



Influence of physico-chemical characteristics of sediment on the in situ spatial distribution of F-specific RNA phages in the riverbed

Blandine Fauvel, Henry-Michel Cauchie, C. Gantzer, Leslie Ogorzaly

► To cite this version:

Blandine Fauvel, Henry-Michel Cauchie, C. Gantzer, Leslie Ogorzaly. Influence of physico-chemical characteristics of sediment on the in situ spatial distribution of F-specific RNA phages in the riverbed. FEMS Microbiology Ecology, 2019, 95 (2), pp.328-340. 10.1093/femsec/fy240 . hal-02921746

HAL Id: hal-02921746

<https://hal.univ-lorraine.fr/hal-02921746>

Submitted on 4 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

RESEARCH ARTICLE

Influence of physico-chemical characteristics of sediment on the *in situ* spatial distribution of F-specific RNA phages in the riverbed

Blandine Fauvel^{1,2,3}, Henry-Michel Cauchie¹, Christophe Gantzer^{2,3} and Leslie Ogorzaly^{1,*},[†]

¹Luxembourg Institute of Science and Technology (LIST), Department of Environmental Research and Innovation (ERIN), 5 Avenue des Hauts-Fourneaux, 4362 Esch-sur-Alzette, LUXEMBOURG., ²Université de Lorraine, Laboratoire de Chimie, Physique et Microbiologie pour les Matériaux et l'Environnement (LCPME), UMR 7564, Faculté de Pharmacie, 5 Rue Albert Lebrun BP 80403 54001 Nancy, FRANCE and ³CNRS, LCPME, UMR 7564, Nancy F-54000, France

*Corresponding author: Luxembourg Institute of Science and Technology (LIST), 41 rue du Brill L-4422 Belvaux, Luxembourg. Tel: +352 275 888 438; Fax: +352 275 885; E-mail: leslie.ogorzaly@list.lu

[†]Leslie Ogorzaly, <http://orcid.org/0000-0001-8393-4818>

One sentence summary: The heterogeneous presence of fine sands, clays and iron-bearing phases has an influence on the *in situ* spatial distribution of faecal phages in riverbed sediment.

Editor: Cindy Nakatsu

ABSTRACT

Riverbed sediment is commonly described as an enteric virus reservoir and thought to play an important role in water column contamination, especially during rainfall events. Although the occurrence and fate of faecal-derived viruses are fairly well characterized in water, little information is available on their presence as their interactions with sediment. This study aimed at determining the main environmental factors responsible for the presence of enteric viruses in riverbed sediment. Using a combination of microbiological and physico-chemical analyses of freshly field-sampled sediments, we demonstrated their contamination by faecal phages. The *in situ* spatial distribution of phages in sediment was mainly driven by sediment composition. A preferential phage accumulation occurred along one bank of the river, where the quantity of fine sands and clay particles smaller than 0.2 mm was the highest. Additionally, a mineralogical analysis revealed the influence of the heterogeneous presence of virus sorbents such as quartz, calcite, carbonates and iron-bearing phases (goethite) on the phage spatial pattern. A more precise knowledge of the composition of riverbed sediment is therefore useful for predicting preferential areas of enteric virus accumulation and should allow more accurate microbial risk assessment when using surface water for drinking water production or recreational activities.

Keywords: F-specific RNA bacteriophages; riverbed sediment; *In situ* spatial distribution; mineralogical composition

Received: 16 July 2018; Accepted: 11 January 2019

© FEMS 2018. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

INTRODUCTION

Viral particles are commonly observed in marine and freshwater sediment. Among them, native aquatic phages (viruses that infect bacteria) form the most represented viral group in number and in diversity (Leroy *et al.* 2008; Borrel *et al.* 2012; Jakubowska-Deredas *et al.* 2012). They are key contributors in aquatic ecosystems, especially for natural bacterial communities by controlling their actions in biogeochemical and ecological processes or by providing them genetic diversity (Danovaro *et al.* 2008). Enteric viruses (phages and human and animal viruses) from faecal origin are also encountered in aquatic ecosystems (Miura *et al.* 2009; Elmahdy *et al.* 2016; Hassard *et al.* 2016; Skrabber *et al.* 2009b). These viruses reach and enter into the environment through sewage discharge or run-off from agricultural activities (Bosch 1998). Contrary to the native aquatic viruses, enteric viruses are unable to multiply in this environment due to the lack of their host cell and therefore should not influence the aquatic microbial communities (Jofre 2009). Data collected until now reports a greater abundance of these faecally derived viruses in sediment than in overlay water column (Gerba *et al.* 1979; Paul *et al.* 1993; Maranger and Bird 1996). Based on these observations, it is generally suspected that sediment acts as reservoir for enteric viruses (Rao, Metcalf and Melnick 1986; Bosch, Pintó and Abad 2006; Staggemeier, Almeida and Spilki 2012). Moreover, it is believed that the association of viral particles with solids (sediment or suspended particles) increases their survival in natural environments. This is achieved through a protective effect against proteolytic enzymes or other degrading factors such as water temperature, pH or solar radiations (Smith, Gerba and Melnick 1978; Liew and Gerba 1980). In addition to abiotic solid surfaces, biofilms are highly present in aquatic ecosystems and represent also a favourable environment for the interaction, protection and storage of viral particles (Mackowiak *et al.* 2018). The attachment of viral particles on different types of aquatic biofilms was previously reported (Skraber *et al.* 2007; Helmi *et al.* 2008; Pelleieux *et al.* 2012; Skrabber *et al.* 2009a). Associated with mineral or plant surfaces, biofilms are a part of this complex environmental matrix that is sediment. In case of a significant accumulation of enteric viruses occurring at the bottom of riverbed, sediment could potentially be an important source of viral contamination of surface water especially during rainfall events. Indeed, the input of enteric phages in surface water following riverbed sediment resuspension has been recently highlighted (Fauvel *et al.* 2016), but this phenomenon is still largely unknown. In the perspective of human health risk assessment, a special attention should be paid to the viral contamination of the sediment compartment (Hassard *et al.* 2016).

Sediment is mainly composed of varying fractions of sand, silt, clay, organic matter and metal oxide contents. All of these fractions may affect the attachment of viruses to sediment differently. Many model granular materials have been examined for their capabilities to interact with viral particles using laboratory-scale columns under well-controlled environmental conditions. Among them, some homogeneous supports such as silica (Bales *et al.* 1993), clays (Tong *et al.* 2012) or sand (Knappett *et al.* 2008) have been recognized as favourable support for virus attachment. However, depending on the surrounding environment, the presence of metal oxide and organic matter can significantly modify, either by favouring or hindering the virus adsorption to the solid particles (Zhuang and Yu 2002; Cao, Tsai and Rusch 2010). The sediment constitutes therefore, a complex matrix composed of many fractions which can affect the attachment of viral particles differently. Due to this complexity, it is difficult to

elucidate the interaction mechanisms between viruses and this heterogeneous environmental matrix. Nevertheless, it is important to understand the reasons behind the presence of enteric viruses in the riverbed sediment, as it would allow the relevant authorities to assess the risk to public health and to establish remediation strategies.

The direct investigation of human pathogenic enteric viruses in aquatic sediment is technically challenging and costly. The sediment sample preparation and the detection assays are time-consuming and there are a lack of appropriate host cell cultures for some epidemiologically significant viruses (Yates 2014). Thus, viral indicators are commonly used to assess the water faecal and viral contamination as well as the presence of pathogenic viruses in the environment (Bosch 1998; Jofre 2007). F-specific RNA bacteriophages (FRNAPHs) have frequently been suggested for this purpose (Havelaar *et al.* 1991). FRNAPHs are able to replicate in *Escherichia coli*, which is highly present in the animal and human gut. They are abundant in surface water (Lucena *et al.* 2003; Ogorzaly *et al.* 2009; Mauffret, Caprais and Gourmelon 2012) and sediment (Haramoto *et al.* 2009; Fauvel *et al.* 2017a) in areas impacted by faecal pollution. The multiplication of FRNAPHs in the environment is quite unlikely and expected to have a no influence on microbial communities (Woody and Cliver 1995; Jofre 2009). Moreover, due to their size, the nature of the genome and the capsid, FRNAPHs are often used as a model to study the behaviour of pathogenic enteric viruses in response to their surrounding environment (Havelaar, Van Olphen and Drost 1993). They constitute therefore a valuable tool to assess the *in situ* presence of enteric viruses in sediment. Although several experimental studies have been conducted on the attachment of FRNAPHs to model granular materials (Torkzaban *et al.* 2006; Syngouna and Chrysikopoulos 2013; Bellou *et al.* 2015) or soil extracts (Dowd *et al.* 1998; Zhao *et al.* 2008; Sasidharan *et al.* 2017) in laboratory conditions, further research is still needed in order to investigate their *in situ* presence in sediment as well as their behaviour under real environmental conditions.

The present study aimed to quantify the presence of FRNAPHs in freshwater sediment samples and evaluate their spatial distribution in the riverbed. For this purpose, fresh riverbed sediment samples were collected under various hydroclimatological conditions (low flow and rainfall event), in accordance to a fine-scale sampling grid covering the entire width of the river. After an elution step to recover viral particles, FRNAPHs were detected and quantified using an infectivity assay. We then investigated the relationship between the occurrence of FRNAPHs, the physical-chemical characteristics of sediments (particle size distribution, specific surface area, mineral composition, organic matter and water content) and the environmental conditions in order to elucidate the distribution pattern.

MATERIALS AND METHODS

Sediment samples

Sediment samples were collected from the Alzette River, one of the main rivers in Luxembourg (Fig. 1). The sampling site (GPS coordinates: 49°35'41.5"N 6°09'39.6"E) was close to the city of Hesperange, hosting about 14 000 inhabitants. A wastewater treatment plant (26 000 inhabitant-equivalents), which is responsible for a continuous point source of viral and faecal pollution, is located 1.5 km upstream of the sampling area. A previous study revealed a viral contamination of the studied river section with an average of $1.4 \pm 0.8 \times 10^3$ plaque forming units (PFU)/100 mL ($n = 54$) of infectious FRNAPHs in surface

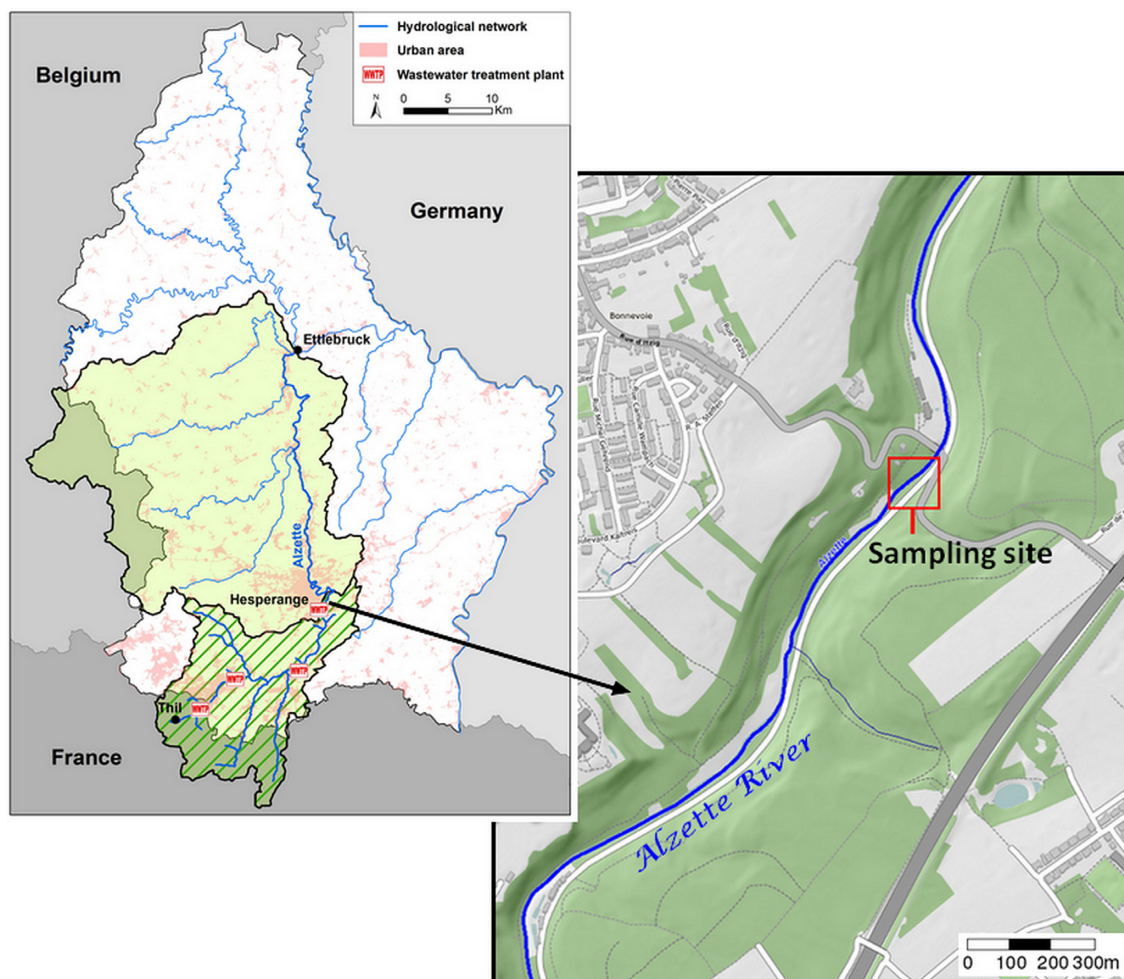


Figure 1. Overview of the sediment sampling site along the Alzette River in Luxembourg. Main drainage network was represented on the map of Luxembourg with the Alzette River watershed (in green) including the studied sub-catchment (striped area).

water (Fauvel et al. 2017a). In order to have a global overview of the bottom of the river, a square grid of nine sampling points (three points on the right bank of the river, three in the middle and three on the left bank of the river) were established at the defined sampling site. A distance of 5.2 m between each sampling point was set to cover the width (10.4 m) of the river. Due to the narrowness of the river at the sampling site, no difference in flow rate between the right and left banks was observed. Moreover, the linearity of the river (no meander) cannot favour a preferential sedimentation along one of the two banks of the river. The surface of the riverbed was vacuumed with a pump system (Fauvel et al. 2017a). In brief, a rigid plastic pipe was connected to a 50-L vacuum container and sediment samples were pumped into a sterile 1-L glass bottle. Four separate campaigns were performed under various hydrological conditions in order to collect a total of 36 sediment samples (Table 1). All samples were transported back to the laboratory in a refrigerated container. The sediment samples were stored for 24 h at 4°C to ensure the settlement of the particles.

Sediment water content

In order to assess the water content in fresh sediment, each sample was oven dried at 105°C until its weight remained constant (ca. 24 h). The percentage of water content (%WC) was

calculated using the following equation: $\%WC = 100 - ((100 \times DW_{105})/WW)$, where DW_{105} represents the dry weight of the sample after heating to 105°C and WW the wet weight of the sample.

Sediment organic matter

The loss on ignition test was used to determine the organic matter content of sediment samples. The difference in weight before and after ignition under specific high temperature represents the amount of the organic matter that was initially present in the sample. After oven-drying of the sediment to constant weight (usually 24 h at 105°C), samples were subjected to combustion at 375°C in a muffle furnace for at least 16 h (overnight). The 375°C temperature was chosen to limit an overestimation of organic matter due to dehydration of clays, hydroxides and other minerals at higher temperature (Beaudoin 2003). After cooling ignited samples in a desiccator to ambient temperature, the dry weight was determined. The percentage of organic matter (%OM) was calculated using the following equation: $\%OM = ((DW_{105} - DW_{375})/DW_{105}) \times 100$, where DW_{105} represents the dry weight of the sample before combustion and DW_{375} the dry weight of the sample after heating to 375°C.

Table 1. Hydrological characteristics of the four sediment sampling campaigns.

Date (dd/mm/yyyy)	Campaign #1 16/09/2014	Campaign #2 20/07/2015	Campaign #3 24/08/2015	Campaign #4 05/07/2016
Flow rate (m ³ /sec)	0.58	0.87	0.87	1.66
Water level (cm)	43	47	47	55
Antecedent rainfall (mm) ^a	0	14.1	2.9	4.8
Hydrological condition	Low flow	Rainfall event / recession phase	Low flow	Low flow

^a 5 days prior the sampling.

Sediment particle size distribution by sieving

The sieve method is a routine procedure used to characterize the particle size distribution of sediment samples. A series of metal sieves with different sized apertures (2 mm, 1 mm, 0.5 mm, 0.2 mm, 0.125 mm and 0.063 mm) were used to separate sediment samples into different sized particles. In order to isolate the mineral particles only and to prevent the sieve overload from branches and foliage, the particle size distribution process was carried out on sediment samples preliminarily heated at 375°C. Each sample was added to sieves arranged in decreasing size and was segregated into different sized particles by washing according to a standard procedure (XP P 94-041:1995). The weight of the sample retained on each sieve was determined after oven-drying at 105°C and after complete evaporation of the water. For particles smaller than 0.063 mm and therefore not retained on the last 0.063 mm aperture sieve, their weight was determined after their settling in the water used to segregate particles and then drying at 105°C. Each measurement retained on each of the respective sieves were reported in terms of percentage of the total initial sediment sample.

Sediment specific surface area

The specific surface area was determined for the nine sediment samples collected during the campaign #4. Each sample represents a point of the grid established during the sampling campaign.

The specific surface area (m²/g) was obtained according to the AFNOR NF 11-621 standard protocol. This method consists of measuring the amount of nitrogen gas adsorbed/ desorbed on the surface of the tested sample as a function of the pressure of nitrogen. Adsorption of the first layer of gas on the material is then modelled according to a theoretical isotherm of multilayer adsorption proposed by Brunauer, Emmett and Teller (1938). The values representing the volume of gas adsorbed in the monolayer allow to determine the specific surface area of sample tested. Successive introductions of liquid nitrogen at -195.8°C (purity of N₂ gas > 99.995%) in a volumetric adsorption measurement instrument (Belsorp-mini II, Bel Japan Inc.) allowed to obtain adsorption-desorption isotherms of each sediment sample. Furthermore, the De Boer method (t-plot, Lippens and de Boer 1965) was used to estimate the microporous and the external surface of the nine sediment samples. To be able to distinguish adsorption onto the external surface from adsorption into the micropores, the experimental isotherms were compared to a reference curve obtained for a non-porous solid, with chemical features and energetic constant as close as possible to those of the studied sample.

Mineralogical composition of sediments by X-ray diffraction

X-ray powder diffraction (XRD) was used for the identification of the main minerals occurring in sediment samples. In order to have a global overview of the sampling site, three samples of the middle, right and left banks of the river were analysed. Samples were grinded to a fine powder before XRD. X-ray diffraction data were obtained using a D8 Discover Bruker diffractometer with a cobalt anode ($\lambda_{\text{CoK}\alpha 1} = 1.79 \text{ \AA}$). The diffractometer is equipped with a linear detector and a generator with the power set at 35 kV and 45 mA. X-ray diffractograms were collected on powder sediment samples in ambient conditions, within the 2θ range [3°, 64°], with 0.03° step and a 3 s collecting time (Montarges-Pelletier et al. 2007).

Enumeration of F-specific RNA bacteriophages

The elution of infectious FRNAPHs from sediments was performed accordingly to the protocol described by Fauvel et al. (2017b). Briefly, 20-g of individual wet sediment samples, were added to 40 mL of pyrophosphate buffer (0.01 M) at pH 7.0. Samples were sonicated for 3 min (37 kHz) on ice and interrupted for 30 sec every minute to mix the samples manually (Danovaro et al. 2001). The mixture was then shaken at 400 rpm for 30 min at 4°C (orbital shaker, KS 260 basic IKA®). After two successive centrifugations at 10 000 g for 10 and 5 min, respectively, supernatant was collected and stored for further analyses. A recovery rate of the elution step was assessed to be of $29.3 \pm 16.0\%$ (average \pm standard deviation) as a function of the sediment matrix (Fauvel et al. 2017b). Ten supernatant aliquots (1 mL each) were used for the determination of infectious FRNAPH concentration by plaque assay using *Salmonella enterica* serovar Typhimurium strain WG49, according to the ISO standard procedure 10705-1:2001. Nalidixic acid was added into culture media to limit the growth of autochthon bacteria flora. Negative and positive (MS2 phage) controls were included in this assay. Plates were incubated overnight at 37°C before PFU (plaque forming units) counting. Taking into account the %WC in fresh analysed sediments, FRNAPH concentrations were expressed in PFU/g of dry sediment. The division of phage concentrations by the respective specific surface area allowed to express the FRNAPH concentrations in PFU/m².

Statistical analyses

Statistical analyses were carried out with XLSTAT (Addinsoft, France) and SigmaPlot 12.5 software. Principal component analysis (PCA) was used to detect the possible relationships

between sediment characteristics and infectious FRNAPHs concentrations. The outcomes being not normally distributed, non-parametric tests (Mann-Whitney, ANOVA on Ranks and Spearman) were applied to compare groups of data or to establish variable correlations.

RESULTS AND DISCUSSION

Heterogenic distribution of F-specific bacteriophages in sediment samples

A total of 36 (9 sampling points \times 4 campaigns) riverbed sediment samples were collected according to the sampling grid established. Infectious FRNAPHs were detected in all sediment samples. Important dispersions appeared among samples ($n = 36$) with a median concentration of 1.8 ± 2.3 (median \pm interquartile interval) \log_{10} PFU/g of dry sediment (eq. $0.7 \pm 1.2 \log_{10}$ PFU/m²) and minimum and maximum concentrations of $0.8 \log_{10}$ and $3.1 \log_{10}$ PFU/g (eq. $-0.6 \log_{10}$ and $2.1 \log_{10}$ PFU/m²), respectively. The occurrence of FRNAPHs in freshwater sediment is thus confirmed, verifying the presence of a faecal contamination in the sediment compartment. In previous studies specifically focused on the infectious FRNAPHs in river sediment, comparable results were reported with concentrations ranging from $0.7 \log_{10}$ to $3.7 \log_{10}$ PFU/g of dry sediment (Haramoto et al. 2009), $1.3 \log_{10}$ to $2.4 \log_{10}$ PFU/g of dry sediment (Skraber et al. 2009b) and $-0.2 \log_{10}$ to $2.7 \log_{10}$ PFU/g of dry sediment (Fauvel et al. 2017a). Each of the reported studies emphasized the large variations in phage concentrations detected in sediment. In the samples analysed here, significant differences of FRNAPH concentrations were observed depending on the sampling time and the sampling area (Fig. 2).

First, the infectious FRNAPH concentrations detected in sediment samples were linked to the hydro-climatological conditions preceding the sampling event. A significant increase of ca. $0.7 \log_{10}$ of FRNAPHs were observed in sediment samples collected during the recession phase of a rainfall event compared to low flow sampling periods (Mann-Whitney Rank test, $P = 0.016$). Recently, a study suggested that the transfer of viral particles from the water column to sediment occurs mainly during the recession phase, when water flow returns to normal conditions (Fauvel et al. 2017b). The authors explained this phenomenon by a higher association of phages with solid settling particles during the later stage of rainfall runoff events. In the present work, the significantly higher viral load detected in sediment, during the recession phase of the rainfall event, supports the hypothesis of a preferential settling of viruses associated with solid particles at this particular time.

Secondly, an *in situ* spatial distribution was identified, with a preferential accumulation of infectious FRNAPHs on one bank of the river irrespective of the hydro-climatological conditions. Indeed, FRNAPH concentrations were significantly higher on the right bank (with concentrations five times superior) than on the middle or the left bank of the river (Mann-Whitney Rank test, $P < 0.001$). In order to explain this spatial phage trend, an accurate characterization of the structure and mineralogy of sediment according to the sampling areas was carried out.

Influence of the structural characteristics of sediment on the distribution of F-specific bacteriophages

The sediment samples collected during the study presented very different colours, odours and textures depending on the sampling points. The organic matter and the water content of sediment samples are reported in Table 2. Near the right bank, the

sediment samples were darker and finer than those collected in the other sampling areas. The %OM in the sediment was found to be stable regardless of the sampling area (median of $5.0 \pm 0.8\%$), whilst a significant difference was observed for the water content on the right bank compared to the other sampling points (ANOVA on Ranks test, $P < 0.001$). A significant difference of particle size distribution was also highlighted for samples of the right bank compared to those from the middle or left bank (Mann-Whitney Rank test, $P < 0.001$). As shown in Fig. 3, the right bank sediment presented large quantities of fine particles with a higher percentage of particles lower than 0.5 mm (74.9% of particles crossed over sieve of 0.5 mm aperture) compared to the other sampling points (33.4% and 38.5% for sediment samples from the middle and left bank, respectively). On the contrary, a similar proportion of coarser particles was determined for sediment samples from the middle and left banks (Mann-Whitney Rank test, $P = 0.267$). A PCA was carried out in order to point out the possible relationships between the concentration of infectious FRNAPHs and the sediment characteristics (Fig. 4). More than 74.0% of the total information of the data can be explained with this PCA (PC1 58.0%; PC2 16.0%) and all the variables are well represented with respect to the correlation circle. Table 3 presents the correlation matrix between all variable parameters including infectious FRNAPHs, organic matter, water content and different sized particles according to sieve apertures. According to the PCA, no correlation was established between phages and organic matter content whereas correlations were determined between FRNAPHs and other variables. First, the FRNAPHs were negatively correlated with large size particles ranging between 2 and 0.5 mm (Spearman correlation test, $r = -0.702$, $P < 0.001$ and $r = -0.621$, $P < 0.001$ for particles ranging between 2 – 1 mm and 1 – 0.5 mm, respectively). Secondly, small particles of less than 0.2 mm present a positive correlation with phage concentrations ($r = 0.740$, $P < 0.001$, $r = 0.775$, $P < 0.001$ and $r = 0.694$, $P < 0.001$ for particles ranging between 0.2 – 0.125 mm, 0.125 – 0.063 and <0.063 mm, respectively). Finally, a positive indirect correlation between infectious FRNAPHs and water content was observed ($r = 0.734$, $P < 0.001$) due to significant correlations between water content and the same size particles as those correlated with phages. These results are consistent with the high densities of infectious FRNAPHs measured in the right bank sediment samples, since they are mainly contained of a large quantity of fine particles. In line with the Wentworth scale, which classify solid particles according to their size (Wentworth 1922), small solid particles positively correlated to FRNAPH concentrations are identified as fine to very fine sands (grain diameter between 0.2 and 0.063 mm). The attachment of viral particles to these classes of solid particles is well documented. Sand is often used for the retention of viral particles in filtration column experiments (Knappett et al. 2008; Treumann et al. 2014) and on a larger scale for the water treatment processes (Lodder et al. 2013; Asami et al. 2016). Nevertheless, results of the present study show a negative correlation between medium to coarse sand (grain diameter between 2 and 0.5 mm) and the FRNAPH concentrations. This finding is in accordance with previously reported data that highlighted a better retention of MS2 bacteriophage in columns filled with fine sand (ca. 0.3 mm) than those filled with medium sand (ca. 0.7 mm) (Knappett et al. 2008). Therefore, beyond sand's intrinsic characteristics as a good virus sorbent, the grain size also seems to have an impact on the attachment of FRNAPHs.

Particles contained in the fraction smaller than 0.063 mm represent clay minerals. With their high ion exchange capacity and their large surface area, these minerals are recognized

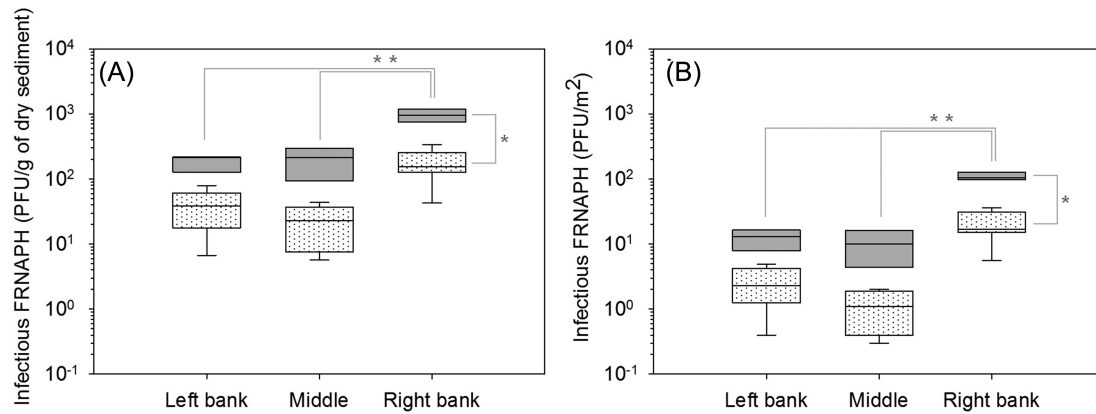


Figure 2. Concentrations of infectious F-specific RNA bacteriophages (FRNAPHs), expressed per gram of dry sediment (A) or per unit area (B), in sediment sampled from the left to the right banks of the river according to hydro-climatological conditions. Dotted box plots represent sediment samples ($n = 9$) collected during low flow periods (3 sampling points \times 3 campaigns), whereas grey box plots ($n = 3$) represent samples gathered during the recession phase of a rainfall event (3 sampling points \times 1 campaign). Boxes represent the 25th and 75th percentiles, whiskers the 10th and 90th percentiles and horizontal line within the box is the median. Stars indicate data significant difference established by a Mann-Whitney Rank test (* $P = 0.016$, ** $P < 0.001$).

Table 2. Characteristics of sediment samples gathered at the middle, right and left banks of the river. For each parameter, the median and the inter-quartile interval (difference between 25th and 75th percentiles) are reported.

	Organic matter (%) ^a ($n = 36$)	Water content (%) ^b ($n = 36$)	Specific surface area (m ² /g) ^c ($n = 9$)
Right bank	5.3 \pm 1.0	53.1 \pm 10.6	9.1 \pm 0.8
Middle	5.0 \pm 0.7	25.7 \pm 7.1	21.3 \pm 1.5
Left bank	4.8 \pm 0.4	24.7 \pm 3.8	16.0 \pm 1.9

^aNo significant difference between the river banks (ANOVA on ranks test, $p = 0.342$), ^bWater percentage contained in sediment samples is statistically different on the right bank compared to the other sampling points (ANOVA on ranks test, $P < 0.001$), ^cSpecific surface area of sediment is statistically different on the right bank compared to the other sampling points (ANOVA on ranks test, $P < 0.001$)

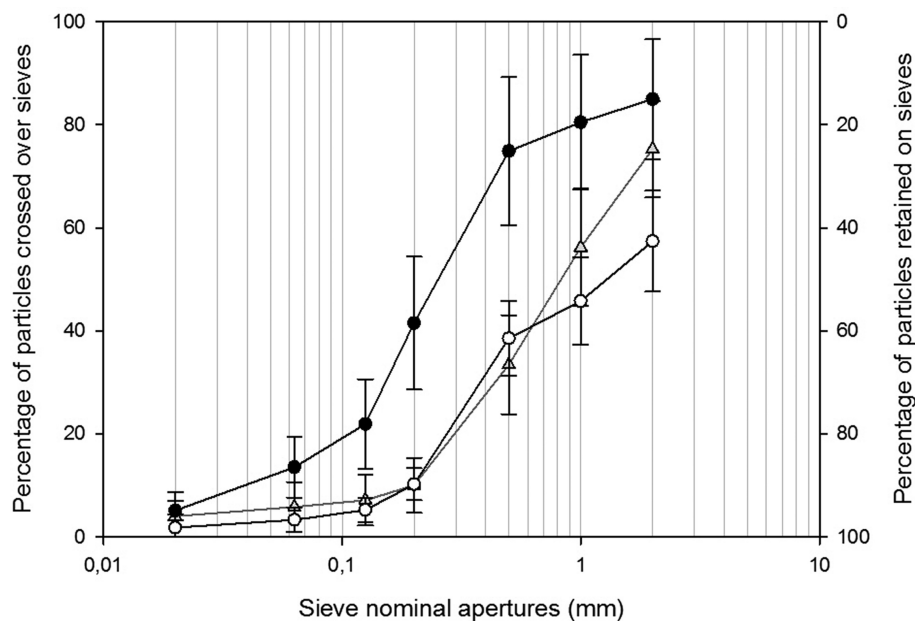


Figure 3. Particle size distribution of right (dark circles), middle (grey triangles) and left (white circles) bank sediment samples according to a series of sieves with different sized apertures (2 mm, 1 mm, 0.5 mm, 0.2 mm, 0.125 mm and 0.063 mm).

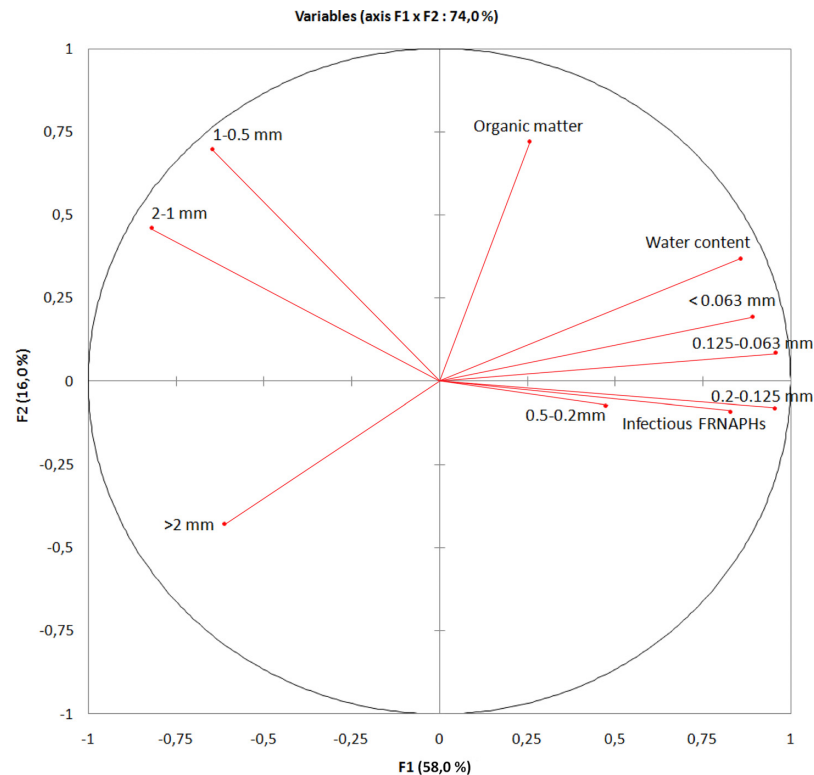


Figure 4. Principal component analysis of the sediment characteristics (organic matter, water content and sized particles according to sieve apertures) and infectious F-specific RNA bacteriophage concentrations (PFU/m²) measured on sediment samples (n = 36).

as strong viral sorbents. Some column studies have emphasized the effectiveness of clay to retain FRNAPHs by adsorption (Chattopadhyay and Puls 1999; Bellou et al. 2015; Syngouna and Chrysikopoulos 2015). In addition, a higher survival rate was observed for adsorbed viruses than those suspended in natural habitat (Babich and Stotzky 1980). The presence of clays in sediment therefore acts in favour of a storage of FRNAPHs in the riverbed and confers protection for viruses from inactivation processes. All data from the literature concerning the attachment capability of viruses on sand and clay particles were assessed in laboratory and controlled conditions. To our knowledge, it is the first time that this result was reported directly from *in situ* analysis of a fresh and complex environmental matrix. The abundance of fine sands and clay particles on the right bank promotes the presence of infectious FRNAPHs and may partially explains the spatial distribution of phages highlighted *in situ*.

A sediment sample predominantly composed of fine particles should potentially offer more available surface area for the attachment of phages than that of a coarse sediment sample. Because of the irregularity and tortuosity of surface, 1 gram of sediment may present a specific surface area of several m². Chrysikopoulos and Aravantinou (2014) highlighted that the attachment of MS2 and ϕ X174 bacteriophages was inversely correlated with quartz sand size. These authors attributed their results to an increase of the surface area available for attachment with the decreasing quartz sand size. In the present study, the specific surface area of sediment samples was analysed because of the large disparity of grain size (Table 2). Significant difference of specific surface areas was observed on the right bank compared to the other sampling points (ANOVA on Rank test, $P < 0.001$). Contrary to what was expected, fine sediments from the right bank of the Alzette River presented the lowest specific surface area compared to coarser sediments from the

middle or left bank of the river. The presence of amorphous iron oxides especially on the middle and left bank of the river could explain this result. A spatial pattern of iron-bearing phases in sediment samples was confirmed by the mineralogical composition established by XRD (see part 3.3). These small iron particles affect sediment properties with an increase of the surface area (Borggaard 1982). In order to avoid the bias of available surface for the attachment of phages to sediment, the FRNAPH concentrations were expressed per unit area (PFU/m²) and ranged from 0.3 to 125.9 PFU/m². To our knowledge, no data expressing a phage concentration per unit area is currently available in the literature. Despite a weaker specific surface area and therefore a weaker contact surface available for phage attachment, the same spatial distribution of FRNAPHs with a preferential accumulation of infectious phages on the right bank of the river was observed (Fig. 2). Therefore, the specific surface area does not appear to be responsible for the higher concentrations of FRNAPHs quantified in sediment samples from the right bank.

Influence of sediment mineralogical composition on the distribution of F-specific bacteriophages

Depending on its mineral composition, riverbed sediment contains certain surface properties (charge and hydrophobicity), which are more or less favourable to interactions with viral particles. Numerous studies have been focused on virus attachment to natural and model soil minerals. The ability to interact with non-biological surfaces depends on many variables such as the physico-chemical properties of viruses and the environment and the nature of the adsorbent (Bitton 1975). As with most viruses found in the aquatic ecosystems, FRNAPHs present

Table 3. Correlation matrix (Spearman correlation coefficient and P-value). Mention of different sized particles according to sieve apertures. The P-values in bold are significant ($P < 0.05$).

Variables	Infectious FRNAPH	Organic matter	Water content	> 2 mm	2 – 1 mm	1 – 0.5 mm	0.5 – 0.2 mm	0.2 – 0.125 mm	0.125 – 0.063 mm	< 0.063 mm
Infectious FRNAPH	1									
Organic matter	0.243 $P = 0.152$	1								
Water content	0.734 $P < 0.001$	0.482 $P = 0.003$	1							
				–0.289 $P = 0.087$	–0.702 $P < 0.001$	–0.621 $P < 0.001$	0.125 $P = 0.466$	0.740 $P < 0.001$	0.775 $P < 0.001$	0.694 $P < 0.001$
				–0.119 $P = 0.486$	0.034 $P = 0.843$	0.217 $P = 0.202$	–0.050 $P = 0.774$	0.224 $P = 0.189$	0.296 $P = 0.080$	0.265 $P = 0.119$
				–0.629 $P < 0.001$	–0.538 $P = 0.001$	–0.323 $P = 0.056$	0.243 $P = 0.153$	0.758 $P < 0.001$	0.835 $P < 0.001$	0.798 $P < 0.001$

a low isoelectric point (IP) in environmental conditions (Langlet et al. 2008). In order to understand the *in situ* distribution of FRNAPHs in sediment samples, a mineralogical characterization using XRD was carried out for three sediment samples from the middle, the right and the left banks of the river (Fig. 5). Four major crystalline phases, i.e. quartz, calcite, goethite and carbonates (dolomite or ankerite), were revealed in all analysed samples, whatever the sampling location. Quartz is the most common mineral compound of sand and known as a favourable support for viral particles (Loveland et al. 1996; Attinti et al. 2010; Chrysikopoulos and Aravantinou 2014). Due to the microscopic chemical heterogeneity of its surface, the quartz exhibits both positive and negative charges allowing the attachment of viral particles (Redman et al. 1997; Foppen, Oklety and Schijven 2006). In the same way, Stevenson et al. (2015) reported the attachment of human adenovirus and PRD1 bacteriophage on a fine granular limestone aquifer material essentially composed of calcite (63%). Goethite is an iron bearing hydroxide mineral which has been reported to have a great potential for controlling the fate and transport of viruses in natural environments (Chu, Jin and Yates 2000; You et al. 2003). In natural soil and at pH values close to neutral, this mineral tends to possess a low positive charge, since it has an IP value of 7.1 (Zhuang and Jin 2008). Goethite is therefore a moderate sorbent for negatively charged viruses in natural aquatic environments taking into account electrostatic interactions (Attinti et al. 2010). Zhuang and Jin (2008) have shown that the attachment of viruses to goethite can be substantially enhanced by the presence of carbonate. This crystalline phase, also observed in Alzette River sediments, is an important oxyanion which is ubiquitous in natural environments. Its presence in sediment can favour the interaction between viruses and metal oxides by the generation of extra sorption sites (addition of protonated surface groups) (Wijnja and Schulthess 2001).

The XRD method is generally considered as a non-quantitative assay, but some differences in the proportion of minerals encountered in the three sediment samples analysed could be determined. On one hand, a lateral distribution of iron-bearing phase quantity contained in fresh sediment samples was revealed. A shift in the intensity of diffracted radiation was observed for the three diffractograms. This variation was especially noticeable with the variable background noise. The presence of iron-bearing phases (such as goethite or other slightly crystallized oxide and hydroxide) could be the reason for this background noise due to the fluorescence of the iron. The analysis of the intensity shift of the three diffractograms can underline a variation of the amount of iron-bearing phases, according to the following ascending order: right bank < middle < left bank of the river. On the other hand, an apparent modification of mineral proportion was observed with the intensity of main characteristic peaks, varying from one sample to another. Indeed, signals corresponding to the quartz were more intense for the right bank sediment sample compared to the others. Moreover, signals corresponding to calcite and goethite were low on the right bank sediment sample whereas they were clearly higher for samples coming from the left bank and the middle of the river. From these observations, the sediment sample from the right bank was different from the others with a higher proportion of quartz and a lower presence of goethite and calcite. This spatial variation in the mineral composition of sediment samples could also explain the *in situ* phage distribution highlighted. Based on studies cited above, quartz, calcite,

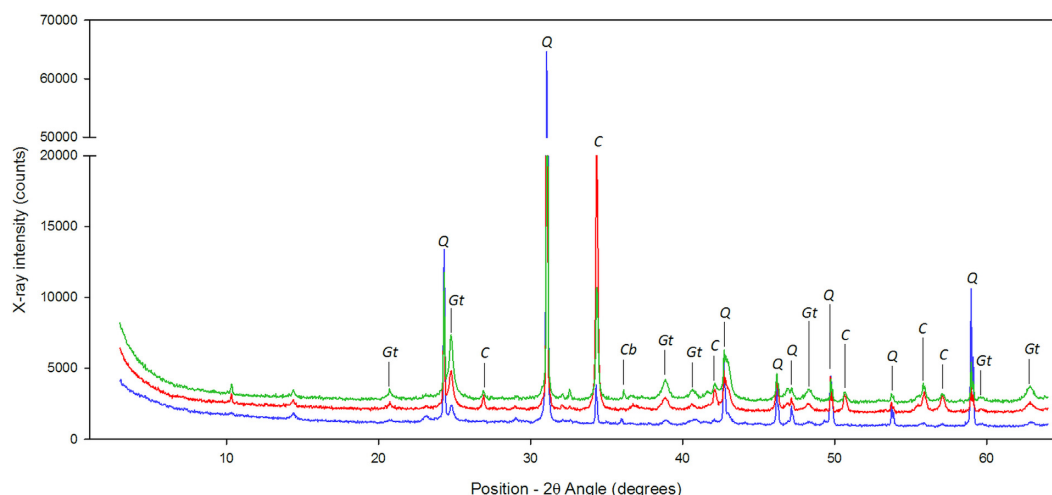


Figure 5. X-ray diffraction patterns obtained from right (blue line), middle (red line) and left (green line) banks of sediment samples. Q quartz; C calcite; Gt goethite; Cb carbonate

goethite and carbonates are all potential sorbents of viral particles. Nevertheless, recent studies demonstrated that the presence of iron oxides in soil can also inactivate viral particles (Chu, Jin and Yates 2000; Schijven and Hassanizadeh 2000; Zhuang and Jin 2008). Zhao et al. (2008) reported an irreversible sorption of viruses on iron oxides as one possible explanation for the greater efficiency in retaining viruses in soil containing iron-bearing phases. Although the exact mechanism involved in adsorption and inactivation of viruses by iron oxides is not fully understood, the disintegration of the virus or the non-infectious fate could be a consequence of a strong attachment between viruses and metal oxides (You et al. 2005). Thus, the higher presence of iron-bearing phases (goethite) in the middle and left bank of the Alzette River sediments could be responsible both for the attachment and inactivation of infectious FRNAPHs. The enumeration of phages by plaque assay is not relevant for the determination of inactivated or non-infectious FRNAPHs explaining perhaps the preferential distribution of infectious FRNAPHs observed in sediment samples from the right bank of the river. To confirm this hypothesis, it will be interesting to quantify total FRNAPHs (i.e. infectious and inactivated/damaged phage particles) through molecular tools. A previous study reported a possible variation of phage surface properties between infectious and non-infectious phages as a consequence of a slower attachment of infectious phages to sediment (Fauvel et al. 2017b). Similarly, a higher adsorption to positively charged beads was reported for infectious MS2 bacteriophage compared to phages inactivated by high temperatures (Brié et al. 2016). Inactivation of phages can cause conformational changes at the capsid modifying the surface properties of phage particles. Thus, the combination of the infectivity assay and genome quantification would refine our understanding of the *in situ* distribution of FRNAPH, and additionally provide interesting information on the FRNAPH genogroups pattern in the riverbed sediment.

In summary, this study provides new insights about the presence and the *in situ* spatial distribution of viral particles in riverbed sediment according to physico-chemical intrinsic characteristics of this complex environmental matrix. At our study site, the heterogeneous presence of fine sands, clays and minerals (quartz, calcite, goethite and carbonate) is assumed to be the main factor responsible for the *in situ* spatial phage distribution. Further studies applying comparable methods to a larger number of samples in more variable locations are still needed

to better understand how enteric viruses interact with sediments of different mineral composition. The mineralogical and physico-chemical study of sediment in rivers should therefore be taken into consideration in order to better evaluate the capability of this environmental matrix to store allochthonous enteric viruses and to prevent an important source of viral contamination in surface water.

ACKNOWLEDGEMENTS

The authors would like to thank Delphine Collard and Cécile Walczak from the LIST for their precious and excellent technical assistance in sampling campaigns and lab experiments. We also thank Dr. Catherine Mulholland (LIST) for the proficient proofreading of the final manuscript. We thank the Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) for the specific surface area measurements and the X-ray diffraction analysis. Special thanks to Emmanuelle Montarges-Pelletier from LIEC and Christophe Hissler from LIST for their valuable advice in the data interpretation.

FUNDING

This work was supported by the National Research Fund [AFR grant number 6035344], Luxembourg. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Conflicts of interest: None declared.

REFERENCES

- Asami T, Katayama H, Torrey JR et al. Evaluation of virus removal efficiency of coagulation-sedimentation and rapid sand filtration processes in a drinking water treatment plant in Bangkok, Thailand. *Water Res* 2016;101:84–94.
- Attinti R, Wei J, Kniel K et al. Virus' (MS2, phi X174, and Aichi) attachment on sand measured by atomic force microscopy and their transport through sand columns. *Environ Sci Technol* 2010;44:2426–32.
- Babich H, Stotzky G. Reductions in inactivation rates of bacteriophages by clay minerals in lake water. *Water Res* 1980;14:185–7.

- Bales RC, Li S, Maguire KM et al. MS-2 and poliovirus transport in porous media: hydrophobic effects and chemical perturbations. *Water Resour Res* 1993;29:957–63.
- Beaudoin A. A comparison of two methods for estimating the organic content of sediments. *J Paleolimnol* 2003;29:387–90.
- Bellou MI, Syngouna VI, Tselepi MA et al. Interaction of human adenoviruses and coliphages with kaolinite and bentonite. *Sci Total Environ* 2015;517:86–95.
- Bitton G. Adsorption of viruses onto surfaces in soil and water. *Water Res* 1975;9:473–84.
- Borggaard OK. The influence of iron oxides on the surface area of soil. *J Soil Sci* 1982;33:443–9.
- Borrel G, Colombet J, Robin A et al. Unexpected and novel putative viruses in the sediments of a deep-dark permanently anoxic freshwater habitat. *The ISME Journal* 2012;6:2119–27.
- Bosch A, Pintó RM, Abad FX. Survival and transport of enteric viruses in the environment, S.M. Goyal. (Ed.) *Viruses in Foods*, in *Food Microbiology and Food Safety*, Springer, US, 2006;151–87.
- Bosch A. Human enteric viruses in the water environment: a minireview. *Int Microbiol* 1998;1:191–6.
- Brié A, Bertrand I, Meo M et al. The effect of heat on the physico-chemical properties of bacteriophage MS2. *Food Environ Virol* 2016;8:251–61.
- Brunauer S, Emmett PH, Teller E. Adsorption of gases in multimolecular layers. *J Am Chem Soc* 1938;60:309–19.
- Cao H, Tsai FT-C, Rusch KA. Salinity and soluble organic matter on virus sorption in sand and soil columns. *Ground Water* 2010;48:42–52.
- Chattopadhyay S, Puls RW. Adsorption of bacteriophages on clay minerals. *Environ Sci Technol* 1999;33:3609–14.
- Chrysikopoulos CV, Aravantinou AF. Virus attachment onto quartz sand: Role of grain size and temperature. *J Environ Chem Eng* 2014;2:796–801.
- Chu Y, Jin Y, Yates MV. Virus transport through saturated sand columns as affected by different buffer solutions. *J Environ Qual* 2000;29:1103.
- Danovaro R, Corinaldesi C, Filippini M et al. Viriobenthos in freshwater and marine sediments: A review. *Freshwater Biol* 2008;53:1186–213.
- Danovaro R, Dell'Anno A, Trucco A et al. Determination of virus abundance in marine sediments. *Appl Environ Microbiol* 2001;67:1384–7.
- Dowd SE, Pillai SD, Wang S et al. Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. *Appl Environ Microbiol* 1998;64:405–10.
- Elmahdy MEI, Fongaro G, Magri ME et al. Spatial distribution of enteric viruses and somatic coliphages in a Lagoon used as drinking water source and recreation in Southern Brazil. *Int J Hyg Environ Health* 2016;219:617–25.
- Fauvel B, Cauchie H-M, Gantzer C et al. Contribution of hydrological data to the understanding of the spatio-temporal dynamics of F-specific RNA bacteriophages in river water during rainfall-runoff events. *Water Res* 2016;94:328–40.
- Fauvel B, Gantzer C, Cauchie H-M et al. In situ of F-specific RNA bacteriophages in a small river: new way to assess viral propagation in water quality studies. *Food and Environ Virol* 2017a;9:89–102.
- Fauvel B, Ogorzaly L, Cauchie H-M et al. Interactions of infectious F-specific RNA bacteriophages with suspended matter and sediment: towards an understanding of FRNAPH distribution in a river water system. *Sci Total Environ* 2017b;574:960–8.
- Foppen JWA, Okletey S, Schijven JF. Effect of goethite coating and humic acid on the transport of bacteriophage PRD1 in columns of saturated sand. *J Contam Hydrol* 2006;85:287–301.
- Gerba C, Smith E, Schaiberger G et al. Field evaluation of methods for the detection of enteric viruses in marine sediments. C.D. Litchfield and P.L. Seyfried (Ed.) in *Methodology for biomass determination and microbial activities in sediments*. American Society for Testing Materials, Philadelphia, 1979;64–75.
- Haramoto E, Kitajima M, Katayama H et al. Application of real-time PCR assays to genotyping of F-specific phages in river water and sediments in Japan. *Water Res* 2009;43:3759–64.
- Hassard F, Gwyther CL, Farkas K et al. Abundance and distribution of enteric bacteria and viruses in coastal and estuarine sediments-A review. *Frontiers in Microbiology* 2016;7:1692.
- Havelaar AH, Farrah SR, Jofre J et al. Bacteriophages as model viruses in water quality control. *Water Res* 1991;25:529–45.
- Havelaar AH, Van Olphen M, Drost YC. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. *Appl Environ Microbiol* 1993;59:2956–62.
- Helmi K, Skrabber S, Gantzer C et al. Interactions of *Cryptosporidium parvum*, *Giardia lamblia*, vaccinal poliovirus type 1, and bacteriophages phiX174 and MS2 with a drinking water biofilm and a wastewater biofilm. *Appl Environ Microbiol* 2008;74:2079–88.
- Jakubowska-Deredas M, Jurczak-Kurek A, Richert M et al. Diversity of tailed phages in Baltic Sea sediment: large number of siphoviruses with extremely long tails. *Res Microbiol* 2012;163:292–6.
- Jofre J. Indicators of waterborne enteric viruses. Bosch A (Ed.), in *Perspectives in Medical Virology*, Elsevier, Barcelona, Spain, 2007;17:227–49.
- Jofre J. Is the replication of somatic coliphages in water environments significant? *J Appl Microbiol* 2009;106:1059–69.
- Knappett PSK, Emelko MB, Zhuang J et al. Transport and retention of a bacteriophage and microspheres in saturated, angular porous media: effects of ionic strength and grain size. *Water Res* 2008;42:4368–78.
- Langlet J, Gaboriaud F, Gantzer C et al. Impact of chemical and structural anisotropy on the electrophoretic mobility of spherical soft multilayer particles: the case of bacteriophage MS2. *Biophys J* 2008;94:3293–312.
- Leroy M, Prigent M, Dutertre M et al. Bacteriophage morphotype and genome diversity in Seine River sediment. *Freshwater Biology* 2008;53:1176–85.
- Liew PF, Gerba CP. Thermostabilization of enteroviruses by estuarine sediment. *Appl Environ Microbiol* 1980;40:305–8.
- Lippens BC, de Boer JH. Studies on pore systems in catalysts: V. The t method. *J Catal* 1965;4:319–23.
- Lodder WJ, van den Berg HHJL, Rutjes SA et al. Reduction of bacteriophage MS2 by filtration and irradiation determined by culture and quantitative real-time RT-PCR. *J Water Health* 2013;11:256.
- Loveland JP, Ryan JN, Amy GL et al. The reversibility of virus attachment to mineral surfaces. *Colloids Surf A* 1996;107:205–21.
- Lucena F, Méndez X, Morón A et al. Occurrence and densities of bacteriophages proposed as indicators and bacterial indicators in river waters from Europe and South America. *Appl Environ Microbiol* 2003;69:1748–52.
- Mackowiak M, Leifels M, Hamza IA et al. Distribution of *Escherichia coli*, coliphages and enteric viruses in water, epilithic biofilms and sediments of an urban river in Germany. *Sci Total Environ* 2018;626:650–9.

- Maranger R, Bird DF. High concentrations of viruses in the sediments of Lac Gilbert, Québec. *Microb Ecol* 1996;**31**:141–51.
- Mauffret A, Caprais MP, Gourmelon M. Relevance of Bacteroidales and F-specific RNA bacteriophages for efficient fecal contamination tracking at the level of a catchment in France. *Appl Environ Microbiol* 2012;**78**:5143–52.
- Miura T, Masago Y, Chan Y-M et al. Detection of bacteria and enteric viruses from river and estuarine sediment. *J Water Environ Technol* 2009;**7**:307–16.
- Montarges-Pelletier E, Jeanneau L, Faure P et al. The junction of Fensch and Moselle rivers, France; mineralogy and composition of river materials. *Environ Geol* 2007;**53**:85–102.
- Ogorzaly L, Tissier A, Bertrand I et al. Relationship between F-specific RNA phage genogroups, faecal pollution indicators and human adenoviruses in river water. *Water Res* 2009;**43**:1257–64.
- Paul JH, Rose JB, Jiang SC et al. Distribution of viral abundance in the reef environment of Key Largo, Florida. *Appl Environ Microbiol* 1993;**59**:718–24.
- Pelleieux S, Bertrand I, Skali-Lami S et al. Accumulation of MS2, GA, and Qbeta phages on high density polyethylene (HDPE) and drinking water biofilms under flow/non-flow conditions. *Water Res* 2012;**46**:6574–84.
- Rao VC, Metcalf TG, Melnick JL. Human viruses in sediments, sludges, and soils. *Bull World Health Organ* 1986;**64**:1–14.
- Redman JA, Grant SB, Olson TM et al. Filtration of recombinant Norwalk virus particles and bacteriophage MS2 in quartz sand: importance of electrostatic interactions. *Environ Sci Technol* 1997;**31**:3378–83.
- Sasidharan S, Bradford SA, Šimůnek J et al. Transport and fate of viruses in sediment and stormwater from a Managed Aquifer Recharge site. *J Hydrol* 2017;**555**:724–735.
- Schijven JF, Hassanizadeh SM. Removal of viruses by soil passage: overview of modeling, processes, and parameters. *Crit Rev Environ Sci Technol* 2000;**30**:49–127.
- Skraber S, Helmi K, Willame R et al. Occurrence and persistence of bacterial and viral faecal indicators in wastewater biofilms. *Water Sci Technol* 2007;**55**:377–85.
- Skraber S, Ogorzaly L, Helmi K, et al. Occurrence and persistence of enteroviruses, noroviruses and F-specific RNA phages in natural wastewater biofilms. *Water Res* 2009a;**43**:4780–9 10.1016/j.watres.2009.05.02019616820
- Skraber S, Schijven J, Italiaander R et al. Accumulation of enteric bacteriophage in fresh water sediments. *J Water Health* 2009b;**7**:372–9 10.2166/wh.2009.09819491489
- Smith EM, Gerba CP, Melnick JL. Role of sediment in the persistence of enteroviruses in the estuarine environment. *Appl Environ Microbiol* 1978;**35**:685–9.
- Staggemeier, Almeida SEM, Spilki FR. Methods of virus detection in soils and sediments. *Virus Reviews and Research* 2012;**16**:1–7.
- Stevenson ME, Sommer R, Lindner G et al. Attachment and detachment behavior of human adenovirus and surrogates in fine granular limestone aquifer material. *J Environ Qual* 2015;**44**:1392.
- Syngouna VI, Chrysikopoulos CV. Cotransport of clay colloids and viruses in water saturated porous media. *Colloids Surf A* 2013;**416**:56–65.
- Syngouna VI, Chrysikopoulos CV. Experimental investigation of virus and clay particles cotransport in partially saturated columns packed with glass beads. *J Colloid Interface Sci* 2015;**440**:140–50.
- Tong M, Shen Y, Yang H et al. Deposition kinetics of MS2 bacteriophages on clay mineral surfaces. *Colloids Surf B* 2012;**92**:340–7.
- Torkzaban S, Hassanizadeh SM, Schijven JF et al. Virus Transport in Saturated and Unsaturated Sand Columns. *Vadose Zone J* 2006;**5**:877.
- Treumann S, Torkzaban S, Bradford SA et al. An explanation for differences in the process of colloid adsorption in batch and column studies. *J Contam Hydrol* 2014;**164**:219–29.
- Wentworth CK. A scale of grade and class terms for clastic sediments. *The Journal of Geology* 1922;**30**:377–92.
- Wijnja H, Schulthess CP. Carbonate adsorption mechanism on goethite studied with ATR-FTIR, DRIFT, and proton coadsorption measurements. *Soil Sci Soc Am J* 2001;**65**:324.
- Woody MA, Cliver DO. Effects of temperature and host cell growth phase on replication of F-specific RNA coliphage Q beta. *Appl Environ Microbiol* 1995;**61**:1520–6.
- Yates MV. Norovirus. in *Microbiology of Waterborne Diseases- Microbiological aspects and risks*, 2nd Edition. Academic Press, College of natural and agricultural sciences, University of California, USA, 2014;515–22.
- You Y, Han J, Chiu PC et al. Removal and inactivation of waterborne viruses using zerovalent iron. *Environ Sci Technol* 2005;**39**:9263–9.
- You Y, Vance GF, Sparks DL et al. Sorption of MS2 bacteriophage to layered double hydroxides. *J Environ Qual* 2003;**32**:2046.
- Zhao B, Zhang H, Zhang J et al. Virus adsorption and inactivation in soil as influenced by autochthonous microorganisms and water content. *Soil Biol Biochem* 2008;**40**:649–59.
- Zhuang J, Jin Y. Interactions between viruses and goethite during saturated flow: effects of solution pH, carbonate, and phosphate. *J Contam Hydrol* 2008;**98**:15–21.
- Zhuang J, Yu G-R. Effects of surface coatings on electrochemical properties and contaminant sorption of clay minerals. *Chemosphere* 2002;**49**:619–28.