77.2	14.1	5.1
7	11-Apr-02	140.19	11.2	14.6	74.3	6.4	2.1

* *Data from the Environment Agency*

Table VI. The flux of SRP calculated from the natural mixed grain-size sediment used in the flume experiments (f_M), and the limiting diffusion flux (f_{Lc}) calculated for a composite sediment, and the limiting flux for each component of the composite. Also, the concentration of soluble reactive phosphorus in the river water at the time of sampling, with the biomass (measured as cholorophyll a), and the number of days since a high discharge event*.

Bentley Mill Way

Exp't	f_M (nmol m^{-2} s^{-1})	Area stones (m^2)	Area gravel (m^2)	Area fines (m^2)	f_{Lc} (nmol m^{-2} s^{-1})	f_{Lc} stones (nmol m^{-2} s^{-1})	f_{Lc} gravel (nmol m^{-2} s^{-1})	f_{Lc} fines (nmol m^{-2} s^{-1})	SRP (μM)	Biomass (mg m^{-2})	Days*	EPC$_0$
1	37.32	0.033	0.020	0.027	15.48	14.8	0.59	0.06	15.7		2	9.51
2	11.91	0.057	0.036	0.028	18.24	17.2	1.02	0.06	32.5	83.6	4	2.76
3	5.63	0.046	0.042	0.032	15.20	13.9	1.20	0.07	5.90	92.2	30	1.16
4	45.34	0.070	0.036	0.014	22.20	21.1	1.03	0.03	57.3	20.8	15	9.07
7	26.35	0.075	0.032	0.013	23.58	22.6	0.92	0.03	45.4	10.3	21	6.30

Sandwell Valley

Exp't	f_M (nmol m^{-2} s^{-1})	Area stones (m^2)	Area gravel (m^2)	Area fines (m^2)	f_{Lc} (nmol m^{-2} s^{-1})	f_{Lc} stones (nmol m^{-2} s^{-1})	f_{Lc} gravel (nmol m^{-2} s^{-1})	f_{Lc} fines (nmol m^{-2} s^{-1})	SRP (μM)	Biomass (mg m^{-2})	Days*	EPC$_0$
1	87.67	0.064	0.030	0.026	184.5	183.5	0.81	0.17	62.8	22.1	8	21.0
2	75.16	0.080	0.027	0.013	231.7	230.9	0.74	0.08	68.1	100.9	7	17.4
3	172.22	0.088	0.021	0.011	252.9	252.3	0.57	0.07	109.2	56.7	26	34.5
4	181.11	0.093	0.023	0.004	267.5	266.8	0.62	0.03	123.4	14.1	14	35.3
7	140.19	0.062	0.017	0.013	179.6	179.0	0.47	0.71	92.0	6.40	21	33.5

Discharge data from the Environment Agency, U.K.
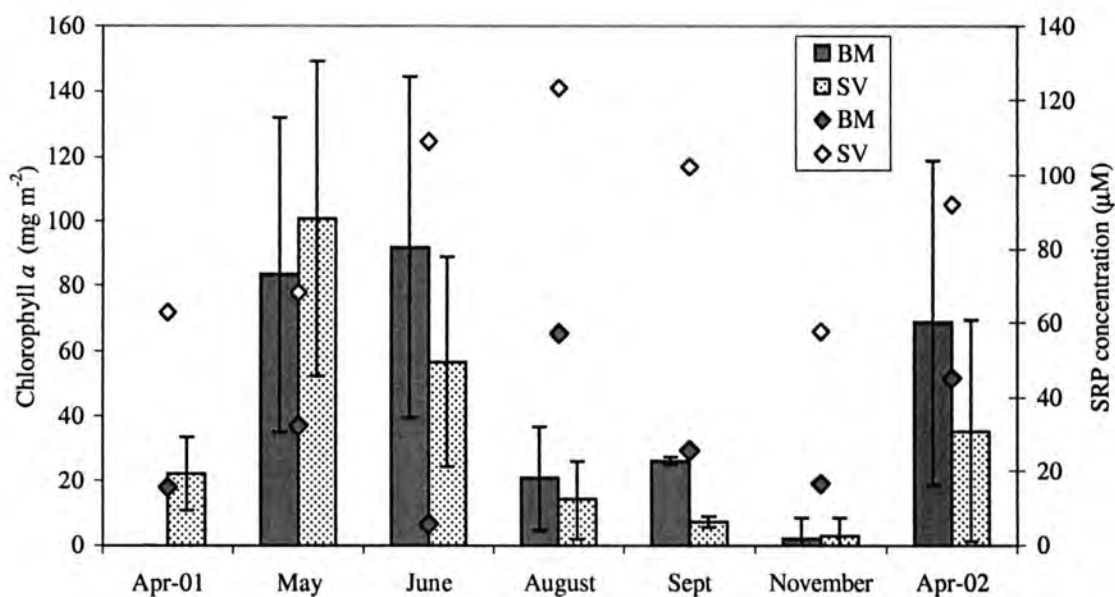
Figure 1. Averages of chlorophyll *a* for both sites over the entire sampling period, estimated from the surface of the sediment collected for each experiment. Error bars signify ± 2 standard deviations. No measurements were made of the chlorophyll *a* in Experiment 1 (BM) in April, 2001. Points represent the soluble reactive phosphorus concentration measured in the river water at the time of sampling - closed diamond BM, open diamond SV.
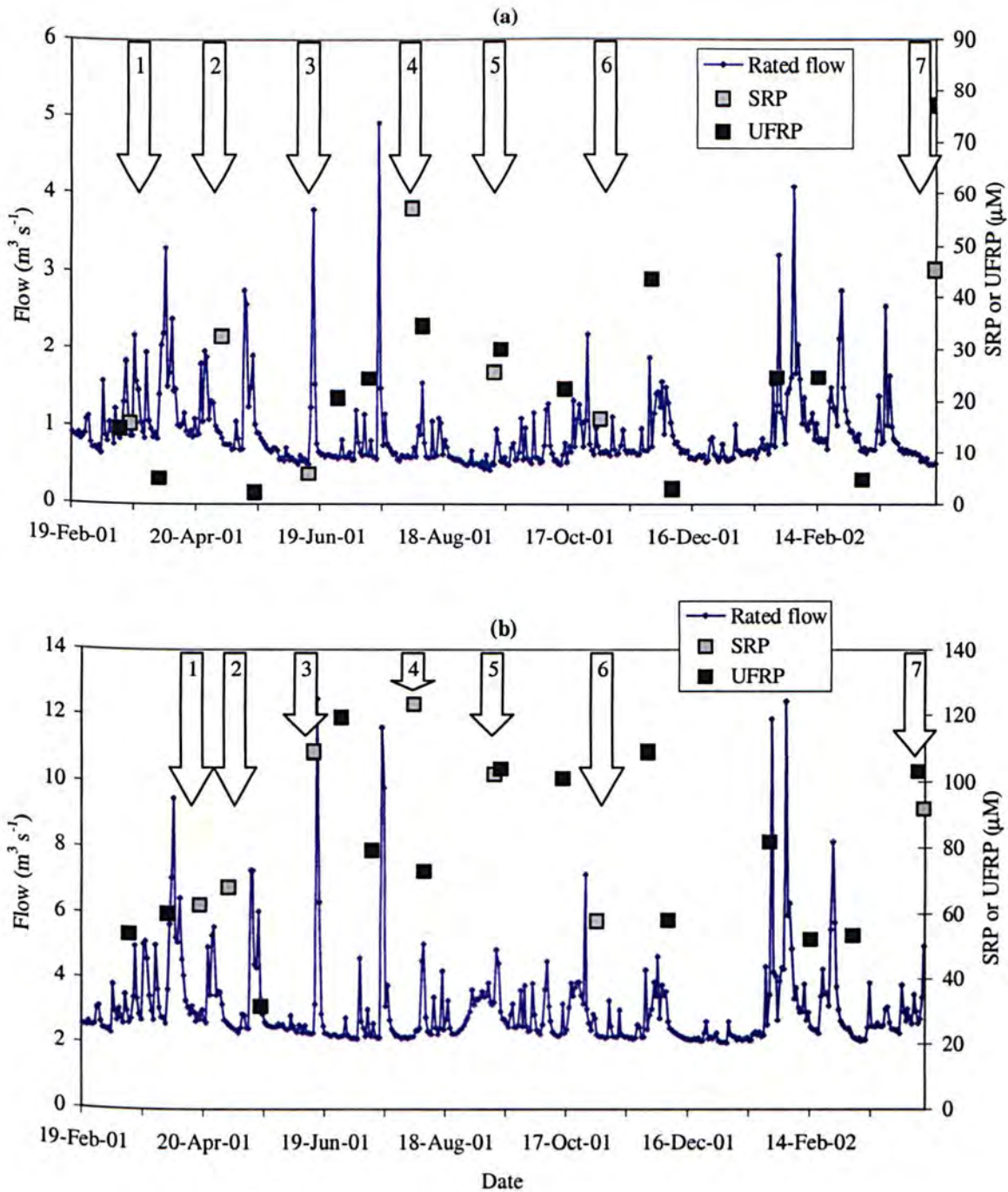
Figure 2. Hydrographs at (a) Bentley Mill Way (BM) and (b) Sandwell Valley (SV) for the year of the sampling period. Arrows indicate the time when the trays were retrieved; the numbers correspond to the experiment numbers (Table I). River water concentrations of SRP (closed points) on sampling dates and unfiltered reactive phosphorus (UFRP) as measured by the EA on the day closest to the sediment sampling dates (shaded symbols) are shown on the second axis. Daily mean Q and spot UFRP concentration data from the Environment Agency, U.K. It should be noted that the scale on the y-axes of these plots differs.
Note: Numbers 5 and 6 represent size fractions only experiments.
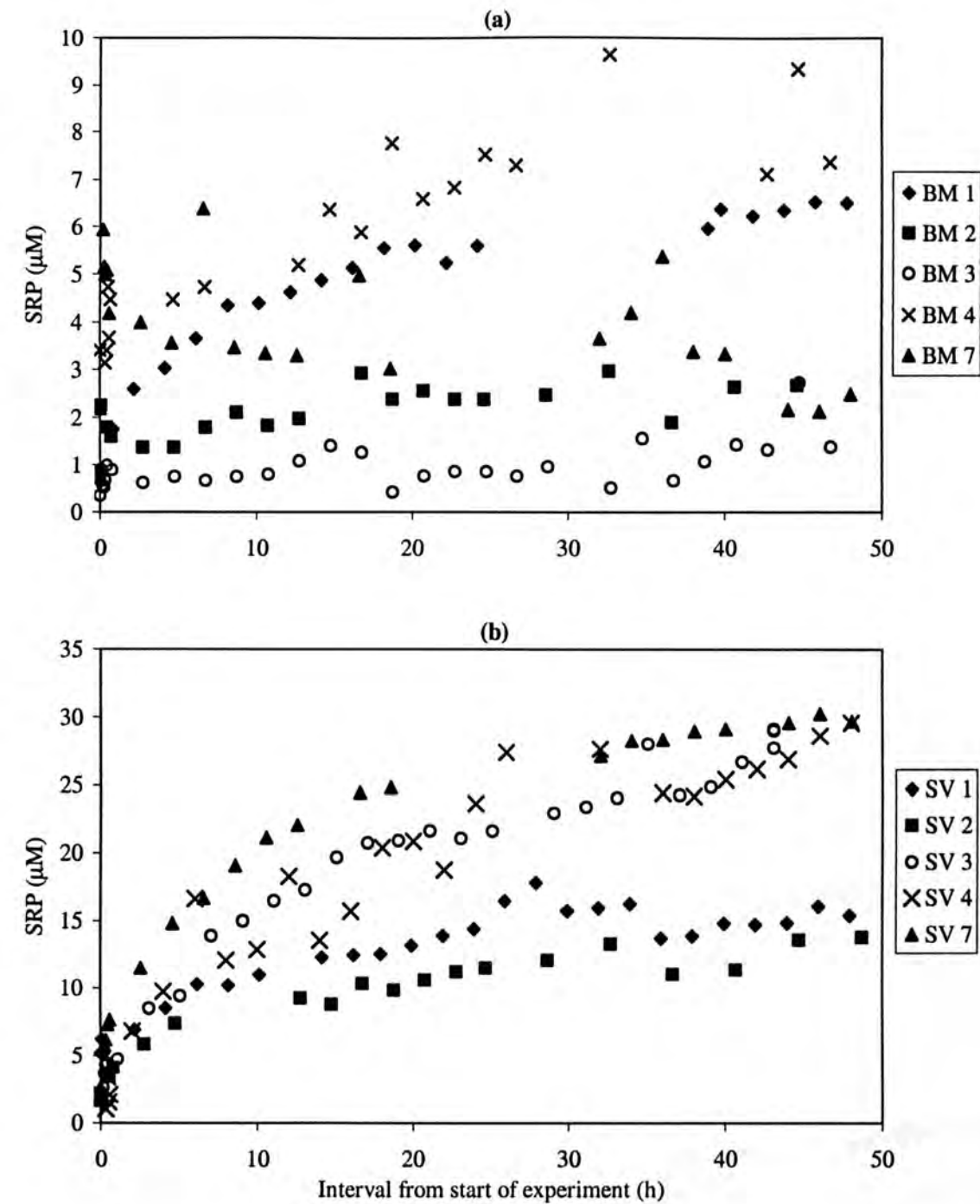
Figure 3. Measured concentrations of SRP released to the overlying solution in Experiments 1 to 4 and 7 utilising natural mixed sediment from a)   Bentley Mill Way (BM) , and b) Sandwell Valley (SV) over a 48 h period.   The samples were collected over the period of one year - spring 2001 to spring 2002.   It should be noted that the scale on the y axis differs between these plots.
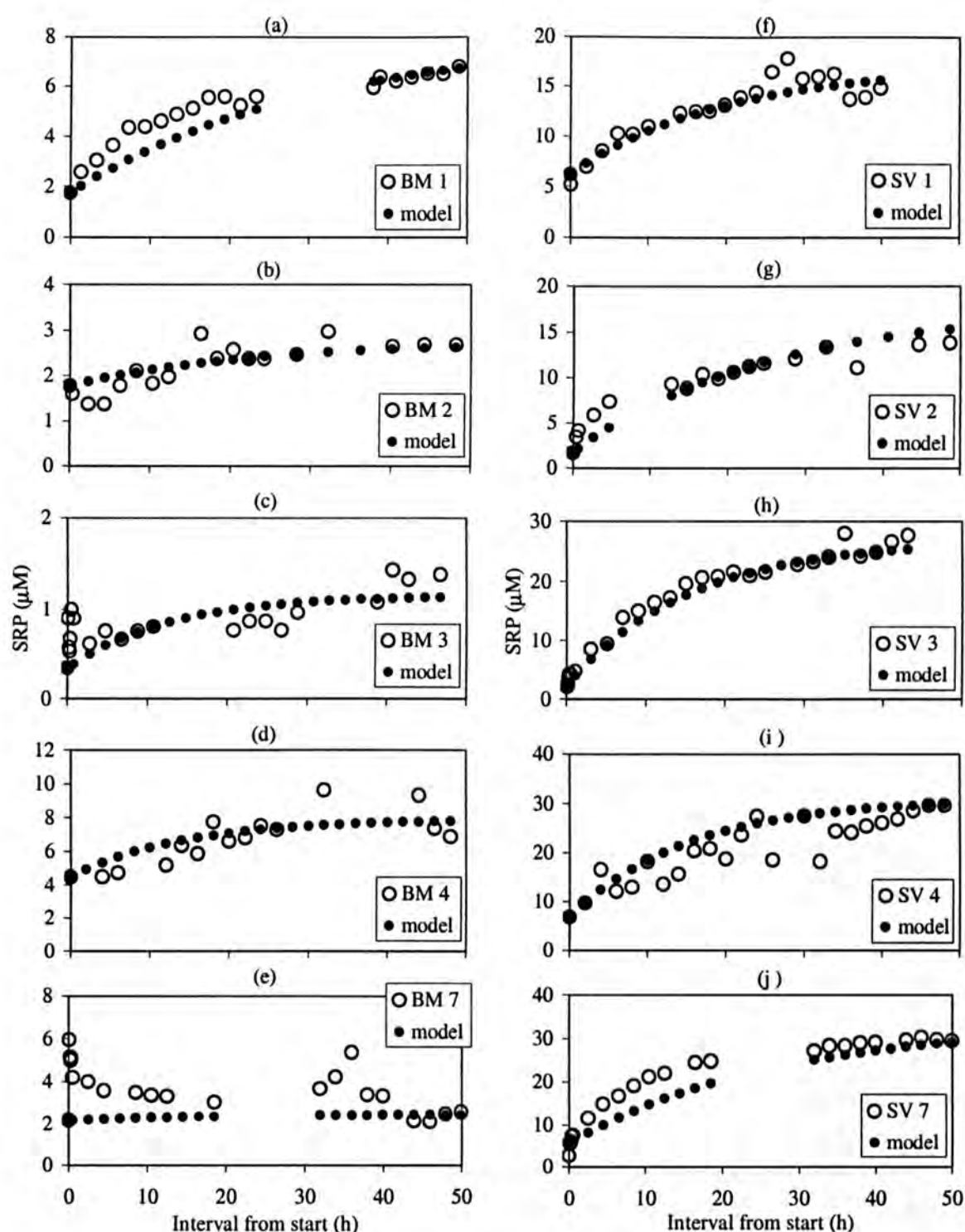
Figure 4. Release of soluble reactive phosphorus to the overlying solution in the flume channel Experiments 1 to 4 and 7 from: (a -e) Bentley Mill Way (BM), and (f - j) Sandwell Valley (SV), using natural mixed sediment samples. The samples were collected over the period of one year - spring 2001 to spring 2002. It should be noted that the scale on the y axis differs between these plots. Comparison of experimental results (open points) and the modelled release (closed points) using the diffusion boundary layer model. It should be noted that the scale on the y-axis of these plots differs.
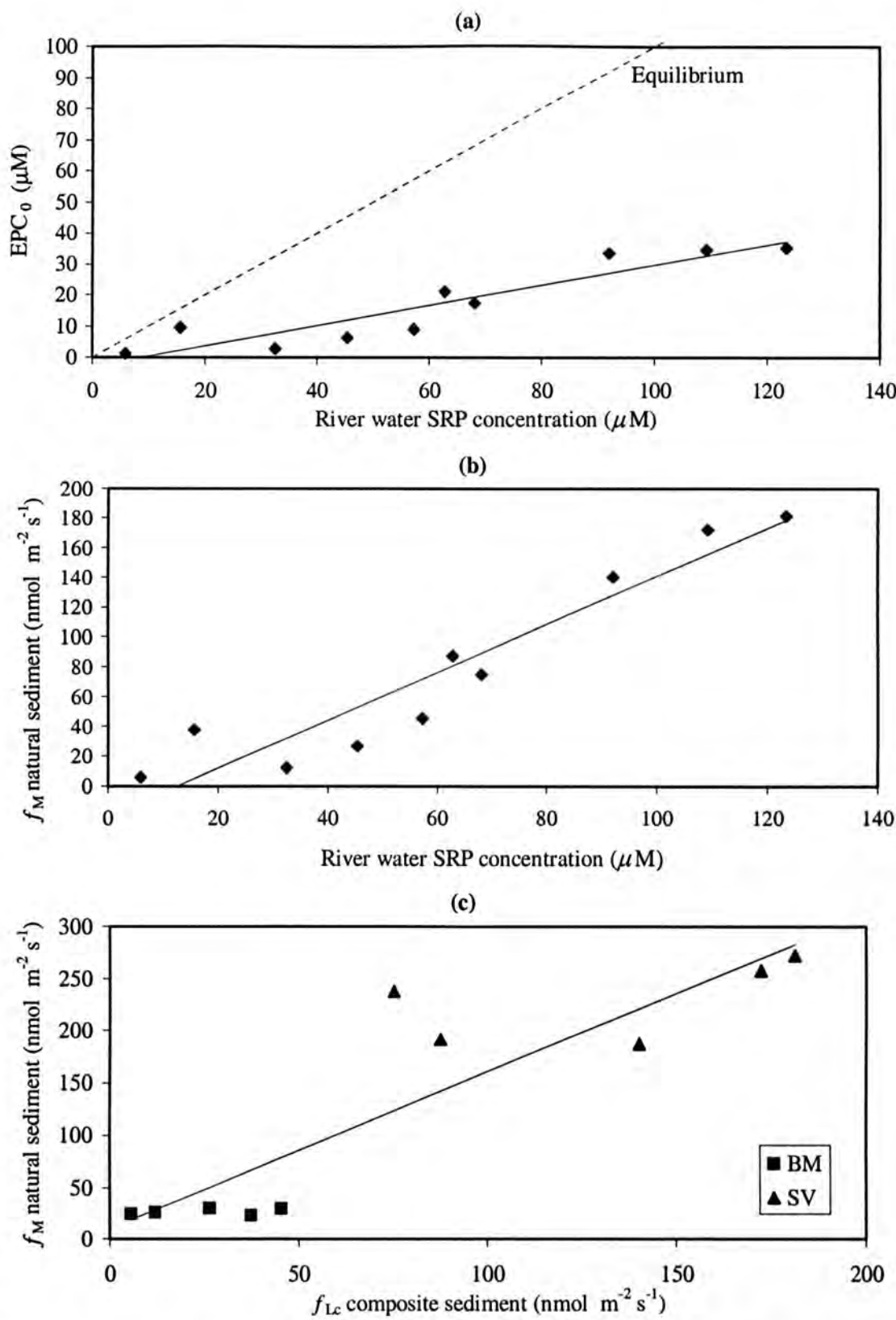
Figure 5. Combined results for the two sites - a) the correlation between the river water concentration of SRP at the time of sampling with the measured EPC_0 for each of the natural mixed grain-size experiments; b) correlation between river water SRP and the measured fluxes for the natural mixed sediment; and c) correlation between measured fluxes from the mixed sediment and the predicted limiting fluxes from the composite sediment comprising three grain-size fractions (< 2mm, 2 - 20 mm, and > 20 mm).

Chapter 5

Copper

5.1: Introduction

A great deal of research has been carried out investigating the trace metals which have health and economic effects both on humans and ecosystems, and in particular copper. The interactions of copper with suspended sediment has been closely examined, and much work has been done in marine and estuarine environments. However, copper is one of the significant trace metal pollutants in fresh water as well (Yan & Pan, 2002), and in urban environments the contribution from sewage and surface run-off (Boller & Steiner, 2002) can be significant.

Much of the copper load in a river is associated with particulate material (Herzl *et al.*, 2003) – adsorbed to surfaces and so not bioavailable (Xue & Sigg, 1993). Thus, because high-flow events usually increase the flux of fine suspended material (Kuhnle, 1992; Xue *et al.*, 2000), this material with its large surface area, can transport adsorbed metals over long distances. Changes in water conditions are required for the copper to be released from the particles (Stumm & Morgan, 1970), and being such dynamic systems the reactions of copper in rivers needs to be well understood.

Although it is a vital micronutrient copper can also affect the growth of plant material and have lethal effects on the animals in aquatic systems. This toxicity is connected to the free Cu^{2+} ions in solution (Yan & Pan, 2002).

In this study changes and variability in the kinetics of dissolved copper sediment-water exchanges were investigated. This was achieved by regular periodic sampling from the river, and maintaining the natural integrity of the bed-sediment samples used in the experiments. Also, the larger size material in a river bed-sediment – the gravel and stones and associated filamentous macroalgæ - was examined for contributions to copper release and uptake. The River Tame has both long-term and contemporary exposure to copper, and, with a heterogeneous mix of sediment grain-size and substantial seasonal algal development, it was found to contribute valuable insight into the effects of disturbance of the sediment.

5.2: Methods and Materials

5.2.1: Field Surveying and Sampling

Field surveying and sampling techniques, and the sampling equipment installed in the river, have been fully described in Chapter 2, Section 2.2.1. The samples for copper analysis were collected on the same dates, and at the same times and locations, as those for the phosphorus analysis.

Environment Agency (EA) river water copper concentration data were obtained using filtered samples from the EA Sampling Points noted in Section 2.2.1.1.

5.2.2: Flume Channels

A complete description of the flume channels and sampling equipment is given in Section 2.2.2. This includes a schematic of the channels and an explanation of the treatment of the sediment in the collection trays at the end of the experiments.

The sample analysis regime differed through the sequence of the experiments. In the first two experiments (BM 1 and 2, and SV 1 and 2) all samples obtained over the 48 h sampling period were analysed for copper concentration. However, as the budget for trace metals analyses was limited, and experience showed that rapid changes occurred at the start of the experiments and concentrations became more stable with time, the number of samples analysed was reduced in the later experiments.

5.2.3: Analytical Techniques

5.2.3.1: Copper

On the day of sampling, the samples were filtered using a 0.45 μm cellulose nitrate membrane. The filtrate was then acidified to 1% to help prevent precipitation/adsorption of the trace metals during transport and storage. Aristar, or Ultrapur, grade concentrated nitric acid was used for acidification.

The dissolved copper concentration was then determined by ICP-MS. Details of the ICP-MS equipment, together with the calibration range and detection limit for copper, are given in Chapter 2, Section 2.3.2.

5.3: Results and Discussion

The physical properties and chemical composition of the river water at the time of sampling for all experiments is given in Table V.I. River discharge was greater at Sandwell than Bentley Mill Way and this is as expected due to the contributions from the Rough and Ford Brooks, diffuse inputs, and sewage treatment works between the sites. With the exception of May, 2001, the water at SV was warmer than at BM, but spring water temperatures at both locations were below the seasonal average. This would have influenced both the water pH and dissolved oxygen (DO) content (Gundersen & Steinnes, 2003). Although mostly in the neutral range, pH, at BM was generally lower than SV, with March, 2001, recording the lowest value. In general DO concentrations were also lower at BM than SV, as was the recorded conductivity of the water. In addition to the experimental material, specimen sediments were taken from each site at the time of sampling and the organic matter (OM) content, porosity, and density of the sediments were measured. The OM content and porosity were lower at SV, but the density of the material is consistent with the mixed grain nature of the sediments at both sites, with a predominance of quartz-type sand.

Concentrations of dissolved copper found in the river water collected for this study are compared with data compiled by the U.K. Environment Agency in Table V.II. It was not possible to compare the two sets of data exactly because the sampling dates do not coincide. The table also contains a breakdown of the particle size composition of the sediment in each experiment - defined here as stones (> 20 mm), gravel (2 - 20 mm), and fines (< 2 mm) - by percentage mass.

A month by month comparison of spot sampled dissolved copper concentrations in the river water over the period of sampling is shown in Figure 5.1 with the river discharge hydrographs for BM and SV for the period April, 2001 to April, 2002. In general copper
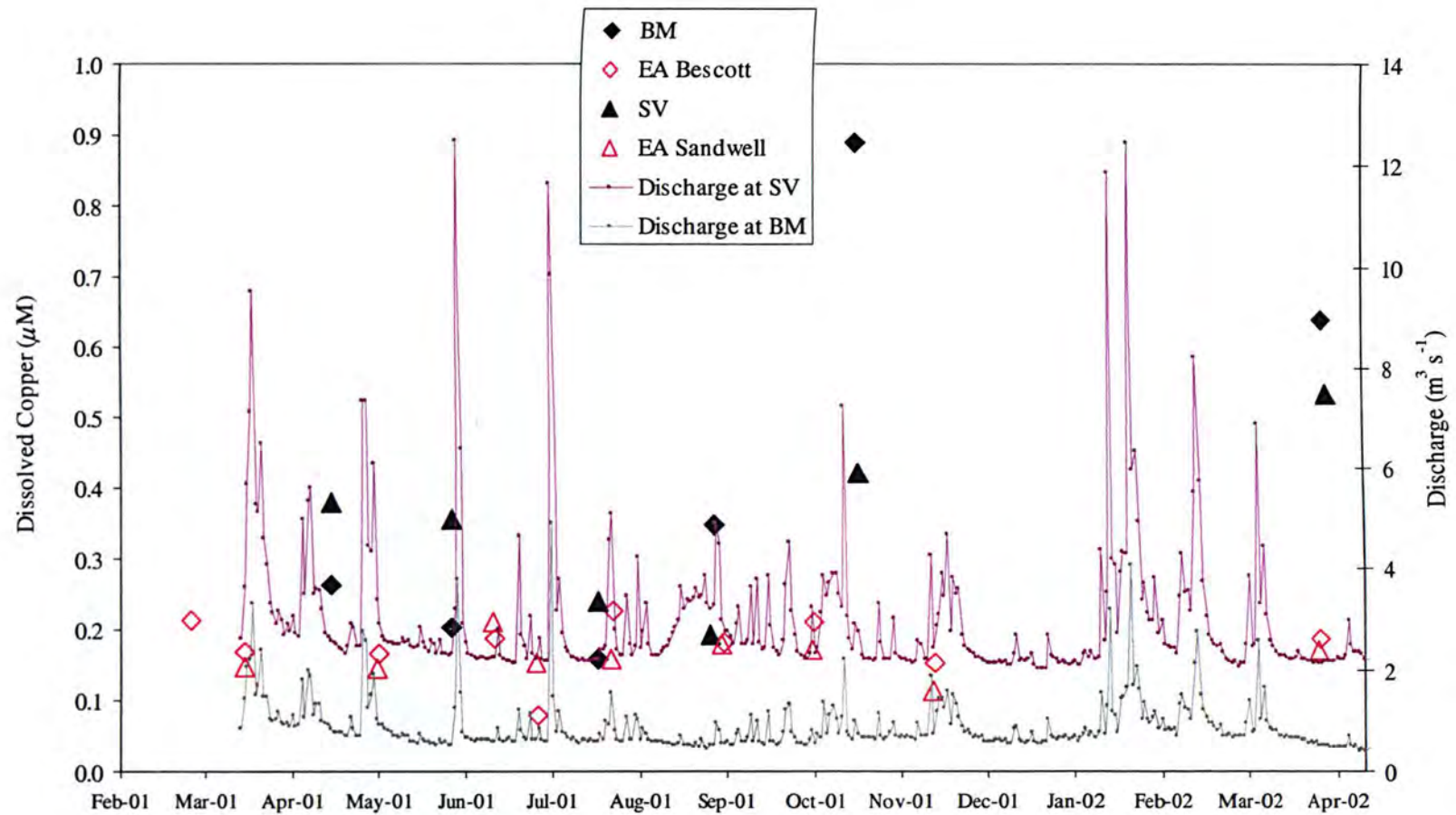
Figure 5.1: River water copper concentrations on dates of sampling from the River Tame, U.K., at Bentley Mill Way (BM) and Sandwell Valley (SV), with corresponding Environment Agency data from the date closest to the sampling date. The Environment Agency sampling point: 59022250 River Tame Bescott (NGR SP0040096200) is the closest available to Bentley Mill Way and is 2.3 km downstream of the BM sampling site. Hydrograph* shows river discharge at SV over the period April, 2001, to November, 2001; data for April, 2002 not available. *Data supplied by the Environment Agency, U.K.
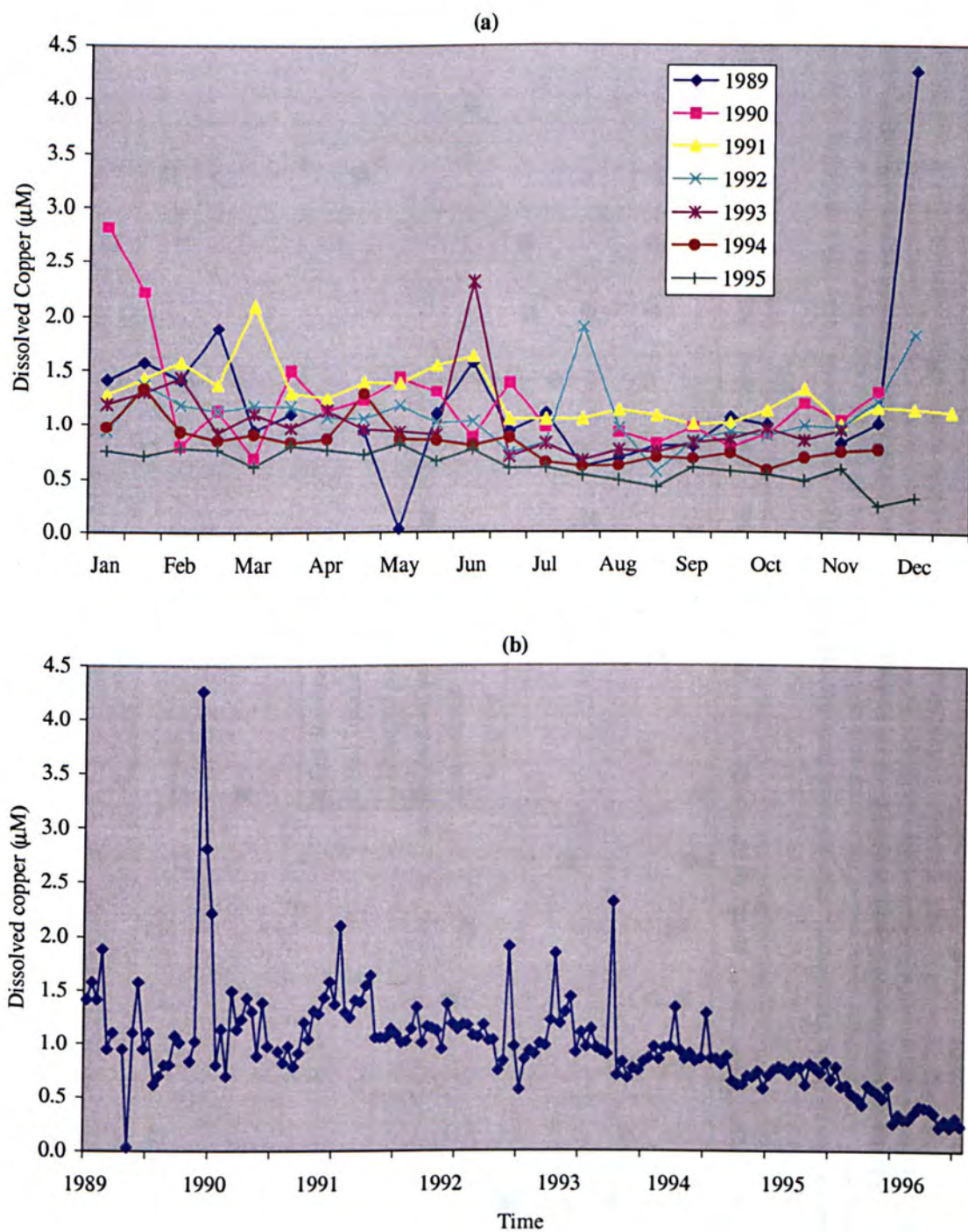
Figure 5.2: Dissolved copper concentration in the River Tame at Sandwell Valley measured bi-monthly between 1989 and 1996; plotted (a) concurrently excluding 1996 data and (b) sequentially including data for 1996. Data supplied by the Environment Agency, U.K.
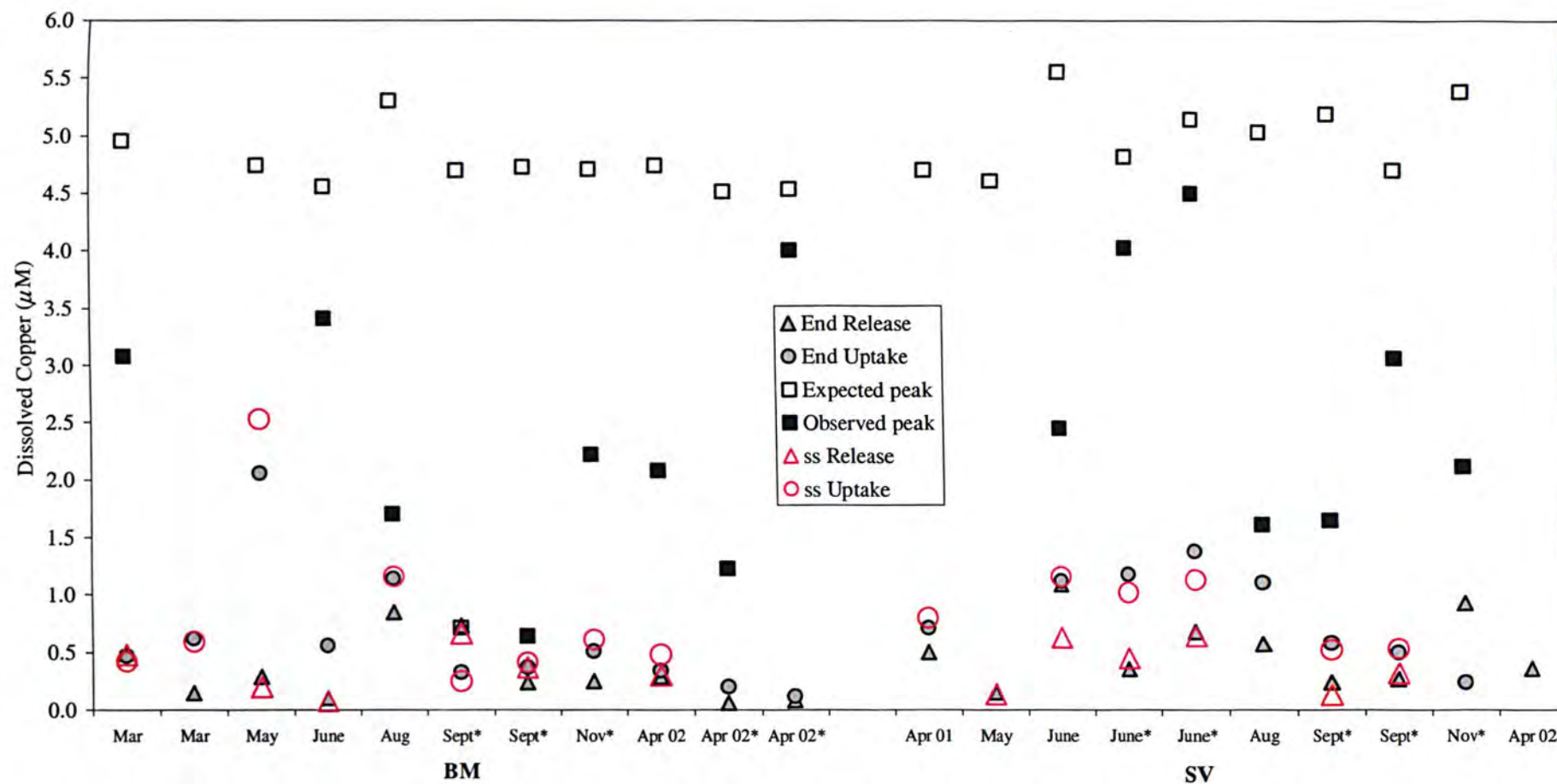
Figure 5.3: Dissolved copper concentrations for all flume experiments, with sediment from Bentley Mill Way (BM) and Sandwell Valley (SV), including those with the individual size fractions, which are marked (*). Concentration at the end of the release part of the experiment, after 48 h, and at the end of the uptake experiment (after a further 48 h) are shown together with the calculated steady state (ss) concentration for each, as the triangular and circular points. The open and closed square points give the expected and observed concentrations after the introduction of a copper standard addition.

Table V.I: Physico-chemical constitution of the river water at Bentley Mill Way (BM) and Sandwell Valley (SV) during the sampling period, March, 2001, to April, 2002, including temperature with temperature deviation from seasonal average (dev), dissolved oxygen (DO), and data for the associated river sediment (†) sampled concurrently.

	Bentley Mill Way (BM)						
Experiment	1	2	3	4	5	6	7
Date sampled	19-Mar	02-May	13-Jun	02-Aug	11-Sep	31-Oct	10-Apr
Discharge (m^3 s^{-1})*	0.9	1.0	0.6	1.6	0.6	0.8	0.5
Temperature (°C) (dev)	8.6 (-3.7)	11.8 (-0.5)	12.6 (-2.5)	15.8 (0.7)	17.2 (5.5)	12.2 (0.5)	10.3 (0.2)
pH	5.6	7	7.2	7	7.2	7.2	7.0
DO (% saturation)	-	84	56	51	-	67	78
Conductivity (μS cm^{-1} at 25°C)	1199	1225	1368	-	1094	841	1380
†Organic matter (%)	-	-	6.6	11.1	6.9	8.3	7.5
†Porosity (% average by site)	-	-	59.2	58.5	48.9	65.5	53.0
†Density (g cm^{-3})	-	-	-	2.5	2.6	2.5	2.5

	Sandwell Valley (SV)						
Experiment	1	2	3	4	5	6	7
Date sampled	18-Apr	02-May	13-Jun	02-Aug	10-Sep	31-Oct	09-Apr
Discharge (m^3 s^{-1})*	3.6	3.4	2.2	5.1	3.0	3.2	2.1
Temperature (°C)	9.8 (-2.5)	10.8 (-1.5)	15.3 (0.2)	17.6 (2.5)	17.8 (6.1)	12.2 (0.5)	12.0 (1.9)
pH	7.1	7.3	7.3	7.4	8	7.6	8.2
DO (% saturation)	-	102	111	85	-	63	85
Conductivity (μS cm^{-1} at 25°C)	-	1305	1437	-	1241	817	1365
†Organic matter (%)	-	-	6.5	3.4	2.9	9.1	4.7
†Porosity (% average by site)	-	-	44.2	35.1	34.2	63.5	47.7
†Density (g cm^{-3})	-	-	-	2.9	2.7	2.2	2.7

*Data from the Environment Agency, U.K.

concentrations measured in samples obtained for this study are higher than those of the EA samples, and there was a bigger difference between the concentrations at the two sites. This may be due to the upper EA sampling site being 2.3 km closer to the Sandwell Valley sampling station and being subjected to additional diffuse and point inputs, though both show variability between sites. There is also some evidence for the elevated concentrations being associated with high flow events (except for those measured in April, 2002) and this may indicate remobilization of fine material or wash-off from overland flow. However, when copper concentration was correlated with discharge no relationship was found. The lack of correspondence in these cases may be due to the position of the sampling time in the rising or falling limb of the hydrographs, and the number of days since a high flow event. These two factors can have a significant effect on the amount of suspended material present (Heidel, 1956; Kuhnle, 1992).

Table V.II: The date of sampling and composition of the sediment samples for each experiment with respect to percentage by mass of the size fractions. Also, the biomass (expressed as chlorophyll *a for each*). The river water dissolved copper concentrations found on each sampling date, along with the Environment Agency data corresponding most closely with that date. Some additional EA data are given where no matching experimental values are available.

Experiment							EA data	
BM	Date	% by mass Fines	% by mass Gravel	% by mass Stones	Biomass (mg m^{-2})	Cu (μM)	EA Bescott	Cu (μM)
1	19-Mar-01	33.6	25.5	40.9	-	-	14-Mar-01	0.21
							02-Apr-01	0.17
2	02-May-01	23.1	29.6	47.3	83.6	0.26	18-May-01	0.17
3	13-Jun-01	26.7	34.9	38.4	92.2	0.20	27-Jun-01	0.19
							12-Jul-01	0.08
4	02-Aug-01	11.9	29.8	58.3	20.8	0.16	07-Aug-01	0.23
5 stones	11-Sep-01	-	-	100	22.8	0.35	14-Sep-01	0.18
6 fines	31-Oct-01	100.00	-	-	3.00	0.89	15-Oct-01	0.21
							26-Nov-01	0.15
7 (+ gravel)	10-Apr-02	11.0	26.5	62.4	10.3 (6.4)	0.64	08-Apr-02	0.19

SV							EA Sandwell	
1	18-Apr-01	21.9	25.0	53.1	22.1		02-Apr-01	0.15
2	02-May-01	10.6	22.6	66.8	100.9	0.38	18-May-01	0.14
3 (+stones)	13-Jun-01	9.4	17.7	73.0	56.7 (62.7)	0.36	27-Jun-01	0.21
							12-Jul-01	0.15
4	02-Aug-01	3.6	19.2	77.2	14.1	0.24	07-Aug-01	0.16
5 gravel	10-Sep-01	-	100	-	8.6	0.19	14-Sep-01	0.18
6 fines	31-Oct-01	100.00	-	-	2.30	0.42	15-Oct-01	0.17
							26-Nov-01	0.11
7	11-Apr-02	11.2	14.6	74.3	6.4	0.54	08-Apr-02	0.17

In Figure 5.2 a the bi-monthly sampled dissolved copper concentrations measured for the years 1989 to 1995 are plotted concurrently, and this indicates there was some variability in the average concentrations observed of between 2.3 and 4.9 μM. Figure 5.2 b plots the annual measurements (including data for 1996) sequentially, and a clear reducing trend is visible over the 7 years of data. Also, fewer high concentration events are observed through the series, and the final concentrations are comparable to those of this study period.

It should be noted that there is limited information about the analytical methods used by the Environment Agency, or whether they have changed over the period discussed, but the trend does not appear to be subject to any abrupt changes.
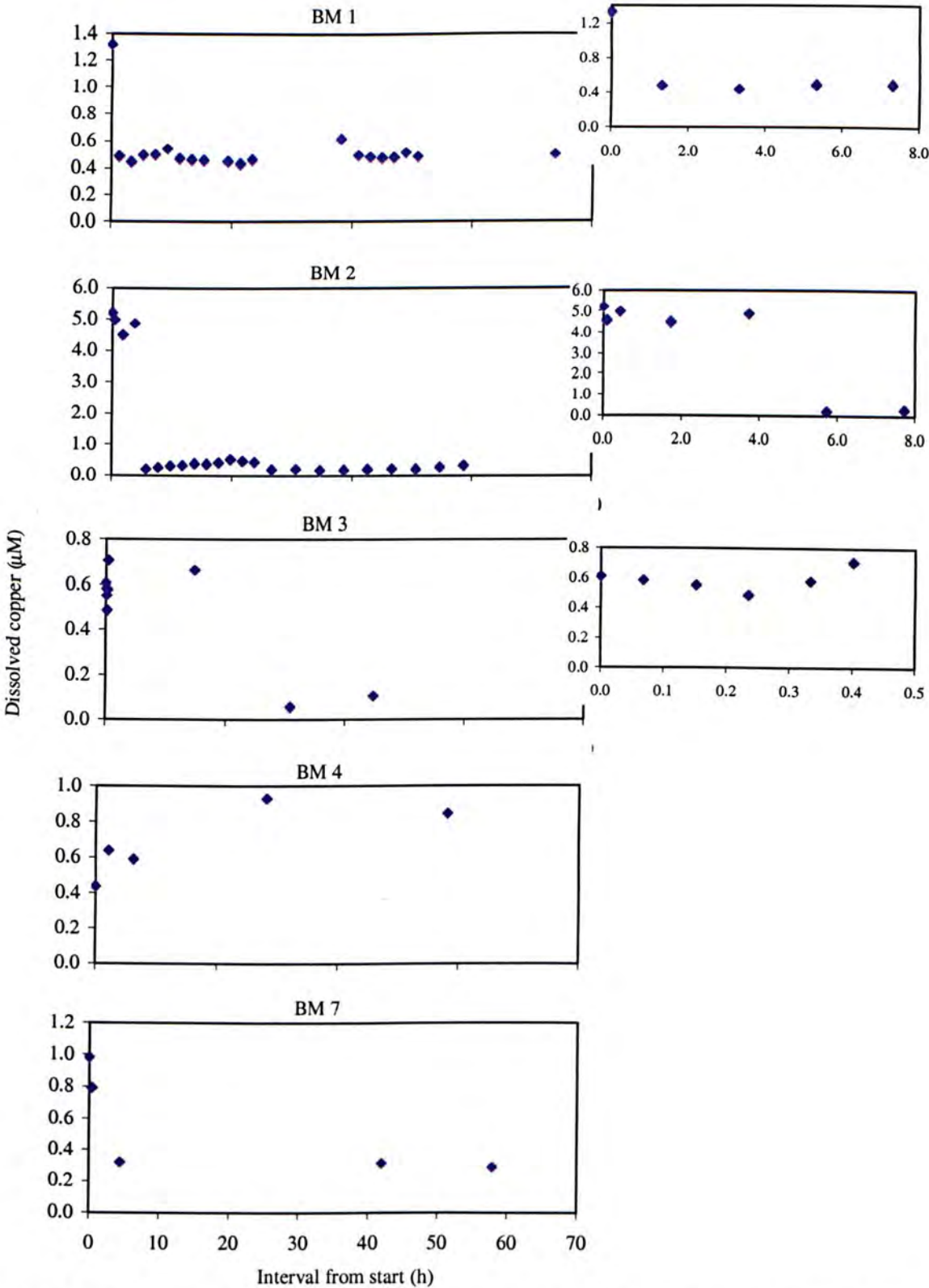
Figure 5.4: Concentrations of dissolved copper released to the overlying solution of simulated river water during the experiments using natural, mixed grain-size sediment from Bentley Mill Way. Insets with expanded scale show initial part of release where reaction occurred rapidly - axes and units are as main plots.
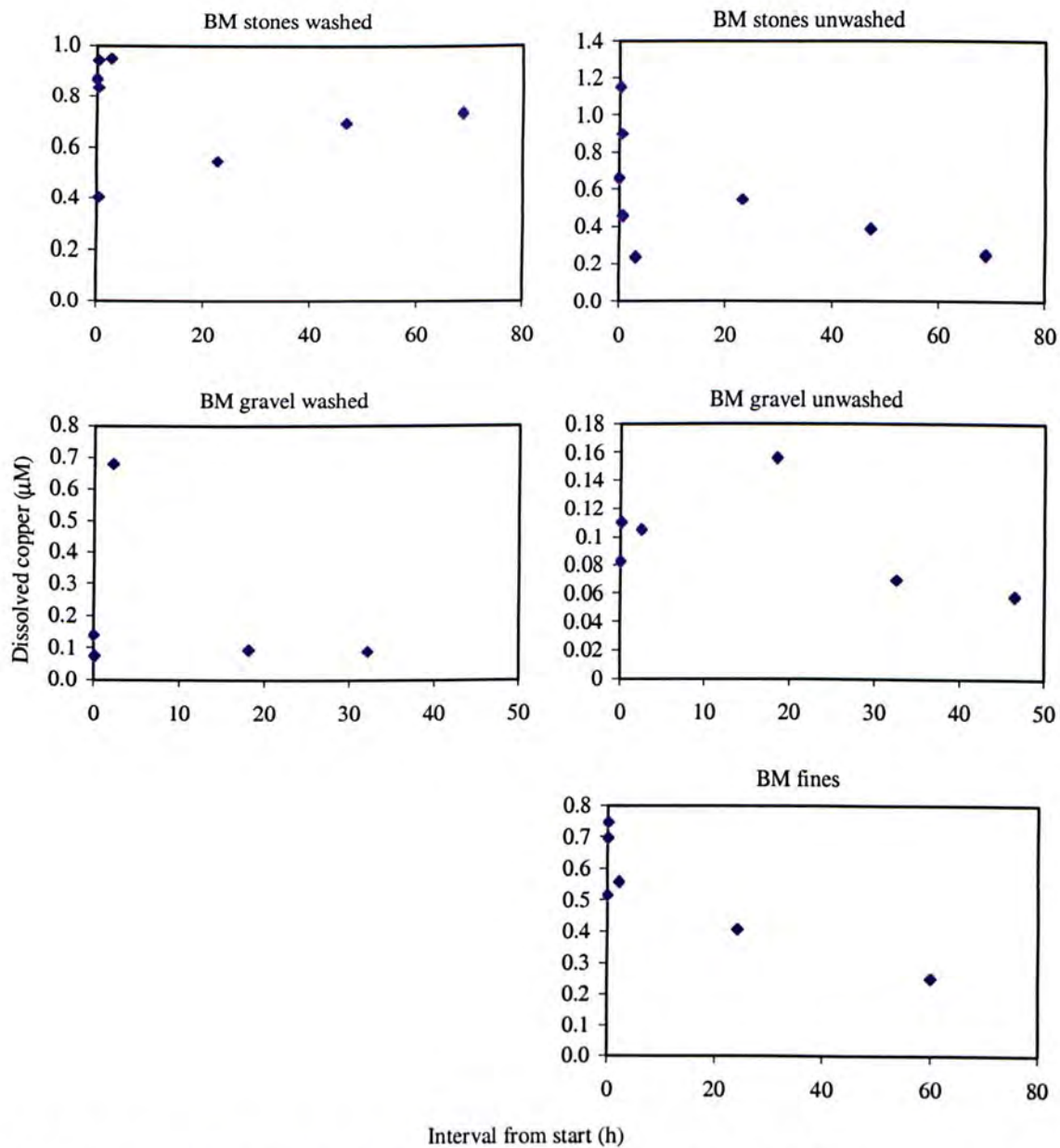
Figure 5.5: Concentrations of dissolved copper released to the overlying solution of simulated river water over the duration of the experiments using the individual grain-size fractions, stones (>20 mm), gravel (2 -20 mm), and fines (<2 mm) from Bentley Mill Way; showing the results from the two different treatments - washed (rinsed to reduce the fine particulates present), and unwashed (as removed from the river).
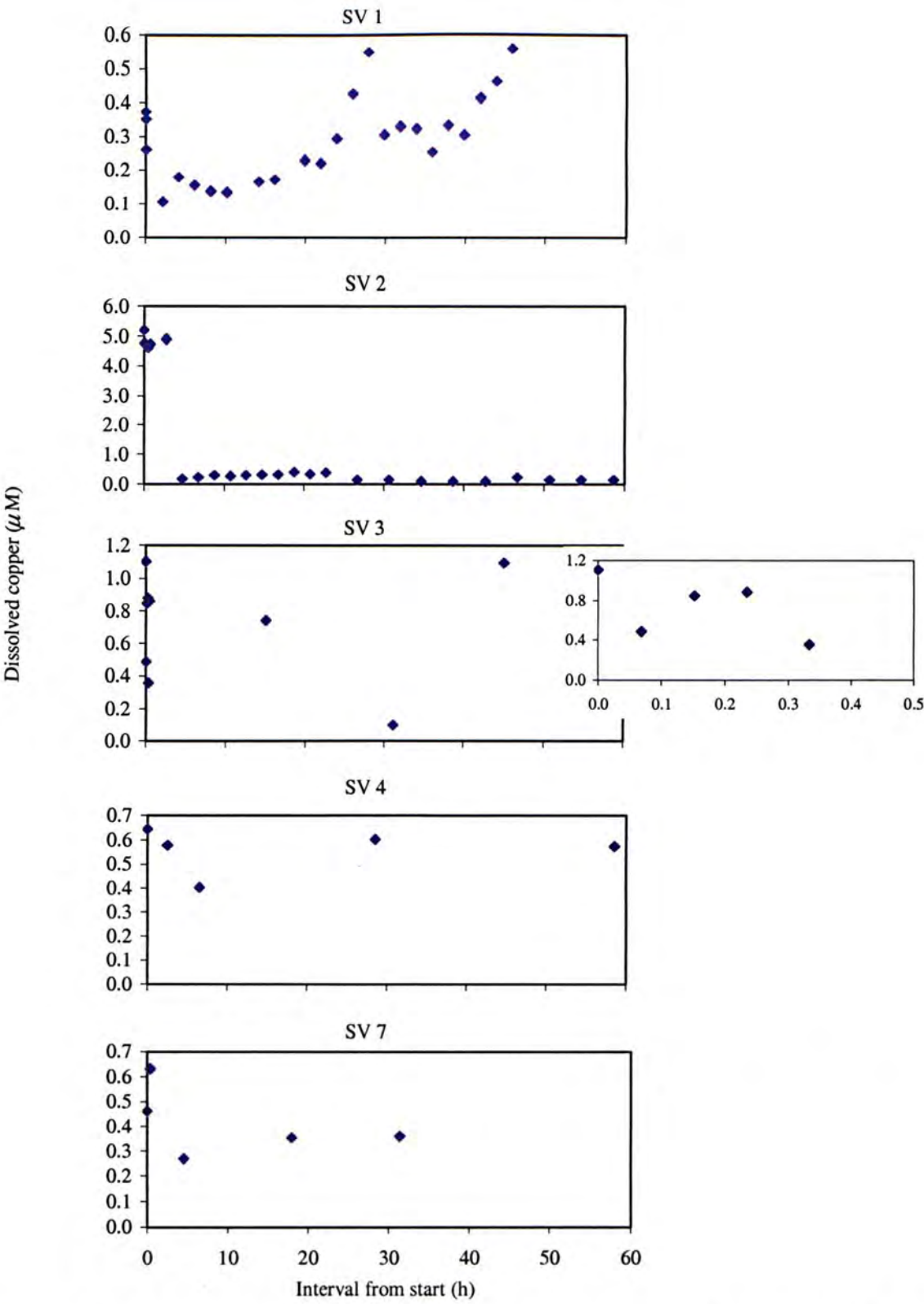
Figure 5.6: Concentrations of dissolved copper released to the overlying solution of simulated river water during the experiments using natural, mixed grain-size sediment from Sandwell Valley, on the River Tame. Inset with expanded scale shows initial part of release where reaction occurred rapidly - axes and units are as main plots.
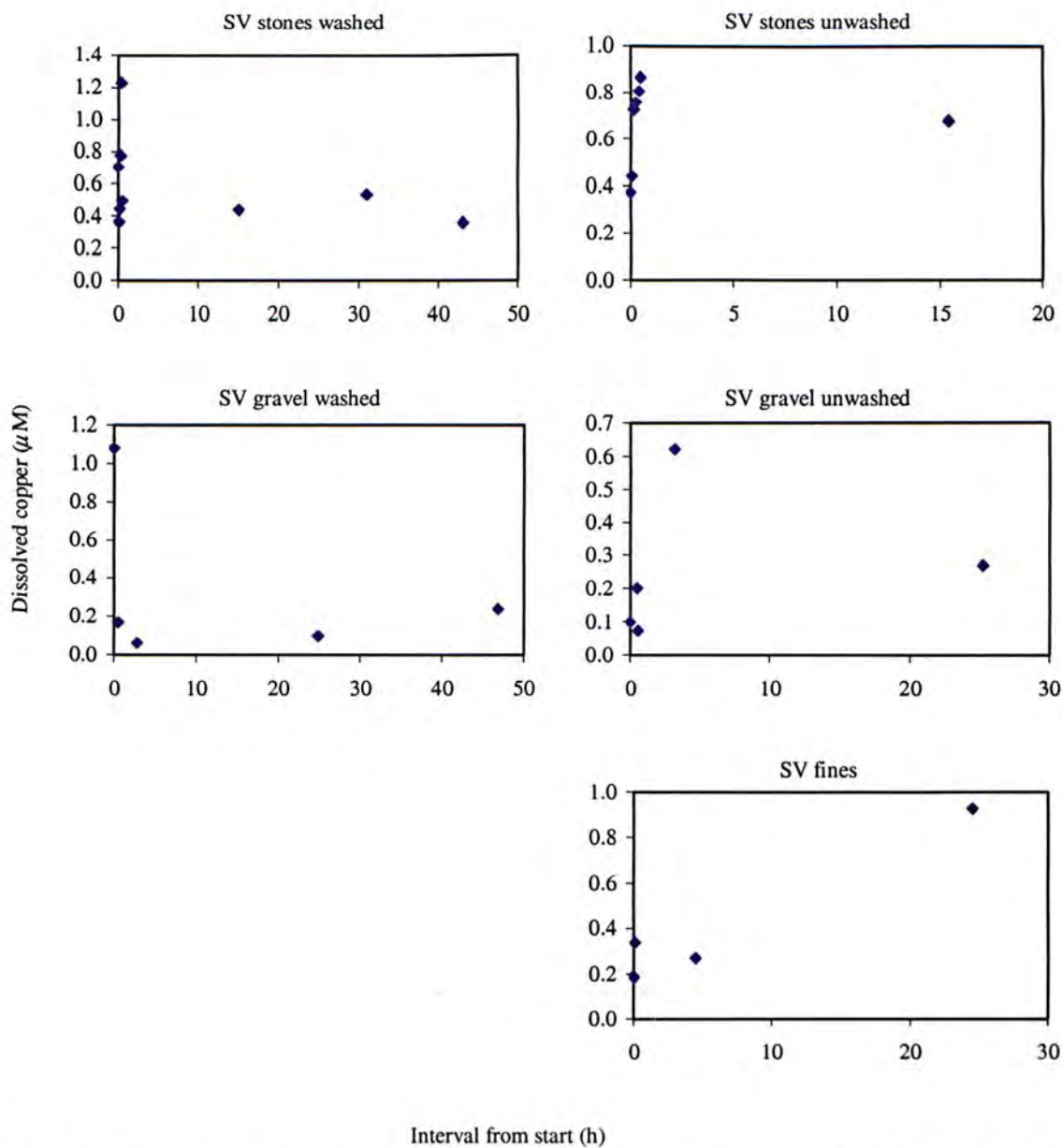
Figure 5.7: Concentrations of dissolved copper released to the overlying solution of simulated river water over the duration of the experiments using the individual grain-size fractions, stones (>20 mm), gravel (2 -20 mm), and fines (<2 mm) from Sandwell Valley; showing the results from the two different treatments - washed (rinsed to reduce the fine particulates present), and unwashed (used as removed from the river).
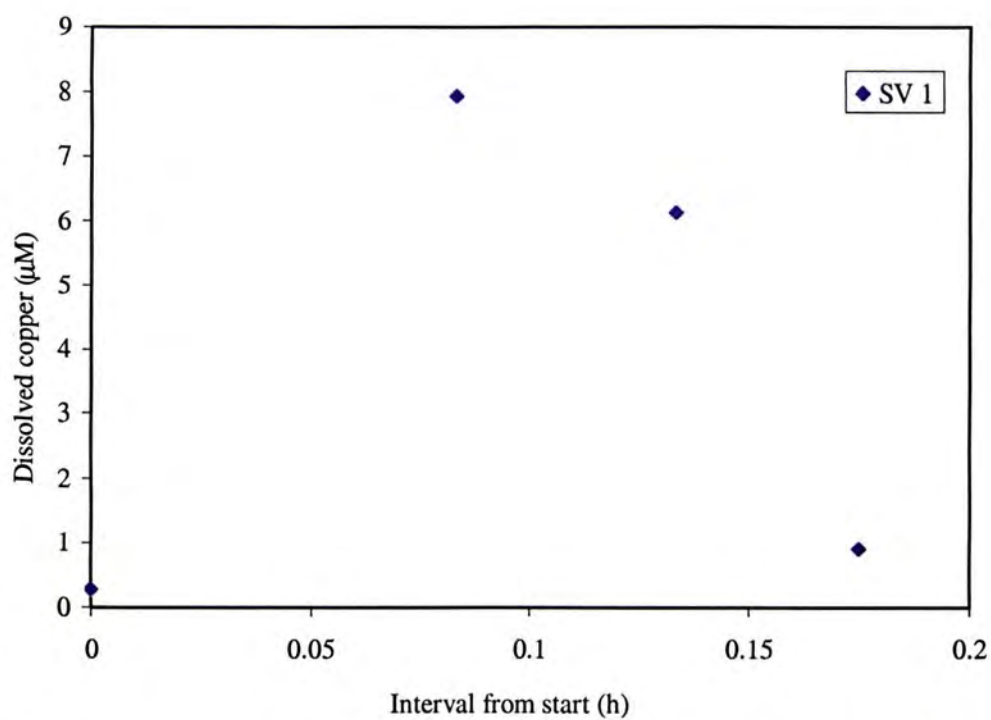
Figure 5.8: An expanded scale plot of the first 0.2 h (12 minutes) of an experiment (SV 1) after addition of the standard copper solution, illustrating the fast stage of uptake and the channel mixing time. In this case less than 6 minutes.

5.3.1: Release Kinetics

Copper concentrations and the nature of any changes in concentration during the flume experiments are shown in Table V.III. For the first part of the experiments, where release was studied, the initial concentration is not zero (indicated as c_0), because immersion of the samples at the start lead to release occurring more quickly than could be measured, *i.e.* faster than the mixing time for the channel. This same effect was seen in the experiments on SRP release as discussed in Chapters 3 and 4. The second part of the experiments, where uptake of dissolved copper by sediment was investigated, is discussed in Section 5.3.2, below.

5.3.1.1: Bentley Mill Way

Figures 5.4 and 5.5 show the concentrations of dissolved copper measured in the overlying solution for all BM experiments with natural sediment, and the three selected size fraction experiments. The insets with the expanded scale illustrate the variability in the initial behaviour between the experiments seen in the changes noted in Table V.III. These varied from rapid (< 10 min) and large (~ 5 μM) changes, to slow and small (< 0.1 μM) fluctuations in concentration. In a number of experiments no release of copper was observed after the initial disturbance and in some cases uptake occurred, *e.g.* Figure 5.4 BM 7 and Figure 5.5 BM fines. Where a steady state was reached the concentration ($[Cu_{ss}]$) has been calculated (the number of points used and standard deviation are given), and these values are in general agreement with the last measured concentrations at the end of the release element of the experiment.

The fast initial release due to submerging trays could not be determined, and where uptake then occurred rapidly this phase could not be measured because of the relatively slow mixing time of the channels. Channel water mixing time has been determined as approximately 10 minutes and therefore uptake was occurring at the same time as mixing.

The maximum concentration measured was for Experiment BM 2 - 5.18 μM. The values for initial concentration (c_0) in the Table V.III indicate that a disturbance of the bed can lead to rapid release into the overlying water. This has implications for the management

Table V.III: River water (r.w.) dissolved copper concentrations for Bentley Mill Way and Sandwell Valley on the day of sampling, and the release and uptake dynamics of all experiments including those using natural mixed grain-size sediment and individual size fractions (stones > 20 mm; gravel 2 - 20 mm; fines <2 mm). For the release section of the experiments - the concentration at the start (c_0), the changes observed, and the calculated steady state (ss) concentration (with number of points and standard deviations), and the observed concentration at the end of the release. For the uptake part of the experiments - the standard addition concentration (spike), the expected concentration peak (nominal concentration) and the observed peak concentration (c_1), with the changes noted, calculated ss, and observed end of experiment concentration. The end of experiment pore water (p.w.) concentrations are included where available.

Experiment	Release (μM)								Uptake (μM)							
	r.w. conc. (μM)	c_0 (μM)	change	pts	ss Rel. (μM)	sd	End rel. (μM)	spike (μM)	nom. conc. (μM)	c_1 (μM)	change	pts	ss Up. (μM)	sd	End Uptake (μM)	p.w. conc. (μM)
BM																
1		1.32	r up ~1.5 h	20	0.48	0.04	0.50	4.46	4.96	3.08	r up ~5 m	17	0.42	0.14	0.47	2.47
							0.15	0		1.20	g up ~12 h	6	0.59	0.04	0.62	
2	0.26	5.18	s ~4 h, r up ~2 h	8	0.20	0.04	0.29	4.46	4.75	8.89	g up~15 h	5	2.53	0.36	2.06	0.43
3	0.20	0.61	rel ~25 m	2	0.08	0.03	0.10	4.46	4.56	3.41	r up ~ 25 m > 0/>up?				0.56	
4	0.16	0.44	g up (c0.93), ss=ins				0.84	4.46	5.30	1.70	s up ~45 m > 0	4	1.16	0.11	1.14	
5 stones wash	0.35	0.87	ss?	3	0.66	0.10	0.73	4.46	4.70	0.71	g up (no peak)	4	0.25	0.12	0.33	
5 stones unwash	0.35	0.65	s, r rel, > up	5	0.37	0.13	0.24	4.46	4.73	0.64	e (no peak)	4	0.41	0.20	0.37	
6 fines	0.89	0.51	d > rel, > up				0.25	4.46	4.71	2.22	r up > ss	4	0.61	0.08	0.51	0.20
7	0.64	0.98	r up > ss	3	0.30	0.02	0.28	4.46	4.74	2.08	ins, con?	2	0.48	0.19	0.34	0.20
7 gravel wash	0.64	0.14	up, ss=ins				0.06	4.46	4.52	1.23	ins				0.2	0.20
7 gravel unwash	0.64	0.08	s rel > up				0.08	4.46	4.54	4.01	up > up, con				0.12	
SV																
1		0.37	up > (c0.13) > rel				0.50	4.46	4.70	7.94	r up	18	0.8	0.10	0.71	0.39
2	0.38	5.19	s ~4 h > r up ~ 2 h	8	0.14	0.04	0.15	4.46	4.61	20.42	r up ~1 h	6	17.9	0.5	18.6	
3	0.36	1.10	e	5	0.63	0.4	1.09	4.46	5.55	2.45	r, s up	3	1.16	0.04	1.12	0.30
3 stones wash	0.36	0.70	s rel (c1.22) 30 m, >s, r u|	4	0.45	0.08	0.36	4.46	4.82	4.03	0 > r up 30 m	4	1.02	0.21	1.18	0.30
3 stones unwash	0.36	0.68	s e, > 0	10	0.64	0.16	0.68	4.46	5.14	4.50	0 > r up	3	1.13	0.37	1.38	0.30
4	0.24	0.64	s up > 0, ss=ins				0.57	4.46	5.03	1.61	r s up > g up				1.11	
5 gravel wash	0.19	1.08	low conc, e, up?	3	0.13	0.09	0.24	4.46	5.19	1.65	r up >ss	6	0.52	0.12	0.58	
5 gravel unwash	0.19	0.37	low conc, e, up?	3	0.32	0.28	0.27	4.46	4.70	3.07	r up >ss	6	0.53	0.12	0.5	
6 fines	0.42	0.44	rel, s up > no ss				0.93	4.46	5.39	2.12	r up , no ss, ins				0.24	
7	0.54	0.46	r rel > up >ss	3	0.33	0.05	0.36	4.46	4.82	4.77	r up > con				0.42	0.54

KEY:

r = rapid change	rel = release	> = leading to/then
g = gradual change	up = uptake	con = continuing uptake
e = erratic response	0 = little or no change	c = concentration
s = small change	h = hours	d = disturbance
ins = insufficient data	m = minutes	ss = steady state

of river systems, for example flood alleviation where dredging is undertaken. This would lead to pulses of copper into the water and thus potentially have an effect on the ecosystem downstream.

The rapid uptake to a lower copper concentration, followed by a slow uptake phase was evident, *e.g.* BM 1 and BM 7, but after the initial reaction to the disturbance concentration generally fell to a steady state concentration of less than 1 μM. Both sites gave similar values for the steady state. In a number of experiments there was an indication of release of copper during the 48 hours in after the periods of uptake - particularly apparent in BM 2. These releases may be due to changes in pH during the experiments because a fall in pH would lead to the release of copper from complexes (Salomans & Förstner, 1984; Jenne & Zachara, 1987). Bioturbation, or other physical disturbances, that move particulate material into the water column could cause release (Matisoff *et al.*, 1985).

River water copper concentrations are similar, *i.e.* < 1 μM, to the concentration of copper at steady state (Cu_{ss}) at the end of release.

5.3.1.2: Sandwell Valley

The concentrations of dissolved copper measured in the overlying solution for all SV experiments with natural sediment, and the three selected size fraction experiments, are shown in Figures 5.6 and 5.7. The inset with the expanded scale demonstrates the variability in the initial behaviour between the experiments seen in the changes noted in Table V.III for SV, and seen in Figure 5.3. As in the BM experiments, the changes in concentration ranged from rapid (< 10 min) and large (~ 5 μM), to slow (~ 4 h) and small. In some cases uptake may have occurred, *e.g.* Figure 5.7 SV stones and gravel unwashed. The steady state concentration ($[Cu_{ss}]$) was calculated (the number of points used and standard deviation are given), and these values are in general agreement with the last measured concentrations. As was the case with the BM experiments, the fast initial release due to submerging trays could not be determined.

The maximum dissolved copper concentration measured was 5.19 μM for SV Experiment

2. Rapid uptake to a lower concentration, followed by a slow release phase was seen only in SV 1, but there was some evidence of release in SV 2 though to a much lower concentration. After the initial reaction to the disturbance SV 2 clearly achieves a steady-state (0.1 μM) and, though there is insufficient data for the later experiments to draw a definitive conclusion, there are indications that steady-state was reached in some of the experiments. Where it was reached both sites resulted in similar steady-state values.

The river water dissolved copper concentrations are similar, *i.e.* < 1 μM, to the concentration of copper at steady state (Cu_{ss}) at the end of the release experiments.

5.3.1.3: Summary of Results

In all experiments the disturbance of fine material during immersion of the sample material caused a relatively large release of copper into the overlying solution, and this occurred so rapidly that no measurement of this phase could be made. This release was followed by a re-adsorption to the sediment, which varied in speed and magnitude, but resulted in copper concentrations at the end of the release experiment similar to that of the river water.

The experiments where the highest initial dissolved copper concentrations were found, BM 2 and SV 2, responded more slowly at the beginning of the experiments, and the rapid uptake phase occurred after ~ 4 h.

Considering the standard deviations for the calculated steady-state concentrations, they were in good agreement with the concentrations recorded at the end of the release part of the experiments. Overall, the sediment from both sites behaved in a similar manner despite the difference in river water copper concentration and discharge.

Due to the nature of the release patterns caused by the disturbance during immersion of the samples, it was not possible to attempt any modelling of the release fluxes.

5.3.2: Uptake Kinetics

For this second part of the experiments, where uptake of dissolved copper by sediment was investigated, a standard solution (spike) of copper was added to the simulated river water overlying the sediment in the channels. The series of samples then taken indicated that the mixing time before peak occurred was approximately 10 minutes. The expanded scale plot in Figure 5.8 is an example of the first 12 minutes of an experiment (SV 1) after addition of the standard copper solution, and illustrates the fast stage of mixing and uptake. In this case mixing time was less than 6 minutes, and the maximum concentration was not observed. However, in this case the recorded peak concentration of 7.9 μM was greater than the calculated nominal concentration of 4.7 μM, *i.e.* the concentration and the end of the release phase plus the concentration of the standard addition.

5.3.2.1: Bentley Mill Way

After introducing the standard solution a nominal concentration peak was expected. However, this peak was not recorded, though Experiments BM 1, 3 and 7 (gravel unwashed) were closest. This nominal concentration peak was not observed because the uptake kinetics were fast compared with the time the solutions took to mix. The dynamics of the changes in uptake are also described in Table V.III and, in general, the uptake of the copper by the sediment was initially rapid with the steady-state being reached within the first hour.

Uptake of dissolved copper took place in all experiments, and generally the steady-state concentration of dissolved copper (Cu_{ss}) for the uptake part of the experiments was greater than the steady-state for the release (Figure 5.3). In these cases the sediment has acted as a buffer and removed copper from the overlying solution, but in doing so has affected the concentration in solution at steady-state. The sediment does not appear to have an infinite capacity to remove copper from the water without affecting the steady-state. This is reflected in the plot of river water concentration over the years 1989 - 1996, where the concentrations measured in 1996 are similar to those found in this study. The change in steady-state concentration suggests that the sediment is responding to reduced river water concentration of dissolved copper. Were it not responding to the change in
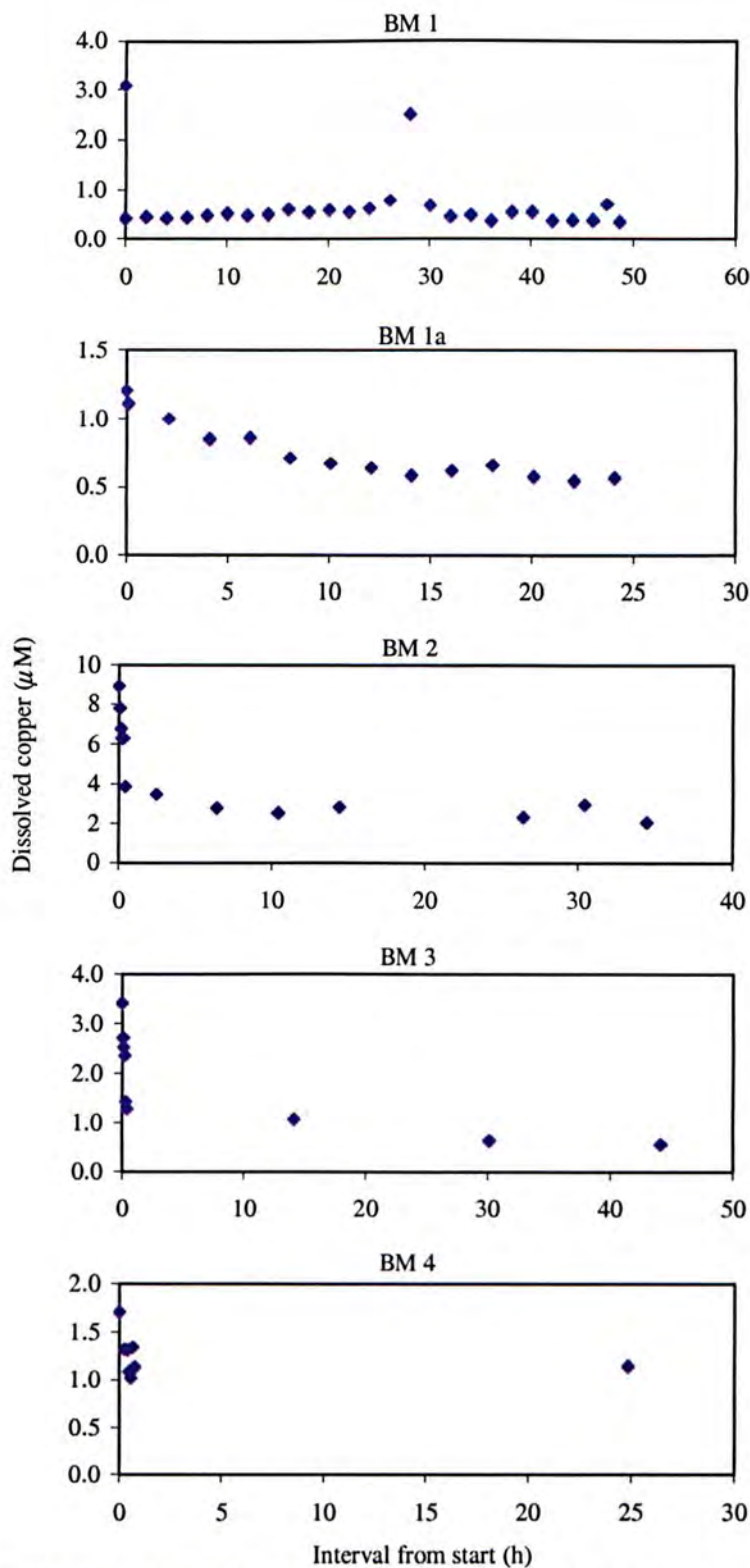
Figure 5.9: Concentrations of dissolved copper taken up by the sediment from the overlying solution of simulated river water during the experiments using natural, mixed grain-size sediment from Bentley Mill Way.

Note: The uptake for Experiment BM1a is shown only as an example. The results are not presented in Figure 5.3 as the experiment was carried out outside the standard 48 hour experimental period.
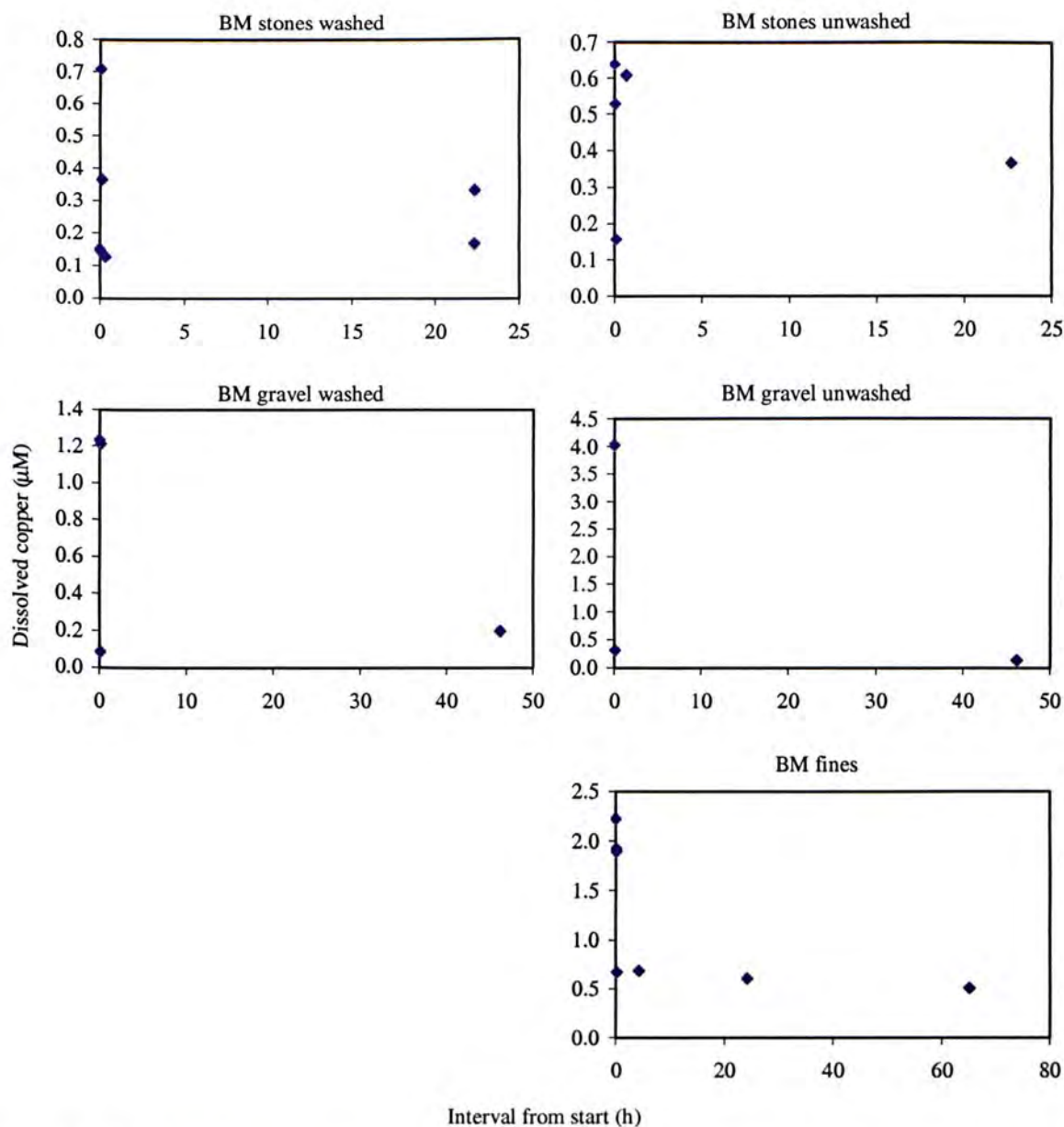
Figure 5.10: Concentrations of dissolved copper taken up by the sediment from the overlying solution of simulated river water over the duration of the experiments using the individual grain-size fractions, stones (>20 mm), gravel (2 - 20 mm), and fines (<2 mm) from Bentley Mill Way; showing the results from the two different treatments - washed (rinsed to reduce the fine particulates present), and unwashed (used as removed from the river).
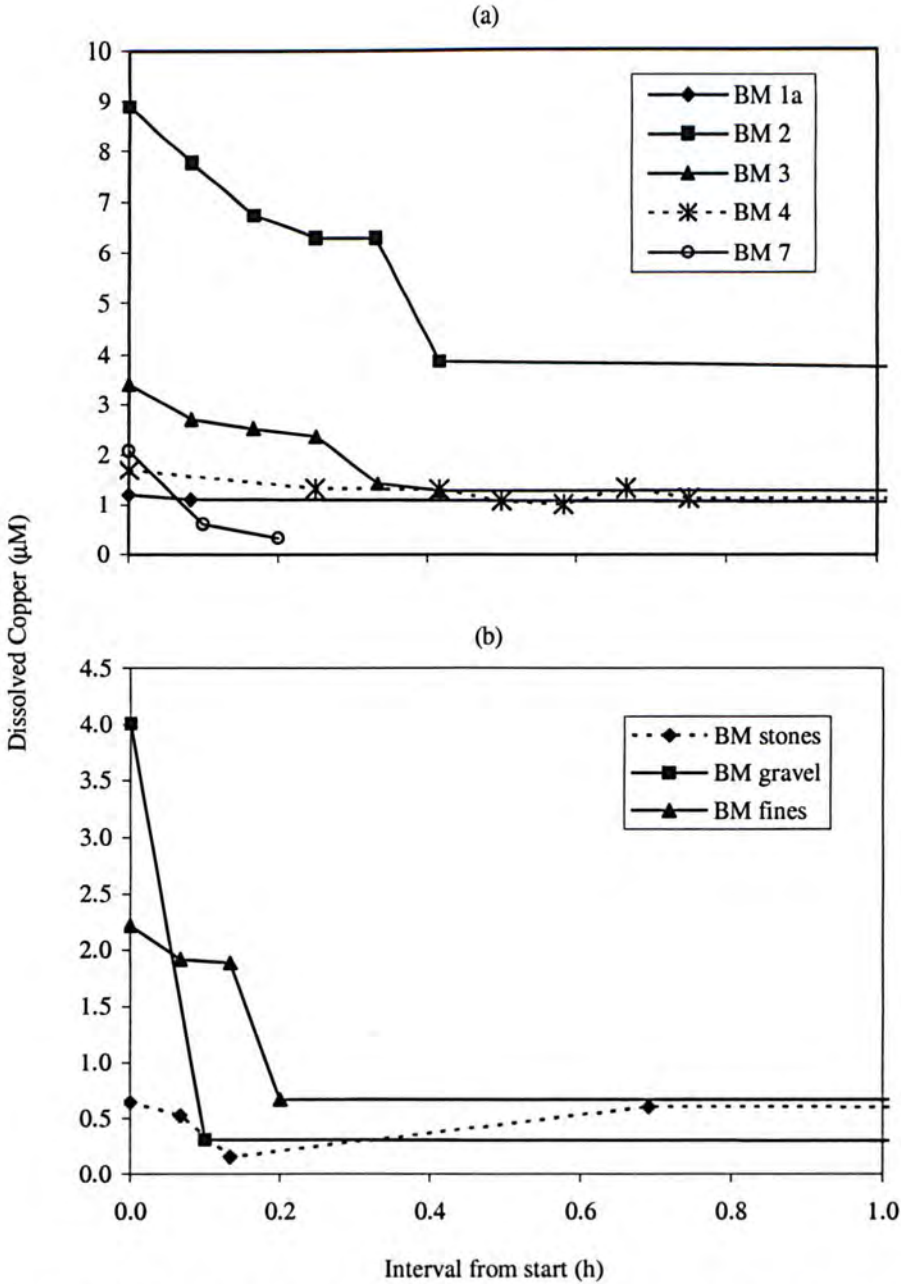
Figure 5.11: Expanded scale plots of the uptake of dissolved copper by the sediment during the first hour of the experiments using (a) natural mixed grain-size sediment, and (b) the unwashed sub-sample of the three size fractions (stones > 20 mm diameter; gravel 2 - 20 mm, and fines < 2 mm) from Bentley Mill Way.
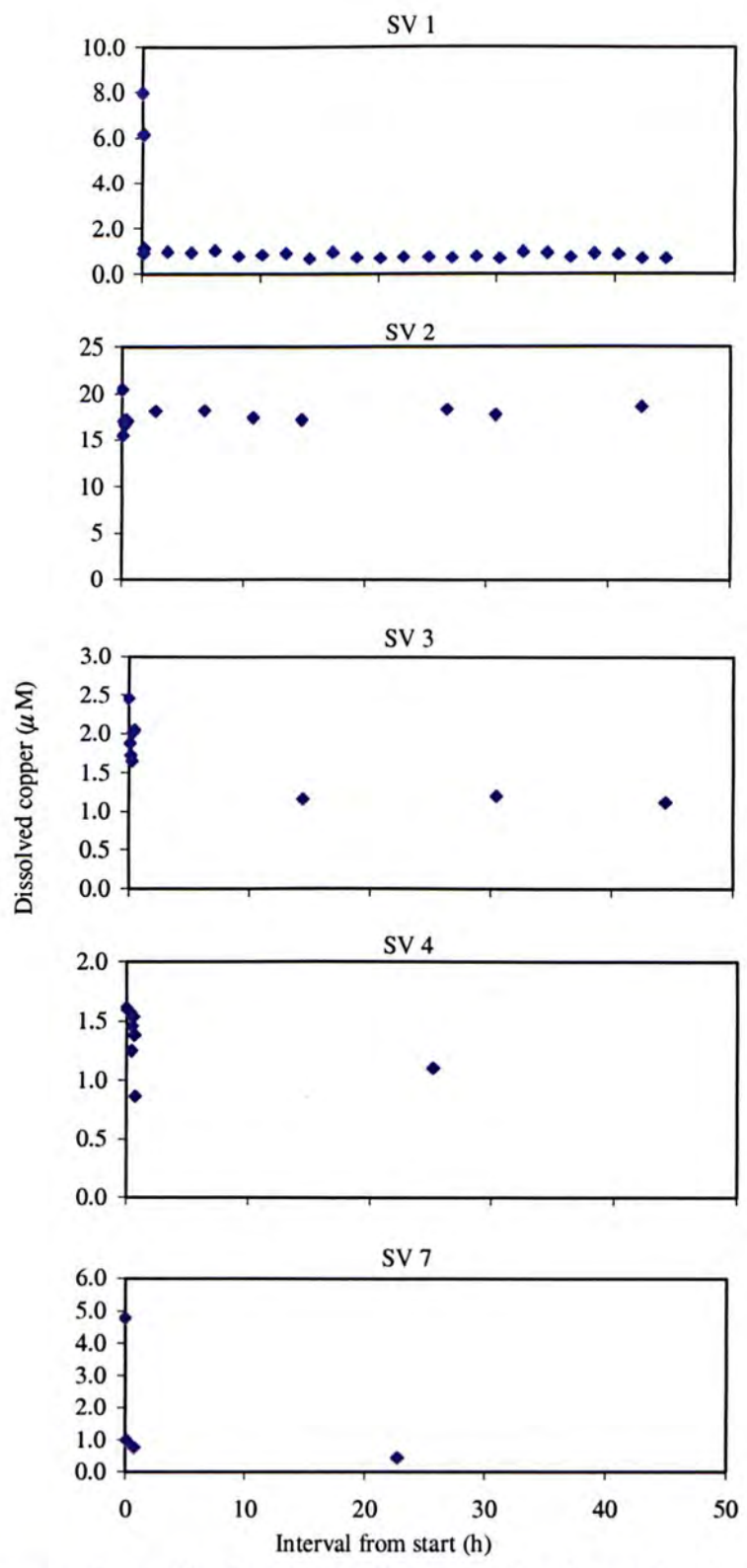
Figure 5.12: Concentrations of dissolved copper taken up by the sediment from the overlying solution of simulated river water during the experiments using natural, mixed grain-size sediment from Sandwell Valley.
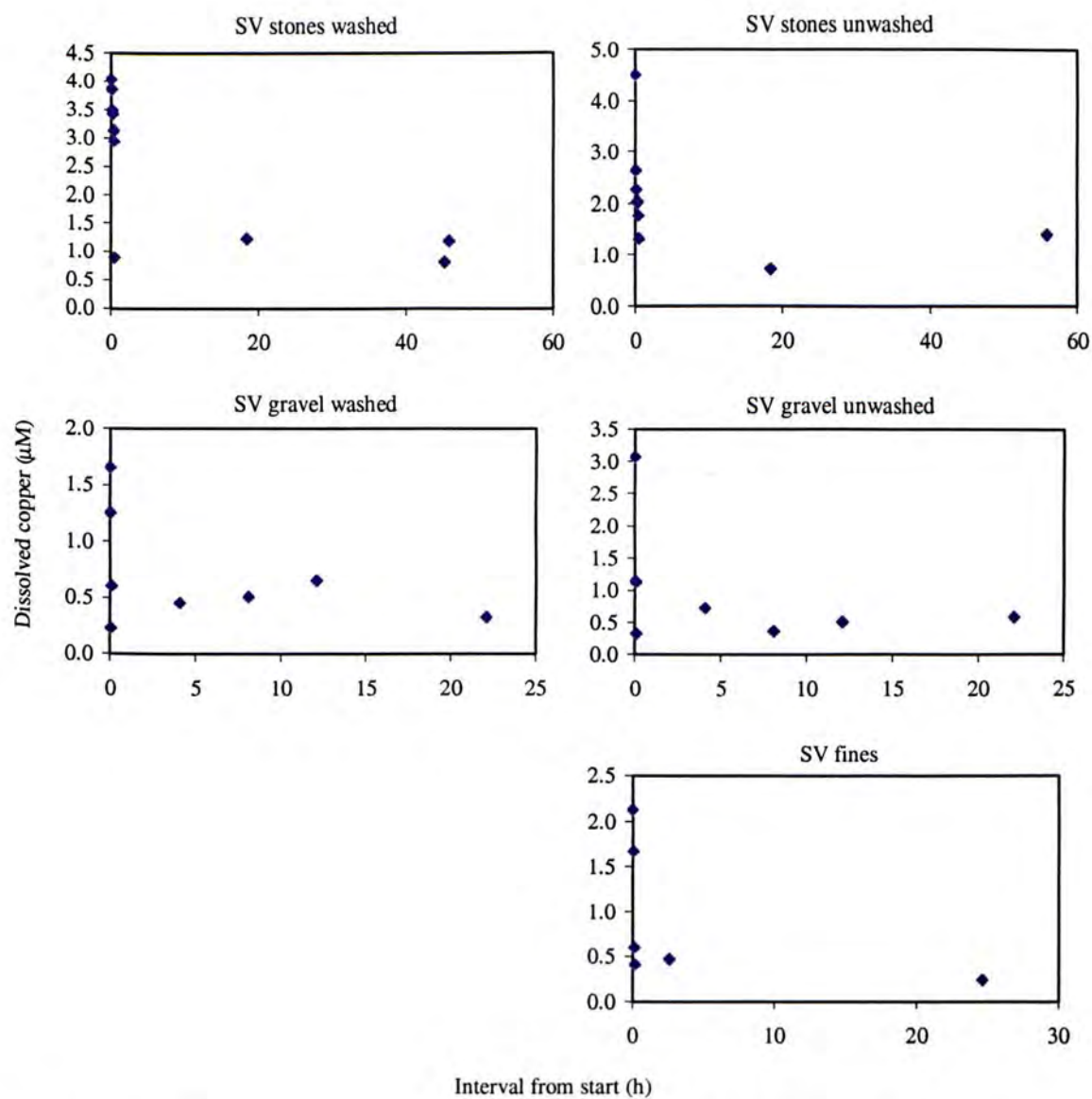
Figure 5.13: Concentrations of dissolved copper taken up by the sediment from the overlying solution of simulated river water over the duration of the experiments using the individual grain-size fractions, stones (>20 mm), gravel (2 - 20 mm), and fines (<2 mm) from Sandwell Valley; showing the results from the two different treatments - washed (rinsed to reduce the fine particulates present), and unwashed (used as removed from the river).
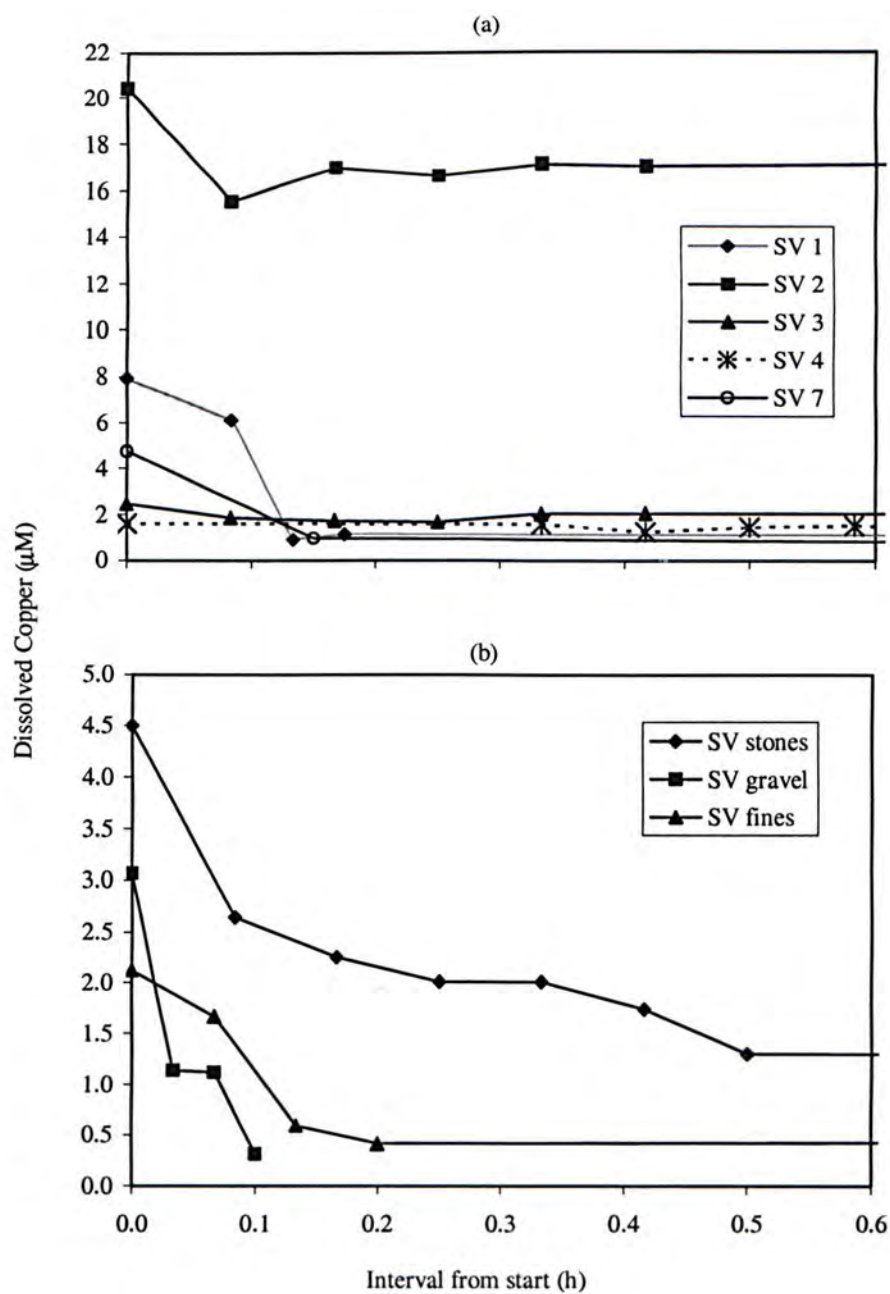
Figure 5.14: Expanded scale plots of the uptake of dissolved copper by the sediment during the 38 minutes of the experiments using (a) natural mixed grain-size sediment, and (b) the unwashed sub-sample of the three size fractions (stones > 20 mm diameter; gravel 2 - 20 mm, and fines < 2 mm) from Sandwell Valley.

concentration, it would have been expected that higher steady-state concentrations, *i.e.* it would have 'remembered' the previous concentration and taken that as the steady-state.

Porewater dissolved copper concentrations approximately corresponded with the river water concentrations, so high levels of organic complexation in porewaters is not evident. This compares with a study of pesticides (Daniels *et al.*, 2000) where concentrations in the porewaters remained high even after removal of the contaminant from the water column. Porewater concentrations of dissolved copper were similar at both sites.

The results of the uptake phase of the flume channel experiments (Figures 5.9 - 5.14) corroborate the findings of the release experiments in that most frequently rapid uptake occurred after the introduction of the copper standard. The exception to this pattern was in BM 1 a (Figure 5.9), which was an extension of the main BM 1 experiment. The system had time to settle and although the uptake is small it exhibits the type of uptake typically associated with silt sediments, *i.e.* a gradual re-adsorption of the dissolved copper into the sediment (Li *et al.*, 2001). Figures 5.11 (for Bentley Mill Way sediments) and 5.14 (for Sandwell Valley sediments) have been included to illustrate more clearly what is happening during the first few minutes of the experiments. Because these uptake reactions happen so rapidly they are not immediately apparent from the overall uptake plots of concentration in solution over time.

The reduced amount of data available in the later experiments was due to cost limitations on the numbers of ICP-MS analyses that could be performed. But, as the evidence from the earlier experiments suggested the rapid initial uptake activity was followed by little or no change over the remainder of the 48 h period, it was deemed that intermediate sampling only would give sufficient information about the latter stages of the experiments.

From the concentrations of dissolved copper in solution measured at set intervals the changes in concentration over time were calculated, and these values were used with coefficients obtained by quadratic smoothing to calculate the flux of copper into the sediment. The fluxes for BM Experiments 1 - 3, normalised with respect to the surface area of the sediment, are given in Table V.IV and the falling rate reflects the reduction in the driving force as the concentration in the overlying solution is diminished over time.
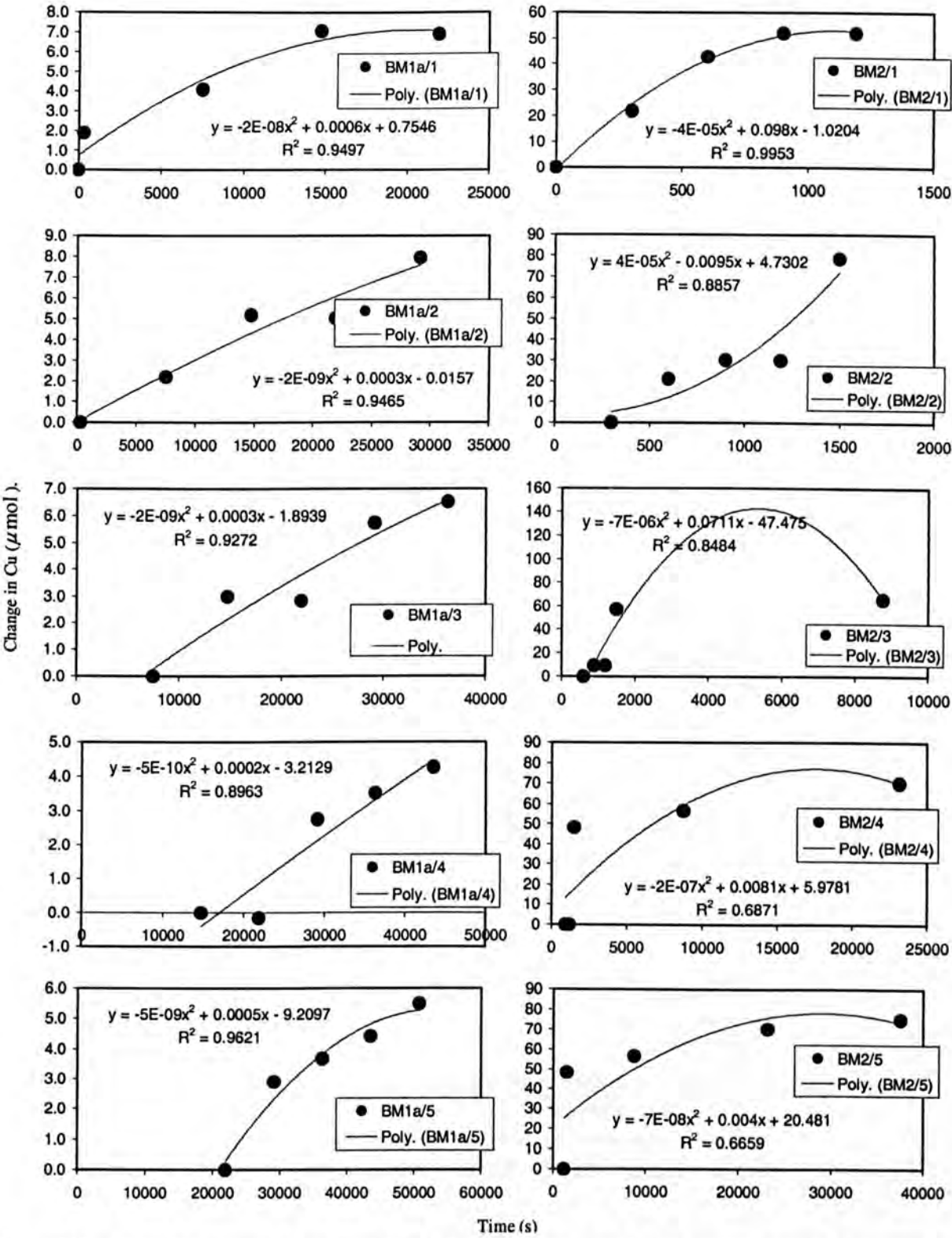
Figure 5.15i: Change in the amount of copper over time for Experiments BM 1a and 2, with polynomial regression trendlline, plus equation and R^2 value.

Figure 5.15ii: Change in amount of copper over time for Experiments BM 3 and fines fraction, with polynomial regression trend line plus equation and R^2 value.

Table V.IV: Copper solution concentrations and the change in concentration over time between sampling for Bentley Mill Way sediment from Experiments BM 1a, 2, 3, and fines fraction, with flux calculated from quadratic smoothing coefficients.

Uptake	Time (s)	[Cu] (μM)	Change in Cu (μM)	Flux (quadratic) (μmol m^{-2} s^{-1})	Uptake	Time (s)	[Cu] (μM)	Change in Cu (μM)	Flux (quadratic) (μmol m^{-2} s^{-1})
BM1a/1	0	1.20	0.000		BM2/4	900	6.28	0.000	
	300	1.11	1.888			1188	6.29	-0.028	
	7500	1.00	4.092	0.004		1500	3.87	48.373	0.063
	14700	0.85	7.081			8758	3.45	56.593	
	21900	0.86	6.924			23159	2.77	70.291	
BM1a/2	300	1.11	0.000		BM2/5	1188	6.29	0.000	
	7500	1.00	2.203			1500	3.87	48.402	
	14700	0.85	5.193	0.003		8758	3.45	56.621	0.004
	21900	0.86	5.036			23159	2.77	70.320	
	29100	0.71	7.947			37558	2.54	74.886	
BM1a/3	7500	1.00	0.000		BM3/1	0	3.41	0.000	
	14700	0.85	2.990			300	2.71	14.163	
	21900	0.86	2.833	0.003		600	2.53	17.625	0.238
	29100	0.71	5.744			900	2.36	21.087	
	36301	0.67	6.515			1200	1.42	39.814	
BM1a/4	14700	0.85	0.000		BM3/2	300	2.71	0.000	
	21900	0.86	-0.157			600	2.53	3.462	
	29100	0.71	2.754	0.002		900	2.36	6.924	0.203
	36301	0.67	3.525			1200	1.42	25.651	
	43501	0.64	4.265			1500	1.28	28.483	
BM1a/5	21900	0.86	0.000		BM3/3	600	2.53	0.000	
	29100	0.71	2.911			900	2.36	3.462	
	36301	0.67	3.682	0.002		1200	1.42	22.189	0.260
	43501	0.64	4.422			1500	1.28	25.021	
	50700	0.58	5.492			50875	1.06	29.522	
BM2/1	0	8.89	0.000		BM3/4	900	2.36	0.000	
	300	7.79	21.956			1200	1.42	18.727	
	600	6.74	42.997	0.417		1500	1.28	21.559	0.002
	900	6.28	52.145			50875	1.06	26.060	
	1188	6.29	52.117			108475	0.63	34.652	
BM2/2	300	7.79	0.000						
	600	6.74	21.041						
	900	6.28	30.189	0.521					
	1188	6.29	30.161						
	1500	3.87	78.563						
BM2/3	600	6.74	0.000						
	900	6.28	9.148						
	1188	6.29	9.120	0.454					
	1500	3.87	57.522						
	8758	3.45	65.741						

Note:

Sediment surface area (m^2) for BM1a only =   0.08

Sediment surface area (m^2) =   0.12

Flume overlying solution volume (L) =   20
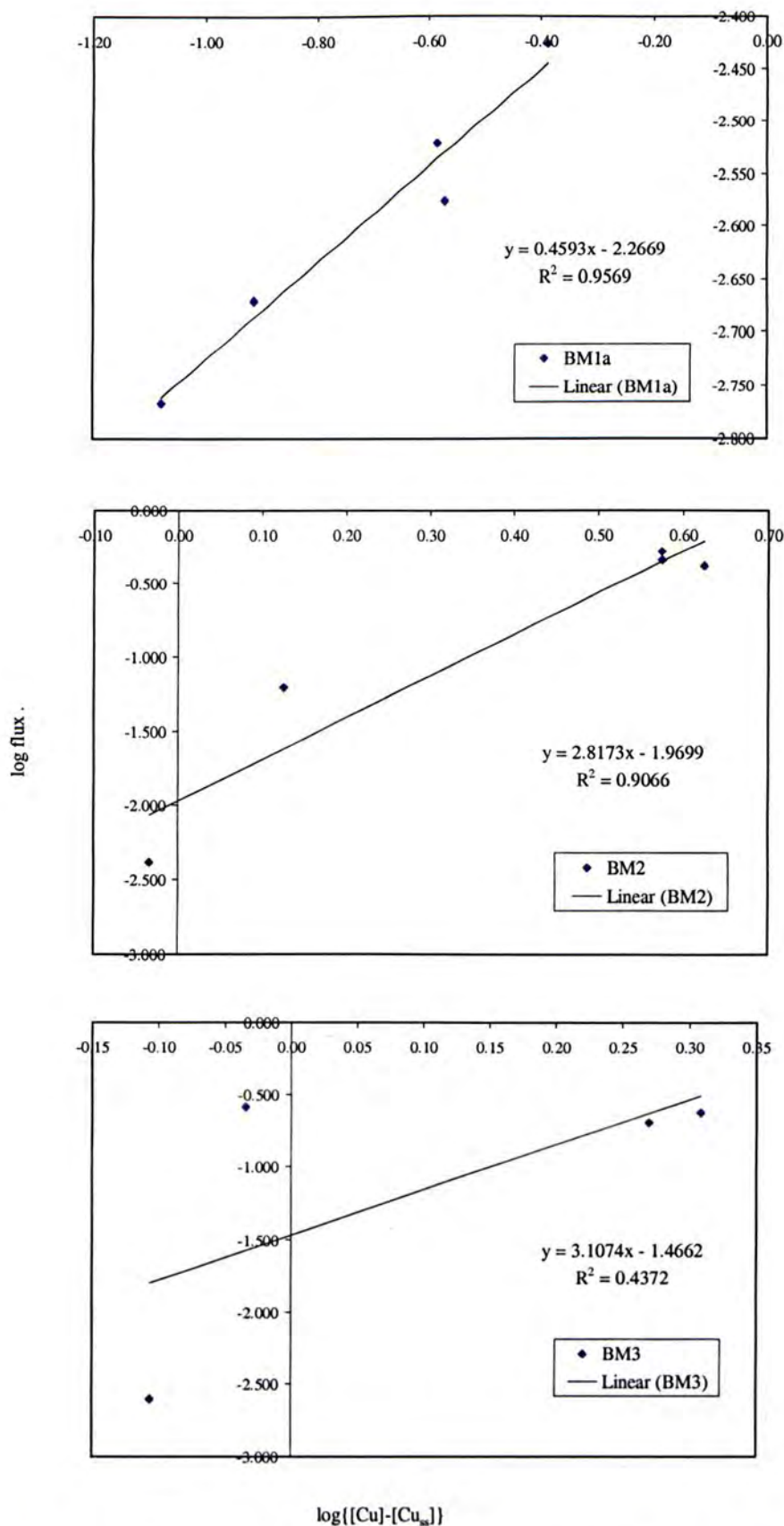
Figure 5.16i: A test of the kinetic model (flux = k {[Cu] - [Cu$_{ss}$]}) for the dissolved copper uptake experiments BM 1, 2, and 3. The values of the reaction order (n) and the rate constant (k) were calculated from the linear regression line shown (Table V.V). Units of flux - μmol m^{-2} s^{-1}, and units of copper concentration - μM.
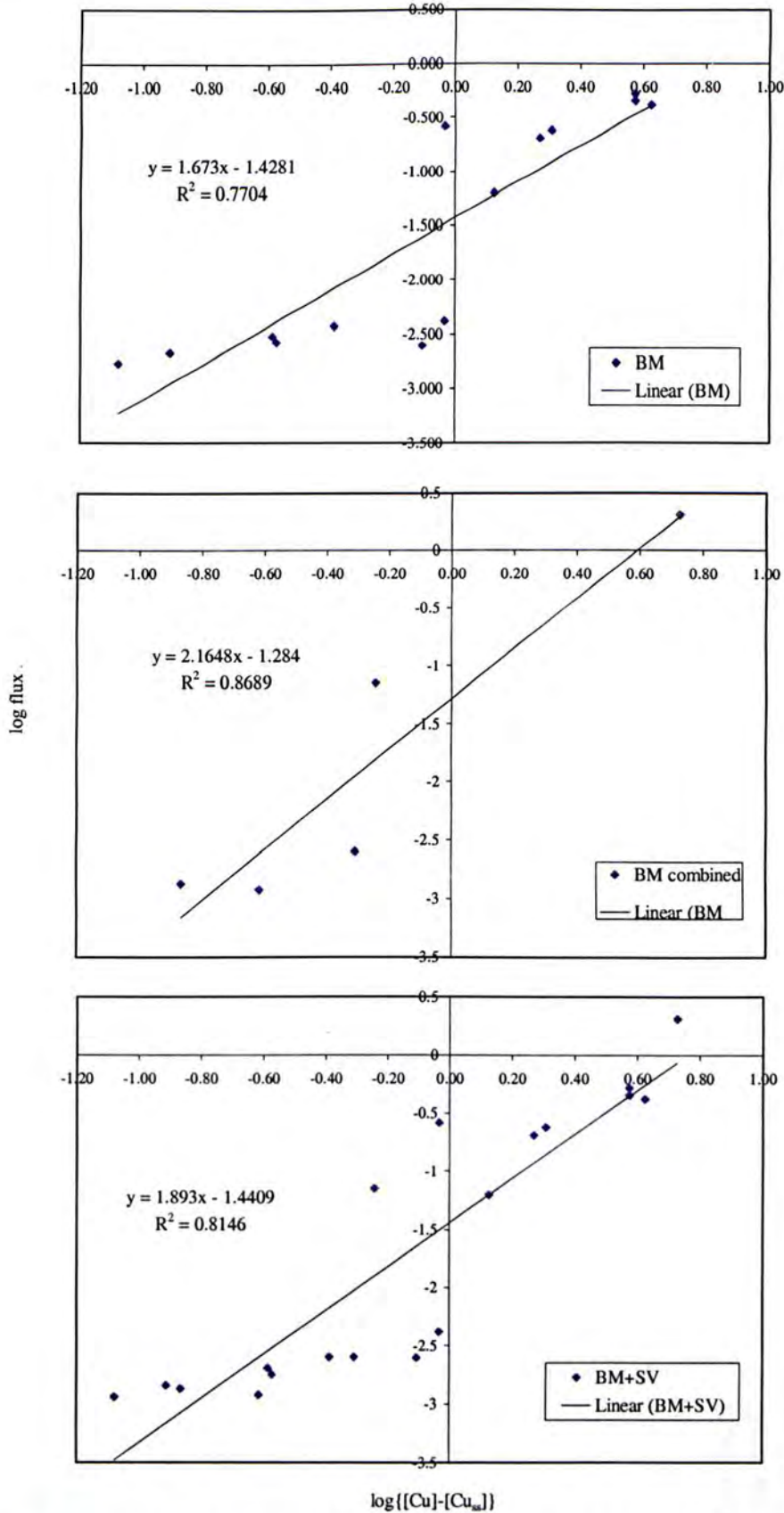
Figure 5.16ii: A test of the kinetic model (flux = k {[Cu] - [Cu$_{ss}$]}) for the dissolved copper uptake experiments for the combined results of the BM Experiments, the SV Experiments, and for both sites amalgamated. The values of the reaction order (n) and the rate constant (k) were calculated from the linear regression line shown (Table V.V). Units of flux - μ mol m^{-2} s^{-1}, and units of copper concentration - μ M.

Table V.V: Kinetic data from the plot of log flux against log concentration (Figure 5.16), for the uptake flux calculated from quadratic smoothing coefficients for Experiments BM 1a, 2, and 3, individually and combined, SV Experiments 1 and 3 combined , and an amalgamation of the results from both sites. Dissolved copper concentration at time ([Cu]) and the calculated steady-state (ss) concentration, with standard deviation (sd) are given. The values of the reaction order (n) and the rate constant (k), with standard error (s.e), were calculated from linear regressions.

BM	Flux (quadratic) (μmol m^{-2} s^{-1})	log flux	[Cu]-[Cu$_{ss}$] (μM)	log {[Cu]-[Cu$_{ss}$]}	[Cu] (μM)	pts	ss (μM)	sd	n	s.e.	k	s.e.
BM1a	0.004	-2.426	0.999	0.000	1.00	6	0.59	0.04	0.5	0.06	5.4	0.5
	0.003	-2.521	0.850	-0.071	0.85							
	0.003	-2.576	0.858	-0.067	0.86							
	0.002	-2.670	0.712	-0.147	0.71							
	0.002	-2.766	0.674	-0.172	0.67							
BM2	0.417	-0.380	6.742	0.829	6.74	5	2.53	0.36	2.8	0.52	11.5	5
	0.521	-0.283	6.284	0.798	6.28							
	0.454	-0.343	6.286	0.798	6.29							
	0.063	-1.204	3.866	0.587	3.87							
	0.004	-2.383	3.455	0.538	3.45							
BM3	0.238	-0.623	2.534	0.404	2.53	-	0.5 [end]		3.1	2.49	59	48
	0.203	-0.692	2.360	0.373	2.36							
	0.260	-0.586	1.424	0.154	1.42							
	0.002	-2.606	1.283	0.108	1.28							
BM combined	-	-	-	-	-	-	-	-	1.7	0.26	39	12
SV combined	-	-	-	-	-	-	-	-	2.2	0.49	65	38
BM + SV combine	-	-	-	-	-	-	-	-	1.9	0.22	37	10

Note: Units of k (mmol^{1-n} m^{3n-2}s^{-1}) if concentration is in μM and the flux is given as μmol m^{-2} s^{-1}.

Figures 5.15i and ii are the plots of the change in dissolved copper concentration against time used to generate the polynomial coefficients for the flux calculations.

The kinetics of the reaction are described by a rate law of the form

$$flux = k \{ [Cu] - [Cu_{ss}] \}^n \qquad\qquad (1)$$

where k is the rate constant, [Cu] is the concentration of copper in the overlying solution at given time, $[Cu_{ss}]$ the calculated steady state concentration of dissolved copper, and the reaction order (n) ~ 2. The rate law describes the way the rate of reaction will vary with changes in the copper concentration.

By plotting the log of the rate (flux) against the log of the copper concentration, which are Figures 5.16 i and ii, values for n and k were generated. This test of the fit of the rate law resulted in good R^2 values for BM 1a and BM 2, which suggests the model is appropriate for these reactions. However, as the fit is not as good in BM 3 it implies that there may be a different mechanism involved than simply diffusion.

The most reliable results are for Experiments BM 1, 2, and 3 and there are indications of a change in the mechanism through the season – BM 1 in later winter to BM 3 in late spring are supported by the n values (Table V.V), because the reaction order increases through the three BM experiments. However, both sites show a similar order for the kinetics, *i.e.* n = 1.7 and 2.2 for BM and SV respectively, from the combined data. Further investigation of the seasonal variability and variability between the samples at the same time of year is warranted here.

The rate constants also increased through the season from ~5 x 10^{-3} – 59 x 10^{-3} ($mmol^{1-n}$ $m^{3n-2}s^{-1}$- see footnote in Table V.V), though this would be expected to some extent as k is temperature dependant and there was a large difference in water temperature between BM 1 and BM3. The SV combined values were higher than BM.

There is no biomass data available for Experiment BM 1, but the composition of the sediment, *i.e.* the higher percentage mass of fine material comprising this sample, may have had an effect on the flux (Table V.II). It is not clear what the reason for the

difference in the n and rate constant values is apart from the time of year. Figure 1 in Chapter 4 shows the biomass, as chlorophyll *a*, determined over the period of sampling and the growth pattern suggests that a seasonal effect on the development of the biofilm may be the cause. Table V.I shows temperatures to have been lower than the seasonal average in spring 2001.

5.3.2.2: Sandwell Valley

As with the uptake experiments using BM sediments, the expected nominal concentration peaks – the combined end of release and standard addition concentrations - were not registered, though Experiments SV 3 (stones) and 7 were closest.

Uptake took place in all experiments and the initial reaction to the spike of additional copper was rapid - within ~12 minutes of peak (observed) concentration being reached the concentration in the overlying solution in most cases had reduced to a steady state (Figures 5.8 and 5.14). This is faster than BM where the response time was twice as long. After the standard solution addition uptake phase had occurred, a new and generally higher steady-state concentration was established, and there appears to have been less subsequent fluctuation in concentration during the later stages of the experiments. This indicates that the sediment responded to changes in copper concentration in solution in the same way as the Bentley Mill Way sediment, by adjusting the steady-state concentration, and in the past when river water concentrations were higher (Figure 5.2) the sediments would have behaved similarly.

Due to the rapidity of the initial uptake reaction, and that sampling points were not frequent enough to generate sufficient data on the flux kinetics of this phase of the experiments, it was not possible for the rate law model to be tested against it. Only the SV experiment using the stones fraction of the sediment resulted in a slower uptake - over ~30 minutes - and the flux calculated from the quadratic smoothing for this experiment was $0.109 \ \mu$mol m^{-2} s^{-1}, a rate comparable to that of BM 3. Both these experiments took place in late spring and had higher biomass values - 92 and 63 mg m^{-2} (as chlorophyll *a*) for BM 3 and SV stones correspondingly. River discharge was low on the day of sampling and the water temperature at BM was below the seasonal average.

Although no rate constants were calculated for individual SV experiments, the combined SV rate constant value was almost double that of BM.

5.3.3: Implications for the River Tame

Because the concentration of the copper at steady state is the same as the river water copper concentration (Table V.III), Eqn (1) yields a method of estimating the flux to the sediment. This could be important for assessing recovery time after a pollution incident, or from major sediment disturbance such as dredging of the river channel. Part of the artificial channel at SV is sometimes dredged to clear accumulated bed material for the flood alleviation mechanism. It can also yield information as to how far downstream the copper concentration would remain elevated, *i.e.* how long it would take the sediment to reduce the copper in a given parcel of water passing down stream.

A commonly used index of the toxicity of trace metals to algae is the effective concentration that inhibits 50% of growth, and is known as the EC_{50} (Yan & Pan, 2002). It is qualified by the number of hours over which the effect is measured, *e.g.* 96 h EC_{50}, and depending on species, the 96 h EC_{50} (for example in the microalgal species *Scenedesmus obliquus, Chlorella pyrenoidosa,* and *Closterium lunula*) has been found to be between 50 and 200 μg l^{-1} (~0.8 and ~3 μM) correspondingly. The concentrations in the River Tame are now at the low end of this range, after the apparent reduction over the last ten years. There is also the no-observed-effect concentration (NOEC), which refers to the highest concentration with a response that is not statistically different from the responses of the control (Shieh *et al.*, 2001). However, there is some dispute about the usefulness of NOEC (Crane & Newman, 2000). Algal sensitivity to copper varies and tolerance may be due to several mechanisms. These mechanisms can include reduced cell membrane permeability excluding the metal ions, and binding of the metal ions to organic complexes (Yan & Pan, 2002). The toxic effects of copper on algæ and bioaccumulation may be linked and tolerance may be acquired (Yan & Pan, 2002). Thus, exposure to elevated levels of copper increases tolerance compared to a biofilm that has not been exposed, and this can lead to changes in the community structure (Soldo & Behra, 2000). For example, a community dominated by *Chlorophyta*. Tolerant and

intolerant strains of alga may be found within a single community, but this observation may be linked to an increasing survival rate for less tolerant species as (toxicity) concentration of copper decreases (Hillebrand & De Vries, 1986). *Cladophora glomerata* is thought of as a species sensitive to trace metals (Hillebrand & De Vries, 1986; Agrawal & Misra, 2002).

It is reported (Yan & Pan, 2002) that there are two classes of uptake processes involved with the bioaccumulation of copper by algæ, a fast, passive and reversible set of processes that include adsorption, complexation, chelation, coordination, and ion exchange, and a second slower and less reversible uptake. These slower processes can include, binding of ions to (enzyme active sites) and proteins, redox reactions, and covalent bonding. In the rapid growth stage, *i.e.* during the spring bloom, alga are able to desorb a significant amount of the copper taken up, and thus less is accumulated, but dead algal cells lose this ability to desorb the copper (Yan & Pan, 2002). Thus in the River Tame when the considerable build-up of filamentous algæ which occurs dies back there may be an export downstream of copper.

In high-flow events most of the copper is in a stream is associated with the suspended particulates (Achterberg *et al.*, 2003), but Xue *et al.* (2000) found that during low-flow periods approximately half the copper load in streams draining an agricultural catchment was in the dissolved form – between 1.87 – 4.90 μg l^{-1} (0.03 – 0.08 μM). During a rain event dissolved copper concentrations were between 3.9 – 5.8 μg l^{-1} (0.06 – 0.09 μM), but concentration was observed to decrease with increasing river discharge (Xue *et al.*, 2000). These concentrations are comparable to those recorded in the River Tame

In neutral waters, such as the River Tame, the majority of the dissolved copper has been found as organic complexes (Xue *et al.*, 2000). Therefore organic carbon, both total (TOC) and dissolved (DOC) may be an important factor in the movement of dissolved copper (Gundersen & Steinnes, 2003).

5.4: Conclusions

- The river water dissolved copper concentrations were generally greater at SV than BM, and this is the same as was found for the SRP concentrations.

- Changes in the steady-state concentration of dissolved copper suggest that the bed sediment is responding to reduced river water concentration and setting a new steady-state.

- The kinetics of the reaction of the sediment to copper are of a similar order, and rate constants are increasing through the season, but are of a similar magnitude for both sites.

- There are differences in the n and rate constant values which indicate a difference in the mechanisms (*i.e.* the order of the kinetics) of uptake through the seasons.

- The kinetics of the uptake reactions in BM sediment are described by a rate law of the form flux = k $\{[Cu] - [Cu_{ss}]\}^{n}$, and because the concentration of the copper at steady state is the same as the river water copper concentration, this rate law yields a method of estimating the flux to the sediment. For example, if during a pollution incident the copper concentration in the water increased so that the copper concentration was much greater than the steady-state concentration of copper, then the rate constant could be used to estimate the initial flux to the sediments. Similarly, if a sediment disturbance led to an increase in copper concentration in the water, the rate law could be used to estimate the time it would take the system to return to a steady state. When the equation is combined with a mass balance, the changes in concentration of copper downstream could also be predicted.

Chapter 6

General Discussion and Conclusions

6.1: Introduction

The results are discussed here in the context of the study and the River Tame, and in the wider perspective of the Natural Environment Research Council Thematic Programme for Urban Regeneration and the Environment (URGENT III).

The aims of URGENT III were to ascertain the extent of the problems involved in the management of contaminated urban areas, and develop generic models or solutions which could be applied elsewhere. One specific target was to gain a better understanding of the factors controlling chemical fluxes to and from contaminated river sediments. This research has examined the effects of both biota, in the form of the filamentous algal biofilm, and the physical composition of a bed sediment on those sediment-water exchanges.

The specific intention of this investigation was to assess the bearing an algal biofilm may have on chemical fluxes at the sediment-water interface and the kinetics of these exchanges. In order to achieve this both field and laboratory elements were employed - field-based observation and sampling on the River Tame, and the use of flume channels to simulate the natural system under controlled conditions.

6.2: Experimental constraints of the flume system

The flume channels were used in these experiments because they were the most practical approach to achieving the aims and objectives of this study given the nature of the River Tame, *i.e.* no feasible alternative was found to do this kind of examination of the system in this river.

The flume channel experiments were designed to study the kinetics of the response of a sediment to a decrease or increase in solution contaminant concentration; in this case SRP and copper. The experiments allowed a steady-state to be reached in the flumes, unlike a river where equilibrium would not be reached because of the relatively slow response of the bed sediment and fine particulates trapped in an algal biofilm to changes in contaminant concentration in the overlying water. Kinetic measurements using

concentration of contaminant require non-equilibrium conditions. The flume channel experiments initially examined non-equilibrium release kinetics. Then an examination of non-equilibrium uptake kinetics was attempted. These experiments were performed over long periods so that a steady-state was approached.

The flumes used in these experiments differ from rivers in that the flumes are closed systems with respect to the exchange of, for example phosphorus, between water and sediment; there are no additional sources of phosphorus either to the overlying solution or to the sediment sample. However, gaseous exchange is unrestricted and the water surface is exposed to daylight. Relatively rapid warming or cooling of the flume contents (because it is smaller than the river) has been minimised in the design by immersing the flume channels in a flowing water using an external source of river water to maintain a river water temperature (Figures 2.4 and 2.5 in Chapter 2).

The closed system design was chosen as it is essential for a mass-balance to be undertaken over an extended period. In an open system a channel in excess of 2 km (House & Denison, 2002), with no additional inputs of contaminants (or monitored inputs) and with continuous monitoring of input and output would be required to detect changes in concentration. In the case of the River Tame this was found to be impractical as the both diffuse and point sources were too abundant to monitor. This would also not be practical for a flume design because of the length of channel required.

In order to simulate the exposure of a water parcel to a sediment to examine the contaminant exchange, the overlying solution circulates and acts as a proxy for a parcel of river water. This achieves an approximation to the continuous exposure of a parcel of water passing down a river would get to a bed sediment. However, the closed system cannot simulate solution exposure to sediments with changing EPC_0 status, *i.e.* in the river the water flows over a succession of types of bed sediment whereas in this closed system one type of sediment is exposed continuously.

An improvement in sediment sampling tray design would have been to allow the vertical exchange of sediment in and out of the trays, both during sampling and experiment, but practical design, operation and cost limitations prevented this.

6.3: Sediment

The sediment sampling method used here maintained the integrity of the sediment as it occurred in the natural river bed. This was an important improvement on previous experimental work (House *et al.*, 1995) in understanding the effects on fluxes across the sediment-water interface because the mixed nature of the sediment as it occurred in the river was preserved. It also allowed the effect of the development of the biofilm through the growth cycle to be considered.

Apart from the Phosphorus Transfer Index discussed in this study (Chapter 2, Section 2.3.8.4, and Chapters 3 and 4), Sallade and Sim (1997) assert that measuring the phosphorus sorption capacity of a fine sediment may be a useful way to assess the potential of the sediment to cause non-point pollution of a system through phosphorus release.

6.4: Soluble Reactive Phosphorus

Because the study reach of the River Tame, like many rivers in the U.K., is subject to sewage effluent that has had no phosphorus removal treatment, SRP is a significant contaminant of the water and the sediment. A study by Gburek *et al.* (2000) found there was little variation in the distribution ratio between the forms of phosphorus (total, dissolved and bioavailable) during downstream transport, but SRP can remain adsorbed in the sediment until a change in the river conditions cause release. Adsorption and desorption processes are most efficient when there is good mixing of the waters (Taylor & Kunishi, 1971), or of the sediment with the overlying water, such as in the irregular flow conditions experienced by the River Tame. Maguire *et al.* (2002) use a phosphorus buffering capacity (PBC) formula, calculated from the amount of adsorbed and water soluble phosphorus derived from a sediment aggregate, to assess the ability of that sediment to release or take up phosphorus from solution. They showed that the fine particles (< 2 mm) behave differently when separated into size fractions, or present as a mixture, and the strongest source/sink of phosphorus can have a disproportionate influence on release and uptake.

In the 1970s Taylor and Kunishi (1971) recorded stream water SRP concentrations of 15

- 20 μg l^{-1} (0.48 - 0.65 μM) during high spring flows, and 40 – 60 μg l^{-1} (1.3 -1.9 μM) in low flows in autumn, in an agricultural catchment. This can be compared with the low flow concentrations in the Tame of between ~16 μM at Bentley Mill Way and ~109 μM at Sandwell Valley to illustrate the impact of the inputs. Tertiary treatment for phosphorus removal from sewage effluent could significantly reduce the load, by around 30 % (Foy & Lennox, 2000), and is increasing in use as a water quality management tool (pers. comm. Severn-Trent Water).

6.5: Copper

Much of the copper load in a lotic system is associated with the dissolved and particulate material (Ramamoorthy & Kushner, 1975; Herzl *et al.*, 2003). Like many urban rivers the Tame has had a high exposure in the past and continues to be subject to high levels of input through sewage and overland flow (Boller & Steiner, 2002). There may be significant amounts stored in the bed sediment available for release when disturbance such as dredging for flow management occurs.

Förstner (1990) states that there is direct (Luther *et al.*, 1980) and indirect (Lu & Chen, 1977) evidence that the concentrations of copper in sulfidic porewaters are determined by dissolution-precipitation processes. In a study of copper in coastal marine sediments (Westerlund *et al.*, 1986) fluxes were linked to the organic matter cycle - earlier investigations of copper in porewater had shown enrichment relative to bottom water; in that study porewater, which was sulfidic, was found to be depleted compared to the ambient water, and therefore the flux would be expected to go from overlying water to sediment. The porewaters of the River Tame sediment were also found to have lower dissolved copper concentrations than the river water, and so a similar response might be expected. The rapid uptake reactions seen in the experiments in this investigation seem to confirm this.

The mechanisms by which trace metals exert toxic effects on algæ include those which affect their nutrient uptake capability, and House and Graupner ((submitted)) also showed photosynthesis to be inhibited by copper. Copper is particularly implicated in enzyme disruption, limiting the uptake of phosphorus by the alga, and interference with the transport of silicon, vital to diatom growth (Price & Morel, 1994).

6.6: Biofilms

Ubiquitous in all natural water systems (Cox, 1988; Kelly, 2000), and even merely damp ones, the biofilm focussed on in this case was the benthic community, and especially the *Cladophora* spp. The River Tame develops a significant growth of this filamentous alga, and it was seen to act as an important trap for a large amount of fine particulate material, as well as epiphytic diatoms (Marks & Power, 2001). This trapping of fines may be an important factor in the release and uptake of contaminants in a polluted river system.

An algal biofilm itself will have a higher pH than the surrounding water during photosynthesis due to the decrease in CO_2, and this creates conditions promoting the removal of contaminants such as phosphorus and trace metals (Liehr *et al.*, 1994; Battin, 2000). The presence of microenvironments also means that variations in the physico-chemical attributes of the biofilm, such as DO, pH, conductivity, micronutrients, and dissolved organic carbon (DOC) concentrations, can take place both spatially and temporally (Dodds & Gudder, 1992; Leadbeater & Callow, 1992; Heath *et al.*, 1993). These differences can occur within very small distances, *i.e.* a few micrometres, but broad time-scales, *i.e.* in response to environmental fluctuations that occur diurnally, seasonally, and even annually. Because of these differences Heath *et al.* (1993) noted that care must be taken with reference to relating bulk solution chemistry to that occurring in the biofilm.

In phosphorus limited systems, studies showed (Peterson *et al.*, 1993a; 1993b) that river fertilisation had a significant impact on the growth of epilithic and filamentous algæ, and hence on all trophic levels of the food web, but that ensuing changes in the grazer community tended to balance the growth pattern. However, in a system such as the River Tame, where phosphorus is not limited at any time of year, such effects would be negligible. Although the growth peaks appear in late spring in this study, Whitton (1970) describes two periods - spring and early autumn - when *Cladophora* growth was observed and suggests temperature and light intensity may also influence growth patterns.

6.7: Macrophytes

It was noted during site visits that in addition to the extensive *Cladophora* growth that developed, there were places within the measured reaches where large clumps of *Potomageton* were present at the height of the growing season. Some research (Sand-Jensen, 1998) indicates that submerged macrophytes have an important effect on sediment composition and trapping, and Aldridge and Ganf, (2003) suggest that macrophytes can also affect the adsorption and desorption of contaminants by influencing the sediment redox and hence affinity. However, the significance of this last effect seems to be limited to emergent species. The *Potamageton*, being a submerged macrophyte, would have little impact, and the cover of emergent species present in the River Tame in the reaches examined was limited. However, this research has shown that the trapping of fine particulates is important, and the *Potomageton* fulfils this rôle in a similar way to the *Cladophora*.

6.8: Macroinvertebrates

Macroinvertebrates inhabit all niches in river systems, and may have effects on contaminant exchanges through their bioturbation of the sediment or grazing of the biofilm. In oxic, fine silt sediments chironomids were found (House *et al.*, 1995a) to enhance fluxes of contaminants, in this case phosphorus. This leads to bioturbation effects being incorporated in measured fluxes, *i.e.* not being distinguishable. It may therefore also be important to distinguish these effects, and to account for seasonal differences in activity, especially those influencing kinetics and burial of surface sorbed contaminants.

Granéli (1979) showed that tubificids are important in the regeneration of phosphorus in freshwater sediments, and chironomids in the sediment-water exchange of dissolved silicon. Chironomids also increase the O_2 uptake of sediments, promote the transport of Si from a sediment, and graze biofilm on the sediment surface. Naturally the effects of these macroinvertebrates are modulated by population densities and these appear to vary dramatically. In one study (Davis *et al.*, 1975) tubificid population numbers in a lake sediment were measured and found to be ~700 worms m^{-2}, but also noted that in eutrophic conditions worms densities can exceed 10,000 m^{-2}. They contend that worms

increase the uptake of phosphorus by the sediment, and cause the redox potential in the uppermost sediment to rise.

Although it had been originally planned to include an assessment of the impact of bioturbation by tubificid oligochætes, and some initial work with mesocosms was undertaken (Appendix 6.1), this was found to be an inappropriate course of action due to the nature of the subtrate in the study reaches, which had very little fine silt sediment. However, a macroinvertebrate census was performed on stones taken from the River Tame as an additional study by Jill Patten. The macroinvertebrate census was executed using methods based on those developed by Marker and Casey (1982). As > 10 species have been found to inhabit *C. glomerata* growths (Whitton, 1970), this method allowed the algæ, and any associated macroinvertebrates, to be removed from the stones in stages, and so give an overall picture of the species present and the preferred habitat. The following macroinvertebrates were found in the samples selected: *Nematoda, Oligochæta, Chironomidæ, Asellidæ,* and *Hirundinea,* and this suggests the community was species poor. However, as *Chironomidæ* were found to dominate the community and are grazers there could be some effect on the biofilm, though Dodds and Gudder (1992) assert that grazers may take other common benthic freshwater algæ in preference to the *Cladophora.*

6.9: Conclusions

This study was the first to be made of the differences in behaviour, with respect to their effects on the fluxes of phosphorus and copper, of the individual size fractions which comprise most natural river sediments, and a heterogeneous, mixed grain-size river sediment.

In the flume experiments sediment from the two sites (Bentley Mill Way and Sandwell Valley) reacted in similar ways, though for both SRP and copper this was over a different concentration range. The river water SRP and dissolved copper concentrations were generally greater at SV than BM. The higher EPC_0s found for SV than BM indicate a greater amount of SRP adsorbed at SV.

6.9.1: Sediment grain size

Sediment grain size distributions in the experiments using natural sediment samples, within the three size fractions defined for this study, were similar for both study sites, but the Sandwell Valley site samples had a greater proportion of stones (> 20 mm).

The biofilm associated with the larger sized material (stones and gravel) was a major control of the limiting flux of SRP in the study reach. The results indicate that the biofilm attached to gravel and stone substrates has an important control over the release of SRP when the overlying water concentration is reduced below the EPC_0 value. These response distinctions were not observed with respect to the copper fluxes.

The presence of fine material trapped in filamentous algal growth on stones influences SRP release into solution, and from this evidence it is concluded that turbulent flow, created by stream-bed surface roughness, and stream velocity contribute to the flux and magnitude through the re-suspension of these fine particulates. The fine sediment from SV released more SRP than the fine material from BM as a result of SV being exposed to higher SRP concentrations.

6.9.2: Kinetics

Although the SRP and copper concentrations at the two sites (Bentley Mill Way and Sandwell Valley) were different, they reacted with the sediment in a similar way when the sediment composition was comparable. Unlike the flume experiments, where SRP and copper in the overlying solution was approaching or at steady-state by the end of the experiments, the river system was not able to equilibrate and was continuously responding to variations in the contaminant concentration. Changes in the steady-state concentration of dissolved copper after the addition of a standard solution to observe uptake by the sediment suggest that the bed sediment is responding to reduced river water concentration and setting a new steady-state.

The measured and calculated fluxes of SRP resulting from the experiments indicate that larger size components in a sediment with a well-developed biofilm present give rise to a greater release than a sediment mainly composed of fine sand and silt. This was observed

at both sites. Differences in behaviour were observed both between the two study sites and the sediment size fractions with respect to phosphorus.

The kinetics of the reactions of the sediment to copper were of a similar order, and rate constants increased through the season, but were of a similar magnitude for both sites. The results indicate a possible faster exchange response mechanism to be in operation, caused by the very rapid interaction of the trapped particulates with the SRP, compared with the diffusion mechanism in the settled bed sediment. The kinetics of these reactions confirms that another mechanism is involved than simple diffusion. There are also differences in the n and rate constant values for copper uptake by the sediment, which points to a difference in the mechanisms (*i.e.* the order of the kinetics) of uptake, though in this case the divergence occurs through the seasons.

Because the concentration of the copper at steady-state in the experiments was the same as the river water copper concentration, the kinetics of the uptake reactions resulted in a method of estimating the flux to the sediment for recovery time after a pollution incident or a major disturbance of the sediment such as remediation work. This method is also able to assess how far downstream the copper concentration would remain elevated after a pollution incident or sediment disturbance.

6.9.3: Summary

The results of the size fraction experiments have shown the significance of the larger size gravel and stones in a river bed because they act as a substrate for the filamentous biofilm growth where fine particulate material is entrapped.

The flux of SRP from the fine particulates trapped within the biofilm associated with stones dominates the limiting diffusion flux from a sediment. However, the concentration of SRP in the river water influences the mechanism controlling the limiting diffusion flux of phosphorus because exposure to high concentrations of SRP will raise the EPC_0 and thus the limiting flux. A comparison of the equilibrium concentration value for a sediment and river water values can establish whether there would be a net influx or efflux of SRP to/from a sediment at that time. This is assuming the sediment is not saturated with SRP, *i.e.* the adsorption is a linear function of concentration as found in

this system.

A rate law describing the flux of copper to a sediment was tested and shown to be a useful method of estimating recovery and downstream reduction in copper concentration in the water in a river with similar characteristics to the study reach, after some major disturbance or polluting incident.

In addition, the output data from this study (in the form of metadata[1] and detailed data) will be catalogued and archived in NERC Data Centres as part of a decision support aid for U.K. planners working in the urban environment.

6.10: Further Work

- Because of the importance of the contribution to fluxes of the fine particulates trapped in the filamentous algæ, finding out how much of this material a filamentous biofilm holds, and how well it holds on to it in different flow conditions, would greatly aid behaviour prediction.

- More work on the seasonal variability in reaction kinetics, and variability between the samples at the same time of year, would enhance the understanding of the driving forces copper fluxes in sediment.

- For further investigation of the fluxes the flume channels systems and methodology need to be developed in such a way as to investigate the rapid initial releases of contaminants on exposure to the solution.

- As natural river systems can be subject to very different flow conditions occurring in the same reaches, it would be very useful to use the artificial flume channels to examine the effects of different flow regimes on fluxes.

---

[1] Metadata describe the content, quality, condition, and other characteristics of data.

- As the size of material in a bed sediment was found to be such an important aspect in influencing contaminant fluxes, further investigation of the changes in bed composition over an annual cycle in a highly heterogeneous system such as the River Tame would allow prediction of behaviour to be more accurate.

- To get a better understanding of the effects of bioturbation and grazing in a mixed grain-size system such as the River Tame it would be valuable to perform a macroinvertebrate census at different times of year would. This idea could be further developed by using the flume channels for study in a controlled environment, *i.e.* remove the invertebrates from a natural sample of bed sediment with a well-developed biofilm to see if there is an effect on fluxes.

- And lastly, it would assist ecosystem management modelling significantly to have a better knowledge of the speciation of copper in river systems – this is an especially interesting aspect because there is little published work on this as yet.

Appendices

Appendix 2.1

Field Record Sheet

FIELDWORK OBSERVATION SHEET

Site: BMW   SV   Date: /   /   Time:
Visit: Routine   Other (specify reason)
Weather conditions:
Overall weather of previous week:
Depth at fixed point: m.

100 metre section (direction of flow →)

```
0
1
2
3
4
5
6
7
8
9
     1      2      3      4      5      6      7      8      9      10
```
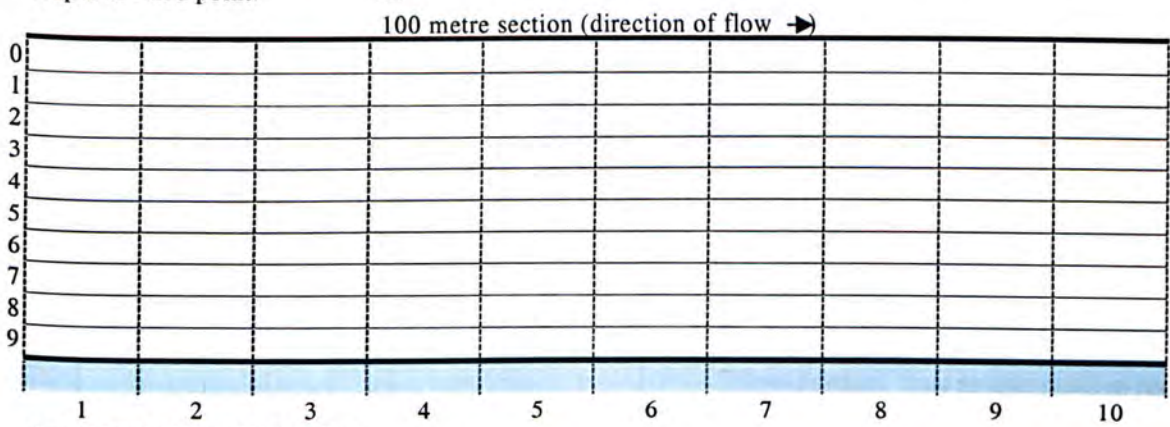
ON SECTION SCHEMATIC:

1. Indicate approximate position, areal coverage and type of main plants present.
         Identify to main plant groups as follows:
         PO = Potamageton   GA = Green algae   MI = Mixed algal cover OC = Other cover
         CL = Cladophora   BR = Brown/grey algae MA = Macrophytes

2. When applicable mark positions samples taken from [x] and the sample codes [letter/date].
3. Mark position of sediment collection trays [5 ⌊__⌡] and which removed and replaced [2 R].
4. Indicate any build-up/erosion of sand/mud bar, including any containing a reed growth.
5. In shaded areas indicate dominant substrate type in that section:
         SS = Sand/silt   GR = Gravel   RO = Stones/large rocks   OS = Other substrate
6. Flow velocity measured at bed depth for each sediment collection tray:

Tray 1: _____	Tray 5: _____	Tray 9: _____
Tray 2: _____	Tray 6: _____	Tray 10: _____
Tray 3: _____	Tray 7: _____	Tray 11: _____
Tray 4: _____	Tray 8: _____	Tray 12: _____

7. Water sampled: Bulk   ml   Pore   ml

ON CHANNEL X-SECTION:

1. Mark current water level and indicate with arrows the position of last high water mark.

Other general observations and comments:
   e.g. colour, odour, disruption or damage, land.

Field Record Sheet - reverse

NOTES for OBSERVATION SHEET

These observations and measurements are required because the river conditions determine the sediment conditions and will inform the experimental work with the sediment in the fluvarium.

Site 1 BMW
100 m section between markers, from bridge downstream.
Site 2 SV
100 m section between markers, where substrates change.

- Observations should be carried out at 2 weekly intervals unless a storm event occurs, when they should be completed as soon as possible after the event.
- In case of any plant/biofilm identity queries, take small sample back to laboratory for identification. Note sample position and number on the Observation Sheet.

Other General Observations and Comments: *e.g.*
- Water colour or turbidity
- Presence of any unusual, distinct odours
- Natural or unnatural disruption of river bed or banks
- Any disruption of equipment, *e.g.* storm damage or vandalism
- Any notable changes in surrounding land appearance or usage

For safety:
- contact EA to check river flow conditions before going to site – Tel: **0906-619-7744**
- do not make site visits alone.
- wear gloves when handling water or sediment.

NOTES for SAMPLING TRAYS

PLACEMENT
Position sample trays in river bed so that the tray is submerged to top of tray edge.

COLLECTION
Make observations, flow velocity measurement at tray position, and take porewater sample from ½ depth inside tray prior to removing from river bed.

TRANSPORTATION
Place trays in transportation boxes and cover with river water; taking as much care as possible not to disturb the sediment in the channels. Transport to laboratory and store at between 4 - 8°C until following day. Carry out treatment of samples.

Appendix 2.2

Measurement of pH Using CIBA-CORNING M90 Field Meter

Initial preparation

1.   Before use remove the wetting cap from the sensor tip and slide the vent sleeve to expose the filler hole at the top of the electrode.
2.   Make sure that the internal electrolyte ("fill solution") is not more than 1 inch below the filler hole. If necessary add KCl solution to fill up to filler hole. *It is important to use the correct filler solution (4 M saturated solution Aristar grade KCl) and not KCl saturated with AgCl.*
3.   Examine the sensor tip to ensure there are no air bubbles at the ceramic junction. If necessary gently tap the sensor to remove any air bubbles at the ceramic junction.
4.   If the sensor is new or has been stored dry, then it is necessary to soak the electrode in pH 7 buffer for about 2 hours before use.

Calibration

1.   In all measurements it is essential that the sensor tip and ceramic frit be immersed in the measuring or standard solutions. Partial immersion will result in unpredictable errors. Temperature control of the solutions is not needed at any stage. Do not use in solutions with a temperature outside the range of 0 °C to 100°C. Do not leave the sensor in organic solvents or strong acid or alkali solutions.
2.   Switch on meter by pressing *mode, read, cal* or *M*.
3.   Wash sensor with distilled water and allow to drain (if necessary blot dry with a tissue but *do not wipe the sensor tip.* Place sensor in pH 7.0 (the buffer pH at 25°C) buffer at the ambient temperature and gently swirl for 2 minutes. Press *cal* (cal 1 is displayed) continue gently swirling until a stable reading is detected and the display automatically updates to the calibration value at the measured temperature.
4.   Wash the sensor with distilled water and allow to drain. Place the sensor in the second calibration buffer at the ambient temperature. This must be either pH 4.0 or pH 10.01 (the buffer pH at 25°C); the choice of the second buffer depends on the expected pH of the natural water to be measured. *It is essential to choose the second buffer so that the measured pH is between the two buffers.* Normally for hardwaters this will be pH 7 and 10.01 and for soft/upland/acid waters pH 7 and 4.0. Gently swirl the sensor for 2 minutes and then press *cal* (cal 2 is displayed) continue gently swirling until a stable reading is detected and the display automatically updates to the calibration value at the measured temperature.

Measurement

1.   Ensure the memory is empty. If the *M* is displayed, press *R* (recall M) until M C is displayed and then *M* to cancel all readings. The *M* on display should disappear.
2.   Wash the sensor with distilled water and allow to drain (see section 7). Place the sensor in the measurement solution and gently swirl for 2 minutes. (If the sensor is placed directly in a flowing river, the sensor may be held still; however it is normally recommended to make the pH measurement in the same conditions as the calibration in section 7 and 8 above.) Press *read*, and wait until a stable reading is detected.

Press *M 1* to store the reading. Repeat the measurement a further 2 times with fresh solutions recording each by pressing *M 2* for the second measurement and *M 3* for the third measurement. Alternatively the readings can be recorded on paper after each measurement.

3. Wash the sensor with distilled water and reposition the vent sleeve to cover the filler hole and replace the wetting cap containing pH 7 buffer.

4. The measurements may be retrieved from memory by pressing *R* to obtain the last saved reading. Press *R* again to recall previously saved readings. The display *M1..F5* indicates which saved measurement is being displayed. To remove stored readings, follow the instructions in section 9 above.

Maintenance

1. Excess KCl crystals in the internal solution or contamination of the internal filling solution. This necessitates that the solution be removed and replace by fresh fill solution. Remove the old fill solution and use warm distilled water to flush out and dissolve excess crystals. Remove water and refill with fresh KCl internal solution.

2. Blocked or damaged ceramic frit. Remove the ceramic frit using tweezers and insert a new junction. Tap to remove air bubble near the frit.

3. Oil contamination of sensor. wash the sensor in 50% water acetone solution; do not soak for more than 2 minutes. Rinse the sensor with distilled water and then soak for 2 hours in pH 7 buffer before recalibration.

4. Slow response of the sensor. Soak in 0.01 M HCl over night. Wash with distilled water and recalibrate.

Appendix 2.3

Oxygen Measurement Using CIBA-CORNING M90 Field Meter

This measurement refers to the use of the Ciba-Corning Mate 90 portable microprocessor based oxygen, temperature meter only. The manufacturers recommend that the samples be stirred at a constant rate of approximately 20 cm s^{-1} during measurements. The tip of the sensor must be immersed to cover the temperature sensor, *i.e.* about 3.5 cm.

Initial preparation

1.   Before use remove the wetting cap from the sensor tip.

2.   The meter should normally be kept connected to the sensor. The sensor may be removed for up to three hours, as a rechargeable battery in the sensor will maintain polarization.

3.   Switch the meter on by pressing *mode, read, cal* or *M*.

Calibration

1.   Wash the sensor with distilled water and allow to drain.

2.   Place the sensor in **fresh** zero oxygen solution (saturated Na_2SO_3 solution). Move the sensor in a gentle circular motion when calibrating or measuring. Continue swirling the sensor for two minutes and then press *cal* (cal 1 is displayed). After the reading has stabilized the display automatically updates to the calibrated value.

3.   Wash the sensor with distilled water and allow to drain.

4.   Place the sensor in water (distilled water or a solution of similar ionic composition to the water to be measured) saturated with air, ie 100% oxygen. A solution with air bubbling through to ensure saturation and equilibrium with the atmospheric oxygen concentration is adequate. No temperature control of the solution is needed. Gently swirl the sensor for two minutes before pressing *cal* (cal 2 is displayed). After the reading has stabilized the display automatically updates to the calibrated value.

Measurement

1.   Ensure the memory is empty. If the *M* is displayed, press *R* (recall M) until M C is displayed and then *M* to cancel all readings. The *M* on display should disappear.

2.   Wash the sensor with distilled water and allow to drain. Place the sensor in the measurement solution and gently swirl for two minutes. (If the sensor is placed directly in a flowing river, the sensor may be held still; however it is better to use the same conditions as in the calibration described above). Press *read*, and wait until a stable reading is detected. Press *M 1* to store the reading. Repeat the measurement twice with fresh solutions recording each by pressing *M 2* for the second measurement and *M 3* for the third measurement. Alternatively the readings can be recorded on paper after each measurement.

3. Wash the sensor with distilled water and replace the wetting cap containing distilled water.

4. The measurements may be retrieved from memory be pressing *R* to obtain the last saved reading. Press *R* again to recall previously saved readings. The display: *M1. M5*, indicates which saved measurement is being displayed. To remove stored readings, follow the instructions in section 8 above.

<u>Maintenance</u>

1. Check for membrane damage; it may be visibly damaged or sufficiently damaged to lead to losses of the DO electrolyte. The membrane should be replaced using the membrane replacement kit (catalogue No. # 473626).

2. Every two weeks of more often as necessary, unscrew the membrane cap from the sensor. If the silver/gold tip is tarnished, clean carefully with particular attention to the gold cathode. A fine abrasive such as silicon carbide paper 166 (English Abrasives) has been found suitable. Rinse tip with DO electrolyte (cat. # 474594) and fill membrane cap avoiding air bubbles. Hold the sensor vertically and gently screw the membrane cap on the sensor allowing surplus electrolyte to run off. Check for air bubbles on the membrane cap by looking up through the membrane from the bottom of the sensor. Fit sensor to the meter and allow at least one hour to polarize.

Appendix .2.4

Conductivity Measurement Using CIBA-CORNING M90 Field Meter

This measurement refers to the use of the Ciba-Corning Mate 90 portable microprocessor based conductivity, temperature meter only. The solution must be above the cell chamber rings and below the vent hole. The clear plastic shield should be in place and the chamber free of air bubbles when measuring.

Initial preparation

1. Ensure the sensor and shield are clean before use.

2. Prepare a standard solution of potassium chloride (0.01 M) by dissolving 0.3728 ± 0.0005 g of AR grade anhydrous potassium chloride (dried for two hours at 110 ± 10°C and subsequently stored in a desiccator) in a 500 ml 'A' grade volumetric flask. Use a glass weighing boat to transfer the KCl to the volumetric flask and rinse the boat with distilled water prior to making the volumetric up to the mark with freshly distilled water. The electrical conductivity of this solution at 25°C is 1413 FS cm^{-1}. Alternatively a 1413 FS cm^{-1} standard solution is available (cat # 473623).

3. Wash the sensor and shield with distilled water and allow to drain.

4. With the sensor held in the air, *i.e.* open circuit, press *cal* (cal 1 is displayed) and wait until stabilized. The display should read 0.0 FS cm^{-1}.

5. Place the sensor in the standard 0.01 M KCl solution. Gently swirl the sensor for two minutes before pressing *cal* (cal 2 is displayed). After the reading has stabilized the display automatically updates to the calibrated value. Independent of the temperature, the updated reading should be 1413 μS cm^{-1}.

Measurement

1. Ensure the memory is empty. If the *M* is displayed, press *R* (recall M) until M C is displayed and then *M* to cancel all readings. The *M* on display should disappear.

2. Wash the sensor and shield with distilled water and allow to drain. Place the sensor in the measurement solution and gently swirl for two minutes. (The measurement is essentially independent of the water flow.) Press *read*, and wait until a stable reading is detected. Press *M 1* to store the reading. Repeat the measurement twice with fresh solutions recording each by pressing *M 2* for the second measurement and *M 3* for the third. Alternatively the readings can be recorded on paper after each measurement.

3. Wash the sensor and shield with distilled water removing the shield if necessary to rinse with water. Ensure that the sensor is clean after use. The sensor must not be left dirty and must be stored in distilled water after use.

4. The measurements may be retrieved from memory by pressing *R* to bet the last saved reading. Press *R* again to recall previously saved readings. The display *M1..M5*

indicates which saved measurement is being displayed. To remove stored readings, follow the instructions in section 6.

5.   Apart from routine and rigorous checks of the cleanliness of the sensor and shield, no other maintenance is necessary.

Appendix 2.5

QBASIC Program

```
'PROGRAM TO PERFORM FLUVARIUM EXPERIMENTS
 'NOVEMBER 1999
DECLARE SUB CALIBRATE (slope1!, slope2!, epot1!, epot2!, E01!, E02!,
E1001!, E1002!)
DECLARE SUB DISKDATA ()
DECLARE SUB PURGE ()
DECLARE SUB per (S)
DECLARE SUB SAMPLE (NS%)
DECLARE SUB INIT ()
DECLARE SUB FLOW (N%)
DECLARE SUB MOVAL ()
DECLARE SUB AICALC ()
DECLARE SUB DISKDATA ()
DECLARE SUB KEYBRD (K)
DECLARE SUB CLEAN ()
DECLARE SUB ROTATE ()
DECLARE SUB ADC ()
DECLARE SUB SETUPADC ()
DECLARE SUB readmv (OPT$, ch1, ch2, ch3, ch4, ch5, ch6, ch7)
DECLARE SUB per (S)
DECLARE SUB mty ()
COMMON SHARED CT, VS, SU, SE, ER, VI, K, CS, CD
DIM SHARED DIGIT%(8), GAIN%(16)
DIM SHARED TT$(50), phT(50), VolT(50), C(50), d(50), S(50)
SCREEN 0, 0, 0: WIDTH 80: CLS : KEY OFF
CALL SETUPADC
'MAIN MENU
epot1 = 1: epot2 = 2: slope1 = 3: slope2 = 4: E01 = 5: E02 = 6: E1001 =
7: E1002 = 8
1500 PRINT "MAIN MENU"
  PRINT : PRINT
  PRINT "1- Start fluvarium run"
  PRINT "2- Turn Valve"
  PRINT "3- Clean syringe pump"
  PRINT "4- Read a data file"
  PRINT "5- Calibrate sensors"
  PRINT "6- Exit the program"
  PRINT "7- Turn sample wheel"
  PRINT "8- Emty syringe"
  INPUT "Which Option do you Require?"; a%
  CLS
  IF a% = 1 GOTO 100
  IF a% = 2 THEN CALL MOVAL: GOTO 1500
  IF a% = 3 THEN CALL CLEAN: GOTO 1500
  IF a% = 4 GOTO 5000
  IF a% = 6 GOTO 6000
  IF a% = 5 THEN CALL CALIBRATE(slope1, slope2, epot1, epot2, E01, E02,
E1001, E1002): GOTO 1500
  IF a% = 7 THEN CALL ROTATE: GOTO 1500
  IF a% = 8 THEN CALL mty: GOTO 1500
100 'Fluvarium experiment menu
  PRINT "FLUVARIUM MENU"
  PRINT : PRINT
  PRINT "1- Perform an Experiment"
  PRINT "2- Return to the Main Menu"
```

```
   PRINT "3- Exit the Program"
   PRINT
   INPUT "Which Option do you Require?"; a%
   CLS
   IF a% = 1 GOTO 160
   IF a% = 2 GOTO 1500
   IF a% = 3 GOTO 6000
   GOTO 100
160 'EXPERIMENTAL DETAILS
   INPUT "Do you want to initialize the stepper driver board (only answer
'Y' if the computer has been switched off or reset since the last
run)?(Y/N)"; a$
   IF a$ = "Y" THEN CALL INIT
   PRINT "Please Enter the Filename ('RUN#+date-dd/mm/yy')"
   INPUT File$
   INPUT "Operator Name?"; name$
   INPUT "Source of sediment for channel 1"; sed1$
   INPUT "Description of channel 1"; des1$
   INPUT "Source of sediment for channel 2"; sed2$
   INPUT "Description of channel 2"; des2$
   W$ = "Date:"
   X$ = "Operator Name:"
   Y$ = "Source of sediment for channel 1:"
   Z$ = "Source of sediment for channel 2:"
   d$ = DATE$
 OPEN "C:\ADC\BASIC\" + File$ FOR OUTPUT AS #2
   PRINT #2, W$
   PRINT #2, d$
   PRINT #2, X$
   PRINT #2, name$
   PRINT #2, Y$
   PRINT #2, sed1$
   PRINT #2, Z$
   PRINT #2, sed2$
   PRINT #2, "Description of sediment 1:", TAB(20); , des1$
   PRINT #2, "Description of sediment 2:", TAB(20); , des2$
   PRINT #2, "Calibration parameters"
   PRINT #2, "epot1="; epot1; "slope1="; slope1; "E01="; E01; "E1001=";
E1001
   PRINT #2, "epot2="; epot2; "slope2="; slope2; "E02="; E02; "E1002=";
E1002
 CLOSE #2
   INPUT "Number of recirculations before each sampling?"; N%
   INPUT "Volume of each Sample?(in ml)"; VS%
   NS% = VS% / 10
180 INPUT "Number of Samples?"; Sam%
   INPUT "Time of each reading in 00 hundred hours?"; Tstart
   Tstart = Tstart * 3600!
   INPUT "Are you ready to start the run?(Y/N)"; a$
   CLS
   IF a$ = "N" GOTO 160 ELSE GOTO 200
200    'AUTOMATED SEQUENCE
   FOR j = 1 TO Sam%
OPEN "$adevice" FOR OUTPUT AS #1
'NOW TAKES SAMPLE FROM CHANNEL 1
   SamTime = TIMER
   PRINT SamTime; "FLUSHING THE SYSTEM"
'FLOW IS THE FLUSHING ROUTINE
 CALL FLOW(N%)
   PRINT "SAMPLE NUMBER ", j, "NOW BEING TAKEN FROM CHANNEL 1"
'SAMPLE is routine to take a sample from the channel
```

```
 CALL SAMPLE(NS%)
 CALL readmv("AUT", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
  pH1 = epot1 + slope1 * ch1
  DO1 = (ch3 - E01) * 100 / (E1001 - E01)
  temp1 = (ch5 * .2461) + .3844
  light = ((-ch7 * .9787 / 4!) + .2521) * 1000! / (4!)
 OPEN "C:\ADC\BASIC\" + File$ FOR APPEND AS #2
  PRINT #2, DATE$ + "   " + TIME$ + "  Channel1   ";
  PRINT #2, pH1; DO1; temp1; light;
 CLOSE #2
  PRINT "NOW PURGING THE SAMPLE LINE"
 CALL PURGE
'NOW TAKES SAMPLE FROM CHANNEL 2
  PRINT "FLOW OPERATING FOR CHANNEL 2"
 CALL FLOW(N%)
  PRINT "SAMPLE NUMBER ", j, "NOW BEING TAKEN FROM CHANNEL 2"
 CALL SAMPLE(NS%)
 CALL readmv("AUT", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
  pH2 = epot2 + slope2 * ch2
  DO2 = (ch4 - E02) * 100 / (E1002 - E02)
  temp2 = (ch6 * .25) + .2159
  light = ((-ch7 * .9787 / 4!) + .2521) * 1000! / (4!)
 OPEN "C:\ADC\BASIC\" + File$ FOR APPEND AS #2
  PRINT #2, DATE$ + "   " + TIME$ + "  Channel2   ";
  PRINT #2, pH2; DO2; temp2; light
 CLOSE #2
  PRINT "NOW PURGING THE SAMPLE LINE"
 CALL PURGE
CLOSE #1
 CALL ROTATE
'MOVES SAMPLE WHEEL READY FOR NEXT SAMPLES
  IF j = Sam% GOTO 220
  'checking how many samples
  PRINT "Sample "; j + 1; "to be taken in "; (NextSam - SamTime) / 60!;
" minutes."
 DO
  LOOP UNTIL TIMER >= Tstart AND TIMER < (Tstart + 5!)
 NEXT j
' CONTINUE RUN
220 INPUT "Do you want to continue the experiment with different time
intervals ?(Y/N) "; a$
  CLS
  IF a$ = "Y" GOTO 180
 OPEN "C:\ADC\BASIC\" + File$ FOR APPEND AS #2
  PRINT 2, DATE$ + "   " + TIME$ + "   end  ", 9999.9, 9999.9, 9999.9,
9999.9;
  PRINT 2, DATE$ + "   " + TIME$ + "   end  ", 9999.9, 9999.9, 9999.9,
9999.9
 CLOSE #2

  GOTO 6000
5000 'READ DATA FILE
  INPUT "Please enter the filename ('Exp#-R or A-dd/mm/yy')"; File$
 OPEN "C:\ADC\BASIC\" + File$ FOR INPUT AS #2
  INPUT #2, W$, d$
  INPUT #2, X$, name$
  INPUT #2, Y$, sed1$
  INPUT #2, Z$, sed2$
  PRINT W$, d$
  PRINT X$, name$
  PRINT Y$, sed1$
```

```
   PRINT Z$, sed2$
   INPUT #2, a1$, des1$
   PRINT a1$, des1$
   INPUT #2, a2$, des2$
   PRINT a2$, des2$
   INPUT #2, c1$
   PRINT c1$
   INPUT #2, c2$, c4$
   PRINT c2$, c4$
   INPUT #2, a$
   PRINT a$
 DO
   INPUT #2, a$, pH1, DO1, temp1, light1, a2$, pH2, DO2, temp2, light2
   PRINT a$, pH1, DO1, temp1, light1, a2$, pH2, DO2, temp2, light2
 LOOP WHILE pH1 <> 9999.9
 CLOSE #2
 OPEN "C:\ADC\BASIC\" + File$ + "Ch10" FOR OUTPUT AS #2
   PRINT #2, W$, d$; X$, name$;
   PRINT #2, Y$, sed1$; a1$, des1$
 CLOSE #2
 OPEN "C:\ADC\BASIC\" + File$ + "Ch20" FOR OUTPUT AS #2
   PRINT #2, W$, d$; X$, name$; Z$, sed2$; a2$, des2$
 CLOSE #2
6000 END


SUB ADC
600 ' PERFORM  A/D CONVERSION for all channels-single sweep  **********
610 '
615 PORT% = &H300                    'SET I/O PORT BASE ADDRESS
618 FOR j = 0 TO 7
620 OUT PORT% + 0, 0
630 ST% = INP(PORT% + 8)
640 IF (ST% AND &H80) = &H80 GOTO 630    'CHECK CONVERSION OK ?
650 DTL% = INP(PORT% + 0)
660 DTH% = INP(PORT% + 1)
670 ADL% = DTL% \ 16
680 ADT% = DTH% * 16 + ADL%
690 CHV% = DTL% - ADL% * 16
695 'PRINT "CURRENT CHANNEL="; CHV%;
700 IF (GAIN%(CHV%) MOD 8) > 3 THEN RES% = ADT% ELSE RES% = ADT% - 2048
710 'PRINT CHV%, RES%
720 DIGIT%(j) = RES%
770 NEXT j
END SUB


   SUB AICALC
 'program to calibrate the sensors
 END SUB

SUB CALIBRATE (slope1, slope2, epot1, epot2, E01, E02, E1001, E1002)
 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
26 PRINT : PRINT
   PRINT "1- start pH calib"
   PRINT "2- start DO calib"
   PRINT "3- start temp check"
   PRINT "4- Print calibration parameters and EXIT"
   PRINT "5- calibration parameters from keyboard"
   INPUT "Which Option do you Require?"; a%
   CLS
   IF a% = 1 GOTO 35
   IF a% = 2 GOTO 99
```

```
    IF a% = 3 GOTO 1100
    IF a% = 4 GOTO 2100
    IF a% = 5 GOTO 2101
35 PRINT "Calibrate pH. Place electrode 1 in pH 7 buffer"
 PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
40 a$ = INKEY$: IF a$ = " " GOTO 42
 GOTO 40
42 PRINT "Taking a reading now !!!"
 CALL per(30)
 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
   bu7el1 = ch1
 PRINT "Place electrode 1 in pH 10 buffer and wait 2 mins"
 PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
49 a$ = INKEY$: IF a$ = " " GOTO 50
GOTO 49
50 PRINT "Taking a reading now !!!"
 CALL per(30)
 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
   bu10el1 = ch1
   slope1 = (bu10el1 - bu7el1) / 3!
   epot1 = bu7el1 - slope1 * 7!
 PRINT "slope="; slope1; "E0="; epot1
 PRINT "Place electrode 1 in pH 7 buffer for checking and wait 2 mins"
 PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
64 a$ = INKEY$: IF a$ = " " GOTO 65
 GOTO 64
65 PRINT "Taking a reading now !!!"
 CALL per(30)
 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
   pH = (ch1 - epot1) / slope1
 PRINT "pH from electrode 1="; pH
 PRINT "Are you happy with that ??! (Y/N)"; a$
 INPUT a$
 IF a$ = "N" THEN GOTO 35
 PRINT "Calibrate pH. Place electrode 2 in pH 7 buffer"
 PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
70 a$ = INKEY$: IF a$ = " " GOTO 72
 GOTO 70
72 PRINT "Taking a reading now !!!"
 CALL per(30)
 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
   bu7el2 = ch2
 PRINT "Place electrode 2 in pH 10 buffer and wait 2 mins"
 PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
80 a$ = INKEY$: IF a$ = " " GOTO 82
 GOTO 80
82 PRINT "Taking a reading now !!!"
 CALL per(30)
 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
   bu10el2 = ch2
   slope2 = (bu10el2 - bu7el2) / 3!
   epot2 = bu7el2 - slope2 * 7!
 PRINT "slope="; slope2; "E0="; epot2
 PRINT "Place electrode 2 in pH 7 buffer for checking and wait 2 mins"
 PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
91 a$ = INKEY$: IF a$ = " " GOTO 92
 GOTO 91
92 PRINT "Taking a reading now !!!"
 CALL per(30)
 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
   pH = (ch2 - epot2) / slope2
```

```
   PRINT "pH from electrode 2="; pH
   PRINT "Are you happy with that ??! (Y/N)"; a$
   INPUT a$
   IF a$ = "N" THEN GOTO 65
   GOTO 26


' DO calibration
99 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
   PRINT "Now to calibrate dissolved oxygen sensor"
   PRINT "Calibrate DO. Place electrode 1 in DOzero soln and wait 30
seconds"
   PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
101 a$ = INKEY$: IF a$ = " " GOTO 110
   GOTO 101
110 PRINT "Taking a reading now !!!"
   CALL per(30)
   CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
     E01 = ch3
   PRINT "Rinse electrode 1 in dH2O and wait 30 seconds to stabilise"
   PRINT " Press 'SPACE' key to continue"
120 a$ = INKEY$: IF a$ = " " GOTO 130
   GOTO 120
130 PRINT "Taking a reading now !!!"
   CALL per(30)
   CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
     E1001 = ch3
   PRINT "Rinse electrode 1 in dH2O for checking and wait 30 seconds"
   PRINT "Press 'SPACE' key to continue"
140 a$ = INKEY$: IF a$ = " " GOTO 142
   GOTO 140
142 PRINT "Taking a reading now !!!"
   CALL per(30)
   CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
     percDO = (ch3 - E01) * 100 / (E1001 - E01)
   PRINT "%DO from electrode 1="; percDO
   PRINT "Are you happy with that ??! (Y/N)"
   INPUT a$
   IF a$ = "N" THEN GOTO 99
145 PRINT "Calibrate DO. Place electrode 2 in DOzero soln and wait 30
seconds"
   PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
151 a$ = INKEY$: IF a$ = " " GOTO 155
   GOTO 151
155 PRINT "Taking a reading now !!!"
   CALL per(30)
   CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
     E02 = ch4
   PRINT "Rinse electrode 2 in dH2O and wait 30 secs to stabilise"
   PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
161 a$ = INKEY$: IF a$ = " " GOTO 162
   GOTO 161
162 PRINT "Taking a reading now !!!"
   CALL per(30)
   CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
     E1002 = ch4
   PRINT "Rinse electrode 2 in dH2O for checking and wait 30 secs"
   PRINT "Put electrode in and PRESS 'SPACE' KEY to continue"
170 a$ = INKEY$: IF a$ = " " GOTO 172
   GOTO 170
172 PRINT "Taking a reading now !!!"
   CALL per(30)
```

```
  CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
    percDO = (ch4 - E02) * 100 / (E1002 - E02)
  PRINT "%DO from electrode 2="; percDO
  PRINT "Are you happy with that ??! (Y/N)"; a$
  INPUT a$
  IF a$ = "N" THEN GOTO 145
  GOTO 26


 'check T sensor
1100 CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
185 PRINT "Check T. Place temp probe + Hg/glass in channel 1 and wait 1
min"
  PRINT "Put probe/Hg-glass in and PRESS 'SPACE' KEY to continue"
190  a$ = INKEY$: IF a$ = " " GOTO 195
  GOTO 190
195 PRINT "Taking a reading now !!!"
  CALL per(60)
  CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
  TChannel1 = ((ch5 / 4) * .9787) + .2521
  INPUT "Hg-glass thermometer reading (C)?"; TCh1
  PRINT " % difference between Hg-glass and PRT="; 100 * (TCh1 -
TChannel1) / TCh1
205 PRINT "Calibrate T. Place temp probe+Hg-glass in Channel 2 and wait
1 min"
  PRINT "Put probe/hg-glass in and PRESS 'SPACE' KEY to continue"
210 a$ = INKEY$: IF a$ = " " GOTO 215
  GOTO 210
215 PRINT "Taking a reading now !!!"
  CALL per(60)
  CALL readmv("MAN", ch1, ch2, ch3, ch4, ch5, ch6, ch7)
    TChannel2 = ((ch6 / 4) * .9787) + .2521
  INPUT "thermometer reading?"; TCh2
  PRINT " % difference between Hg/glass and PRT="; 100 * (TCh2 -
TChannel2) / TCh2
GOTO 26
'calibration values added manually
2101 INPUT "slope1, slope2 ?"; slope1, slope2
     INPUT "epot1, epot2 ?"; epot1, epot2
     INPUT "E01, E02 ?"; E01, E02
     INPUT "E1001, E1002 ?"; E1001, E1002
2100 CLS : PRINT "slope1="; slope1, "slope2="; slope2, "epot1="; epot1,
"epot2="; epot2, "E01="; E01, "E02="; E02, "E1001="; E1001, "E1002=";
E1002
END SUB

SUB CLEAN
  INPUT "DO YOU WISH TO INITIALIZE(only necessary if 1st run) (Y/N)?";
a$
  IF a$ = "N" THEN GOTO 1
  CALL INIT
1 OPEN "$adevice" FOR OUTPUT AS #1

  'FILL SYRINGE
 PRINT "FILLING SYRINGE"
 PRINT #1, "ACTIVE(2)"
 PRINT #1, "SET 2;CCW;S(90 90 3680)"
 PRINT #1, "TRANSFER(2)"
 PRINT #1, "RUN(2)"
 CALL per(45)

   'EMPTY SYRINGE
```

```
  PRINT "EMTYING SYRINGE"
  PRINT #1, "ACTIVE (2)"
  PRINT #1, "SET 2;CW;S(90 90 3680)"
  PRINT #1, "TRANSFER (2)"
  PRINT #1, "RUN (2)"
  CALL per(45)
  CLOSE #1
END SUB
```

Appendix 2.6

Determination of Soluble Reactive Phosphorus in Water

In a suitably acidified solution, phosphate reacts with molybdate to form molybdophosphoric acid, which is then reduced to the molybdenum blue complex and determined spectrophotometrically.

Reagents

1. Carefully add 140 ml of concentrated sulphuric acid to 700 ml of distilled water, cool and make up to 1000 ml with distilled water.

2. Dissolve 15 g of ammonium molybdate in 500 ml of distilled water.

3. Dissolve 5.4 g of ascorbic acid in 100ml of distilled water. Prepare fresh each day.

4. Dissolve 0.34 g of potassium antimonyl tartrate in 100 ml of distilled water.

5. Working reagent - 200 ml. Prepare fresh each time:
    i. 100 ml sulphuric acid solution.
    ii. 40 ml ammonium molybdate solution.
    iii. 40 ml ascorbic acid solution.
    iv. 20 ml potassium antimonyl tartrate solution.

6. Standard phosphate solution (3mM)
    Dissolve 0.4082g of potassium dihydrogen phosphate (dried at 105°C for 2hrs) in distilled water and make up to 1 dm^3 in a volumetric flask.

7. Working standard solution (30μM)
    Dilute solution (No.6) x 100. Prepare this solution fresh each time it is used.

Procedure

i. Transfer 20 ml of filtered sample to a 25 ml volumetric flask, add 4 ml of working reagent reagent at 1 minute intervals, and make up to 25 ml with distilled water, mix well.

ii. Prepare a blank of 4 ml reagent made up to 25 ml with distilled water to measure sample absorbances against.

iii. Prepare a suitable calibration graph from standards 0.3, 1.5, 3, 6, 9, 12 μmol dm^{-3}, *i.e.* transfer 1, 5, 10, 20, 30, 40 ml of working standard solution (No. 7) to 100 ml volumetric flasks and make up to 100 ml with distilled water. Then put 20 ml of each into 25 ml volumetric flasks, add 4 ml of working reagent, and make up to 25 ml with distilled water.

iv. After exactly 10 minutes measure absorbance of the samples, including the blank, in a 4 cm quartz cell at 880 nm.

Authors: W.A. House, F.H. Denison.

References:

Murphy, J. & Riley, J.P.(1962). A modified single solution method for the determination of phosphate in natural waters. Analytica chim. Acta, 27, 31-36

Stephens, K.(1963). Determination of low phosphate concentrations in lakes and marine waters. Limnol. Ocanogr. 8, 361-362

Example of Typical Calibration Line:

Calibration

$y = 0.10941x - 0.00133$

$R^2 = 0.99956$

Corrected Absorbance

Conc standard in 25ml volumetric
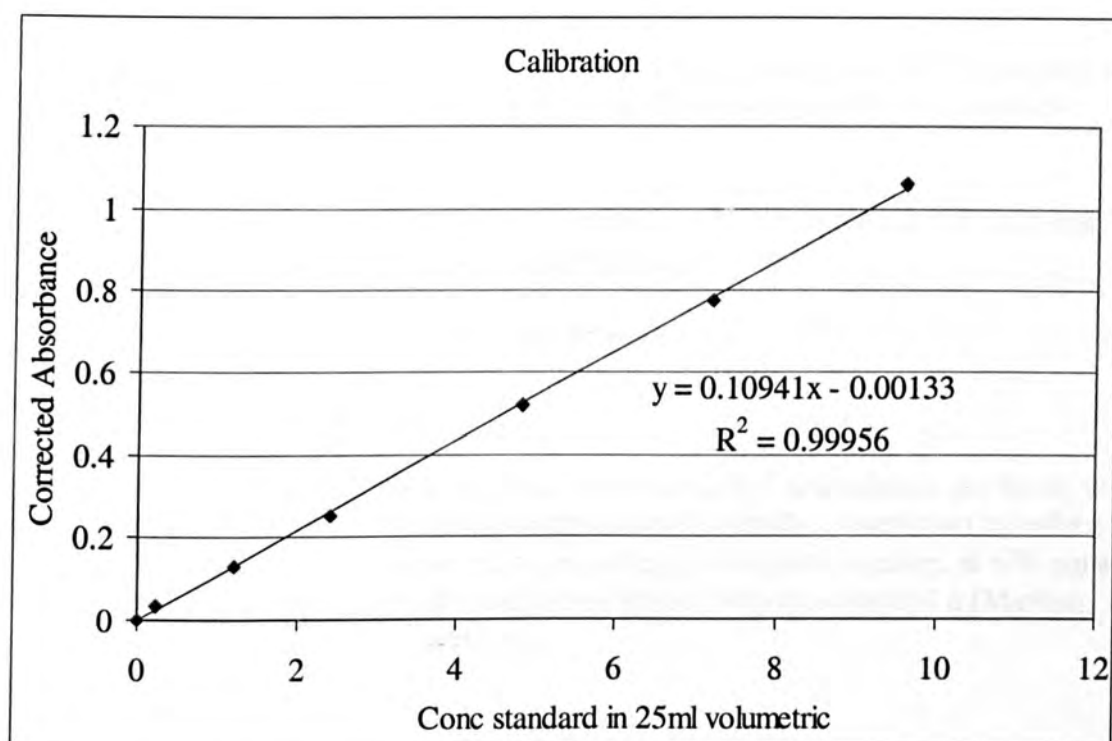
Appendix 2.7

Methanol Extraction of Chlorophyll *a*

Procedure

1.   Filter aqueous solution of any mud or silt. Discard filtrate.

2.   Immerse larger fraction (gravel, rocks or stones) and filter papers in a suitable volume, *i.e.* to cover contents, of 100% methanol in a wide-necked vessel; seal with airtight film and lid.

3.   Agitate by gentle shaking (<100 cycles per minute) in the dark at 5°C overnight.
4.   Ensure as much extract is collected as possible; by draining stones very thoroughly and squeezing filters against vessel rim using forceps.

5.   Centrifuge methanol extract in a stoppered tube, or cover with film, to prevent loss, until a clear solution of pigment is obtained; 5 minutes at 3500 rpm is usually sufficient.

6.   Decant the clear supernatant extract carefully, so as not to disturb the sediment, using a pipette. Let total volume of this extract be v ml.

7.   Reserve extract for absorbance measurements.

Spectrophotometric Analysis

As chlorophylls *b* and *c* (plus other pigments and chlorophyll degradation products) will be extracted along with chll *a*, the determination should include a correction to reduce interference error. This can be achieved by including absorbance readings at 630 nm and 645 nm, correcting these for turbidity with a reading at 750 nm as for chll *a* (Marker, 1972), and including this in the calculation.

Calculation

Chll *a* $(\mu g\ cm^{-2})$ = ((11.6($a_1 - a_4$)-1.31($a_2 - a_4$)-0.14($a_3 - a_4$)x total volume of extract (ml) (v))/(cell path length (d) x sample area (cm^2) (A))

Where a_1 is the absorbance at 630 nm, a_2 the absorbance at 645 nm, a_3 the absorbance at 665 and a_4 is the absorbance at 750 nm.

Absorbances

i.   Absorbance at 665 nm should fall within 0.050 – 0.700 units range.

ii.   Absorbance at 750 nm should not exceed 0.020 in a 40 mm pathlength cell, *i.e.* 0.005 units per 10 mm of path length.

References:

Marker, A.F.H. 1976 The Benthic Algæ of some streams in southern England. I. Biomass of the epilithon in some small streams. J. Ecol.Vol.**64**, pp359-373.

Marker, A.F.H. and Casey, H. 1982 The population and production dynamics of benthic algæ in and artificial re-circulating hard-water stream. Phil. Trans. Soc. (B) **298**, pp265-308.

HMSO, 1983 The Determination of Chlorophyll a in Aquatic Environments 1980. Methods for the Examination of Waters and Associated Materials. London.

Appendix 2.8

Determination of the Volume of a Sample Vial

For the most accurate determination of a small volume vessel measure use ethyl alcohol, *i.e.* density 0.7893, because it has less meniscus than water.

1. Weigh vial empty, fill dropwise to completely level, and re-weigh.

2. This gives mass of ethyl alcohol from which volume may be calculated, *i.e.* Volume = mass/density

3. Calculate relative percentage error

    i. Calculate difference between replicated volume measurements
    ii. Divide difference by lowest measured volume (relative error)
    iii. Multiple by 100 (percentage error)
    e.g. 11.715 ml ± 0.317 (coefficient of variance = 2.7%).

Appendix 2.9

Measurement of Porosity

The porosity of a sediment is the sum of all pores present in a unit volume of sediment. Grain size, sorting and packing of particles all affect the porosity of a sediment, and porosity determines the volume of water a particular sediment holds.

To measure porosity:

1. Weigh vial of known volume empty and note weight.

2. Fill to level with wet sediment and re-weigh. Dry at $50^{\circ}C$ for 6 hours, to avoid steam pushing sediment out of vial, and then at $105^{\circ}C$ overnight. Weigh dry.

3. Calculate mass of water

    i. Calculate volume of water from density (=0.998 at $20^{\circ}C$) and mass.
    ii. Difference between volume of container and volume of water is the porosity.
    n.b. a porosity of 1.0 would be pure water.

4. Calculate porosity

    (bulk volume (volume of container) - solids volume (of sediment))/bulk volume (volume of container)

Calculations

1. Mass water (g) = (weight wet sediment (g) – weight vial (g)) - (weight dry sediment (g) -weight vial (g))

2. Volume water (ml) = mass water (g)/density water

3. Volume sediment (ml) = volume vial (ml) – volume water (ml)

4. Porosity = (volume vial – volume sediment)/volume vial

Appendix 2.10

Measurement of Density

The density of a solid shows the character of the material. For example, a sediment composed primarily of sand would have a value of ~2.6 g cm^{-3}, *i.e.* the density of quartz and steel has a density of ~8.0 g cm^{-3}.

To measure density:

1. Weigh vial of accurately known volume empty and note weight.

2. Fill to level with wet sediment and re-weigh. Dry at 50°C for 6 hours, to avoid steam pushing sediment out of vial, and then at 105°C overnight. Weigh dry.

Calculation

Density (g ml^{-1}) = dry weight sediment (g)/volume sediment (ml)

Appendix 2.11

Determination of Organic Matter (OM) in Sediment

<u>To measure OM</u>

1. Dry evaporating dishes (crucibles) in oven at 100°C for 1 hour, and then put in a dessicator to cool.

2. Weigh dry crucible, note weight on crucible and in laboratory book. If necessary, store in the dessicator until required.

3. Use 10 – 30 g of sediment depending on the OM content, *i.e.* for better accuracy use more sediment if OM content is low. Use sediment that has been previously dried at 105°C and stored in a dessicator. Place crucibles on furnace tray and make a plan of the positions of each sample and its code.

4. Re-weigh crucible with sediment and cover with foil.

5. Set furnace at 550°C and fire sediment 12 h.

6. Place in dessicator with release valve to cool for ~1 hour, then re-weigh.

7. To get ash weight re-weigh immediately (without foil covers), or remove to dessicator until re-weighing, making sure to record positions and sample numbers.

<u>Calculations</u>

OM content (g) = ((<u>dry sediment weight – ashed sediment weight</u>)/(dry sediment weight)) x 100

Dry/Ash sed wt (g) = crucible and sed wt – crucible wt

<u>Error</u> – Above 550°C $CaCO_3$ (as calcite) decomposes to CaO leading to a potential error with this method if that temperature is exceeded. This is a slow but progressive reaction that accelerates above 550°C.

Appendix 2.12

Determination of Suspended Solids in Water

Depending on the concentration of suspended solids in the water, larger sample volumes will give a better suspended solids to filter paper ratio and reduce weighing errors. Where possible sample volumes of between 500 - 1000 ml are recommended.

Preparation of filters

1. Dry a batch of filter papers (Whatman GF/F 42 mm or 90 mm) by placing in thin stacks on glass petri dishes and heating to 105°C for 12 h.

2. Remove from oven and store in a desiccator to cool until required.

3. When handling filter papers it is advisable to use blunt ended forceps or wear disposable latex gloves to avoid transfer of moisture from hands to filter paper.

Samples

Water samples should be thoroughly mixed, before sub-samples for filtering are taken, to ensure a homogenous sample is obtained.

Prior to filtering samples can either be measured out using a measuring cylinder, or alternatively the volume can be obtained by subtracting the sample container weight from the weight of the container plus sample. In most cases it can be assumed that 1 g is equivalent to 1 ml. Using a balance weighing to 1 g accuracy, 1 litre of sample will result in an error on weighing of $< 0.1\%$.

Procedure

1. Remove a filter paper from the desiccator, label with a soft-leaded pencil, *e.g.* 2B, along the edge of the filter, carefully weigh using a 4-place balance and note the weight.
(NB. When weighing dried filter papers care must be taken to keep the time between removal from the desiccator and weighing the filter paper to a minimum to avoid uptake of moisture from the air).

2. Carefully place filter on filtration holder base, put glass reservoir on base and clamp in position.

3. Place the filtration apparatus onto either a side arm vacuum flask of suitable size, or the stainless steel filtration manifold.

4. Connect vacuum flask, or manifold, to a vacuum pump - making sure there is a second vacuum flask 'in line' to prevent water entering the pump. Switch on pump.

5. Carefully pour some de-ionised water into apparatus to check for leaks in the seal between the reservoir and the base.

6. Shake the sample thoroughly, to ensure a homogenous solution, and filter the weighed (or measured) sample immediately. Rinse out sample container twice with de-ionised water and filter washings.

7. Rinse any residual solids from the sides of the filter unit carefully with deionised water.

8. Allow the vacuum to dry the filter paper for a few seconds before removing top of filtration unit.

9. Remove the damp filter paper from the base very carefully to avoid damaging it. Dry the filter paper for ~ 12 h at a temperature of 70 - 105°C.

10. Place dried filter papers in a desiccator to cool before reweighing.

11. If suspended solids concentration in a sample is high further filters may be required. Repeat steps 1 – 11.

12. Run a blank filtration by filtering 500 ml of deionised water as above.

Note:
After filtering samples the filtration units should be soaked for 24 hours in a phosphate-free surface active cleaning agent, *e.g.* Decon90, thoroughly rinsed with de-ionised water, and dried. The vacuum flasks should also be emptied and rinsed out.

<u>Calculation</u>

Total Suspended Solids (g l^{-1}) = ((A - B) x 1000)/C

Where A is the weight of dried sample and filter paper (g), B is the weight of filter paper (g), and C is the sample volume (ml), or weight (g)

Results should be corrected for any blank value.

<u>Health and Safety</u>.

• Inspect vacuum glassware for any chips or cracks, and if present dispose of the glassware.

• Safety glasses must be worn at all times when the using the vacuum system

Appendix 2.13

Photographic Records

An example of the photographic record made of the appearance of each tray in each experiment when in position in the flume channels. These images are shown smaller than actual size.

These photographs show trays from Experiment 1 for Sandwell Valley sediment sampled in April, 2001.

Appendix 2.14

Acetate Tray Plans

An example of the acetate tray plans used to record appearance of sediment sampling trays when in position in the flume channels. This is shown smaller than actual size. Each tray from each experiment was recorded in this manner.

On each plan sheet the larger sized material is outlined and the presence of filamentous algal growth indicated. Areas of fine sand and silt are marked ss (sand/silt). The date recorded and direction of flow are also marked on the sheet.

This plan shows Tray 1 from Experiment 1 for Sandwell Valley sediment sampled in April, 2001.
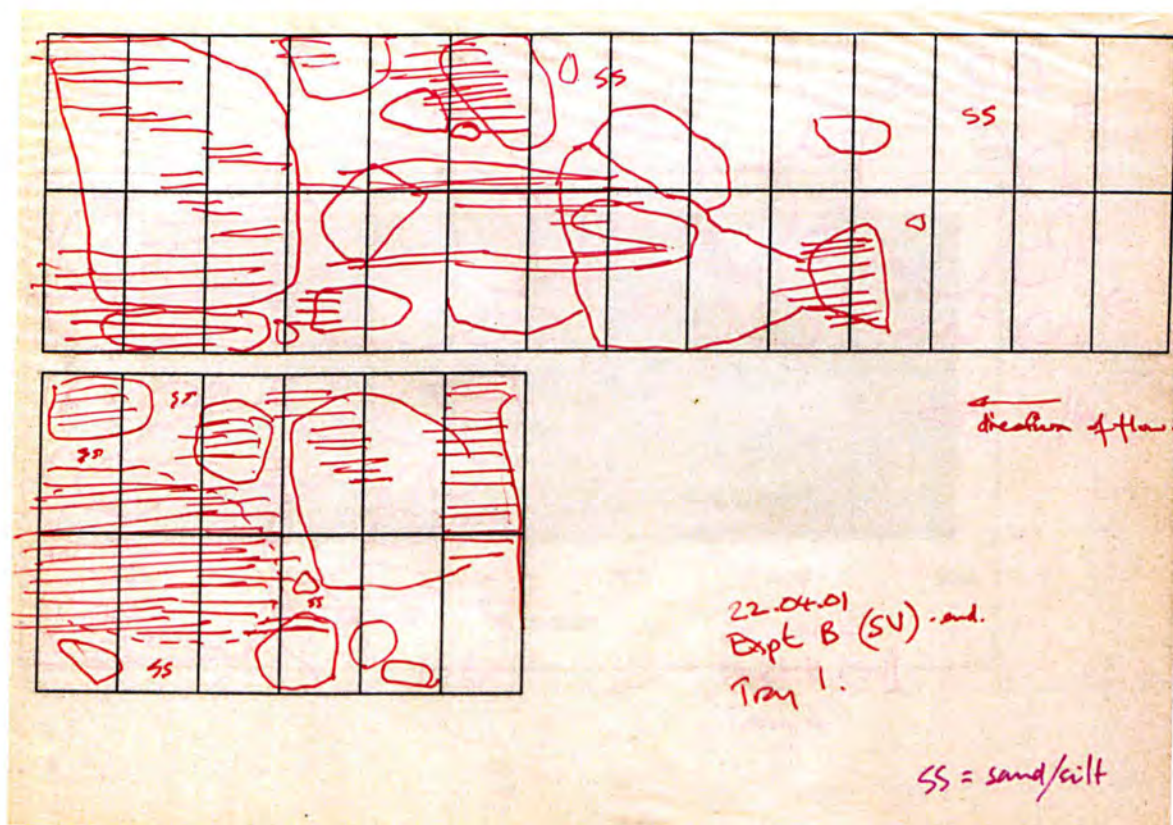
Appendix 2.15

Gravimetric Measurements

Comparison of the results of the gravimetric measurements for the stones fraction and the percentage areal cover for the stones from the acetate maps.

SITE	EXPT	Average % areal cover	Average% mass stones
BM	1	25.3	40.9
	2	36.9	47.3
	3	31.0	26.5
	4	39.0	58.3
	7	35.0	62.4
SV	1	43.3	48.8
	2	46.3	66.8
	3	41.1	50.1
	4	42.3	77.2
	7	33.6	51.8

$y = 1.5177x + 0.2032$

$R^2 = 0.5445$
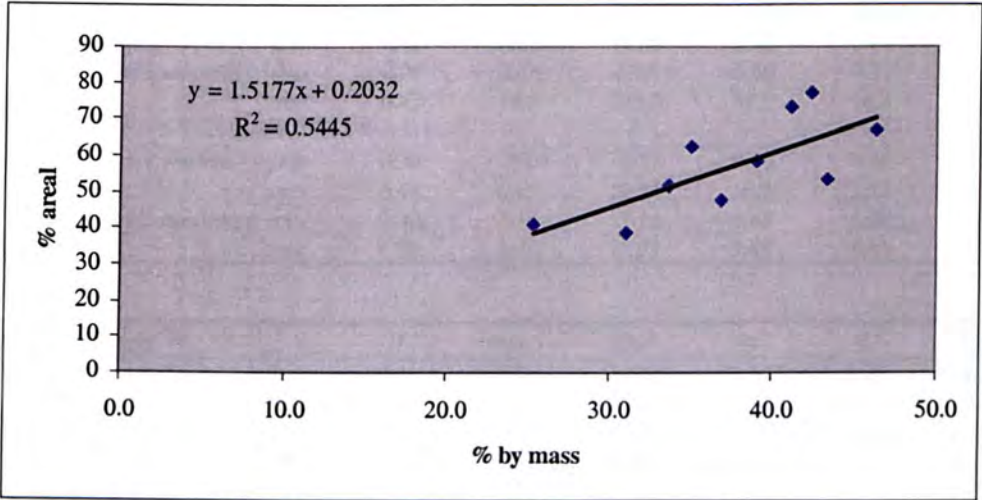
Appendix 2.16

Major Ions

Example summaries of major ions analyses for Experiments 6 (BM and SV fines), 7 (BM and SV natural mixed sediment), and 7a (BM gravel), showing minimum and maximum values occurring during the experiments. Units of ion concentration mg L^{-1}. Key: ND - not detected; see Chapter 2, Section 2.3.5 for detection limits.

Expt 7

BM + SV			F^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
Release	ch1 BM	min	0.11	2.01	2.05	0.20	0.68	1.00	66.0
		max	0.28	2.01	31.79	12.00	4.79	13.4	79.0
	ch2 SV	min	0.09	1.90	0.95	0.10	0.68	0.90	67.0
		max	0.24	4.13	18.14	9.90	5.97	8.90	73.0
Uptake	ch1 BM	min	0.23	ND	32.34	12.30	5.14	13.7	64.13
		max	0.29	ND	48.40	13.83	7.92	16.0	73.64
	ch2 SV	min	0.21	2.74	2.62	2.00	0.55	0.30	67.22
		max	0.55	42.1	217.4	106.5	17.5	44.1	116.0

Expt 7a

BM Gravel			F^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
Release	ch1 washed	min	0.17	2.12	4.15	2.20	0.26	0.30	63.0
		max	0.30	3.30	14.39	8.48	5.11	11.0	76.0
	ch2 unwashed	min	0.09	2.33	6.33	5.60	0.97	1.90	59.0
		max	0.42	36.6	237.2	87.9	14.7	58.2	121.0
Uptake	ch1 washed	min	0.33	2.36	15.82	9.80	4.49	9.86	57.47
		max	0.41	6.45	19.34	10.5	7.53	11.1	62.45
	ch2 unwashed	min	0.30	2.34	17.14	8.68	5.08	11.4	60.28
		max	0.38	6.49	21.53	9.58	8.43	12.9	63.34

Expt 6

BM + SV Fines			F^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
Release	ch1 BM	min	0.40	ND	8.98	0.66	0.85	0.60	5.17
		max	0.76	ND	39.43	5.34	2.04	4.41	83.95
	ch2 SV	min	0.47	ND	5.71	0.78	0.83	0.24	51.61
		max	0.95	ND	64.32	3.52	1.99	2.22	81.58
Uptake	ch1 BM	min	0.23	ND	34.75	5.56	2.18	4.91	72.83
		max	0.43	ND	62.99	9.61	3.46	8.67	100.2
	ch2 SV	min	0.23	ND	5.80	4.31	2.43	3.48	67.19
		max	0.59	ND	8.32	6.04	3.27	5.07	91.34

Appendix 3.1

Udden-Wentworth grain-size scale

Millimeters	μm	Phi (φ)	Wentworth size class	
		-20		
4096		-12	Boulder (-8 to -12φ)	
1024		-10		
256		-8	Pebble (-6 to -8φ)	Gravel
64		-6		
16		-4	Pebble (-2 to -6φ)	
4		-2		
3.36		-1.75		
2.83		-1.50	Gravel	
2.38		-1.25		
2.00		-1.00		
1.68		-0.75		
1.41		-0.50	Very coarse sand	
1.19		-0.25		
1.00		-0.00		
0.84		0.25		
0.71		0.50	Coarse sand	
0.59		0.75		
1/2 — 0.50	500	1.00		Sand
0.42	420	1.25		
0.35	350	1.50	Medium sand	
0.30	300	1.75		
1/4 — 0.25	250	2.00		
0.210	210	2.25		
0.177	177	2.50	Fine sand	
0.149	149	2.75		
1/8 — 0.125	125	3.00		
0.105	105	3.25		
0.088	88	3.50	Very fine sand	
0.074	74	3.75		
1/16 — 0.0625	63	4.00		
0.0530	53	4.25		
0.0440	44	4.50	Coarse silt	
0.0370	37	4.75		
1/32 — 0.0310	31	5	Medium silt	
1/64 0.0156	15.6	6	Fine silt	
1/128 0.0078	7.8	7	Very fine silt	Mud
1/256 — 0.0039	3.9	8		
0.0020	2.0	9		
0.00098	0.98	10		
0.00049	0.49	11	Clay	
0.00024	0.24	12		
0.00012	0.12	13		
0.00006	0.06	14		

Udden-Wentworth grain-size scale for siliciclastic sediment.
(After Wentworth, 1922).

Appendix 4.1

Phosphorus Uptake

The second phase of each experiment was to examine the response of the sediments to the addition of a standard phosphorus solution. However, as river water concentrations of phosphorus were high and the amount of phosphorus released from the sediment during the first phase of the experiments was high, it was found that in most cases the standard additions were insufficient to show the uptake response clearly; the exceptions being Experiments 5a, 7 and 7a, the latter two having a standard addition of higher SRP concentration. Without a more complete sequence of uptake response results, the seasonal effects of the biofilm growth on the phosphorus flux over that cycle could not be evaluated. Where no uptake was evident, the data from this phase of the experiments was amalgamated with the first phase and the whole considered as release, *i.e.* the release kinetics dominated for the entire experiment.

The data from the phosphorus uptake experiments, where uptake occurred, are given on the following pages along with plots of the data as illustration. Experiment 7 was performed in April 2002, using natural mixed sediment sampled using the emplaced sediment trays from both Bentley Mill Way (BM) and Sandwell Valley (SV). Experiments 7a and 5a are those where the gravel size fraction alone was employed, and these were carried out in April 2002 and September 2001 respectively. A complete description of all sampling methods and experimental treatments are given in Chapter 2, Section 2.2.1.

Comparing steady-state concentrations reached at the end of these uptake experiments with concentrations at the end of the release component, it was noted that after prolonged exposure to higher solution concentrations of SRP, the sediment settled at a higher EPC_0. This is most clearly shown in Experiment 7a, where little variation occurred in the concentration over the last 4 samplings over ~ 6 hours. The unwashed gravel fraction settling at a concentration an order of magnitude higher - ~6.5 μmol l^{-1} to ~17.2 μmol l^{-1}. The SRP concentrations appear to have still been decreasing slowly in the SV sample of Experiment 7 and in both washed and unwashed fractions of the SV gravel (Experiment

5a). However, this approach to steady-state was at also at a higher concentration than at the end of release.

The following table appears in Chapter 2, section 2.2.2.2. Standard additions were added in 20 ml aliquots.
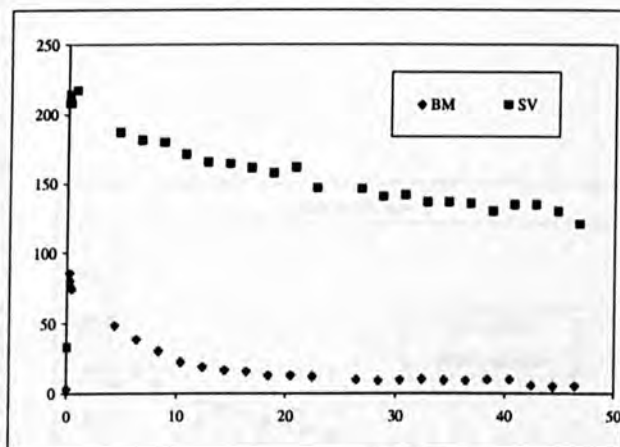
Table II.II: Concentrations (μM) of standard additions of SRP as KH_2PO_4 used in each of the flume channel uptake experiments, including those using separate sediment size fractions (numbered a or b). Key: BM – Bentley Mill Way; SV – Sandwell Valley; SRP – soluble reactive phosphorus.

		1	2	3	3a	4	5a	5b	6	7	7a
BM	SRP	2.94	5.88	5.88	-	5.88	2.94	-	5.88	90.0	90.0
SV	SRP	5.88	5.88	5.88	5.88	11.76	-	96.8	5.88	180.0	-

Data from SRP Uptake phase of Experiments 7, 7a and 5a.

Expt 7 (BMW + SV) - SRP

Code	Interval from start (h)	SRP Conc (µmol/l)	SRP Conc (µg/l)	Code	Interval from start (h)	SRP Conc (µmol/l)	SRP Conc (µg/l)
BM				**SV**			
7BM.1	0.00	3.08	95.28	7SV.1	0.00	33.52	1038
7BM.2	0.22			7SV.2	0.08		
7BM.3	0.25	85.87	2659.82	7SV.3	0.12	208.21	6449
7BM.4	0.28	80.41	2490.59	7SV.4	0.15	214.39	6640
7BM.5	0.32			7SV.5	0.18		
7BM.6	0.35	76.37	2365.51	7SV.6	0.22	208.69	6464
7BM.7	0.42	74.95	2321.36	7SV.7	0.77	217.71	6743
7BM.8	2.42			7SV.8	2.77		
7BM.9	4.42	48.6	1505.15	7SV.9	4.77	187.54	5809
7BM.10	6.42	38.67	1197.75	7SV.10	6.77	181.63	5626
7BM.11	8.42	30.40	941.59	7SV.11	8.77	179.97	5575
7BM.12	10.42	22.37	692.74	7SV.12	10.77	171.47	5311
7BM.13	12.42	19.06	590.28	7SV.13	12.77	165.56	5128
7BM.14	14.42	16.46	509.77	7SV.14	14.77	164.38	5091
7BM.15	16.42	15.75	487.81	7SV.15	16.77	161.31	4996
7BM.16	18.42	12.91	399.98	7SV.16	18.77	157.76	4887
7BM.17	20.42	12.87	398.68	7SV.17	20.77	162.08	5020
7BM.18	22.42	11.96	370.37	7SV.18	22.77	147.00	4553
7BM.19	24.42			7SV.19	24.77		
7BM.20	26.42	9.90	306.67	7SV.20	26.77	146.54	4539
7BM.21	28.42	8.99	278.36	7SV.21	28.77	140.83	4362
7BM.22	30.42	9.6	298.03	7SV.22	30.77	142.20	4405
7BM.23	32.42	10.1	312.19	7SV.23	32.77	136.95	4242
7BM.24	34.42	9.32	288.60	7SV.24	34.77	136.95	4242
7BM.25	36.42	9.01	279.16	7SV.25	36.77	136.03	4213
7BM.26	38.42	9.85	305.11	7SV.26	38.77	130.55	4044
7BM.27	40.42	10.00	309.83	7SV.27	40.77	135.12	4185
7BM.28	42.42	5.66	175.36	7SV.28	42.77	135.12	4185
7BM.29	44.42	4.98	154.12	7SV.29	44.77	130.32	4037
7BM.30	46.42	5.36	165.92	7SV.30	46.77	121.18	3753

Expt 7a (BM gravel) - Uptake

Code	Interval from start (h)	SRP Conc (µmol/l)	SRP Conc (µg/l)	Code	Interval from start (h)	SRP Conc (µmol/l)	SRP Conc (µg/l)
Ch1 -washed				Ch2 - unwashed			
7a(w).1	0.00	2.03	63.00	7a(u).1	0.00	6.51	202
7a(w).2	0.03	1.82	56.23	7a(u).2	0.03	6.08	188
7a(w).3	0.07			7a(u).3	0.07		
7a(w).4	0.10	102.20	3165.40	7a(u).4	0.10	89.53	2773
7a(w).5	0.13	84.45	2615.62	7a(u).5	0.13	87.89	2722
7a(w).6	0.17			7a(u).6	0.17		
7a(w).7	0.20	91.93	2847.38	7a(u).7	0.20	83.19	2577
7a(w).8	0.23	80.90	2505.68	7a(u).8	0.23	79.26	2455
7a(w).9	0.27			7a(u).9	0.61		
7a(w).10	2.27	43.06	1333.89	7a(u).10	2.61	79.31	2456
7a(w).11	4.27	33.07	1024.22	7a(u).11	4.61	44.68	1384
7a(w).12	6.27	26.23	812.47	7a(u).12	6.61	38.43	1190
7a(w).13	8.27	22.70	703.17	7a(u).13	8.61	34.76	1077
7a(w).14	10.27	19.54	605.26	7a(u).14	10.61	32.41	1004
7a(w).15	12.27	18.36	568.83	7a(u).15	12.61	30.35	940
7a(w).16	14.27	16.67	516.46	7a(u).16	14.61	29.24	906
7a(w).17	16.27	14.91	461.81	7a(u).17	16.61	27.04	838
7a(w).18	18.27	12.48	386.54	7a(u).18	18.61	24.73	766
7a(w).19	20.27	12.15	376.19	7a(u).19	20.61	23.22	719
7a(w).20	22.27			7a(u).20	22.61		
7a(w).21	24.27	10.92	338.26	7a(u).21	24.61	21.22	657
7a(w).22	26.27	10.70	331.36	7a(u).22	26.61	20.05	621
7a(w).23	28.27	10.20	315.84	7a(u).23	28.61	19.99	619
7a(w).24	30.27	10.36	321.01	7a(u).24	30.61	19.10	592
7a(w).25	32.27	9.64	298.60	7a(u).25	32.61	18.71	580
7a(w).26	34.27	10.20	315.84	7a(u).26	34.61	18.44	571
7a(w).27	36.27	8.58	265.84	7a(u).27	36.61	18.16	562
7a(w).28	38.27	8.81	272.73	7a(u).28	38.61	17.49	542
7a(w).29	40.27	8.36	258.94	7a(u).29	40.61	17.21	533
7a(w).30	42.27	8.50	263.33	7a(u).30	42.61	17.43	540
7a(w).31	44.27	8.01	248.06	7a(u).31	44.61	17.26	535

Expt 7a (BM gravel)
◇ Ch1 -washed
■ Ch2 - unwashed
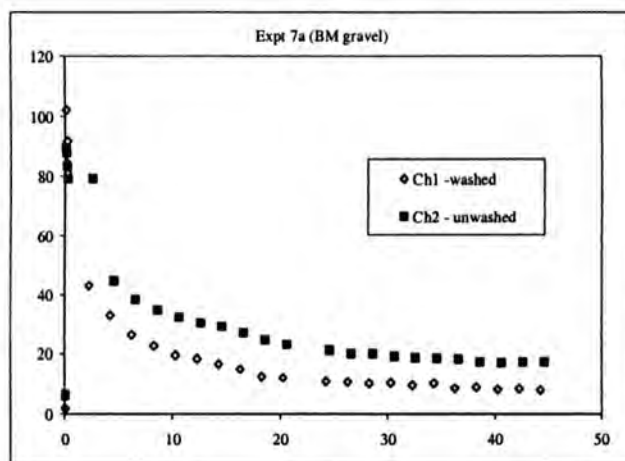
Expt 5a (SV gravel) - Uptake

Code	Interval from start/h	SRP Conc (µmol/l)	SRP Conc (µg/l)	Code	Interval from start/h	SRP Conc (µmol/l)	SRP Conc (µg/l)
Ch1 -washed				Ch2 - unwashed			
5a(w).1	0.00	7.14	221.13	5a(u).1	0.00	8.50	263
5a(w).2	0.03	114.69	3552.50	5a(u).2	0.03	97.26	3012
5a(w).3	0.07	117.18	3629.65	5a(u).3	0.07	100.65	3118
5a(w).4	0.10	114.47	3545.49	5a(u).4	0.10	99.75	3090
5a(w).5	0.13	114.69	3552.50	5a(u).5	0.13	97.71	3026
5a(w).6	0.17	109.49	3391.19	5a(u).6	0.17	97.03	3005
5a(w).7	0.20	80.96	2507.50	5a(u).7	0.54	73.71	2283
5a(w).8	2.20	81.18	2514.52	5a(u).8	2.54	78.69	2437
5a(w).9	4.20	70.77	2191.90	5a(u).9	4.54	74.62	2311
5a(w).10	6.20	63.75	1974.48	5a(u).10	6.54	69.41	2150
5a(w).11	8.20	58.54	1813.18	5a(u).11	8.54	66.92	2073
5a(w).12	10.20	53.56	1658.88	5a(u).12	10.54	64.43	1996
5a(w).13	12.20	51.29	1588.75	5a(u).13	12.54	61.71	1911
5a(w).14	14.20	50.39	1560.69	5a(u).14	14.54	58.99	1827
5a(w).15	16.20	47.44	1469.52	5a(u).15	16.54	58.77	1820
5a(w).16	18.20	44.50	1378.34	5a(u).16	18.54	54.46	1687
5a(w).17	20.20	42.01	1301.20	5a(u).17	20.54	53.78	1666
5a(w).18	22.20	39.52	1224.05	5a(u).18	22.54	51.23	1587
5a(w).19	24.20	38.39	1188.98	5a(u).19	24.54	49.61	1537
5a(w).20	26.20	37.03	1146.90	5a(u).20	26.54	48.23	1494
5a(w).21	28.20	35.44	1097.81	5a(u).21	28.54	47.07	1458
5a(w).22	30.20	34.76	1076.77	5a(u).22	30.54	48.00	1487
5a(w).23	32.20	32.76	1014.83	5a(u).23	32.54	45.46	1408
5a(w).24	34.20	32.99	1021.98	5a(u).24	34.54	45.69	1415
5a(w).25	36.20	31.38	971.94	5a(u).25	36.54	48.92	1515
5a(w).26	38.20	29.99	929.05	5a(u).26	38.54	43.38	1344
5a(w).27	40.20	30.46	943.35	5a(u).27	40.54	43.38	1344
5a(w).28	42.20	29.53	914.75	5a(u).28	42.54	43.15	1337
5a(w).29	44.20	16.43	864.71	5a(u).29	44.54	41.77	1294

Expt 5a (SV gravel)
◇ Ch1 -washed
■ Ch2 - unwashed
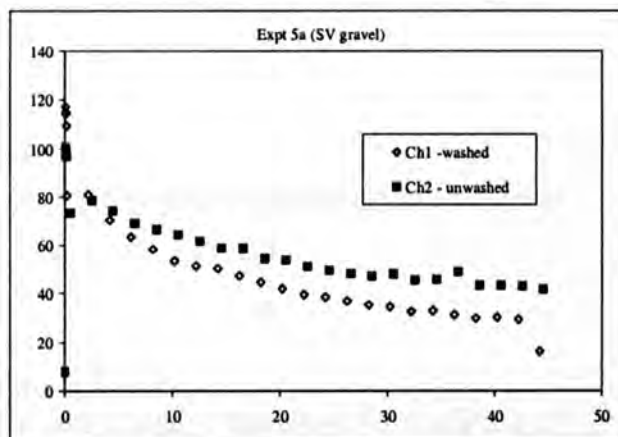
Appendix 6.1

Benthic Macroinvertebrate Husbandry

Tubificids were collected locally to the IFE River Laboratory in order to propagate and husband them for use in the channel experiments *i.e.* there should be an ample and readily available supply of tubificid worms at hand to populate the experiments with. It was thought that in suitably anoxic conditions the worms that would proliferate would be the resilient species, able to survive in conditions found in the River Tame sediments.

Laboratory Notes

Sediment samples were also collected from several locations in the vicinity of the River Laboratory. The sediments were first coarsely sieved to remove large gravel and debris. Then, using progressively finer sieves (250 μm and 150 μm) the sediments were sorted into sand and silt portions, and any visible fauna removed. The tubificids were collected and placed into tanks containing sieved sediment, obtained from the laboratory water butt, and tap water.

3.11.99

Tank A was inoculated with ~30 tubificids extracted from Derek's Pond and the Mill Stream (IFE River Laboratory).

4.11.99

Tank B was inoculated with ~30 worms from Field Pond.
Tank C was inoculated with ~100 worms from Experimental Channels Ditch

5.11.99

Tank D was inoculated with ~30 worms from Derek's Pond
Tank E was inoculated with an unspecified number of individuals from Bovi Stream (recommended location from P. Armitage)

Husbandry notes:
• a cardboard light-shield was installed 9.11.99
• each few days the surface of the tanks was cleaned by removing any scum and water levels were topped-up with more tap water.

1.12.99

Collection of sediment and water samples from River Tame.
1 x 10L sediment sample from each site collected and stored in fluvarium with æration of surface water.

2.12.99

Small sub-sample of Site 1 sediment sieved through mesh down to 150 μm and examined for presence of animals. Found included:

- leeches – disposed into Millstream flood channel
- alder fly larvæ – also disposed into flood channel
- asellus   }
- snail   } collected and put into sediment tanks
- tubificids }

3.12.99

Extra sub-sample of sediment taken from both Sites 1 and 2 samples in order to use for tubificid husbandry. Sieved through 600μm mesh to remove any animals and collect worms. Two new tanks set up:

<u>Tame Site 1 Tank</u> containing River Tame sediment, River Tame tubificids, but using tapwater.

<u>Tame Site 2 Tank</u> as above but many more worms found from this site.

8.12.99

It was decided to see if centrifuging (for porewater analysis) killed animals and left the sediment macroinvertebrate free and suitable for use in the mesocosm experiments.
n.b. spun sediment means most porewater was removed by the centrifuging.

Two batches were spun:
<u>Batch 1</u> was only partially spun as the settings on the centrifuge were wrong initially.
<u>Batch 2</u> spun 10 minutes at ~8000 g

Unfortunately, there was both Site 1 and Site 2 sediments in both batches. This meant that spun and part spun sediments were mixed during the sieving and put into baths together. This was also sieved, through 600 μm, using tap water, which would certainly change the nature of the porewater.

Baths set up to settle sediment and check for the presence of tubificids:

<u>Tame Site 1 Bath</u> – mixed spun/part-spun sediment from Site 1
<u>Tame Site 2 Bath</u> – ditto from Site 2

9.12.99

- Tubificids found in Bath 1 and 2.

- Full-spun sediment from Site 2 sieved (600 μm), set to settle to check for worms. Tap water used as rinse in sieving.

- Petri dishes set up to try to ensure all macroinvertebrates clear from before introducing River Frome worms to test survival in River Tame sediment.

Petri 1 – Site 1 part spun   }
Petri 2 – Site 2   "   "   } with tap water
Petri 3 – Site 2 full spun   }
Petri 4 – Site 2 full spun (20 minutes at 8000 g), sieved with River Tame water

Further batch spun:
Batch 3 - Site 2 sediment only was spun 20minutes at 8000 g and sieved, 600 μm, using River Tame water.

10.12.99

- Leech noted in Tame Site 1 – therefore sieving at 600 μm may not take out all predators and the two Baths (Tame Site 1 and 2) were then abandoned.

Petri 3 – inoculated with 10 Frome (Tank C – Experimental Channels) worms of assorted sizes, plus a cocoon.
Petri 4 – inoculated with worms only as above.

16.12.99

- During routine cleaning and topping up of tanks worms were noted living in the scum formed on the top of the Bovi Stream Tank E.

20.10.00

- The silvery scum which formed on the surface of the water was identified as a bacterial growth.
- Several tanks containing local sediment have developed colonies of *Daphnia* and others have copepods.
- Asellus found in Tank C removed to small pot containing asellus and snail originally collected from the Frome sediments.
- Tame 1 has many copepods but Tame 2 has none.
- Tanks A + B were given small quantities, *i.e.* 2-3 flakes, of dissolved fish food.

24.01.00

- The clouding in the water in Tame 1 found to be a massive ciliate bloom.
- Stentors also found in a number of tanks.

8.02.00

Tank B looking very depleted of worms, and surface quite jellified. Few cladocerans swimming about. Therefore action taken:
  i.   Sediment sieved and 2 x large mature Alder Fly larvæ found, also a Phantom Midge larvæ (*Chaioborus*). These were removed.
  ii. Sediment recycled with fresh tap water and the ~75 worms, mostly very small individuals, found remaining in the tank re-introduced to the sediment.

Note: the fact that ~75 worms were found, and only ~30 were originally introduced, seems to indicate that in spite of predation by the fly larvæ, either reproduction or growth/development, has been occurring.

27.03.00

Tank B still forming some sort of growth on sediment surface despite thorough mixing during last couple of water surface cleanings. However, worms still active.
Other Tanks clear in water column again, except Tame Site 2, which still shows some signs of the cloudiness caused by a ciliate bloom.

At this point the mesocosm experiments were abandoned and the tubificid husbandry programme discontinued.

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