ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Solar disinfection of viruses (SODIS): inactivation of coliphages MS2 and phiX174, human adenovirus and echovirus in water from Switzerland and India

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Master Project
Spring semester 2013
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Résumé

L'accès à l'eau potable reste un défi dans de nombreuses régions du monde. De nouvelles méthodes pour traiter l'eau potable sont constamment développées et améliorées à la fois dans les pays industrialisés et dans les pays en développement. SODIS est une technique intéressante de désinfection de l'eau à petite échelle qui consiste simplement à mettre de l'eau dans une bouteille en PET et de l'exposer au soleil pendant au moins 6 heures. Les bactéries sont efficacement inactivées par la lumière du soleil avec cette méthode, mais l'efficacité de SODIS sur l'élimination de virus est beaucoup moins bien connue.
Dans ce travail, l'inactivation des bactériophages MS2 et phiX174 ainsi que les virus humain. echovirus et l'adénovirus a été étudiée dans trois eaux différentes, deux d'Inde et une de Suisse. Une bonne diminution de la concentration de MS2 (4 logs en 6 heures à 22 ° C et un taux irradiance de 1.34 kJ/cm^2) dans l'eau du robinet en Suisse a été atteinte, alors que l'inactivation a été moins efficace pour MS2 et echovirus (1 log en 6 heures à 22 ° C et à un taux d'irradiance de 1,34 kJ/cm^2) dans les deux eaux indiennes. Les virus phiX174 et adénovirus furent beaucoup plus résistantes lors de l'usage de SODIS comme système de désinfection, moins de 1 log d'inactivation a été observé dans l'eau du robinet suisse pour phiX174. L'inactivation n'était pas significativement différente du témoin effectuée dans la pénombre pour les expériences avec phiX174 dans les eaux indiennes ainsi que dans aucune des expériences faites avec adénovirus. L'inactivation de echovirus fut similaire à celle de MS2 et l'inactivation d'adénovirus aussi lente que pour phiX174. Une augmentation de la température au cours de SODIS améliora substantiellement l'inactivation observable; On peut s'attendre à une inactivation de 6 logs en 6 heures à 45 ° C et avec un taux d'irradiance de 1,34 kJ/cm^2.
L'inactivation indirecte solaire fut le principale mécanisme responsable de l'inactivation MS2, tandis que le mécanisme d''inactivation directe via l'absorption de la lumière du soleil par le génome n'était pas responsable de l'inactivation des virus lors de l'utilisation de SODIS. D'après les expériences menées, il semblerait que le fer pourrait être responsable de la formation de dérivés réactifs de l'oxygène qui réagiraient avec les virus et les inactiveraient. L'inactivation lente dans les eaux indiennes est probablement due à l'effet de consommation des dérivés réactifs de l'oxygène par la matière organique présente dans l'eau. L'usage de SODIS pour inactiver echovirus pourrait suffire (4 logs en 6 heures), en particulier à des températures élevées et avec une faible teneur en matière organique de l'eau traitée. En revanche, SODIS n'est suffisamment pas efficace pour inactiver adénovirus totalement ou au de 4 logs.

Abstract

Access to safe drinking water remains a challenge in many regions of the world. New methods to treat drinking water are constantly developped and improved in both in industrialised countries and in developing countries. SODIS is an interesting household-level disinfection technique that simply consists of pouring water into a PET bottle and exposing it to sunlight for at least 6 hours. Bacteria are efficiently inactivated by sunlight during SODIS, but the efficiency of SODIS on virus removal is much less studied.
In this work, the inactivation of bacteriophages MS2 and phiX174, and human echovirus and adenovirus was studied in three different waters, two from India and one from Switzerland. Good removal (4 logs in 6 hours at 22°C and a fluence rate of 600 1.34 kJ/cm^2) of MS2 was achieved in Swiss tap water, while less efficient inactivation was achieved in both Indian waters for MS2 and echovirus (1 log in 6 hours at 22°C and a fluence rate of 1.34 kJ/cm^2). Both phiX174 and adenovirus were much more resistant to SODIS disinfection and less than 1 log of inactivation was obtained in Swiss tap water for phiX174. However, inactivation was not significantly different from the dark control in Indian water and for all experiments with adenovirus. Echovirus was inactivated similarly than MS2 and adenovirus was equally slow as phiX174. An increased temperature during SODIS enhanced inactivation substantially; 6 logs in 6 hours could be expected at 45 °C and with a fluence rate of 1.34 kJ/cm^2.
Indirect-solar inactivation was responsible for the MS2 removal, while direct inactivation via sunlight absorption of genome was not occurring during SODIS. We argue that iron could be responsible for the formation of reactive oxygen species which react with the viruses and yield their inactivation. The slower inactivation in Indian waters was probably due to the quenching effect of the higher concentration of organic matter. Removal of echoviruses during SODIS could be sufficient (4 logs in 6 hours) particularly at higher temperatures and for water with a low organic matter content. In contrast, SODIS may not be effective to remove Adenoviruses.

Acknowledgment

I would like to thank Tamar Kohn who supervised this work and who was always available to answer any of my questions. I also would like to thank Michael Jon Mattle who followed me and help throughout this project. His comments and advices have been very beneficial for this work. Those months spent in the LCE were very pleasant thanks to the whole team, which included Anna Carratalà Ripollès, Florence Bonvin, Heather Bischel, Julien Omlin, Loic Decrey, Qingxia Zhong and Thérèse Sigstam. I thank them for their good mood and for helping me out in different situations.
I also want to thank David Cordier, Pascal Stalder, Raphaelle Fustier and all others who have made the lunch breaks pleasant moments of relaxation and pleasantry. I thank my family for their support in different ways throughout this project and especially thank my sister, Marisa Dionisio, who took the time to read my report.
Finally, I express my very special thanks to Ludivine Clément, who joined me for some long weekend of work

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1 Introduction

Problems related to water are today on the main stage of world global issues. Two major aspects are water resource management and drinking water. About 11% of the world population in 2010 had no access to an improved source of drinking water (Figure 1)[1]. Additionally, access is not equal in each part of our planet and particularly limited in developing nations. Due to lack of safe drinking water, these people are exposed to a higher risk of attracting waterborne diseases. For example, diarrheal diseases cause 1.8 million deaths per year. Furthermore, there are 1.5 million new cases of clinical hepatitis A every year. According to the UN, a reduction in diarrhoea morbidity between 6% and 25% could be achieved by providing these people with an improved source of drinking water[2].

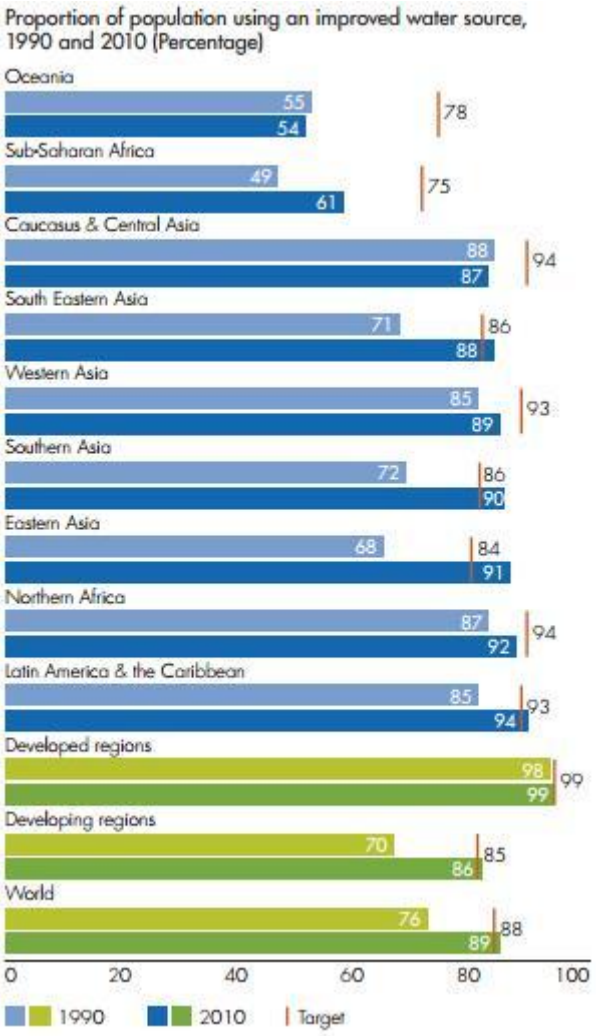

Figure 1 : Proportion (in percent) of population using an improved water source in 1990 and 2010[1].

An interesting way of improving the drinking water supply, without the need of constructing big and expensive installations of water treatment plants and maintaining them at high cost, is the implementation of treatment systems at household level. For instance, drinking water can be boiled or chlorinated but these methods have some serious disadvantages. Boiling of water requires a lot of fire wood and chlorination can be dangerous, if an inadequate quantity of chlorine is used. Therefore chlorination demands appropriate skills and training. A very cheap and simple alternative to treat drinking water is solar water disinfection (SODIS). This

household-based treatment technology only requires PET bottles and sunlight, both broadly available in most developing nations.

1.1 SODIS

Solar water disinfection was mentioned for the first time in 1984 by Atim Acra in a booklet from UNICEF[3]. Later, this method has been further investigated using PET bottles and implemented in many countries by a research team from Sandec (Water and Sanitation in Developing Countries) at Eawag (Swiss Federal Institute of Aquatic Science and Technology).
SODIS is a simple and sustainable way to treat drinking water at a household level. The water is poured into a PET bottle and then exposed to sunlight for at least 6 hours (Figure 2). Furthermore, on cloudy days it is recommended to leave the PET bottles exposed to the sun for at least two consecutive days (2 times 6 hours).

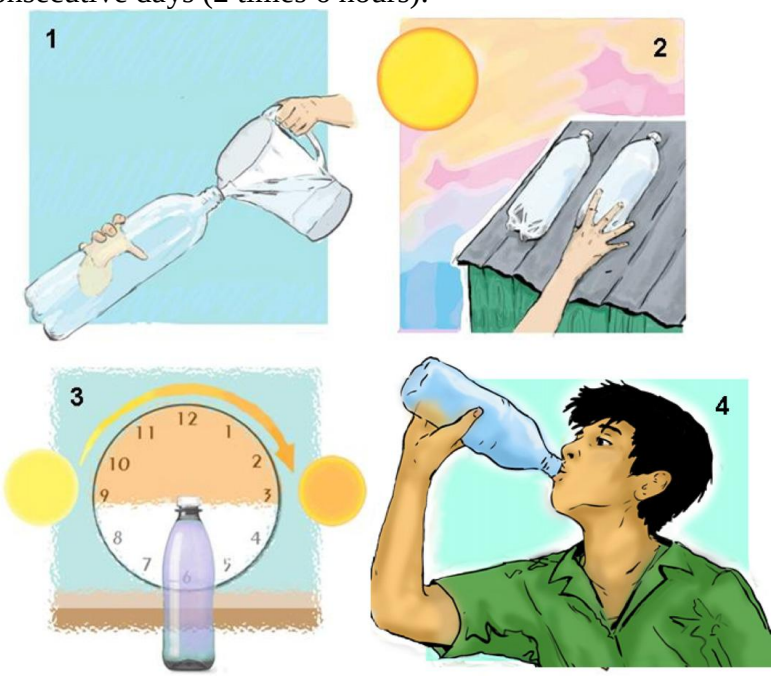

Figure 2: Graphical description of the solar disinfection (SODIS) household level water treatment technique[4].

SODIS is designed to disinfect small quantities of water with low turbidity. In order to improve the efficiency of SODIS, water can for example be filtered by a simple piece of cloth or bottles can be placed on a refracting surface (for example aluminium or a roof made with corrugated iron sheets). This leads to faster inactivation of the microorganisms[3]. Moreover SODIS can also be improved by using additional equipment[4], as for example reflectors that concentrate the solar irradiation on the bottle; or different containers than PET. The problem with these modifications is that they make SODIS more complicated and will also increase the costs, which could render the treatment unaffordable for many households.
During the SODIS treatment UV-A radiation and the increase in temperature[3] may lead to disinfection of infectious microorganisms. Additionally, previous studies have shown that there is a synergistic effect of the UV-A and the increase in water temperature[4–6]. This means that the inactivation of the microorganisms is faster when there is the combination of both UV-A and high temperatures compared to the sum of the two individual phenomena.

Solar disinfection of bacteria is well documented in the literature and many studies have shown that bacteria are highly sensitive to UV-A and are therefore easily inactivated[4]. In contrast, there is only little information regarding viruses. Previous studies[4] with SODIS

demonstrated that viruses are much more resistant than bacteria (partially due to their capsid)[7]. However, the importance of waterborne diseases caused by viruses is not negligible (as discussed below). Therefore it is fundamental to better understand the efficiency of virus inactivation during SODIS. Furthermore, the elucidation of the inactivation mechanisms may help in the future to predict for which water types SODIS may be efficient at inactivating viruses.

1.2 Viruses

A virus is a microorganism that can only replicate inside a living cell by infecting its host[7]. They either consist of a single- or double-stranded DNA or a single- or double-stranded RNA. The genetic material is enveloped in a structure of proteins, which is called capsid[7]. Furthermore, in some cases the capsid may also contain others macromolecules[7]. Viruses are able to infect all types of microorganisms, prokaryotes and eukaryotes. These infections can cause serious infections and diseases, either due to the modification of the genetic material of the infected host, or by using the cellular machinery of the host to promote virus replication[7]. Several severe illnesses related to the consumption of unsafe drinking water are due to viral infections[8]. Additionally viruses are among the most numerous biological entities and new ones are discovered every year[9]. For all these reasons it is important to study and understand viruses,

The most common pathogenic waterborne viruses are Rotavirus, Hepatitis A, Adenovirus, Poliovirus, Echovirus, Coxsackievirus and Coronavirus[7,10,11]. All these viruses can be transmitted by unsafe drinking water. For instance, Hepatitis A virus can cause serious damages to the liver. Additionally, it is an endemic virus in some regions of the world where hygiene and sanitation condition are usually poor[12]. Rotavirus and Adenovirus are two important pathogens that can cause diarrheal diseases and are a major cause of infant and children mortality in the world[13]. Adenovirus is of concern in both industrialized and developing countries. It can cause gastroenteritis with the same symptoms as Rotavirus. It can be particularly dangerous (even mortal) for elderly people and children without access to safe drinking water and proper care[11]. Echovirus, is a common enteric virus and can cause various diseases such as encephalitis, respiratory diseases or exanthema and is predominantly spread by the fecal-oral route[10,14].

A major part of this study was conducted with two bacteriophages, which are viruses that infect bacteria: MS2 bacteriophage composed of a single-stranded RNA and phiX174 composed of a single-stranded DNA. Both have an icosahedral symmetry, which means that they are composed of a symmetric structure with twenty faces which form a spherical structure (Figure 3). MS2 has been chosen for this study because it has a similar structure as many pathogenic enteric viruses, in particular those belonging to the genus enterovirus (e.g., Polio or Echovirus). It has therefore been used in many previous studies as a surrogate for enteric human viruses[15,16]. PhiX174 has been chosen because it is also a surrogate for enteric human viruses but unlike MS2 it is more sensitive to direct UV inactivation and more resistant to oxidants[17].

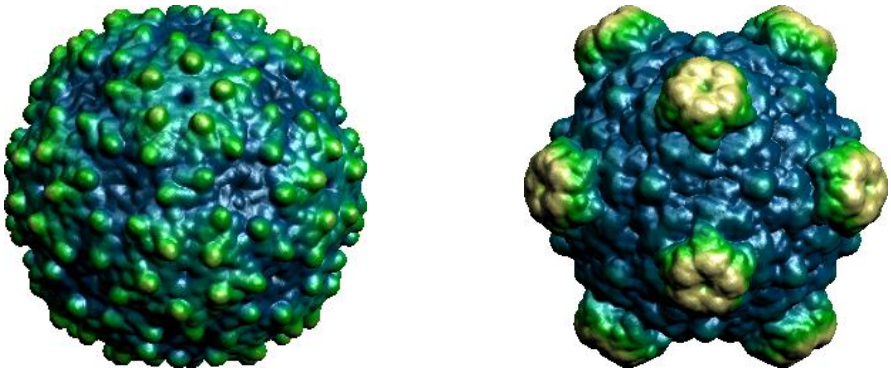

Figure 3: Capsid figures of bacteriophages MS2 (on the left) and PhiX174 (on the right)[18]

There are many benefits of using bacteriophages in comparison to human viruses: Firstly, they are easy to handle as they are not dangerous to humans. Secondly, the time needed for the enumeration process is much shorter than for human viruses, which allows to obtain experimental results in only one or two days as compared to one or two weeks for human viruses. Furthermore, the bacteriophages also serve as surrogates for human viruses for which no enumeration techniques exist.
The use of these bacteriophages, allowed us to define the experimental methodology of this study in relatively short time and they permitted to predict the inactivation of human viruses.
In order to obtain more relevant data for SODIS users, two human viruses have also been studied: Adenovirus (Figure 4) a double-stranded DNA virus and Echovirus (Figure 4) a single-stranded RNA virus. Adenovirus is one of few enteric viruses with a double-stranded DNA and is known to be stable and persistent in aquatic environments[11].

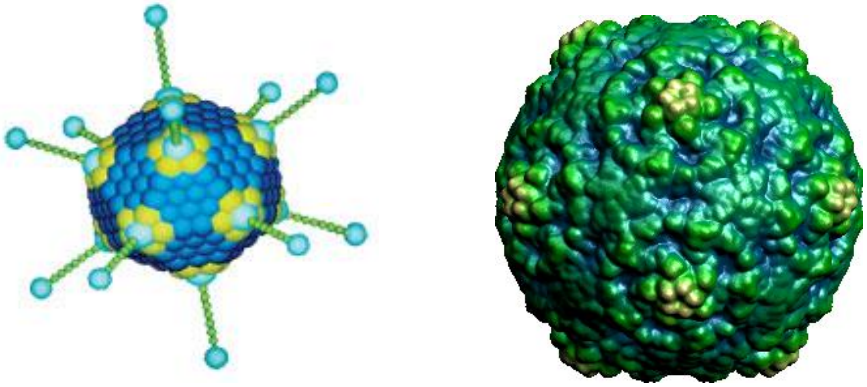

Figure 4: Capsid figures of Adenovirus[19] **(on the left) and Echovirus (on the right)**[18]

1.3 Sunlight-mediated inactivation

Research on disinfection of microorganisms by sunlight started around 1877 with a rigorous study of Downe and Blunt[20] which showed that there was no or little growth of bacteria in tubes (with Pasteur's solution) when exposed to sunlight. In contrast, bacteria grew in the same tubes but wrapped in sheet-lead that excluded sunlight[20]. Later in 1980, Aftim Acra et al.[22] investigated the effect of solar disinfection for drinking water at the University of Beirut. Their first experiments consisted of exposing artificially contaminated water in different transparent containers to sunlight for different time periods (15-30 minutes to few hours) before enumeration[21]. Their results allowed them draw conclusions about the main factors influencing the rate of inactivation. These factors included the sunlight intensity, the kind of

bacteria exposed, the characteristics of the containers and the clarity of the water. Later, as mentioned previously, a team of scientists from Eawag started working on solar disinfection (SODIS).

Sunlight-mediated inactivation involves sunlight which contains UV-B (λ=280-320 nm), UV-A (λ=320-400 nm) and visible light (λ= 400-700 nm)[22,23]. Solar disinfection results by two main processes: direct inactivation and indirect inactivation.
For direct inactivation, it is the UV-B that plays the most important role. After being absorbed by the DNA or RNA of the microorganisms, it causes modifications to the genome by producing photoproducts such as thymine dimers or bond breakage of the nucleic acid[15]. As a consequence it will inhibit the reproduction of the viruses.
Indirect inactivation can be divided in two categories: indirect endogenous inactivation and indirect exogenous inactivation. In both cases, the inactivation is characterized by the formation of reactive oxygen species (ROS), which can oxidize certain elements of microorganisms. When indirect endogenous inactivation occurs, it is an internal element of the pathogen, which absorbs photons and forms ROS through electron or energy transfer[22]. Bacteria often contain chromophores which act as sensitizers for the formation of ROS. These ROS may further react with cell constituents and lead to a loss of the biological activity and ultimately to the death of the cell[13]. UV-A radiation is very efficient in inactivating bacteria but much less effective against viruses, because they rarely have such chromophores. Finally, as for direct inactivation also indirect inactivation is generally faster with lower wavelengths[24].
For the exogenous-indirect inactivation, it is an external chromophore present in the water that absorbs photons and produces ROS. For example, organic matter (OM) can absorb light and produce reactive oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radical ($OH^\bullet$)[6]. OM can produce ROS by photoreactions, but it also consumes ROS, especially $OH^\bullet$ [25].

Another photochemical process contributing to the formation of ROS may occur in the presence of iron. Iron (II) may react with hydrogen peroxide (H_2O_2) and generate a hydroxyl radical ($OH^\bullet$), which is a strong oxidant and may contribute to the inactivation of viruses. The process that forms $OH^\bullet$ by the decomposition of H_2O_2 catalysed by iron is called Fenton process, shown in equations (1) and (2). Equation (2) is known as the Fenton reaction[26].

$$Fe(III) + H_2O_2 \rightarrow Fe(II) + HO_2^\bullet + H^+ \quad (1)$$
$$Fe(II) + H_2O_2 \rightarrow Fe(III) + HO^- + HO^\bullet \quad (2)$$

The Fenton process can produce as much as 50% of the formation of $OH^\bullet$ in surface waters[27]. This process may therefore be an important one for indirect exogenous inactivation and could contribute to virus inactivation during SODIS.

1.4 Goals of the study

Several studies have been conducted on sunlight-mediated inactivation, in particular for bacteria. However, inactivation of waterborne viruses has been less studied and only very little data is currently available on the inactivation of human viruses in PET bottles placed in sunlight. It can be deduced from previous experiments that SODIS is less efficient for viruses than for bacteria[4]. Furthermore, the processes leading to virus disinfection during SODIS treatment are greatly unknown. As viruses also cause serious waterborne diseases, they should also be inactivated during an effective water treatment. Therefore, this study aims to

investigate the inactivation of both phages and human viruses in sunlit PET bottles to better evaluate the effectiveness of SODIS for virus removal.

The objectives of this master project were to determine the efficiency of SODIS for different viruses, and to evaluate the influence of different water types. Additionally, we aimed to elucidate the potential inactivation mechanisms of viruses in PET bottles by sunlight. The overall goal of this study was to obtain a better estimate of the effectiveness of SODIS for virus inactivation in real-world applications.

2 Methodology and materials

2.1 SODIS inactivation experiments

Virus inactivation during SODIS treatment was studied in ground and tap water from India (Chennai) and tap water from Switzerland (Lausanne). Viruses were spiked into these waters and placed into PET bottles before being exposed to natural or simulated sunlight. Aliquots were withdrawn after certain time periods and enumerated.

2.1.1 Sunlight source

Experiments with simulated sunlight were conducted using a solar simulator (Abet technologies, Sun 2000) (Figure 5) equipped with a 1000 W Xenon lamp, an AirMass (AM) 1.5 filter and a 2 mm thick Atmospheric edge (AE) filter to mimic the solar radiation spectrum (left side of Figure 6 and Figure 7**Erreur ! Source du renvoi introuvable.**).

Figure 5 : experimental set-up. Solar simulator (left) and PET bottle with a beaker inside exposed to the light from the simulator (right)

Erreur ! Source du renvoi introuvable. Inactivation experiments were also performed under natural sunlight (Figure 6 right side of Figure 6 and Figure 7) in Lausanne (N 46.52°, E 6.57°).

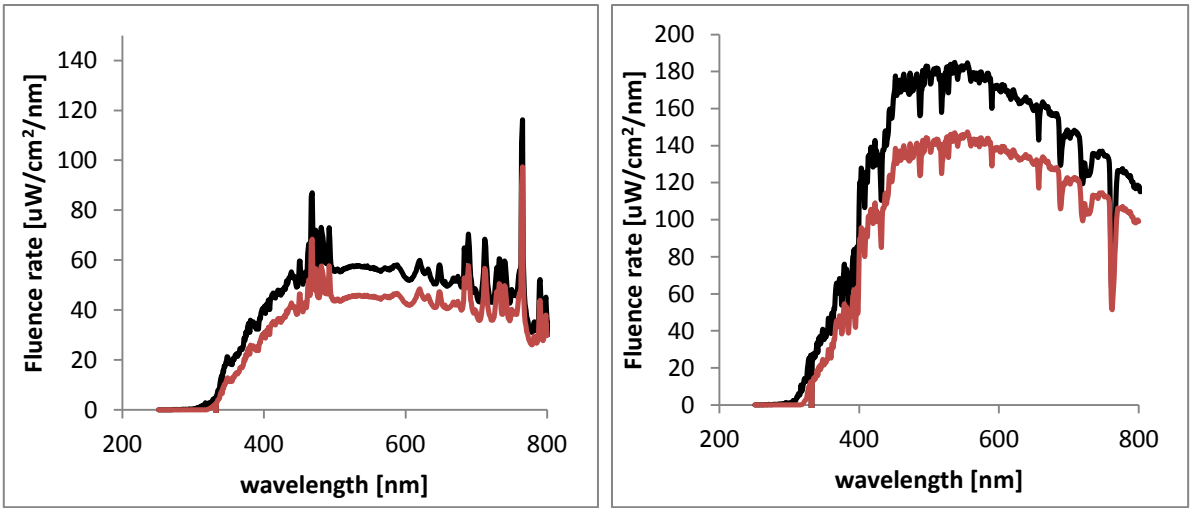

Figure 6 : fluence rate of solar simulator [uW/cm2/nm] between 200 nm and 800 nm outside (black line) or inside (red line) the PET bottle (left side); fluence rate of sunlight [uW/cm^2/nm] between 200 nm and 800 nm outside (black line) or inside (red line) the PET bottle, measured the 27.05.2013 at 02:30 PM in Lausanne (N 46.52°, E 6.57°) (right side)

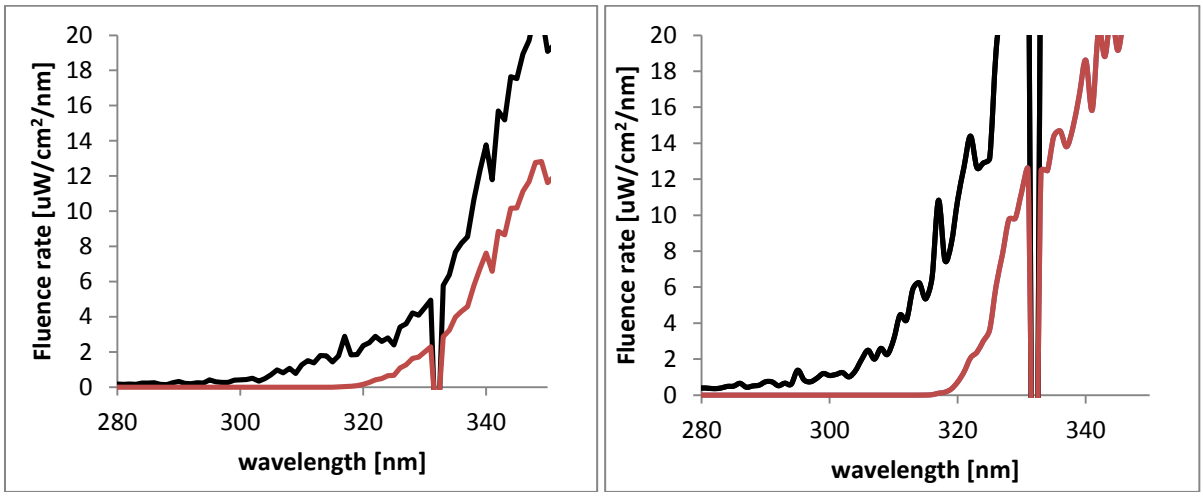

Figure 7 : fluence rate of solar simulator [uW/cm2/nm] between 280 nm and 350 nm outside (black line) or inside (red line) the PET bottle (left side); fluence rate of natural sunlight [uW/cm^2/nm] between 280 nm and 350 nm outside (black line) or inside (red line) the PET bottle, measured the 27.05.2013 at 02:30 PM in Lausanne (N 46.52°, E 6.57°) (right side)

The fluence rate was measured at the end of each experiment (for each position used below the solar simulator) with a spectroradiometers ILT 900-R (InternationalLight Technologies, Massachusetts). The average fluence rate was 249 ± 14 W/m^2, which resulted in a fluence received by the bottle of 0.54 kJ/cm^2 and 2.15 kJ/cm^2 after 6 and 24 hours of irradiation, respectively. Whereas the mean fluence rate during the experiments conducted under natural sunlight was 618 ± 41 W/m^2, which resulted in a fluence received by the bottle of 1.34 kJ/cm^2 after 6 hours of irradiation.
The absorbance of the PET bottles was measured with a UV-Vis spectrophotometer (UV-2550, Shimadzu) and the fluence rates within the bottles were computed (Figure 6 and Figure 7). Most of the UVB radiation was removed due to the PET bottle absorbance. However, the rest of the sunlight spectrum was only slightly affected.

2.1.2 Experiments conducted under simulated sunlight

Two different experimental set-ups were tested and compared to the inactivation kinetics of SODIS. Two different containers were examined, namely a PET bottle (0.5 L, CocaCola) and an open beaker (30 mL) (Figure 8). The containers were filled with 500 mL and 20 mL water,

respectively. The viruses were spiked into the water to give an initial virus concentration between 10^4 and 10^7 pfu/mL for MS2, 10^5 and 10^7 pfu/mL for phiX174, 10^4 and 10^6 pfu/mL for adenovirus and 10^2 and 10^3 pfu/mL for echovirus. The beaker was also put in a PET bottle (1.5 liters, CocaCola) to remove the same wavelengths from the sunlight as in real SODIS applications. The containers were placed under the solar simulator, and were stirred continuously with a magnetic stirrer at 150 rpm to ensure good mixing. The containers were kept at constant temperature (15, 22, or 27°C) by a water cooler (Julabo).
Samples were taken approximately every two hours (150 µL diluted in 450 µL phosphate buffered saline (PBS: 5mM phosphate, 10mM NaCl, pH 7.5)) and then stored at 4°C before enumeration on the same day. For experiments that showed very slow virus inactivation, further samples were withdrawn on two consecutive days of irradiation.

Experiments were also performed using PBS instead of water to determine the direct inactivation of viruses during SODIS. Furthermore, experiments without stirring were also conducted in order to study the necessity of the stirring. Finally, dark controls were obtained under the same conditions as the irradiated samples but with aluminium foil wrapped around the containers.

Figure 8: Containers. From left to right, PET bottle (0.5 L) and open beaker

2.1.3 Experiments conducted under natural sunlight

Experiments under natural sunlight were conducted in 0.5 L PET bottles with water from Lausanne (Figure 9). MS2 was spiked into the water to give an initial virus concentration of $6 \cdot 10^6$ pfu/mL. The bottle was then placed at a 30° angle and was always oriented toward the sun during 6 hours (from 10:30 AM to 04:30 PM) on a clear day in May. The fluence rate and the temperature inside the bottle were measured each hour. Samples (150 µL in 450 µL PBS) were withdrawn every hour and stored at 4°C before enumeration on the same day. A dark control was conducted in an open beaker wrapped in aluminium foil, over the same time period. The initial concentration of MS2 for this experiment was $2 \cdot 10^5$ pfu/mL.

Figure 9 : Setup of the experiment done outside. Spiked water in a PET bottle and in a beaker as a dark control exposed to sunlight

2.2 Virus enumeration

2.2.1 MS2

The MS2 (DSMZ No. 13767) stock used in this study was propagated and purified as described in Pecson et al.[15]. The concentration of the stock was about $2 \cdot 10^{13}$ pfu/ml and it was conserved in PBS. Enumeration after experiments was performed using the double-layer agar method, as described elsewhere[28].

2.2.2 Phi

The PhiX174 (ATCC no. 13706-B1) stock used in this study was propagated and purified as described in Pecson et al., 2009[15]. The concentration of the stock was about $2 \cdot 10^{13}$ pfu/ml and it was conserved in PBS. Enumeration after experiments was performed using the double-layer agar method, as described elsewhere [28].

2.2.3 Adenovirus

The Adenovirus used for the experiments was human adenovirus type 2 (HAdV-2; kindly provided by R. Girones, U of Barcelona). It was cultivated and propagated on A549 human lung cell adenocarcinoma epithelial cells in high-glucose Dulbecco's modified Eagle's medium (DMEM) (Invitrogen n°31966021) containing penicillin (50 U/ml), streptomycin (50µg/ml), and 10% fetal bovine serum (FBS) and was incubated at 37°C in 5% CO_2 and at 95% humidity.

To propagate the HAdV-2, A549 cells were cultivated to 95% confluence in a 10 ml culture flask. The medium was then discharged and replaced by DMEM containing 2% FBS. Viruses (~10^8 pfu) were added to the culture. Cultures were incubated until the cytopathogenic effect indicated the dying of the cells and 80% of the cell layer was detached. This took 2-3 days. Afterwards, three freeze/thaw cycles were done to lyse the cells (freezing at -20°C, thawing at room temperature). Debris was centrifuged down at a speed of 6.5 rpm for twenty minutes. The supernatant was membrane filtered (0.22 µm; Millipore), the viruses were washed three times with PBS and concentrated with 15ml centrifugal membrane filter units. The virus stock (10^9 pfu/ml) was stored at 4°C.

To quantify the viruses, a most probable number assay was used. The procedure was as follows: A 95% confluent A549 cell monolayer was prepared in 96-well plates with flat-bottom wells. Adenovirus samples were diluted over a 10-fold dilution series in DMEM containing 2% FBS. The medium on cell monolayers was removed and replaced with 150 µl of DMEM with 2% FBS and 100µl of virus dilutions. After an incubation time of 12-14 days, the cytopathogenic effect could be observed and used to identify positive and negative wells. Finally, the adenovirus concentration was evaluated using a most probable number (MPN) table (MPNCU/ml).

2.2.4 Echovirus

The Echovirus used for the experiments was a human echovirus type 11 (E11). It was cultivated and propagated on buffalo green monkey kidney (BGMK) cells in minimum essential media (MEM) (Invitrogen n°31095029) containing penicillin (50 U/ml), streptomycin (50µg/ml), and 10% fetal bovine serum (FBS) and was incubated at 37°C in 5% CO_2 and at 95% humidity.

The propagation of echovirus was done identically as for adenovirus. The amount of viruses added to the cell culture was (~10^5 pfu). The virus stock was (10^6 PFU/ml) was stocked at 4°C.

For quantification of echovirus the same procedure as for adenovirus was followed. The only difference was the shorter time of incubation (4-7 days) needed to observe a cytopathogenic effect.

2.3 Water collection, storage and analysis

Three different waters were used for this study. One was taken from the tap in the LCE laboratory (at EPFL, Lausanne, Switzerland) and the other two waters were brought from India by the Sandec team from Eawag. One of the Indian waters was groundwater and the other one was tap water from the city of Chennai (capital city of the Indian state of Tamil Nadu). The majority of the Indian water was frozen at -30°C and a small amount (~0.5 L) was stored at 5°C for immediate experiments. When more water was needed, part of the frozen stock was thawed at room temperature. Swiss tap water was collected (one liter), stirred overnight in an open beaker to volatilise any remaining chlorine and then stored at 5°C.

The three waters were chemically analysed by the Central environmental laboratory from EPFL (GR-CEL). Total organic carbon (TOC) and inorganic carbon (IC) were analysed by a Shimadzu TOC-V_{CPH} analyser. Iron and copper cations were analysed by a ParkinElmer ICP-MS Elan DRC II. Anions (chloride, nitrite, bromide, nitrate, phosphate and sulphate) were determined by a Dionex ICS 3000. In addition, the three waters were checked for residuals of free chlorine using the spectrophotometric method described in "Standard methods for the examination of water and wastewater"[28]. In all waters the content of residual free chlorine was below detection limits (< 0.5 mg/L).

The absorption spectrum of the three waters (Figure 10) was measured with a UV-Vis spectrophotometer (UV-2550, Shimadzu). All three waters only absorb very little light above 320 nm, therefore they hardly influence the light intensity within the bottle.

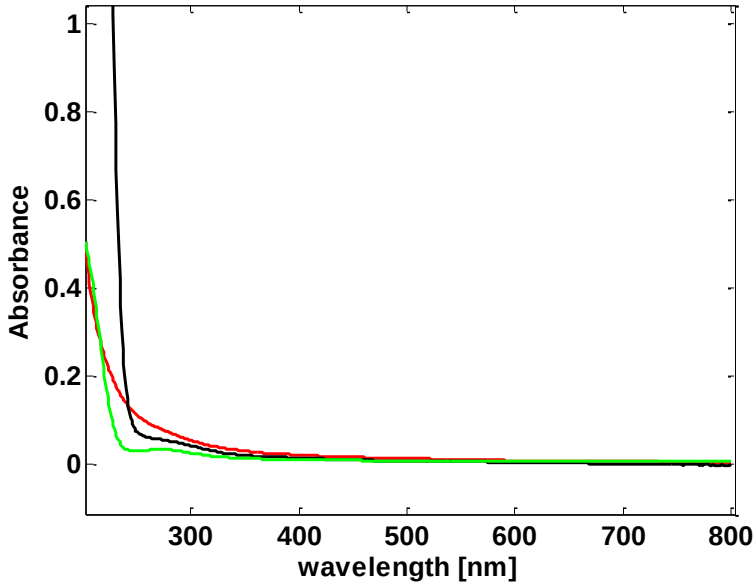

Figure 10 : Absorbance of the waters used in this study. The red, black and green lines represent the absorbance of the Indian groundwater, the Indian tap water and the Swiss tap water, respectively

2.4 Data analysis

2.4.1 Inactivation kinetics

Inactivation of all viruses was approximated by a first order exponential decay law:

$$C = C_0 \cdot e^{-k \cdot t} = C_0 \cdot e^{-\kappa \cdot F} \qquad (3)$$

where C_0 is the initial infective virus concentration (pfu/mL), C is the remaining infective virus concentration (pfu/mL) after irradiation time t (h) and k is the time-based, first-order inactivation rate constant (h^{-1}). The remaining virus concentration C can also be expressed as function of the fluence F (kJ/cm^2) received at the surface of the PET bottle; here, κ (cm^2/kJ) is the fluence-based inactivation rate constant.

Equation (3) can also be reformulated as:

$$\ln(C/C_0) = -k \cdot t = -\kappa \cdot F \qquad (4)$$

Therefore, if we graph $\ln(C/C_0)$ versus the fluence received, the slope corresponds to -κ.
The inactivation rates and the 95% confidence intervals were calculated in Excel using the linear regression tool applied to equation (4). The results were statistically compared using the ANCOVA analysis[29] to evaluate if there was a significant difference between two given inactivation rate constants. The ANCOVA analysis was mainly used to determine if the inactivation rate constants for SODIS experiments were significantly different from the dark controls.
To determine the inactivation rate constants for the dark control, the regression was done based on several dark control experiments conducted under identical conditions. This was done in order to improve the precision of the inactivation rate constants, as small inactivation was very difficult to measure. Moreover, all the values of inactivation rate constants or values derived from it mentioned in Chapter 3 are corrected for dark inactivation, unless indicated otherwise. All the details and inactivation rate constants for all experiments are reported in Table I in the Appendixes

2.4.2 Dependence of the inactivation rate constant on temperature

The effect of temperature on inactivation was investigated with a set of experiments with varying temperatures (15, 22 and 27°C) of the water. The dependence of the inactivation rate constant on temperature should follow an Arrhenius-type relationship[30]:

$$\kappa = A \cdot e^{-E_a/(R \cdot T)} \qquad (5)$$

where κ is the virus inactivation rate constant (cm^2/kJ), A is a pre-exponential factor (cm^2/kJ), E_a is the activation energy (J/mol), R is the universal gas constant ($J/K^{-1}mol^{-1}$) and T is the temperature (K). If the natural logarithm of κ is plotted versus the inverse of the temperature a straight line should be obtained and the slope of this line equals $-E_a/RT$ and the intercept ln(A).

3 Results and discussion

3.1 Development of the experimental setup

3.1.1 Developing a set-up to study disinfection during SODIS in lab experiments

Only few previous studies have investigated solar inactivation of viruses inside PET bottles. For example, Fisher et al.[31] used entire PET bottles. However, Heaselgrave et al.[32] replaced the PET bottles by polystyrene microtitre plates, which absorbed less in the UV-B range than PET.

The use of normal PET bottles for the inactivation experiments was not feasible for two major practical reasons: Firstly, a major source of water used in the inactivation experiments came from a region in India where SODIS is implemented. Hence, the complete study had to be conducted with less than 2 L of water brought from this region. Secondly, spiking large volumes of water with viruses requires large amounts of virus stock solutions. Therefore, it was preferable to conduct the inactivation studies in smaller containers in which the inactivation was similar to an entire PET bottle. Nevertheless, we did not want to exchange the PET by another material as a different light absorption may strongly influence the inactivation kinetics. Hence, in this study, we compared the use of another container with a 0.5 L PET bottle: a small open beaker was placed inside a 1.5 L empty PET bottle. The absorbance spectra of both PET bottles (1.5 L and 0.5 L PET bottles) did not show any important differences over the whole spectrum of solar radiation (Figure 11). Therefore, it was assumed that both set-ups could be used interchangeably for water types that did not absorb greatly in solar light transmitted through the PET.

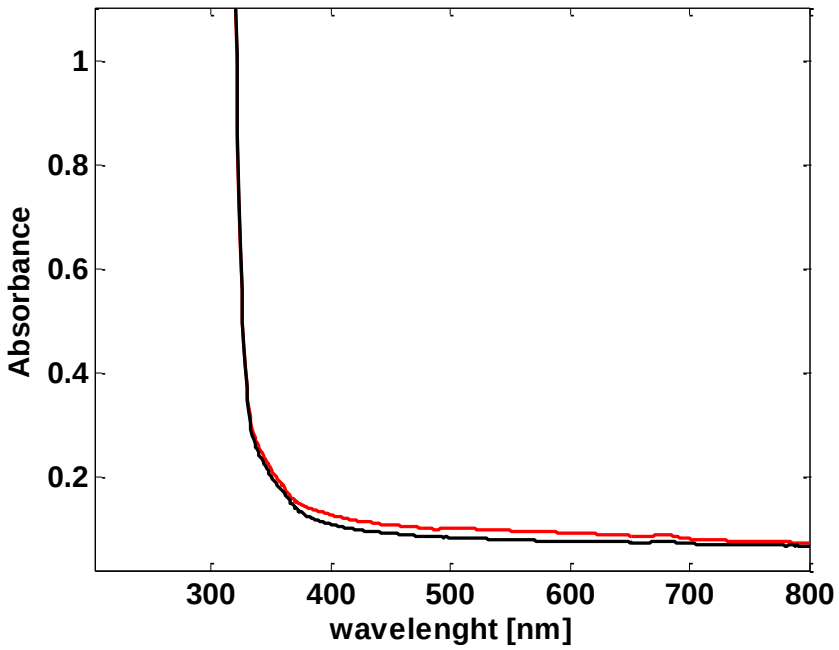

Figure 11 : Absorbance spectrum of the 0.5 L PET bottle (red line) and of the 1.5 L PET bottle (black line)

The inactivation in the open beaker placed in a PET bottle was compared to inactivation in a 0.5 L PET bottle (Figure 12). The inactivation rate constants for the open beaker and the 0.5 mL PET bottle were very similar for Swiss tap water, namely 6.40 ± 2.13 cm^2/kJ, 6.45 ± 1.24 cm^2/kJ (values not corrected for dark inactivation), respectively. However, the inactivation rate constant for the dark control was higher in the open beaker (1.97 ± 2.05 cm^2/kJ) than in

the 0.5 L PET bottle (0.26 ± 0.53 cm^2/kJ), though neither was significantly different from zero. The dark inactivation rate constant in the beaker is surprisingly high, we expect that this is due to high surface to volume ratio and relatively vigorous stirring[33].
After correction of the inactivation rate constants for dark inactivation, the open beaker slightly underestimated the inactivation that occurred in the PET bottle (4.43 ± 2.95 cm^2/kJ and 6.19 ± 1.35 cm^2/kJ for the open beaker and the PET bottle, respectively).

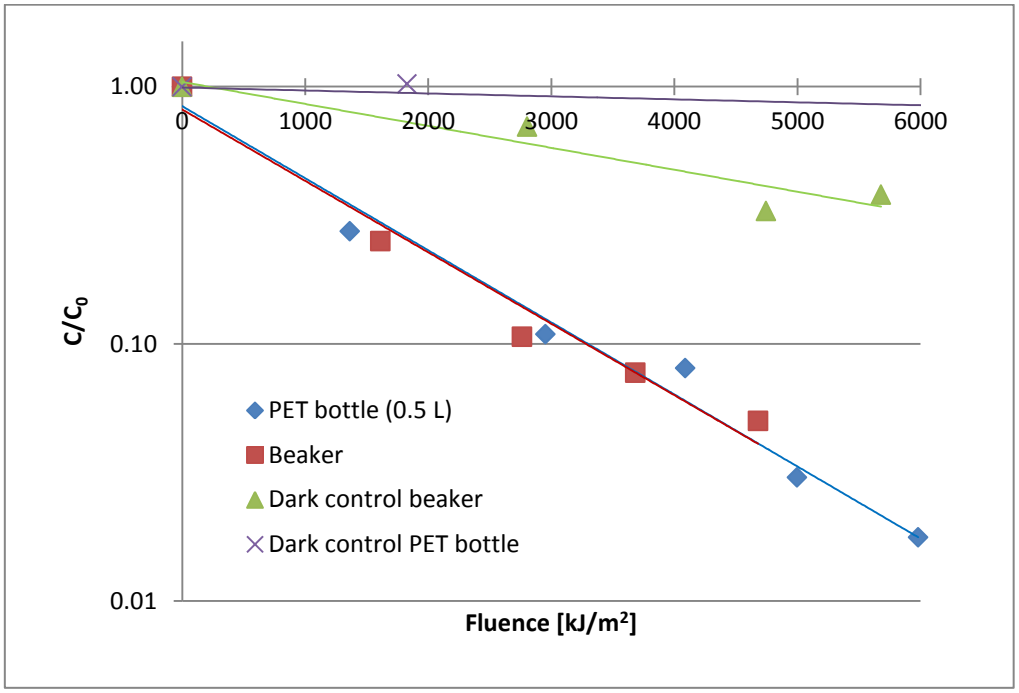

Figure 12 : MS2 inactivation in Swiss tap water conducted in a 0.5 L PET bottle or in a beaker placed in a PET bottle. The initial virus concentrations were $6 \cdot 10^6$ pfu/ml and $4 \cdot 10^5$ pfu/ml, respectively. The total fluence received was 0.60 kJ/cm^2 except for the dark control of the PET bottle, which received a total fluence of 2.50 kJ/cm^2. The initial virus concentrations were $2 \cdot 10^5$ pfu/ml and $6 \cdot 10^5$ pfu/ml, in the dark control done in a beaker and in the dark control done in the PET bottle, respectively Values were not corrected for dark inactivation. No duplicate was produced for these experiments.

An identical experiment was repeated with the same set-up but with tap water collected on a different day. The inactivation rate constants were higher in this water but the inactivation rates constants of the beaker and of the PET bottle experiments were again similar. In this experiment the inactivation in the beaker was slightly faster than in the PET bottle, though not at a statistically significant level (p-value: 0.06) (Figure 13).

Combined, these experiments show us two things; firstly, the inactivation rate constants in the beaker and the PET bottle were in good agreement. Hence, the open beaker could be used to represent the full PET bottle. This should hold true for water used in this study, which is sufficiently clear not to importantly influence the light intensity within the bottle. Secondly, the inactivation rate constants for the same water varied from day to day even for the same sample location. Therefore only water collected at the same moment could be compared across experiments.

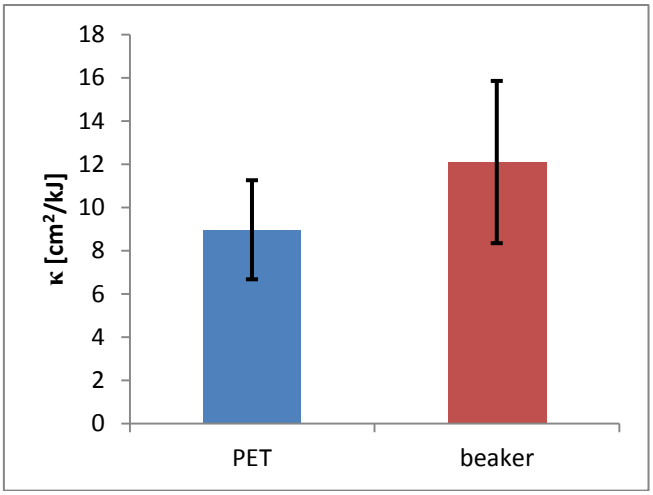

Figure 13: MS2 inactivation in Swiss tap water conducted in a 0.5 L PET bottle or in a beaker placed in a PET bottle. The initial virus concentration was $4 \cdot 10^5$ pfu/ml in the two containers. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation. No duplicate were performed for these experiments.

3.1.2 Comparison between natural and simulated sunlight

To verify the relevance of our setup to natural conditions, an experiment with exposure to natural sunlight was done in parallel to one conducted under the solar simulator. A 0.5 L PET bottle and a beaker wrapped in an aluminium foil (dark control) were exposed to direct sunlight and another 0.5 L PET bottle, a beaker placed in a PET bottle and a beaker wrapped in an aluminium foil were exposed to the solar simulator light (Figure 14 and Figure 15).

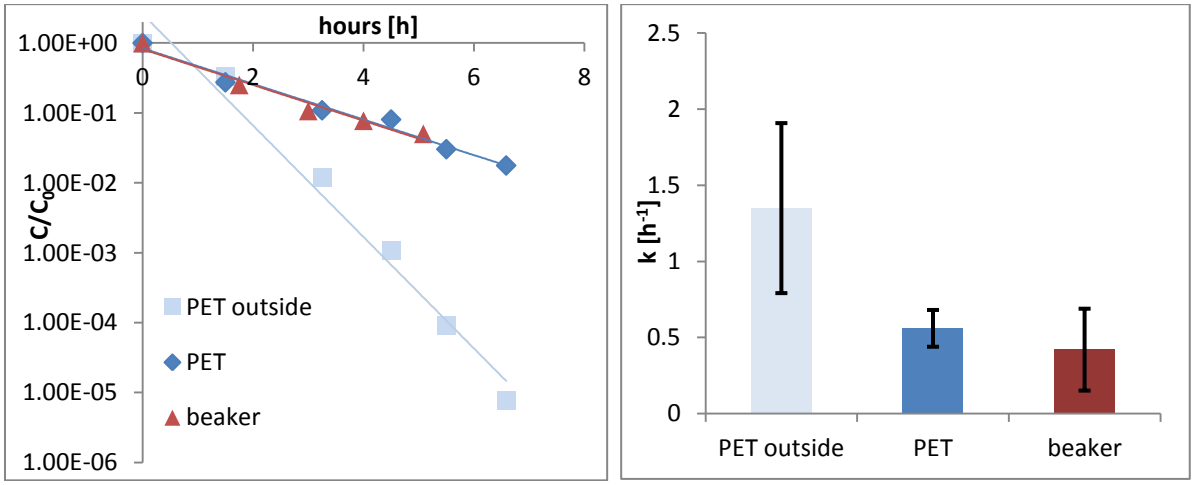

Figure 14 : MS2 inactivation in Swiss tap water conducted in a 0.5 L PET bottle exposed to sunlight, a 0.5 L PET bottle and a beaker placed in a PET bottle exposed to solar simulator light. The initial virus concentrations were $6 \cdot 10^6$ pfu/ml and $4 \cdot 10^5$ pfu/ml in the PET bottles and in the beaker, respectively Error bars indicate 95% confidence intervals. Values of k were corrected for dark inactivation. No duplicates were produced for these experiments

For the same duration of exposition to light, the total amount of viruses inactivated was higher for the PET bottle exposed to natural sunlight compared to that under the solar simulator. In approximately six hours an inactivation of five logs (99.999%) was observed for the PET bottle placed outside whereas the PET bottle under the solar simulator showed only two logs (99%) of inactivation. However, the fluence of the sun (650 W/m^2) was higher than the fluence of the solar simulator (250 W/m^2), and the temperature of the water inside the PET bottle exposed to natural sunlight was higher (maximum temperature of 32°C in the middle of the day compared to 22°C under the simulator). This explains why a higher inactivation rate constant was observed for the PET bottle exposed to natural sunlight in comparison to the two containers placed under the solar simulator. To directly compare the data of the two

experiments, the inactivation rate constants were calculate based on the fluence received rather than simply based on time exposed to sunlight (Figure 15). The time-based inactivation rate constants k (h^{-1}) were therefore converted to the fluence-based inactivation rate constants κ (cm^2/kJ) (Figure 15).

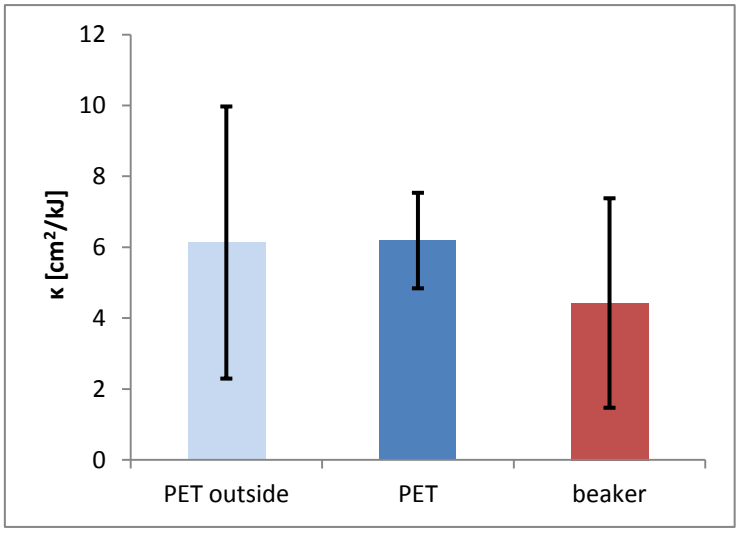

Figure 15 : MS2 inactivation in Swiss tap water in a 0.5 L PET bottle exposed to natural sunlight (PET outside), a 0.5 L PET bottle (PET) and a beaker placed in a PET bottle exposed to solar simulator light (beaker). The initial virus concentrations were $6 \cdot 10^6$ pfu/ml and $4 \cdot 10^5$ pfu/ml in the PET bottles and in the beaker, respectively. Error bars represent 95% confidence intervals. Values were corrected for dark inactivation. No duplicates were conducted for these experiments.

Reconsidering our data based on fluence received, similar inactivation rate constants were observed in the three different experiments. These results indicate that the solar simulator is a good tool to simulate what happens outside in natural sunlight.

These remarks on the difference of a time-based analysis and a fluence-based analysis are essential and give a good explanation why it is better to report the inactivation rate constants based on the fluence instead of the exposure time. The rate constant will always remain constant independently of the location on the earth where SODIS is or will be applied. The geographic position will only influence the time of exposure needed to reach a certain amount of energy received per square meter (which correspond to the notion of fluence (kJ/cm^2)), but not the inherent sensitivity of the virus to inactivation. The energy received will ultimately tell us how many logs of virus inactivation had been achieved.

3.1.3 Influence of stirring

Another important aspect that had be taken into account in our set-up development was the fact that in practice when SODIS is applied in the field the bottles are not or only rarely stirred or mixed when they are exposed to sunlight. However, in the experimental set-up used herein, experiments were always stirred by means of a magnetic stirrer inside the PET bottle or the beaker. Therefore, an experiment was conducted to compare the inactivation of MS2 in a stirred and a non-stirred PET bottle. The inactivation rate constants were 9.23 ± 2.23 cm^2/kJ for the stirred bottle and 8.91 ± 1.47 cm^2/kJ for the unstirred one (Figure 16).

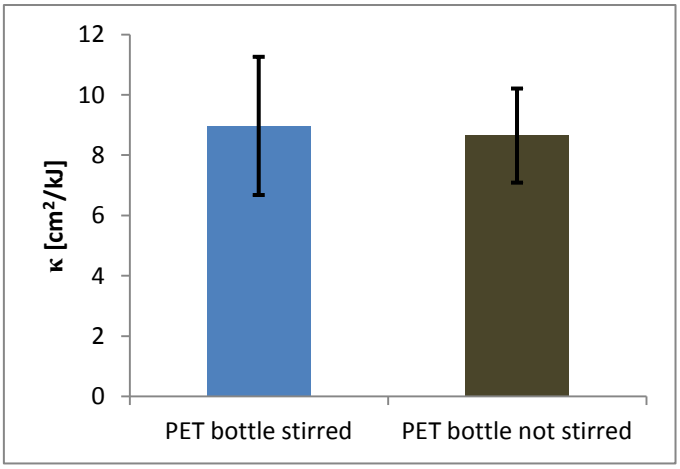

Figure 16 : MS2 inactivation in Swiss tap water cconducted in 0.5 L PET bottles. The initial virus concentrations was $4 \cdot 10^5$ pfu/ml in both experiments. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation. No duplicates were conducted for these experiments.

Similarly, the inactivation rate constants were 1.67 ± 0.54 cm^2/kJ, 1.70 ± 0.90 cm^2/kJ for the stirred and the unstirred beaker, respectively (Figure 17, these experiments were performed in Indian ground water. The inactivation was slower than in the Swiss tap water, this aspect will be discussed in more detail further in Chapter 3.3). These results indicate that there was no observable influence of the stirring on the inactivation rate constants either conducted in PET bottles or beakers. Hence, to ensure good homogeneity of the virus concentration in the beakers and PET bottles, the experiments were always conducted under magnetic stirring.

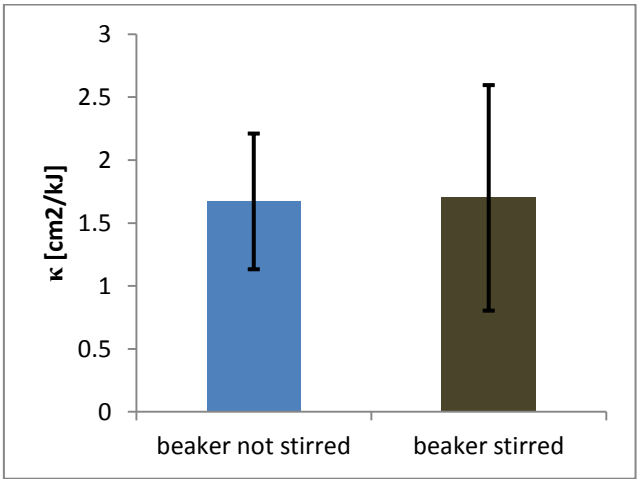

Figure 17 : MS2 inactivation in Indian groundwater conducted in beakers placed in PET bottle. The initial virus concentration was $4 \cdot 10^5$ pfu/ml for both experiments. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation. The inactivation rate constants presented here are the mean of two duplicates.

3.2 Initial virus concentration

The effect of the initial virus concentration spiked into our samples was also investigated. This was done to ensure that the virus solution spiked into the waters did not influence the steady-state concentration of ROS, and consequently lower the rate of indirect inactivation. An increased initial virus concentration also resulted in a higher concentration of impurities which were potentially present in the virus stock solution. These impurities could also quench the ROS and result in a decreased steady-state concentration. This issue became particularly apparent when non-purified MS2 virus stock was used for SODIS experiments. The non-purified stock led to a much slower inactivation of MS2 compared to what was observed

previously for purified stocks (Figure 18). This is consistent with the non-purified virus stock reducing ROS concentrations and therefore also the virus inactivation.

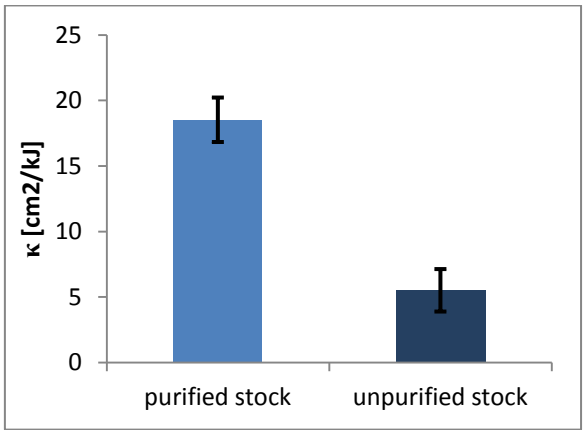

Figure 18 : MS2 inactivation from two different virus stocks (one purified the other not) in Swiss tap water conducted in beakers placed in PET bottles. The initial virus concentration was $6 \cdot 10^7$ pfu/ml. Error bars indicated 95% confidence intervals. Values were not corrected for dark inactivation. No duplicates were produced for these experiments.

Several experiments were performed with varying initial virus concentrations (from the purified virus stock). These experiments were conducted in all waters for this study to make sure that the purified stock did not influence the ROS steady-state concentrations.

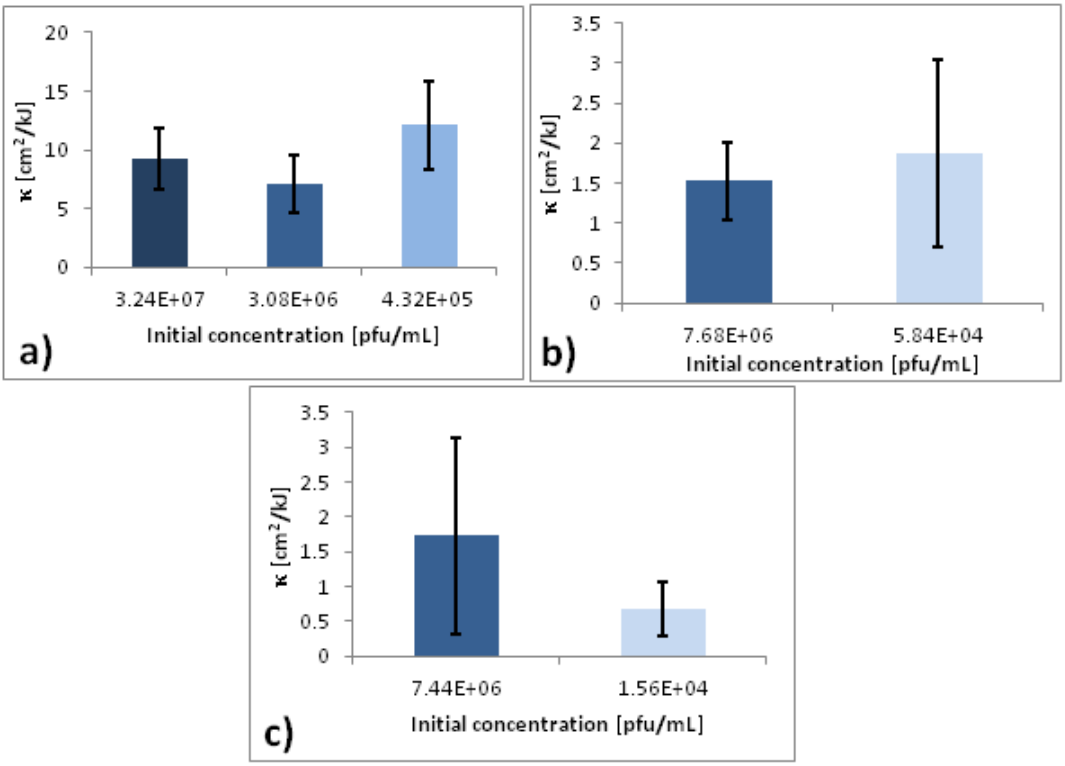

Figure 19 : MS2 inactivation for different initial concentrations contained in beakers. a) virus spiked into Swiss tap water, b) virus spiked into Indian groundwater, c) virus spiked into Indian tap water. Error bars are the 95% confidence interval. Rate constants are corrected for dark inactivation. No duplicates were conducted for these data.

Each water showed similar inactivation rate constants independent of the initial virus concentration added (Figure 19). No statistically significant difference for the same water was observed. In Swiss tap water, compared to the sample with the smallest initial virus concentration the p-values from the ANCOVA analysis were 0.56 and 0.85. For Indian

groundwater the p-value was 0.28 and for Indian tap water the p-value was 0.07. These results indicate that the initial virus concentration spiked into the water did not influence the inactivation rate constants. Therefore, the addition of the viruses did not lower the ROS steady-state concentrations, which means that we studied a 'natural' system which was not changed by the addition of the viruses. As already observed before, different waters resulted in different inactivation rates, which will be discussed in Chapter 3.3

3.3 Solar disinfection of MS2 in Swiss and Indian water: kinetics and mechanisms

In this study three different waters (one Swiss and two Indian) were compared. In this section, only experiments performed with MS2 will be presented and analysed. Results of other viruses will be discussed in detailed in Chapter 3.5

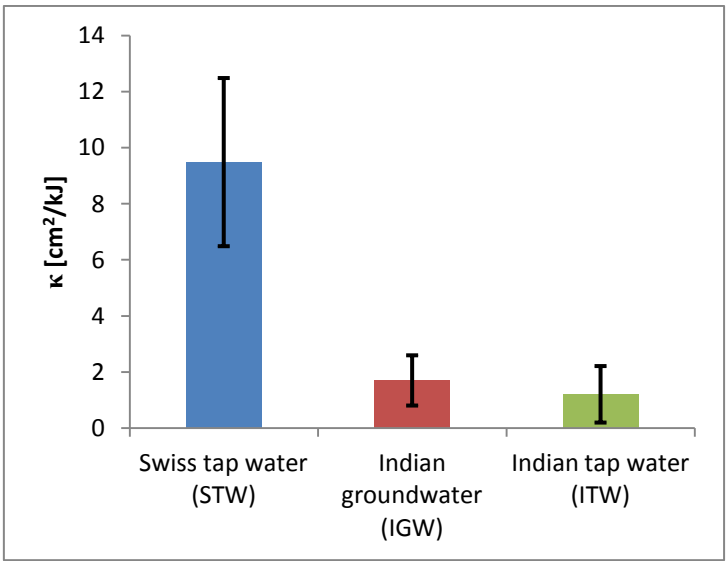

Figure 20 : MS2 inactivation rate constants obtained in different waters. Experiments were conducted in beakers placed in PET bottles. The inactivation rate constants for Swiss tap water (STW) and both Indian waters are the mean of three and two experiments, respectively. The initial virus concentrations were $1 \cdot 10^7$ pfu/ml, $4 \cdot 10^6$ pfu/ml and $4 \cdot 10^6$ pfu/ml in the STW, Indian groundwater (IGW) and Indian tap water (ITW), respectively. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation.

The inactivation rate constant in Swiss tap water (STW, 9.49 ± 3.00 cm^2/kJ) was much greater than in the two water from India: 1.70 ± 0.90 cm^2/kJ and 1.21 ± 1.01 cm^2/kJ for the Indian groundwater (IGW) and the Indian tap water (ITW), respectively (Figure 20). The inactivation was actually more than five time faster in STW than in the two other waters. However, IGW and ITW showed similar inactivation rate constants. At a fluence of 1.34 kJ/cm^2, which corresponds to 6 hours exposed to natural Swiss light, a reduction of 5.5 logs, 1.0 logs and 0.7 logs was observed in STW, IGW and ITW, respectively. In comparison, SODIS data from Fisher et al[31] showed a 3 log inactivation of MS2 in 33 hours, which corresponded to 5.11 kJ/cm^2 for their experimental set-up. This means that they observed an inactivation rate constant of 1.35 cm^2/kJ for MS2 in wastewater diluted in PBS (40 mL wastewater to 800 mL PBS), which corresponds well to our values for the two Indian waters.

MS2 inactivation was also studied in phosphate buffered saline (PBS). However, almost no inactivation was observed under these conditions (Figure 21) and the order of magnitude of the inactivation rate constant was similar to the one observed in the dark control (p-value from the ANCOVA analysis: 0.15). As no reactive oxygen species (ROS) are expected to form in PBS, this observation indicates that no or only negligible direct inactivation or endogenous indirect inactivation occurs during SODIS. Hence, the only mechanism leading to MS2

inactivation during SODIS is indirect exogenous. SODIS of MS2 thus strongly depend on the ROS formed in the water.

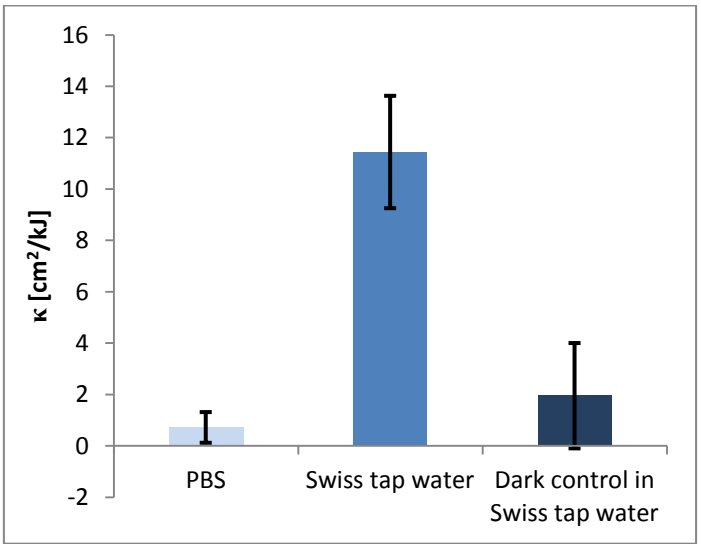

Figure 21 : MS2 inactivation in PBS and Swiss tap water conducted in beakers place in PET bottles. The inactivation rate constant in the Swiss tap water and in his dark control are the mean of three and two experiments, respectively. No duplicate was conducted in PBS. The initial virus concentrations were $4 \cdot 10^6$, $1 \cdot 10^7$ and $2 \cdot 10^5$ pfu/ml in PBS, Swiss tap water, and in the dark control, respectively. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation.

As the main mechanism responsible for the inactivation of viruses has to be indirect exogenous solar disinfection, the composition of the water may have a strong effect on the virus inactivation. Therefore, the three waters used were chemically analysed (Table 1 and Table 2).

Table 1 : Ions content in the different waters. * detection limits are 1 µg/L for iron and copper and 0.4 mg/L, 0.8 mg/L, 0.8 mg/L, 0.1 mg/L, 0.2 mg/L, 0.2 mg/L for nitrate, sulphate, chloride, bromide, nitrite and phosphate, respectively

	Swiss tap water (STW)	Indian groundwater (IGW)	Indian tap water (ITW)
Ions			
Fe (µg/L)	99.6	77.8	below detection limits*
Cu (µg/L)	7.7	4.8	7.3
NO_3^- (mg/L)	3.3	4.6	52.1
SO_4^{2-} (mg/L)	44.0	53.3	91.3
Cl^- (mg/L)	10.7	92.9	81.1
Br^- (mg/L)	below detection limits*	0.1	0.1
NO_2^- (mg/L)	below detection limits*	below detection limits*	below detection limits*
PO_4^{3-} (mg/L)	below detection limits*	below detection limits*	below detection limits*

Table 2: Content in iron and copper cations and in total organic carbon and inorganic carbon in the different waters

	Swiss tap water (STW)	Indian groundwater (IGW)	Indian tap water (ITW)
TOC (mg/L)	1.8	8.4	4.7
IC (mg/L)	-	33.3	56.4

In both waters from India the total organic carbon (TOC) was higher than in the water from Switzerland. TOC is a measure of the organic matter (OM) present in the water. OM can contribute to the formation of ROS but OM can also consume them[25]. Therefore, the lower

inactivation rate constants observed for Indian waters may be due to their higher content of OM, which may consume a lot of ROS in the water. Nitrate can also be a source of hydroxyl radicals (a very reactive ROS) when excited by UV-B radiation[25]. However, as PET bottles cut short wavelength radiation, this mechanism does not or only minimally occur during SODIS. Therefore, the higher nitrate concentration in the ITW did not translate into faster inactivation of MS2.
Both STW and IGW have a relatively high content of iron, but the iron in ITW was not measurable because it was below the detection limit (< 1µg/L). Iron can produce hydroxyl radicals by decomposition of hydrogen peroxide (formed during irradiation of OM). Hence, in the STW and IGW, iron could be a good source of $OH^{\bullet}$. These hydroxyl radicals could then lead to the inactivation the viruses. As hydroxyl radicals are quickly quenched by OM, the higher content of OM in IGW than in STW could result in lower steady-state concentrations in the IGW. This could be the reason explaining the much faster inactivation in the STW than in the IGW. This hypothesis is only based on chemical analysis of the waters and to confirm this theory it would be necessary to determine the steady-state concentrations of the ROS present in the water during the experiments. By combining these concentrations with the second-order inactivation rate constants, one could compute the inactivation due to each ROS. The analysis of the ROS would therefore permit to more precisely understand all the mechanisms that play a role in the virus inactivation during SODIS.

3.4 Dependence of MS2 inactivation on temperature

The inactivation of MS2 in Indian groundwater was investigated at three different temperatures (15°C, 22°C and 27°C). The inactivation rate constants κ obtained were 1.02 ± 0.81 cm^2/kJ, 2.25 ± 0.97 cm^2/kJ and 2.64 ± 4.15 cm^2/kJ at temperatures of 15°C, 22°C and 27°C, respectively (Figure 22). The higher the temperature, the higher was the observed inactivation rate constant. At 27°C the inactivation rate constant had a large 95% confidence interval. This is due to the dark control used to correct the value which had a high 95% confidence interval.

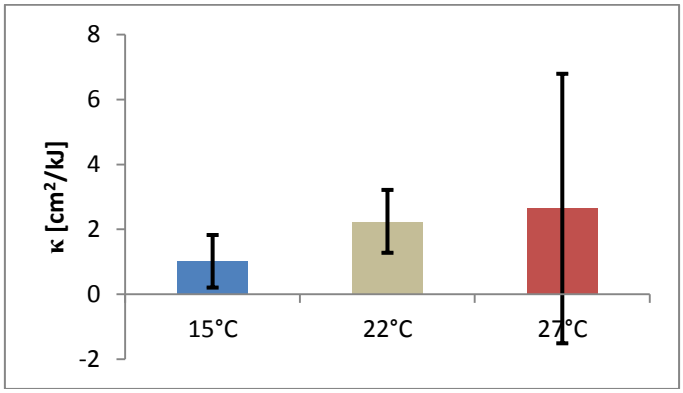

Figure 22: MS2 inactivation at 15°C, 22°C and 27°C in Indian groundwater. The inactivation rate constants are the mean of two and sixteen experiments at a temperature of 15°C and 22°C, respectively. Only one experiment was performed at 27°C. The average initial virus concentrations were $2 \cdot 10^6$ pfu/ml, $7 \cdot 10^6$ pfu/ml and $3 \cdot 10^6$ pfu/ml at 15°C, 22°C and 27°C, respectively. Error bars indicate the 95% confidence intervals. Values were corrected for dark inactivation

From the Arrhenius plot (Figure 23) the activation energy of the overall processes leading to inactivation was calculated to be 58'775 J/mol and A to be equal to $4.94 \cdot 10^{10}$ cm^2/kJ. These values may not reflect actual physical-chemical parameters associated with specific reactions, as the underlying mechanisms are not understood at a molecular level. Nevertheless, this information can be used to estimate the inactivation rate constant of MS2 at higher or lower

temperatures. For example, if a PET bottle with Indian groundwater is exposed to sunlight in Chennai (India), the temperature of the water could easily reach 45°C. Under these conditions, the inactivation rate constant κ would be 10.95 cm^2/kJ according to the Arrhenius equation and our data. This increase in temperature increased the inactivation rate constant by a factor of 5 compared to 22°C. At 45°C and an exposure of 6 hours of sunlight in Lausanne (1.34 kJ/cm^2) an inactivation of MS2 of 6 logs could be expected, whereas at 22°C approximately only 1 logs were observed. Therefore the inactivation should be a sensibly higher in Chennai (India) where the fluence rate is also higher. Hence, this clearly shows how important an increased temperature is to achieve sufficient virus inactivation. Nevertheless, these results should be taken with caution as it is an extrapolation and our Arrhenius equation is based on only three points. More experiments at higher temperature should be conducted to confirm the E_a calculated, and to define its applicable range before heat-mediated inactivation processes begin to dominate.

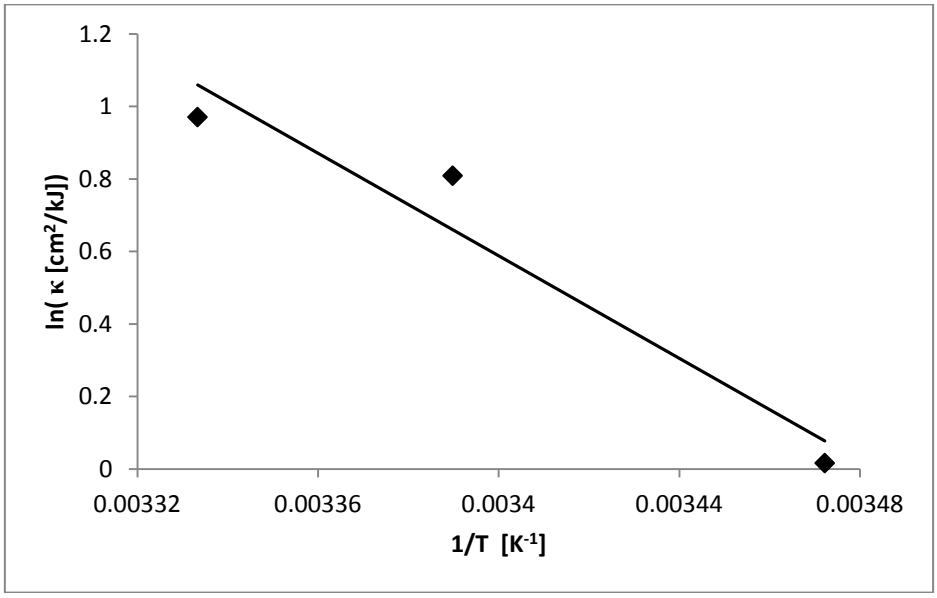

Figure 23: plot. ln (κ) versus 1/T based on three κ values at 15°C, 22°C and 27°C (trendline equation: -7069.3 x + 24.62)

3.5 Inactivation of other viruses

All inactivation studies with different viruses were conducted in the presence of MS2. The comparison of the inactivation rate constants gives an indication of how well other viruses are inactivated compared to MS2. MS2 is an interesting reference virus because it is a widely used bacteriophage, which is easily measured. Using the comparison with MS2 as a benchmark will permit in the future to extrapolate virus inactivation for different waters based only on the inactivation kinetics of MS2.

Moreover, the addition of MS2 allowed us to determine whether the presence of the other virus influenced the ROS concentrations. Hence, control experiments with different initial virus concentrations did not have to be repeated.

Firstly, the influence of the addition of other viruses on the inactivation rate constant of MS2 was studied. In the case of Swiss tap water, no effect could be observed (Figure 24), as the inactivation rate constants varied only slightly due to the addition of other viruses: 9.49 ± 3.00 cm^2/kJ, 8.89 ± 2.62 cm^2/kJ and 7.48 ± 3.92 cm^2/kJ when MS2 was alone, mixed with phiX174 or adenovirus, respectively.

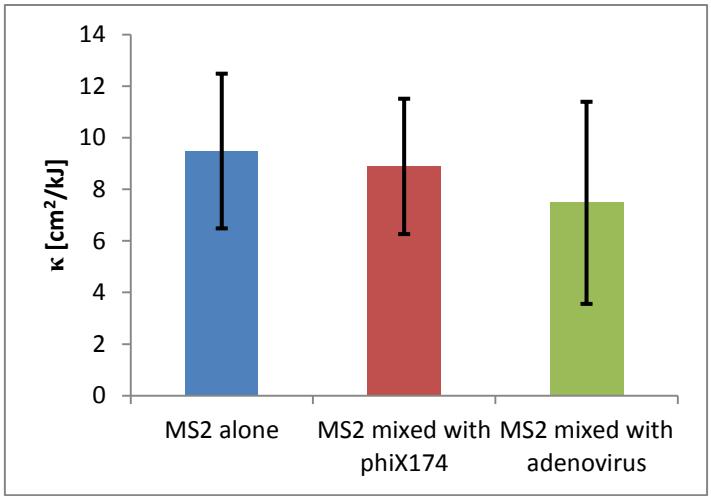

Figure 24 : Inactivation of MS2 alone and in the same container with phiX174 or adenovirus in Swiss tap water. The inactivation rate constants for MS2 are the mean of three, two and four experiments conducted alone, with phiX174 or adenovirus, respectively. The initial MS2 concentrations were $3 \cdot 10^6$ pfu/ml, $2 \cdot 10^7$ pfu/ml and $4 \cdot 10^6$ pfu/ml for experiments conducted alone, mixed with phiX174 or adenovirus, respectively. The initial concentrations of phiX174 and adenovirus for experiments conducted with MS2 were $2 \cdot 10^7$ pfu/ml and $2 \cdot 10^6$ pfu/ml, respectively. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation.

Analogous experiments were conducted in Indian groundwater (Figure 25), which yielded greater variation among the different experiments: 1.70 ± 0.90 cm^2/kJ, 3.12 ± 0.93 cm^2/kJ, 2.30 ± 1.38 cm^2/kJ and 1.86 ± 0.42 cm^2/kJ when MS2 was alone, mixed with phiX174, adenovirus or echovirus, respectively. However, in the Indian groundwater the inactivation was much slower and therefore more difficult to measure, which explains the large 95% confidence intervals. Nevertheless, taking into account the large confidence intervals, the inactivation rate constant values for the different experiments were not significantly different.

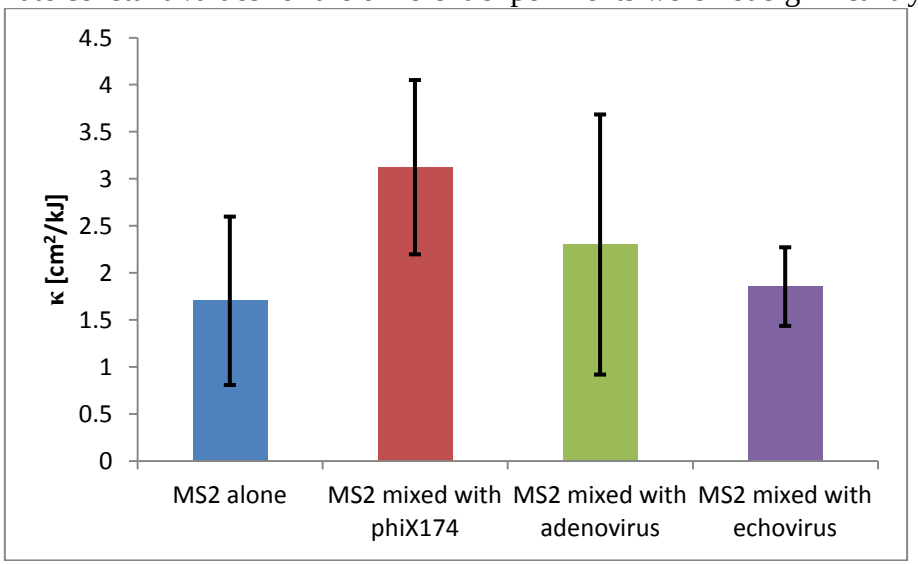

Figure 25 : Inactivation of MS2 alone and in the same container with phiX174, adenovirus and echovirus in Indian groundwater. The inactivation rate constants for MS2 are the mean of two, five, seven and two experiments for experiments conducted alone, with phiX174, adenovirus or echovirus, respectively. The initial MS2 concentrations were $1 \cdot 10^6$ pfu/ml, $4 \cdot 10^6$ pfu/ml, $1 \cdot 10^6$ pfu/ml and $6 \cdot 10^5$ pfu/ml for experiments conducted alone, with phiX174, adenovirus or echovirus, respectively. The initial concentrations of phiX174, adenovirus, echovirus for experiments conducted with MS2 were $2 \cdot 10^6$ pfu/ml, $1 \cdot 10^5$ pfu/ml and $1 \cdot 10^3$ pfu/ml, respectively. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation.

The addition of adenovirus had no influence on the inactivation of MS2 in Indian tap water (Figure 26), as the κ value changed only from 1.21 ± 1.03 cm^2/kJ to 1.19 ± 1.02 cm^2/kJ.

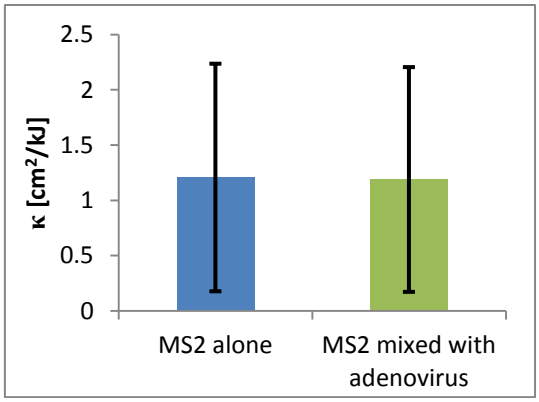

Figure 26 : Inactivation of MS2 alone and in the same container with adenovirus in Indian tap water. The inactivation rate constants for MS2 are the mean of two experiments. The initial MS2 concentrations were $4 \cdot 10^6$ pfu/ml and $6 \cdot 10^5$ pfu/ml for experiments conducted alone or mixed with adenovirus, respectively. The initial concentration of adenovirus for experiments conducted with MS2 was $1 \cdot 10^5$ pfu/ml. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation.

These three sets of experiments show that the addition of other viruses at the concentrations used had no influence on the inactivation rate constants observed and therefore did not affect the ROS concentrations in solution.

3.5.1 Inactivation of phiX174

The inactivation of phiX174 was investigated in Swiss tap water and in Indian ground water (Figure 27). While inactivation could be observed in the case of Swiss tap water (0.95 ± 0.25 cm^2/kJ), the inactivation was negligible in the Indian ground water (0.08 ± 0.56 cm^2/kJ). Several remarks can be made on these values. Firstly, in both waters the order of magnitude of κ is much smaller than the one observed for MS2, 9 and 39 times in the case of STW and IGW, respectively. This can be explained by the fact that phiX174 is known to be more resistant to ROS[17], which is the main inactivating agent during virus inactivation by SODIS, as discussed previously (Chapter 3.3). Secondly, the inactivation rate constant observed for phiX174 in IGW is 12 times smaller than in STW. In comparison, MS2 was inactivated 3 times slower in IGW than in STW. Finally, the value of κ was so low in the case of IGW that it was actually not significantly different from the dark control (according to ANCOVA analysis the p-values of the 5 experiments done in IGW compared to the dark control were 0.12, 0.23, 0.39, 0.51 and 0.53). This implies that solar inactivation cannot be held responsible for the inactivation of phiX174 observed in Indian groundwater. Furthermore, even with Swiss tap water and light intensity of a sunny day in Lausanne, only 0.5 logs of inactivation of phiX174 was observed in 6 hours. This is far less than the 4 logs that should be achieved for drinking water treatment. Unfortunately, it indicates that SODIS alone may not be sufficient for viruses, which are resistant against oxidants.

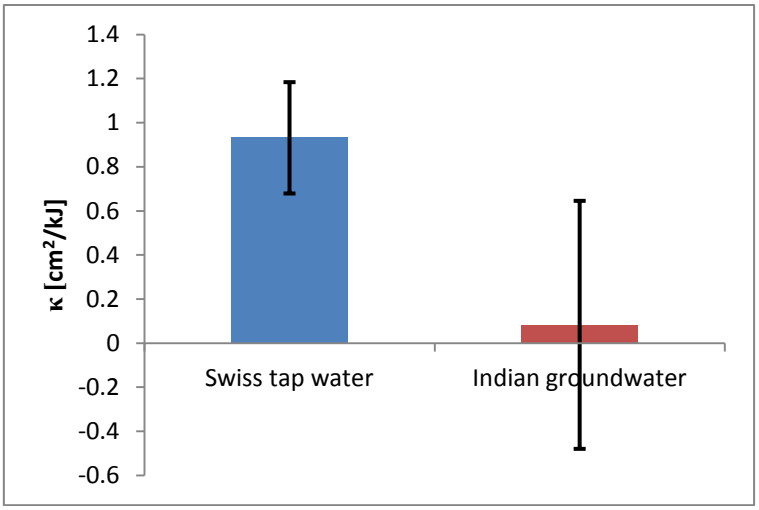

Figure 27 : Inactivation of phiX174 conducted in the same container with MS2 in Swiss tap water and Indian ground water. The inactivation rate constants reported are the mean of two experiments performed in Swiss tap water and five experiments in Indian groundwater. The initial concentrations of phiX174 were $2 \cdot 10^7$ pfu/ml and $2 \cdot 10^6$ pfu/ml, respectively. The initial MS2 concentrations were $2 \cdot 10^7$ pfu/ml and $4 \cdot 10^6$ pfu/ml, respectively. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation.

3.5.2 Inactivation of Adenovirus

As all experiments (except one) with adenovirus were done on a short period of 6 hours As the inactivation observed was very small, all 95% confidence intervals are relatively high (Figure 28). The inactivation rate constants observed were 1.45 ± 11.74 cm^2/kJ, -0.16 ± 5.47 cm^2/kJ and -0.54 ± 6.91 cm^2/kJ in Swiss tap water, Indian groundwater and Indian tap water, respectively. Based on the ANCOVA analysis, all the inactivation rate constants observed were not significantly different from the dark inactivation rate constant (p-values: 0.12, 0.22, 0.33 for STW, IGW and ITW.) Our experiments were thus performed over a too short duration to demonstrate a significant difference with the dark control. This is why negative values of the inactivation rate constant can be observed when corrected for dark inactivation. Moreover, adenovirus experiments frequently exhibited samples that were impossible to enumerate precisely, either because the concentration of viruses was too high to count, or below the detection limit. A worst case regression was therefore performed. This implies that we included samples below the detection limit, assigning them the concentration corresponding to the detection limit. These worst case regressions were used for all experiments in Swiss tap water and it was used for one experiment in Indian tap water.

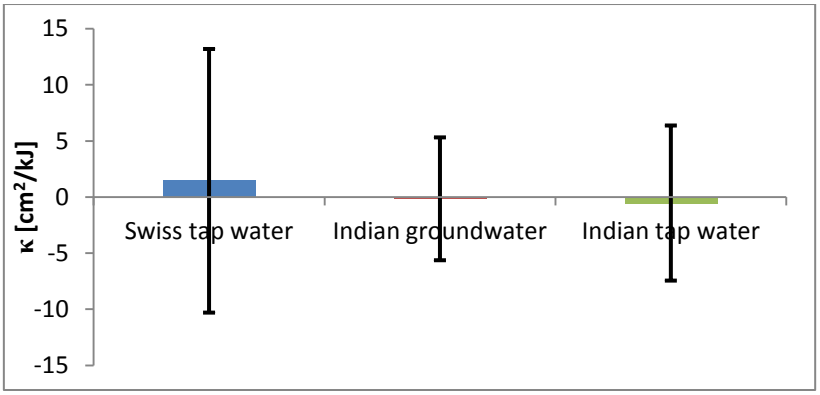

Figure 28 : Inactivation of adenovirus conducted in the same container with MS2 in Swiss tap water, Indian ground water and Indian tap water. The inactivation rate constants reported are the mean of three, two and two experiments, respectively. The initial concentrations of adenovirus were $2 \cdot 10^6$ pfu/ml, $1 \cdot 10^5$ pfu/ml and $1 \cdot 10^5$ pfu/ml, respectively. The initial concentrations of MS2 mixed were $4 \cdot 10^6$ pfu/ml, $1 \cdot 10^6$ pfu/ml and $6 \cdot 10^5$ pfu/ml, respectively. Error bars indicate 95% confidence intervals. Values were corrected for dark inactivation.

One experiment was conducted for a period of time greater than 6 hours (Figure 29). Note that the points at 23.5 and 26 hours, there was not enough viruses to be quantified precisely. The given values are the maximum that could be expected taking into account the detection limit. Even exposed for 25 hours, however, no significant difference of the inactivation rate constants between the dark control and the sample exposed to the solar simulator could be determined (p-value: 0.20). Surprisingly, the inactivation rate constants observed in the dark controls are relatively high. We expect that adenovirus is particularly sensible to the fact that there is a high surface to volume ratio and relatively vigorous stirring in the open beaker. To improve the data on adenovirus inactivation, it would be interesting to conduct experiments using a PET bottle instead of the beaker and to conduct experiments for at least 24 hours.

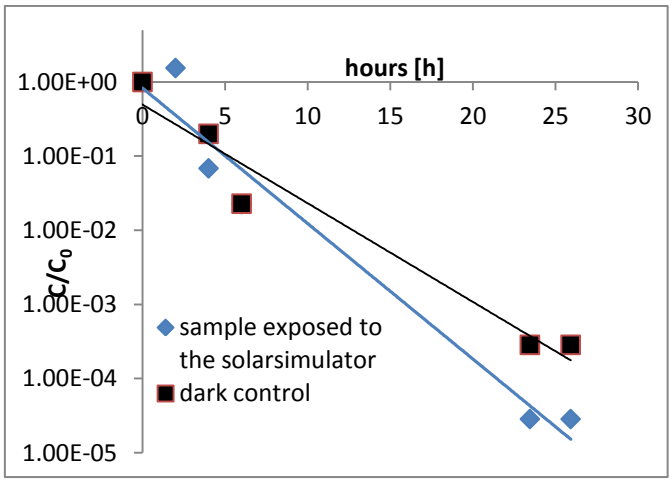

Figure 29 : Comparison of adenovirus inactivation in Indian tap water exposed to the solar simulator or protected from light by aluminum foil (dark control). The initial virus concentration was $4 \cdot 10^5$ pfu/ml in both samples. Error bars indicate 95% confidence intervals. No duplicates were conducted for these experiments

Bosshard et al.[11] observed an adenovirus inactivation of 1 log after one day of exposure to a solar simulator (identical to our) in Swiss tap water. Our experiments showed a similar inactivation of 1.36 logs after 24 hours of exposition to the solar simulator, corresponding to a total fluence rate of 2.15 kJ/cm^2 (inactivation rate constant observed: 1.45 ± 11.74 cm^2/kJ). In both cases the inactivation of adenovirus in Swiss drinking water was slow. Additionally, we observed that inactivation in Indian water was even lower. Hence, as for phiX174, Adenovirus is not sufficiently quickly inactivated by SODIS to yield sufficiently high inactivation after six hours of exposure. Nevertheless, is seems that SODIS inactivates Adenovirus to some extent, which is certainly better than no inactivation at all.

3.5.3 Inactivation of Echovirus

The inactivation rate constant of echovirus in Indian groundwater (1.92 ± 0.98 cm^2/kJ) was similar to MS2 (1.86 ± 0.42 cm^2/kJ, Figure 30). Contrary to Adenovirus and phiX174, the rate constant was relatively high and clearly different to the dark inactivation.

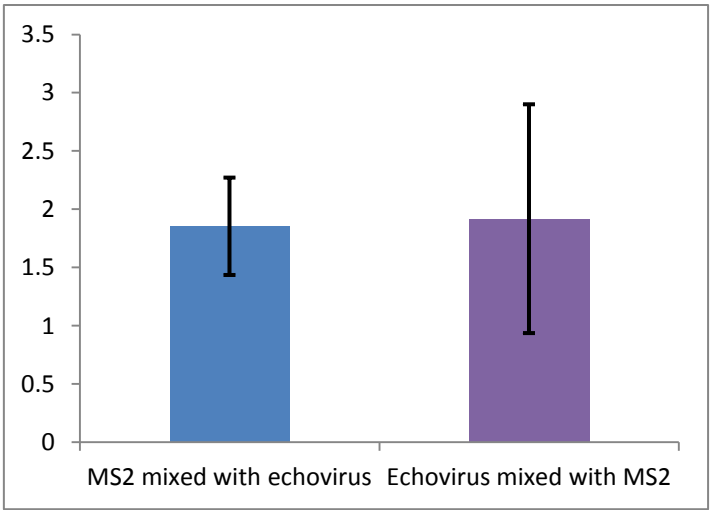

Figure 30 : Inactivation of MS2 and echovirus mixed together in the same container in Indian groundwater. The inactivation rate constant for MS2 and echovirus are the mean of two experiments. The initial concentrations of MS2 and echovirus were $6 \cdot 10^5$ pfu/ml and $1 \cdot 10^3$ pfu/ml, respectively. Error bars indicate 95% confidence intervals. Values corrected for dark inactivation.

In our experiments, 1.1 logs of inactivation could be calculated for a fluence of 1.34 kJ/cm^2 (6 hours exposure in May in Switzerland). In comparison, Fujioka et al.[10] observed an inactivation of 3 logs for 4 hours exposure to sunlight in summer and only 1 log in winter. Hence, particularly in summer they observed much faster inactivation than in our experiments. However, their viruses were directly exposed to sunlight without the UVB cut-off of a PET bottle. Therefore a direct comparison to our results is not possible. Nevertheless, the higher inactivation can be explained by the higher fluence in the UV-B range of direct sunlight and therefore more significant direct inactivation probably occurred during the experiments of Fujioka et al. Silverman et al.[22] investigated inactivation of poliovirus in different coastal waters. Poliovirus is similar to echovirus and they observed different inactivation rate constants for each water investigated. The higher inactivation observed in their study was 0.4 logs for 6 hours exposed to a solar simulator with a UV-B blocking filter and a fluence rate of 187 W/m^2. This inactivation is lower than the results we got for echovirus but in our case the fluence rate of solar simulator was higher (249 W/m^2). Moreover Silverman et al. obtained different results for each water investigated and as they had not analysed the composition of their water and we do not know if it was similar to our water, it is difficult to directly compare their results with ours.
So far inactivation of echovirus in Swiss tap water or Indian tap water was not yet investigated. However, from the experiments with MS2 it can be expected that echovirus would be inactivated similarly in Indian tap water as in Indian groundwater. However, inactivation of echovirus should be much faster in Swiss tap water.

3.6 Summary of results of phiX174, adenovirus and echovirus compared to MS2

The ratios of $\kappa_{virus}/\kappa_{MS2}$ were always close to zero for adenovirus and phiX174, but close to 1 in the case of echovirus (Table 3). Our data suggests that MS2 may be a good indicator for echovirus, whereas phiX174 may be an appropriate indicator for adenovirus. However, further experiments will have to be conducted to confirm these hypotheses.

Table 3 : Ratio of inactivation rate constant of other viruses and MS2. Values were corrected for dark inactivation. The ratios were calculated based on two and five experiments for phiX174/MS2 in STW and IGW,respectively. Three, two and two experiments for adenovirus/MS2 in STW, IGW and ITW, respectively and two for echovirus/MS2 in IGW.

	Swiss tap water	Indian groundwater	Indian tap water
$\kappa_{phiX174}/\kappa_{MS2}$ [-]	0.09 ± 0.04	0.03 ± 0.25	-
$\kappa_{adenovirus}/\kappa_{MS2}$ [-]	0.03 ± 2.01	-0.04 ± 1.39	0.17 ± 4.44
$\kappa_{echovirus}/\kappa_{MS2}$ [-]	-	1.05 ± 0.59	-

In order to summarise all the inactivation data collected, the logs of inactivation were calculated for a fluence of 1.34 kJ/cm^2 (6 hours of exposition on a typical sunny day in May in Lausanne, Switzerland, N 46.52°, E 6.57°) based on our experimental data (Table 4).

Table 4 : Logs of inactivation calculated for an exposition of 1.34 kJ/cm^2 (6 hours exposure in May in Switzerland). Values were corrected for dark inactivation

	Swiss tap water	Indian groundwater	Indian tapwater
MS2	4.75 ± 1.45	1.41 ± 0.43	0.69 ± 0.44
phiX174	0.54 ± 0.11	Inactivation not significantly different from dark inactivation	-
adenovirus	Inactivation not significantly different from dark inactivation	Inactivation not significantly different from dark inactivation	Inactivation not significantly different from dark inactivation
echovirus	-	1.11 ± 0.43	-

Hence, in Swiss tap water a reduction of more than 99.99% (4 logs) and 90% (1 log) were observed for MS2 and phiX174, respectively. However, the inactivation in Indian water was much lower; 90% (1 log) was observed in groundwater for both MS2 and echovirus and less than 90% (1 log) of inactivation was observed for MS2 in Indian tap water.
To conclude, viruses that are susceptible to oxidants are inactivated well by SODIS in 'reactive' water like Swiss tap water, but inactivation is substantially reduced (90 %) in less 'reactive' waters, like the waters from India. In contrast, viruses resistant to oxidants, like phiX174 and adenovirus, are only affected (< 90 %) by 'reactive' waters but no inactivation could be observed in less 'reactive' waters.

4 Conclusion

Viruses cause problematic health issues related to unsafe drinking water consumption. At a household level, SODIS is an effective solution to remove bacteria from drinking water, but its effectiveness against viruses has been much less investigated. Due to broad diversity of virus structures the response to solar disinfection could vary widely for different viruses. Furthermore, the inactivation mechanisms of viruses during SODIS are not yet understood.

In water from Switzerland 5 logs of MS2 were inactivated after receiving 1.34 kJ/cm^2 of sunlight (6 hours of exposition in Switzerland). In comparison, Berney et al.[34] observed 6 logs of inactivation of *Escherichia coli* for a fluence of only 0.24 kJ/cm^2. Hence, MS2 was 6 times more resistant than bacteria. This comparison highlights the difficulty to inactivate viruses during SODIS, particularly as MS2 seems to be a relatively sensitive virus. Nevertheless, SODIS was effective to inactivate echovirus efficiently, a reduction of 99.99% (4 logs) was achieved in 6 hours (with Swiss tap water under natural sunlight). In contrast, adenovirus, which is structurally very different from echovirus, was not susceptible to SODIS. Moreover, this study also showed that the systematic use of MS2 as a point of comparison when testing other viruses was a good reference value and the addition of MS2 into all SODIS experiments with virus should be incorporated in future studies.
Bacteriophage MS2 proved to be a good surrogate for echovirus and could be used to predict the response of echovirus, and potentially other enteroviruses, to SODIS in different waters. PhiX174 could potentially serve as a surrogate for adenovirus, but further experiments need to be conducted to confirm this behavior. The inactivation potential of SODIS can greatly vary in function of the water type, but as MS2 and phiX174 seem to be good surrogates for echovirus and adenovirus, respectively, different waters could be analyzed simply by using these two bacteriophages.
Iron, an element often found in water, was suggested as a potentially important water constituent that could be responsible for the formation of reactive oxygen species that cause virus inactivation. In contrast, organic matter was suggested to also act as a quencher of reactive species. Hence, these two water constituent should always be analysed for future SODIS experiments, as they may be used in future to estimate SODIS efficiency for virus inactivation.
Another important aspect of SODIS is the effect of the water temperature on the inactivation. The higher the temperature, the greater will be the extent of inactivation. In most countries where SODIS is or will be implemented, the ambient temperature is much higher than in Switzerland; moreover the fluence rates are often higher, too. The effectiveness of SODIS could therefore be considerably higher, and sufficient inactivation of echovirus should be possible in many cases, whereas adenovirus will probably only be slightly reduced in most cases. Hence, even though viruses are more resistant than bacteria to SODIS disinfection, particularly echovirus removal should be efficient as long as water temperatures are relatively high (above 40 °C) and organic matter content low.

4.1 Future work

This study is a first initiative to elucidate the effectiveness of SODIS on waterborne viruses. Our results and the results from other studies clearly show that inactivation of viruses is substantially slower than of bacteria. However, further experiments should be conducted to better understand the inactivation mechanisms during SODIS. Additional waters should be used under the same set-up to better understand which components of the water (e. g. content of organic matter, iron, copper) influence virus inactivation. It would also be particularly interesting to analyse which reactive oxygen species are formed in the water during SODIS

disinfection. The use of MS2 and phiX174 as surrogates for human echovirus and adenovirus, respectively, should be confirmed with further experiments. Inactivation of additional human viruses should also be investigated to obtain a more general understanding of human virus removal during SODIS disinfection. Moreover, viruses should be investigated from a structural point of view to understand better why certain viruses are inactivated more slowly than others.

Particularly inactivation of phiX174 and adenovirus was very slow, therefore for future work it will be important to conduct experiments over periods of at least 24 hours and withdraw a sufficiently high number of aliquots (at least 6) to effectuate a more precise analysis of the inactivation.

In general, in future work the choice of the container will be particularly important. As the material can greatly influence the light spectrum within the container, the inactivation rate constant observed is also strongly influenced. We therefore suggest to always use PET bottles and no other materials to study SODIS disinfection. Nevertheless, a smaller open container can be placed within the PET bottle to reduce the volume of water and the quantity of virus needed to conduct experiments.

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6 Appendixes

Table I: Details and inactivation rate constants observed in all experiments. Grey cells are experiments conducted with Swiss tap water collected in the same day.

Water type	1st virus	2nd virus	container	Initial concentration of the 1st virus [pfu/ml]	Initial concentration of the 2nd virus [pfu/ml]	comments	k [h^{-1}] ± confidence interval 95%	κ [cm^2/kJ] ± confidence interval 95%
Swiss tap water	MS2	-	PET	$6.6 \cdot 10^8$	-		**1.34 ± 0.53**	**13.03 ± 5.17**
Swiss tap water	MS2	-	PET	$4 \cdot 10^5$	-		**0.84 ± 0.20**	**9.23 ± 2.23**
Swiss tap water	MS2	-	PET	$4.68 \cdot 10^5$	-	Not stirred	**0.82 ± 0.14**	**8.91 ± 1.47**
Swiss tap water	MS2	-	PET	$6.08 \cdot 10^5$	-	Dark control	**0.03 ± 0.05**	**0.26 ± 0.53**
Swiss tap water	MS2	-	PET	$7.00 \cdot 10^6$	-		**0.59 ± 0.11**	**6.45 ± 1.24**
Swiss tap water	MS2	-	PET	$5.20 \cdot 10^6$	-	Natural sunlight	**1.84 ± 0.39**	**8.41 ± 2.84**
Swiss tap water	MS2	-	Beaker	$1.80 \cdot 10^5$			**0.59 ± 0.20**	**6.40 ± 2.13**
Swiss tap water	MS2	-	Beaker	$2.08 \cdot 10^5$		Dark control	**0.17 ± 0.18**	**1.97 ± 2.05**
Swiss tap water	MS2	-	Beaker	$1.80 \cdot 10^5$		Dark control, natural sunlight	**0.49 ± 0.40**	**2.27 ± 2.58**
Swiss tap water	MS2	-	Beaker	$3.84 \cdot 10^7$	-		**2.3 ± 1.48**	**22.40 ± 14.35**
Swiss tap water	MS2	-	Beaker	$3.24 \cdot 10^7$	-		**0.96 ± 0.14**	**11.21 ± 1.64**
Swiss tap water	MS2	-	Beaker	$3.08 \cdot 10^6$	-		**0.77 ± 0.11**	**9.06± 1.34**
Swiss tap water	MS2	-	Beaker	$5.88 \cdot 10^7$	-		**1.62 ± 0.15**	**18.54 ± 1.70**
Swiss tap	MS2	-	Beaker	$4.32 \cdot 10^5$	-		**1.17 ± 0.26**	**14.07 ± 3.14**

water								
Swiss tap water	MS2 not purified	-	Beaker	$6.92 \cdot 10^7$	-		**0.48 ± 0.14**	**5.53 ± 1.62**
Swiss tap water	MS2	phiX174	Beaker	$3.08 \cdot 10^6$	$3.92 \cdot 10^6$		**1.07 ± 0.14**	**12.76 ± 1.68**
Swiss tap water	MS2	phiX174	Beaker	$4.56 \cdot 10^7$	$2.64 \cdot 10^7$		**0.62 ± 0.29**	**7.01 ± 3.29**
Swiss tap water	MS2	phiX174	Beaker	$4.92 \cdot 10^7$	$1.92 \cdot 10^7$	Dark control	**0.08 ± 0.29**	**0.93 ± 3.24**
Swiss tap water	MS2	phiX174	Beaker	$1.48 \cdot 10^6$	$1.44 \cdot 10^6$	Dark control	**0.10 ± 0.02**	**1.02 ± 0.17**
Swiss tap water	MS2	Adenovirus	beaker	$7.92 \cdot 10^6$	$\approx 10^6$		**1.23 ± 0.37**	**12.32 ± 3.72**
Swiss tap water	MS2	Adenovirus	Beaker	$8.76 \cdot 10^6$	$4.00 \cdot 10^5$		**0.98 ± 0.62**	**10.50 ± 6.70**
Swiss tap water	MS2	Adenovirus	Beaker	$6.64 \cdot 10^6$	$4.00 \cdot 10^5$	Dark control	**-0.20 ± -**	**-2.10 ± -**
Swiss tap water	MS2	Adenovirus	Beaker	$2.56 \cdot 10^5$	$1.96 \cdot 10^5$		**0.40 ± 0.06**	**4.83 ± 0.68**
Swiss tap water	MS2	Adenovirus	Beaker	$2.56 \cdot 10^5$	$6.80 \cdot 10^5$	Duplicate	**0.52 ± 0.13**	**6.14 ± 1.49**
Swiss tap water	MS2	Adenovirus	Beaker	$1.40 \cdot 10^4$	$2.16 \cdot 10^6$	Dark control	**0.46 ± -**	**5.65 ± -**
Swiss tap water	Adenovirus	MS2	Beaker	$4.00 \cdot 10^5$	$8.76 \cdot 10^6$		**0.57 ± 1.36**	**6.13 ± 14.62**
Swiss tap water	Adenovirus	MS2	Beaker	$4.00 \cdot 10^5$	$6.64 \cdot 10^6$	Dark control	**-0.27 ± -**	**-2.79 ± -**
Swiss tap water	Adenovirus	MS2	Beaker	$1.96 \cdot 10^5$	$2.56 \cdot 10^5$		**0.14 ± 0.19**	**1.69 ± 2.28**
Swiss tap water	Adenovirus	MS2	Beaker	$6.80 \cdot 10^5$	$2.56 \cdot 10^5$	Duplicate	**0.19 ± 0.81**	**2.19 ± 9.50**
Swiss tap water	Adenovirus	MS2	Beaker	$2.16 \cdot 10^6$	$1.40 \cdot 10^4$	Dark control	**0.11 ± -**	**1.33 ± -**
Swiss tap water	phiX174	MS2	Beaker	$2.64 \cdot 10^7$	$4.56 \cdot 10^7$		**0.08 ± 0.02**	**0.91 ± 0.20**
Swiss tap	phiX174	MS2	Beaker	$1.92 \cdot 10^7$	$4.92 \cdot 10^7$	Dark control	**0.02 ± 0.02**	**0.26 ± 0.26**

water								
Swiss tap water	phiX174	MS2	Beaker	$3.92 \cdot 10^6$	$3.08 \cdot 10^6$		**0.13 ± 0.02**	**1.51 ± 0.25**
Swiss tap water	phiX174	MS2	Beaker	$1.44 \cdot 10^6$	$1.48 \cdot 10^6$	Dark control	**0.03 ± 0.02**	**0.30 ± 0.24**
Swiss tap water	MS2	-	Beaker	$3.96 \cdot 10^6$		75% PBS	**0.23 ± 0.12**	**2.83 ± 1.49**
Swiss tap water	MS2	-	Beaker	$3.24 \cdot 10^6$		50% PBS	**0.24 ± 0.22**	**2.95 ± 2.74**
Swiss tap water	MS2	-	Beaker	$2.44 \cdot 10^6$		25% PBS	**0.65 ± 0.52**	**7.64 ± 6.12**
PBS	MS2	-	PET	$1.9 \cdot 10^6$	-		**0.01 ± 0.09**	**0.07 ± 0.91**
PBS	MS2	phiX174	Beaker	$3.8 \cdot 10^6$	$9.08 \cdot 10^5$		**0.07 ± 0.06**	**0.73 ± 0.60**
PBS	phiX174	MS2	Beaker	$9.08 \cdot 10^5$	$3.8 \cdot 10^6$		**0.06 ± 0.02**	**0.66 ± 0.24**
Indian ground water	MS2	-	Beaker	$7.68 \cdot 10^6$	-		**0.27 ± 0.04**	**2.72 ± 0.37**
Indian ground water	MS2	-	Beaker	$5.84 \cdot 10^4$	-		**0.29 ± 0.11**	**3.06 ± 1.12**
Indian ground water	MS2	-	Beaker	$3.60 \cdot 10^6$	-	75% PBS	**0.07 ± 0.03**	**0.75 ± 0.30**
Indian ground water	MS2	-	Beaker	$4.16 \cdot 10^6$	-	50% PBS	**0.08 ± 0.02**	**0.91 ± 0.23**
Indian ground water	MS2	-	Beaker	$4.36 \cdot 10^6$	-	25% PBS	**0.14 ± 0.01**	**1.50 ± 0.12**
Indian ground water	MS2	-	Beaker	$7.76 \cdot 10^6$	-	Not stirred	**0.26 ± 0.04**	**3.24 ± 0.54**
Indian ground water	MS2	-	Beaker	$7.68 \cdot 10^6$	-	Duplicate Not stirred	**0.21 ± 0.02**	**2.49 ± 0.29**
Indian ground water	MS2	-	Beaker	$8.60 \cdot 10^6$	-	Dark control Not stirred	**0.10 ± 0.03**	**1.19 ± 0.32**
Indian ground water	MS2	phiX174	Beaker	$5.28 \cdot 10^6$	$2.20 \cdot 10^6$	15°C	**0.09 ± 0.03**	**1.14 ± 0.33**
Indian ground water	MS2	phiX174	Beaker	$3.72 \cdot 10^6$	$1.80 \cdot 10^6$	15°C, duplicate	**0.09 ± 0.04**	**1.09 ± 0.49**
Indian ground water	MS2	phiX174	Beaker	$5.84 \cdot 10^6$	$2.08 \cdot 10^6$	15°C, dark control	**0.01 ± 0.06**	**0.10 ± 0.69**
Indian ground	MS2	phiX174	Beaker	$5.96 \cdot 10^7$	$4.28 \cdot 10^7$		**0.69 ± 0.14**	**7.60 ± 1.58**

water								
Indian ground water	MS2	phiX174	Beaker	$4 \cdot 10^6$	$1.06 \cdot 10^6$		**0.18 ± 0.02**	**1.89 ± 0.24**
Indian ground water	MS2	phiX174	Beaker	$3.8 \cdot 10^6$	$2.88 \cdot 10^6$		**0.21 ± 0.02**	**2.32 ± 0.26**
Indian ground water	MS2	phiX174	Beaker	$3.24 \cdot 10^6$	$1.1 \cdot 10^6$	duplicate	**0.36 ± 0.08**	**4.05 ± 0.95**
Indian ground water	MS2	phiX174	Beaker	$5.2 \cdot 10^6$	$2.6 \cdot 10^6$	duplicate	**0.24 ± 0.04**	**2.52 ± 0.44**
Indian ground water	MS2	phiX174	Beaker	$9.04 \cdot 10^6$	$3.04 \cdot 10^6$	27°C	**0.26 ± 0.09**	**3.04 ± 1.01**
Indian ground water	MS2	phiX174	Beaker	$1.72 \cdot 10^6$	$9.20 \cdot 10^5$	Dark control	**0.03 ± 0.04**	**0.27 ± 0.44**
Indian ground water	MS2	phiX174	Beaker	$3.96 \cdot 10^7$	$2.2 \cdot 10^7$	Dark control	**0.08 ± 0.05**	**0.84 ± 0.52**
Indian ground water	MS2	phiX174	Beaker	$6.72 \cdot 10^6$	$1.80 \cdot 10^6$	27°C, dark control	**0.03 ± 0.32**	**0.40 ± 4.02**
Indian ground water	MS2	Adenovirus	Beaker	$1.01 \cdot 10^7$	$\approx 10^6$		**0.17 ± 0.02**	**1.85 ± 0.19**
Indian ground water	MS2	Adenovirus	Beaker	$6.16 \cdot 10^6$	$\approx 10^4$		**0.23 ± 0.17**	**2.30 ± 1.70**
Indian ground water	MS2	Adenovirus	Beaker	$6.04 \cdot 10^6$	$\approx 10^4$	Duplicate	**0.22 ± 0.09**	**2.31 ± 0.97**
Indian ground water	MS2	Adenovirus	Beaker	$1.56 \cdot 10^5$	$4.00 \cdot 10^5$		**0.28 ± 0.11**	**3.32 ± 1.25**
Indian ground water	MS2	Adenovirus	Beaker	$1.86 \cdot 10^5$	$2.00 \cdot 10^6$	Duplicate	**0.18 ± 0.06**	**2.17 ± 0.69**
Indian ground water	MS2	Adenovirus	Beaker	$1.71 \cdot 10^5$	$2.80 \cdot 10^6$	Dark control	**0.016 ± 0.209**	**0.20 ± 2.61**
Indian ground water	MS2	Adenovirus	Beaker	$4.64 \cdot 10^5$	$9.20 \cdot 10^4$		**0.48 ± 0.13**	**5.41 ± 1.47**
Indian ground water	MS2	Adenovirus	Beaker	$4.80 \cdot 10^5$	$1.96 \cdot 10^5$	Duplicate	**0.23 ± 0.14**	**2.48 ± 1.48**
Indian ground water	MS2	Adenovirus	Beaker	$4 \cdot 10^5$	$1.96 \cdot 10^5$	Dark control	**0.07 ± 0.01**	**0.82 ± 0.05**
Indian ground	MS2	Echovirus	Beaker	$6.24 \cdot 10^5$	$1.96 \cdot 10^3$		**0.23 ± 0.02**	**2.45 ± 0.20**

water								
Indian ground water	MS2	Echovirus	Beaker	$6.20 \cdot 10^5$	$9.20 \cdot 10^2$	Duplicate	**0.20 ± 0.04**	**2.24 ± 0.41**
Indian ground water	MS2	Echovirus	Beaker	$4.40 \cdot 10^5$	$1.32 \cdot 10^3$	Dark control	**0.04 ± 0.02**	**0.49 ± 0.27**
Indian ground water	Echovirus	MS2	Beaker	$1.96 \cdot 10^3$	$6.24 \cdot 10^5$		**0.18 ± 0.09**	**1.92 ± 0.98**
Indian ground water	Echovirus	MS2	Beaker	$9.20 \cdot 10^2$	$6.20 \cdot 10^5$	Duplicate	**0.22 ± 0.05**	**2.40 ± 0.57**
Indian ground water	Echovirus	MS2	Beaker	$1.32 \cdot 10^3$	$4.40 \cdot 10^5$	Dark control	**0.02 ± 0.05**	**0.24 ± 0.57**
Indian ground water	Adenovirus	MS2	Beaker	$9.20 \cdot 10^4$	$4.64 \cdot 10^5$		**0.10 ± 0.52**	**1.09 ± 5.86**
Indian ground water	Adenovirus	MS2	Beaker	$1.96 \cdot 10^5$	$4.80 \cdot 10^5$	Duplicate	**0.29 ± 0.32**	**3.15 ± 4.15**
Indian ground water	Adenovirus	MS2	Beaker	$1.96 \cdot 10^5$	$4 \cdot 10^5$	Dark control	**0.12 ± 0.88**	**1.44 ± 10.22**
Indian ground water	phiX174	MS2	Beaker	$2.20 \cdot 10^6$	$5.28 \cdot 10^6$	15°C	**0.06 ± 0.03**	**0.68 ± 0.33**
Indian ground water	phiX174	MS2	Beaker	$1.80 \cdot 10^6$	$3.72 \cdot 10^6$	15°C, duplicate	**0.04 ± 0.02**	**0.43 ± 0.22**
Indian ground water	phiX174	MS2	Beaker	$2.08 \cdot 10^6$	$5.84 \cdot 10^6$	15°C, dark control	**0.02 ± 0.03**	**0.21 ± 0.38**
Indian ground water	phiX174	MS2	Beaker	$2.88 \cdot 10^6$	$3.8 \cdot 10^6$		**0.04 ± 0.01**	**0.40 ± 0.11**
Indian ground water	phiX174	MS2	Beaker	$1.1 \cdot 10^6$	$3.24 \cdot 10^6$	Duplicate	**0.03 ± 0.02**	**0.37 ± 0.19**
Indian ground water	phiX174	MS2	Beaker	$2.6 \cdot 10^6$	$5.2 \cdot 10^6$	Duplicate	**0.03 ± 0.01**	**0.33 ± 0.15**
Indian ground water	phiX174	MS2	Beaker	$4.28 \cdot 10^7$	$5.96 \cdot 10^7$		**0.04 ± 0.05**	**0.48 ± 0.49**
Indian ground water	phiX174	MS2	Beaker	$1.06 \cdot 10^6$	$4 \cdot 10^6$		**0.03 ± 0.04**	**0.34 ± 0.46**
Indian ground water	phiX174	MS2	Beaker	$3.04 \cdot 10^6$	$9.04 \cdot 10^6$	27°C	**0.06 ± 0.24**	**0.74 ± 2.82**
Indian ground	phiX174	MS2	Beaker	$9.20 \cdot 10^5$	$1.72 \cdot 10^6$	Dark control	**0.00 ± 0.05**	**-0.02 ± 0.50**

water								
Indian ground water	phiX174	MS2	Beaker	$2.2 \cdot 10^{7}$	$3.96 \cdot 10^{7}$	Dark control	**0.07 ± 0.16**	**-0.76 ± 1.66**
Indian ground water	phiX174	MS2	Beaker	$1.80 \cdot 10^{6}$	$6.72 \cdot 10^{6}$	27°C, dark control	**-0.03 ± 0.19**	**-0.42 ± 2.32**
Indian tap water	MS2	-	Beaker	$7.44 \cdot 10^{6}$	-		**0.20 ± 0.14**	**2.05 ± 1.39**
Indian tap water	MS2	-	Beaker	$1.56 \cdot 10^{4}$	-		**0.09 ± 0.03**	**1.01 ± 0.33**
Indian tap water	MS2	-	Beaker	$3.76 \cdot 10^{6}$	-	75% PBS	**0.05 ± 0.02**	**0.54 ± 0.25**
Indian tap water	MS2	-	Beaker	$4.76 \cdot 10^{6}$	-	50% PBS	**0.06 ± 0.01**	**0.68 ± 0.12**
Indian tap water	MS2	-	Beaker	$4.24 \cdot 10^{6}$	-	25% PBS	**0.06 ± 0.02**	**0.65 ± 0.21**
Indian tap water	MS2	Adenovirus	Beaker	$6.12 \cdot 10^{5}$	$9.60 \cdot 10^{4}$		**0.17 ± 0.12**	**2.01 ± 1.38**
Indian tap water	MS2	Adenovirus	Beaker	$4.48 \cdot 10^{5}$	$3.16 \cdot 10^{5}$	Dark control	**-0.00 ± 0.41**	**-0.05 ± 4.93**
Indian tap water	MS2	Adenovirus	Beaker	$3.60 \cdot 10^{5}$			**0.09 ± 0.02**	**1.01 ± 0.27**
Indian tap water	MS2	Adenovirus	Beaker	$4.48 \cdot 10^{5}$		Dark control	**0.04 ± 0.01**	**0.45 ± 0.13**
Indian tap water	MS2	phiX174	Beaker	$2.12 \cdot 10^{6}$	$1.56 \cdot 10^{6}$	Dark control	**-0.00 ± 0.01**	**-0.04 ± 0.14**
Indian tap water	Adenovirus	MS2	Beaker	$9.60 \cdot 10^{4}$	$6.12 \cdot 10^{5}$		**0.12 ± 0.83**	**1.39 ± 9.61**
Indian tap water	Adenovirus	MS2	Beaker	$3.16 \cdot 10^{5}$	$4.48 \cdot 10^{5}$	Dark control	**0.24 ± 1.29**	**2.84 ± 15.49**
Indian tap water	Adenovirus	MS2	Beaker	$1.40 \cdot 10^{5}$	$3.60 \cdot 10^{5}$		**0.42 ± 0.12**	**4.72 ± 1.30**
Indian tap water	Adenovirus	MS2	Beaker	$1.40 \cdot 10^{5}$	$4.48 \cdot 10^{5}$	Dark control	**0.31 ± 0.12**	**3.36 ± 1.32**
Indian tap water	phiX174	MS2	Beaker	$1.56 \cdot 10^{6}$	$2.12 \cdot 10^{6}$	Dark control	**-0.00 ± 0.02**	**-0.01 ± 0.22**