On-farm filtration technology for pathogen reduction

Reuse of low hygienic quality water for vegetable irrigation

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Cover: Wastewater-irrigated lettuce plot
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Abstract

Reusing wastewater for irrigation is an effective way to recirculate plant nutrients and water, particularly in arid and semi-arid regions. However, wastewater reuse in agriculture poses several hazards for human health, because of potential introduction of pathogens into agricultural production systems. Risks are higher in developing countries, where treatment plants face several challenges in adequately treating the wastewater. In order to feasibly address such risks, a new management approach has been posed in which alternative measures act as barriers along the farm-to-fork pathway. The concept is that a cumulative effect of these barriers reduces exposure to pathogens. The overall aim of this study was to evaluate the hygienic quality of produce from agricultural systems using irrigation water contaminated with wastewater and to assess suitability of an on-farm filtering in this system.

To achieve this objective, the concentration of bacteriophages, *E. coli* and helminth eggs was measured in lettuce, water and soil during one cropping season in an agricultural system that uses wastewater for irrigation of vegetables in Cochabamba, Bolivia. Five riverbank wells and the associated river were sampled every two weeks during the cropping season. Soil samples were taken from the five plots that were irrigated with the monitored wells when the lettuce was planted and when harvested. Composite lettuce samples were taken when harvested. In the laboratory, the reduction of bacteriophages (ϕX174 and MS2), *E. coli*, *Enterococcus* spp. and *Saccharomyces cerevisiae* by charcoal filters was investigated in relation to three grain diameter of filtering media. The tested parameters and levels were: two hydraulic loading rates (200 and 400 L m^{-2} d^{-1}), three grain diameters of biochar (Ø = 1.4, 2.8 and 5 mm), and two inflowing levels of electric conductivities (500 and 1000 µS cm^{-1}).

The microbial concentrations found in soil, lettuce and water sources of agricultural system evidenced high probabilities of fecal contamination along the system. Two types of riverbank filtration wells were identified: protected and unprotected. Both types exhibited significant levels (*circa* 4 $\log_{10}$ *E. coli*, 2 $\log_{10}$ bacteriophages, 1 $\log_{10}$ protozoa cysts and 70 % helminth eggs) of microbial reduction. Protected wells had significantly higher reduction rates for all microorganisms except virus. Results from biochar filters showed 1 $\log_{10}$ unit removal of all the monitored microorganisms, however, only for the smallest grain diameter (1.4 mm). No difference was found in microbial removal with either tested hydraulic loading rates nor with the tested electric conductivities. Grain diameter and uniformity of filtering media were identified as main factors for microbial removal for the two tested filtration technologies. Full-scale implementation of both is considered extremely context-dependent due to need of specific geological characteristics for riverbank filtration and due to large area requirement for biochar filters.

Keywords: biochar filtration, pathogen reduction, semiarid/arid cultivation, wastewater irrigation.

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Sammanfattning

Avloppsvattenbevattning är ett effektivt sätt att återanvända växtnäringsämnen och vatten, särskilt i torra och halvtorra områden. Återvinning av avloppsvatten i jordbruket innebär dock flera risker för människors hälsa på grund av risken för spridning av smitta till livsmedelskedjan. Riskerna är högre i låg-, och medelinkomstländer där avloppsvattenreningen ofta är bristfällig eller obefintlig. För att hantera dessa risker behövs en ny hanteringskedja där fler barriärer introduceras längs produktionskedjan. Barriärerna minskar risken för att konsumenterna exponeras för sjukdomsalstrande mikroorganimser (patogener). Det övergripande syftet med denna studie var att utvärdera den hygieniska kvaliteten hos produkter från jordbrukssystem som använder avloppsvattenbevattning och att utvärdera möjligheten att införa en barriär i form av filtrering i anslutning till fälten. För att utvärdera systemet mättes koncentrationerna av bakteriofager, E. coli och parasitägg i sallad, vatten och jord under en odlingssäsong med avloppsvattenbevattning. Vattnet som användes togs från brunnar i flodbanken. Försöken genomfördes i ett grönsaksproduktionssystem i Cochabamba, Bolivia. Fem brunnar i flodbanken och floden provtogs varannan vecka under studien. Jordprover togs från de fem fälten som bevattnades vid plantering och vid skörd av salladen. Salladen provtogs i samband med skörd. Utvärdering av biokolfilter som barriär för behandling av avloppsvatten gjordes genom att reduktionen av ϕX174 och MS2, E. coli, Enterococcus spp. och Saccharomyces cerevisiae utvärderades för filtermedia av tre olika partikelstorlekar. Utöver detta utvärderades två hydrauliska belastningsflöden och två salthalter i avloppsvattnet. Den mikrobiella koncentrationen som återfanns i jord, sallad och vatten visade hög sannolikhet för fekal kontaminering. Två typer av brunnar användes i systemet: skyddade och oskyddade. Båda typerna uppvisade signifikanta nivåer av mikrobiell reduktion jämfört med flodvattnet. De skyddade brunnarna hade signifikant högre reduktion av alla mikroorganismer utom virus. Biokolfiltren gav en tiopotens reduktion av alla undersökta mikroorganismer för den minsta partikelstorleken. De två vattenflödena och salthalterna gav ingen skillnad i reduktion av mikroorganismer över filtren. Partikelstorleken och enhetligheten i filtermedierna påvisade påverka reduktionen av mikroorganismer i filtrerna. Effektiviteten i fullskaledrift bedömdes vara mycket sammanhangsberoende, då flodbankfiltreringen kräver specifika geologiska förutsättningar och biokolfiltren kräver stor area för effektiv behandling.

Nyckelord: avloppsbevattning, biokolfilter, grönsaksodling, patogenreduktion, torra/halvtorra

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Perez-Mercado, L.F.***, Lalander, C., Joel, A., Ottoson, J., Iriarte, M., Oporto C. and Vinnerås, B. (2017). Pathogens in crop production systems irrigated with low-quality water in Bolivia (submitted)

II **Perez-Mercado, L.F.***, Lalander, C., Joel, A., Ottoson, J. and Vinnerås, B. (2017). Biochar filters as on-farm treatment to reduce pathogens for wastewater irrigation (manuscript)

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The contribution of Luis F. Perez Mercado to the papers included in this thesis was as follows:

I Vinnerås and Perez-Mercado planned the study. Perez-Mercado performed the field work. Perez-Mercado and Iriarte performed the laboratory work. Perez-Mercado and Vinnerås wrote the study with revisions by the co-authors.

II Vinnerås, Lalander and Perez-Mercado planned the study. Perez-Mercado performed the laboratory work. Perez-Mercado and Lalander carried out the statistical analysis. Perez-Mercado and Lalander wrote the paper with revisions by the co-authors.

1 Introduction

In society today, there are many linear flows of substances. One obvious flow is that of plant nutrients from mining to mineral fertilisers, which go onto agricultural fields into plants, ending up in human excreta and eventually, in the environment as a pollutant. Several different systems have been introduced for closing the plant nutrient loop. Wastewater is a major reliable source of nutrients that can be reused in agriculture, providing the potential to reduce pressure on the production of chemical fertilisers (Hamilton et al., 2007), while reducing the risk of eutrophication in the environment. In addition, the reuse of wastewater reduces the pressure on other water sources (Toze, 2006, Keuckelaere et al., 2015), which is especially important in arid and semi-arid areas where wastewater is permanently generated and is therefore a reliable water source (Hamilton et al., 2007). However, wastewater reuse in agriculture poses several hazards for human health, environmental quality and food safety, because of potential introduction of pathogens and toxic compounds into agricultural production systems (Scott et al., 2004). This situation is worse in developing countries, where wastewater is poorly treated or not treated at all (Hamilton et al., 2007), in particular in dry areas where the need for irrigation to ensure agricultural production determines the use of all available water sources. Thus, the use of wastewater and manure in agricultural systems is desirable, but also poses threats to public health and the environment which must be properly addressed.

A major hazard associated with the use of wastewater for irrigation is the increase in the burden of infectious diseases due to ingestion of pathogens through food (Scott et al., 2004). Conventional secondary wastewater treatment systems are inadequate in removing microorganisms (Gerba, 2008), so there are risks of infection from the high load of pathogens even when wastewater is released to a receiving water body prior to irrigation (Ottoson et al., 2006). Infectious diseases prevent the consumption of food and hamper the ability of body to metabolise nutrients, leading to malnutrition or undernutrition (Fanzo,

2014). Together, these account as the greatest factor – representing 16% of global disability-adjusted life years (DALY) – in the global burden of disease (Lopez et al., 2006). If this burden is added to that due to unsafe water, sanitation and hygiene (3% global DALY), the total result is 19% global DALY (Lopez et al., 2006). Therefore, pathogens still represent a major hazard in wastewater use in agriculture.

The Stockholm framework has been promoted world-wide by World Health Organization (2006) to address the microbiological risks from reusing wastewater, based on the notion that the effectiveness of pathogens in causing disease depends on both epidemiological characteristics and environmental barriers that the pathogens must cross to infect humans. The risks of disease can be managed by knowing and handling the relevant characteristics of each wastewater reuse system. Consequently, many microbiological risk assessments of water reuse in agriculture have been carried out using such an approach, but several information gaps in these have been identified (Keuckelaere et al., 2015). In Bolivia, the use of wastewater for irrigation is a known practice and its potential in disease transmission has been recognised (Huibers et al., 2004). However, there is a lack of data on microbial prevalence in agricultural systems that use wastewater for irrigation of vegetables. Therefore, more detailed studies are needed on the prevalence of microbes in such agricultural systems.

Regarding risk management, there are various strategies available for reuse at farm level to reduce microbial contamination of produce (*e.g.* die-off until harvest, river bank filtration) (Huibers et al., 2004, Verbyla et al., 2016). Successful implementation of on-farm measures is highly dependent on adaption to site characteristics and practices, such as plot size, irrigation method, water quality, vegetables grown, *etc.* (Keraita et al., 2014). Evaluation of the efficiency of microbial (virus, bacteria and protozoa) removal by on-farm measures in real-life irrigation conditions is also necessary in order to know the feasibility of their implementation in peri-urban semi-arid regions.

2 Objectives

The overall aim of this study was to evaluate the hygienic quality of produce from an agricultural system that uses wastewater-contaminated irrigation water and to assess the suitability of an on-farm treatment using this system in terms of both improvement in the microbiological quality of water and the requirements for on-farm implementation for the purposes of safe re-use of water and nutrients from flush sanitation by irrigation.

The specific objectives were: (1) to investigate and analyse the existing agricultural system in terms of hygienic quality of produce, and (2) to evaluate on-farm filtration technologies for treatment of water from a domestic wastewater-polluted stream in relation to the reduction of pathogenic microbes.

To accomplish these objectives, concentrations of pathogens and indicator organisms in lettuce and water from several field-scale plots were measured. Furthermore, reduction rates in the concentration of pathogens and indicator organisms in water were studied using different filtering media and flow rates, at laboratory scale, to estimate microbial reduction potential under on-farm conditions.

3 Background

3.1 Pathogens in domestic wastewater

Origin

The human body is a reserve for numerous pathogens. There are several ways in which pathogens can be transmitted: from person to person, through contaminated water and aerosols, through living vectors, and by ingestion of contaminated food. Gastrointestinal pathogens are excreted in the feces at high concentrations (up to 10^{12} g^{-1}) even without symptoms of disease (Bitton, 2005). The prevalence and survival of pathogens depend on climate and local public-health status which, in turn, will define the impact of pathogens on public health (World Health Organization, 2006, Jiménez et al., 2010). For instance, in places where sanitary conditions are poor, intestinal helminths frequently pose the greatest health risks (World Health Organization, 2006). Wastewater can become a reservoir that allows the pathogens from excreta and hygiene to survive, to multiply and to transport (Bitton, 2005).

Microbial pathogens and health risks

The pathogenic organisms found in wastewater can be classified into four broad categories: bacteria, protozoa, helminths and viruses (Bitton, 2005). Bacterial pathogenic organisms of human origin typically cause gastrointestinal diseases.

Domestic wastewater contains a wide variety of nonpathogenic and pathogenic bacteria (Metcalf et al., 2003). Bacteria are excreted in feces and represent approximately 9% of wet weight; some species can multiply in the environment (Bitton, 2005, Kadlec and Wallace, 2008). Where sanitation is

poor, *Salmonella typhi*, *Vibrio cholerae*, *Shigella* and the enterotoxigenic strain of *Escherichia coli* are common causes of disease due to fecal contamination (Ashbolt, 2004, Bos et al., 2010, Uyttendaele et al., 2015). The minimal infective dose depends on the bacterial species and ranges from 10^2 of *Escherichia coli* O157:H7 up to 10^7 of *Salmonella* spp. (Bitton, 2005).

Viruses have a rather simple structure, are submicroscopic, and consist of nucleic acid enclosed in a sheath made of protein (*i.e.* metabolism capacity and reproductive structures) (Schijven and Hassanizadeh, 2000, Kadlec and Wallace, 2008). Therefore they are unable to multiply outside their host cells and consequently are typically found in fewer numbers than bacteria (Bitton, 2005). In human feces, more than 100 different types of enteric viruses that can cause infection are excreted (Metcalf et al., 2003). Based on their pathogenesis, viruses from feces are classified as enteropathogenic viruses (astroviruses, calciviruses and rotaviruses) and non-enteropathogenic (hepatitis A/E viruses, enteroviruses and most adenoviruses), depending on whether or not they infect the gastrointestinal system (Guardabassi et al., 2003). The infective dose is generally lower than bacterial pathogens *e.g.* 10^1 for Hepatitis A virus and echovirus-12 (Bitton, 2005). Despite the typically low concentrations of viruses, their facility of transportation due to reduced size (20-200 nm for most species) and low infective dose make of viruses relevant disease agents in wastewater (Schijven and Hassanizadeh, 2000, Nordin, 2007).

Protozoa is a group of eukaryotic organisms considered as animals due to their capacity of movement (World Health Organization, 2006). Most of the protozoan parasites coat the large intestine of the host and produce cysts, which is an infectious-dormant state where the organism is able to survive under adverse environmental conditions (Bitton, 2005). An individual infected with protozoa excretes cysts in the feces. The protozoan are responsible for the majority of enteric diseases, and both *Cryptosporidium parvum* and G*iardia lamblia* are considered of great concern because of their impact on health of immunocompromised people, such as young children and elderly (Metcalf et al., 2003, World Health Organization, 2006). The minimum infective dose is in the range of $10^1 - 10^2$ (Bitton, 2005).

Helminth infections are considered a major concern, especially in regions with inadequate sanitation (Bitton, 2005, Nordin, 2007). The eggs constitute the infective stage of parasitic helminths; they are highly resistant to environmental stress and, similarly to protozoa, excreted in feces (Bitton, 2005). Most of the helminths are part of one of three phyla: Nematoda (*e.g. Ascaris lumbricoides*, *Trichuris trichuria*), Platyhelminthes (*e.g. Taenia* spp., *Schistosoma* spp.) and Annelida (segmented worms) (Metcalf et al., 2003). *Ascaris lumbricoides* is considered as the most prevalent parasitic infection

worldwide, which probably is associated with its low minimum infective dose (≤ 10 eggs) and high capacity of producing eggs (one adult can produce up to 20000 eggs d^{-1}) (Metcalf et al., 2003, Bitton, 2005). Size of helminth eggs ranges between 10 to 100 µm, which enables their removal by physical mechanisms such as straining and sedimentation (Metcalf et al., 2003).

Resulting restrictions for wastewater reuse in irrigation

The use of insufficiently treated wastewater may result in disease transmission due to crop contamination (World Health Organization, 2006, Verbyla et al., 2016). The traditional approach to manage such risks was based on reduction of contaminants – pathogen or chemical - through wastewater treatment plants. Such reduction is intended to reach defined threshold levels, depending on which, water is classified into three specific agricultural uses (*i.e.* unrestricted irrigation, irrigation of fodder crops, or no irrigation). This classification was criticized for being excessively restrictive and also for lacking context-sensitivity to different agricultural systems. Besides, the implementation of wastewater treatment plants (*i.e.* the basement for traditional risk management) has seldom been successful in developing world (Qadir et al., 2010, Cossio et al., 2017), and hence, more feasible management approaches had to be explored.

Multiple-barrier approach

In order to realistically address microbiological risks from reusing wastewater, a new management approach (Multiple-barrier approach) has been proposed that is based on multiple barriers along the pathway (*i.e.* exposure route) that pathogens must follow to reach the population at risk. This approach proposes management by considering the whole exposure route and identifying the most effective measures/strategies to reduce the exposure, whether they are feasible in the given context, and if they could be implemented along the route (World Health Organization, 2006, Olivieri et al., 2014). The objective is the management of health risks by the accumulative effect of these barriers in terms of pathogens reduction. This new approach is evidence-based, leads to more flexibility than the traditional approach, and enables contextualized risk management (Keuckelaere et al., 2015).

A barrier can be defined as an event or a physical barrier that prevents transmission, reduces infectivity or decreases pathogens concentration (Nordin, 2007). Some examples of on-farm barriers for wastewater irrigation are wastewater treatment, on-farm wastewater treatment, drip irrigation, on-farm

produce rinsing, etc. For their implementation, the efficiency of the barrier must be studied and verified (World Health Organization, 2006).

The multi-barrier approach has been applied for wastewater irrigation to estimate current health risks and also to estimate risks in potential scenarios (Keuckelaere et al., 2015, Dalahmeh et al., 2016, Verbyla et al., 2016). Several information gaps were identified in many of them: i) lack of data on prevalence of pathogens in irrigation water and fresh produce, ii) use of little site-specific data, and iii) few studies included pathogens of the four basic groups of microbes (i.e. viruses, bacteria, protozoa and helminths) (Keuckelaere et al., 2015). Besides risk estimation, application of the approach for actual decision making regarding wastewater irrigation has been rather limited (Olivieri et al., 2014).

3.2 On-farm wastewater treatment systems

The processes that reduce pathogens from wastewater on farms have been called "no-treatment" or "on-farm measures" (World Health Organization, 2006). These on-farm measures can be divided into: i) on-farm water treatments and ii) water handling measures. Examples of water handling measures, described by Keraita et al. (2010), can be irrigation methods that minimize contact between wastewater and crops and scheduling of water application to allow die-off of microbes. On the other hand, on-farm treatments are based on processes used in conventional treatments, although their features – *i.e.* values of design parameters and pollutant removal capacity - widely differ (Keraita et al., 2014). Keraita et al. (2014) divided them as i) on-farm pond treatments and ii) on-farm filtration systems.

3.2.1 On-farm filtration systems

There are many different filter types that can be used for on-farm implementation. They can be grouped according to the type of filtering material in i) organic filters, ii) slow sand filters, and iii) riverbank filters (Keraita et al., 2014). Sand is the most commonly used filtration media, due to uniform removal rates of several pollutants; however, clogging occurrences caused by the implementation of suboptimal sand grain diameter have encouraged the search for other filtration media with similar performance level, widely available and lower risk of clogging (Dalahmeh et al., 2011, Keraita et al., 2014). Occurrence of clogging is minimal with organic filters because the effective diameter of filtering media can be carefully selected; however, it has been reported that a studied organic material (*i.e.* pine bark) is biodegradable in

the long-term (Dalahmeh et al., 2014). All the advantages and disadvantages should be considered in relation to their suitability for the on-farm context and the required pathogen reduction levels.

3.2.2 Riverbank filtration

Riverbank filtration is a technology used to treat surface water through physicochemical and biological processes that occur as water passes through riverbank soil. It consists of shallow wells for water extraction located close to a river and recharged by river water (Verbyla et al., 2016). Wastewater from the stream infiltrates the surrounding soil and is filtered on its way to the well. The wells can vary from fairly simple structures (hand-dug wells) to highly complex installations at a depth of several hundred metres (Levantesi et al., 2010, Freitas et al., 2017). It is considered a robust contaminant removal system because it can remove pathogens, bulk organic matter and several micropollutants (Sharma and Kennedy, 2017).

Since riverbank filtration (RBF) relies on soil for water treatment, the characteristics of the particular soils play a major role in its feasibility. The main soil requirement is a predominance of sand and gravel materials (Tufenkji et al., 2002). A high proportion of sand in the treatment zone is favourable since it provides adequate levels of both filtration efficiency and permeability (Tufenkji et al., 2002, Sprenger et al., 2014). The presence of these materials is typical in alluvial valley aquifers, however the type of original rock and degree of fluvial action also affect the composition of soils, resulting in the occurrences of clay layers or heterogeneity in the soil material (Tufenkji et al., 2002). Clay layers can prevent the flow of water, while a heterogenic soil type can lead to preferential flows through larger pores, reducing or nullifying treatment efficiency. When the river water is highly polluted, anoxic zones in the soil can be formed in which reducing conditions (*i.e.* low redox potential) predominate. Such reducing conditions can affect the stability of mineral surface coatings, which can decrease the filtering effect of the soil (Tufenkji et al., 2002). The travel time of water (*i.e.* the distance from the river to the extraction well) can also play a role as a longer distance will mean longer contact between the river water and the soil acting as a filter, and therefore higher removal of pollutants. Nonetheless, the presence of appropriate material is the predominant condition because most of the water treatment occurs in the first few metres if appropriate material is present (Sprenger et al., 2014).

3.2.3 Biochar filtration

Biochar as filtering material cannot be fully considered as an organic material, despite its vegetal origin. Biochar is the resulting material from charring of forestry or agricultural by-products at elevated temperatures under oxygen absence conditions (Dalahmeh, 2016). Charring (pyrolysis) produces a material (biochar) with characteristics to remove pollutants more efficiently than sand (e.g. specific surface area of biochar $\geq$ 170 m^2 g^{-1} and porosity porosity $\geq$ 60 %, compared to 0.15 m^2 g^{-1} and 34 % porosity of sand) (Dalahmeh, 2016). An additional advantage is that grain diameter of biochar can be selected from a wider range than sand; therefore clogging risks can be minimized. Research about the use of biochar as filtering media for wastewater treatment has recently started, but not yet for on-farm wastewater treatment (Dalahmeh, 2016).

3.3 Pathogen removal mechanisms in filters

Filters are rather complex systems in which several mechanisms and interactions take place. Pathogens are reduced in filters through two steps: retention and elimination (Stevik et al., 2004, Keraita et al., 2014). As reviewed by Stevik et al. (2004), straining and adsorption are the main mechanisms for retention of pathogens, and elimination depends on biotic and abiotic factors.

3.3.1 Straining

Straining can be defined as the physical blocking of pathogens movement through pores smaller than the size of the pathogens (Stevik et al., 2004). Consequently, removal of pathogens through straining is dependent on the size and shape of pathogens, as well as pore size of the filtering media. When biofilm grows, it forms a “sticky” layer on the filtering media, which reduces pore size and enables straining of smaller pathogens (Kadlec and Wallace, 2008). Besides the size of both pathogens and pore, the hydraulic loading rate can affect effectivity of straining for pathogen removal. High flow rates result in greater flow through bigger pores, which reduces possibilities of straining (Stevik et al., 2004).

3.3.2 Adsorption

Adsorption is the dominant mechanism of retention when the pathogens are smaller than the pore size (Stevik et al., 2004, Lalander et al., 2013).

Adsorption can be defined as the accumulation or concentration of substances – pathogens, in the present case – at the surface of filtering media, and it occurs as a result of the active forces of surface boundaries of both filtering media and pathogens (Çeçen and Aktaş, 2011). Both electrostatic and van der Waals forces are responsible for adsorption of pathogens to filtering media (Stevik et al., 2004). Consequently, any factor that alters such forces, either in pathogens or in filtration media, can influence adsorption. Therefore, characteristics of filtering media (*i.e.* size of pore, charge), pathogens (*e.g.* hydrophobicity and electrostatic charges on pathogen surface) and aqueous environment (pH, ionic strength, flow velocity) have direct influence on adsorption of pathogens to filtering media (Stevik et al., 2004). However, wastewater filtering adds some additional factors whose influence increases with the time of operation: organic matter content and biofilm development (Çeçen and Aktaş, 2011). The biofilm acts as an additional sorbent providing additional adsorption sites on the filtration media (Stevik et al., 2004). Role of organic matter in relationship to adsorption of pathogens is double sided. On the one hand, it promotes the development of biofilm by providing nutrients for bacterial growing; on the other hand, dissolved organic matter competes with pathogens for adsorption sites, eventually reducing their removal (Stevik et al., 2004).

3.3.3 Elimination

When pathogens are retained by either straining or adsorption, most are not able to survive in the environment. As reviewed by Stevik et al. (2004), biotic and abiotic mechanisms eliminate the retained pathogens in filtration media. The abiotic factors are moisture content (*i.e.* lower survival while drier environment), pH (*i.e.* higher survival at pHs close to neutral), temperature (*i.e.* lower survival while higher temperatures), and organic matter content (*i.e.* some pathogens might survive and even reproduce if they access nutrients from the organic matter). The biotic factors refer to both different survival times of different type of microorganisms and action of predators on retained pathogens.

4 Methodology

The first study in this thesis (PAPER I) described the agricultural system that uses diluted wastewater for lettuce irrigation in order to characterise the likely flows of pathogens along the system and therefore determine feasible interventions when adopting the multi-barrier approach. Furthermore, both studies (PAPERS I & II) analysed pathogen reduction from wastewater by filtration under on-farm conditions. Information about microbial removal rates from both filtration systems was used to analyse the feasibility of their implementation in similar contexts by analysing the requirements for implementation of the assessed technologies and likely changes in water management at farm level.

PAPER I characterised the agricultural system and assessed pathogen flows along it. The paper described the agricultural system according to likely pathogen flows, and also determined concentrations of pathogens and indicators in the components of the system (*i.e.* water sources, soil and produce). These data were also used to estimate the microbial removal rate of the existing riverbank filtration wells as part of the agricultural system.

PAPER II studied the reduction of microbial (*i.e.* bacteria, virus and oocysts) indicators from wastewater using biochar filters in the laboratory. The filters worked at loading regimes intended to emulate the field conditions characterised in PAPER I (*i.e.* high hydraulic loading rates and high electrical conductivity).

4.1 Riverbank system (PAPER I)

4.1.1 Description

The study site was located on the River Rocha (in Cochabamba's Sacaba municipality in Bolivia). The RBF wells were introduced in 2007 in the face of

growing contamination of the Rocha River and pressure from the authorities to prohibit vegetable irrigation with water from that river. The flow in the river is significantly impacted by both partially treated and untreated domestic wastewater from human settlements. The test site consisted of five production wells (Fig. 1). Three of them were classified as unprotected riverbank filtration (U-RBF) wells as they consisted of excavations with wellhead diameters greater than 5 m with no protection against external factors such animals or surface runoff (PAPER I). The remaining two were classified as protected riverbank filtration (P-RBF) wells, as the walls of these wells were lined with concrete rings (diameter ~1 m), surrounded by a layer of gravel/sand and with a lid covering the top (PAPER I). The monitored wells were located between 5 and 70 m from the river. The water table was approximately 4 m below soil level.

During this study, the wells were operated by farmers following their traditional operational schemes, *i.e.* constant pumping (every 2, 3 or 4 d) until almost complete depletion of their available volume for the irrigation of lettuce. Although the incoming flow was not measured, information provided by farmers and the volume of protected wells were used to estimate a flow of 130 L m^{-2} d^{-1} in the recharge zone.

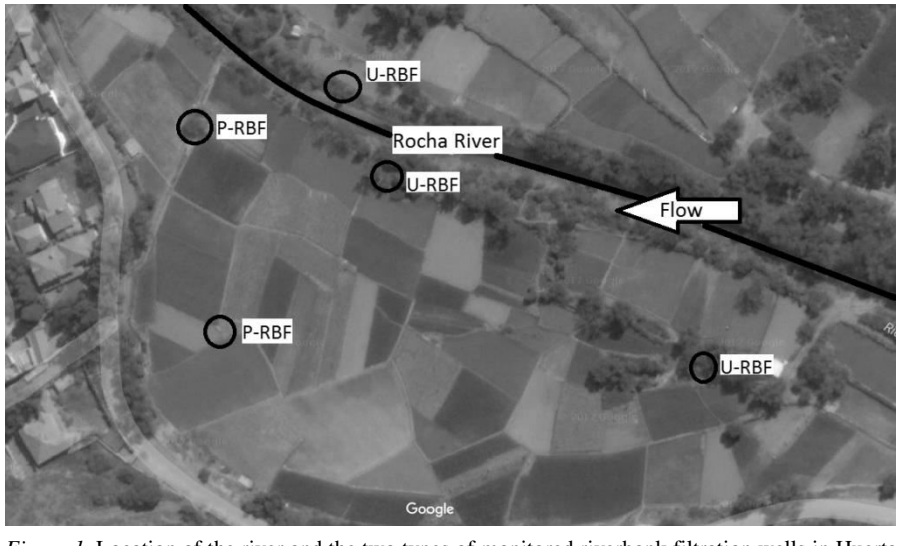

Figure 1. Location of the river and the two types of monitored riverbank filtration wells in Huerta Mayu community (Sacaba municipality, Bolivia). U-RBF: unprotected wells; P-RBF: protected wells

4.1.2 Selection of studied pathogens and microbial indicators

Somatic and F^+ specific bacteriophages were used as virus indicators because they include several groups of viruses that have different characteristics. Compared with enteroviruses, they are found in at least the same quantities, are approximately the same size as enteroviruses, and their movement in soils is similar. However, they can reproduce in the environment and lack correlation with all enteroviruses due to their wide range of sizes and isoelectric points (*Table 1*), producing both false positives and false negatives (Leclerc et al., 2000). In addition, bacteriophage assays are routinely used for environmental water research in Cochabamba (Verbyla et al., 2016).

E. coli were used as an indicator of enterobacteria. *E. coli* exhibit longer survival in the environment compared to many other pathogens and are specific to faecal contamination. This makes them a good indicator, although with a certain trend of overestimating actual concentrations of bacterial pathogens (Pachepsky et al., 2016).

Table 1. *Dimensions and relevant properties of studied microorganisms*

Organism	Dimensions (µm)	Relevant electrical properties	Hydrophobicity / Hydrophilicity
MS2	0.03 D	IP: 3.5	Hydrophobic
ϕX174	0.03 D	IP: 6.6 to 6.8	Weakly hydrophobic
Bacteriophages	0.02-0.09 D 0.006 W x 0.8 L	Variable according to strains	
Escherichia coli	1.1-1.5 W x 2-6 L		Variable depending on the strain
Enterococcus spp.	0.6-2.5 D		Depending on the strain: Most *E. faecalis* are hydrophobic Most *E. faecium* are hydrophilic
Saccharomyces cerevisiae	1-7 W x 5-10 L	IP: 3.9 to 4.2 Zeta potential: -30 -40 at pH 7.0	Variable depending on the strain

D: diameter, W: width, L: length, IP: isoelectric point
Sources: Jann et al. (1981), van Haecht et al. (1982), Zare et al. (1997), Schijven and Hassanizadeh (2000), Jin et al. (2001), Chang and Chang (2002), Shields and Farrah (2002), Willey et al. (2009), Feldmann (2011)

Giardia spp. cysts and *Cryptosporidium* spp. oocysts were used as indicators of pathogenic protozoa. The cysts and oocysts can remain latent for months at environmental conditions (Olson et al., 1999). Some species of both protozoa are human pathogens. However, the methods used in this study do not allow identification as to whether the species are human pathogens.

Helminth eggs were used as indicators of pathogenic helminths due to their long survival in the environment as eggs (Feachem et al., 1983). As with the

monitored protozoa, some species of helminths are human pathogens, but the methods used in this study do not enable them to be identified as such.

4.1.3 Sampling and analysis

Water samples for microbiological analysis were collected from the river and the selected wells twice a month throughout the study (*i.e.* prior to the rainy season in October-December 2014 and after the rainy season in March-May 2015). In the sampling before the rainy season, the samples were analysed for *E. coli*, bacteriophages (somatic coliphages and F+RNA), *Giardia* spp. cysts, *Cryptosporidium* spp. oocysts and helminth eggs (*Ascaris* spp. and *Taenia* spp.). In the post-rainy season, only bacteriophages and helminth eggs were analysed.

Composite soil samples were collected from five plots at planting (October 2014) and harvesting (December 2014). Each plot corresponded to each RBF well monitored. Also at harvesting, composite lettuce samples were collected from every plot. Soil and lettuce samples were analysed for bacteriophages, *E. coli* and helminth eggs. Triplicates of lettuce samples were analysed for helminth eggs only.

4.2 Biochar filters (PAPER II)

4.2.1 Experimental set-up of biochar filters and sampling

The performance of three different grain diameters (*i.e.* d10 of 1.4, 2.8 and 5 mm) of biochar filters were compared in terms of microbial removal at three different loading regimes. The loading regimes were determined by combining two different hydraulic loading rates (HLR) (*i.e.* 200 and 400 L m^{-2} d^{-1}) and two different electrical conductivities (*i.e. ca.* 500 and 1000 µS cm^{-1}). For the purposes of analysing microbial removal from wastewater, influent and effluent samples were collected and model organisms analysed after reaching steady-state conditions in terms of BOD_5 removal (PAPER II) (Dalahmeh et al., 2014). In order to collect data to explain microbial removal from wastewater, influent samples were collected and biochemical oxygen demand and electrical conductivity were analysed.

A mix of wastewater was used in this experiment (PAPER II). The "basic" diluted wastewater was prepared by dissolving 25 % (v/v) raw wastewater from the Kungsängen municipal sewage treatment plant (Uppsala, Sweden) in tap water. Salt, bacteriophages ϕX174 and MS2, and yeast were inoculated into

this mixture to ensure stable concentrations accordingly to the defined loading regimes, whereas both bacterial populations and organic load came exclusively from wastewater. All the samples were taken on a weekly basis. The first loading regime was carried out for 58 days at a hydraulic loading rate of 200 L m^{-2} d^{-1} and electrical conductivity of around 500 µS cm^{-1}. The second loading regime was performed for 42 days at a hydraulic loading rate of 200 L m^{-2} d^{-1} and an electrical conductivity of around 1000 µS cm^{-1}. The third loading regime was carried out for 34 days at a hydraulic loading rate of 400 L m^{-2} d^{-1} and an electrical conductivity of around 1000 µS cm^{-1}.

Physicochemical data were used to calculate inflowing electrical conductivity and the organic loading rate. Microbiological data were used to estimate the inflowing microbial rate and the reduction of each parameter on a weekly basis. Principal component analysis (PCA) was applied to identify the factors that have the greatest influence on the removal of the studied microbes. This identification was based on the graphic relationships between microbial reduction and the remaining factors, which were obtained when the two main principal components were plotted. In addition, generalised linear regression was used to quantitatively assess the influence of factors identified with PCA for both dataset types. All the graphical plots and analyses were carried out using R software (R Foundation for Statistical Computing, Vienna, Austria).

4.2.2 Selection of model organisms

To simulate virus removal through filtration, phage MS2 is considered a worst-case virus model due to its low isoelectric point (*Table 1*). Conversely, phage ϕX174 is considered easier to remove (*i.e.* due to its isoelectric point being close to neutral). Therefore, the use of both viruses as models provides a more accurate representation of actual pathogenic virus behaviour (Schijven and Hassanizadeh, 2000).

With regard to bacterial models for filtration, it has been demonstrated that hydrophobicity is a major factor in bacteria attachment to organic and inorganic surfaces, and therefore explains several processes for bacteria removal through filtration (Magnusson and Davies, 1980, Jann et al., 1981, Stevik et al., 2004). *E. coli* are considered an appropriate indicator for filtration experimentation because in addition to the above-mentioned characteristics, it includes many strains with different characteristics, including a wide range of hydrophobicity (*Table 1*). However, *Enterococcus* spp. also comprise a number of species and strains with different hydrophobicities, but with a more rounded shape than *E. coli* (*Table 1*). Hence, *E. coli* and *Enterococcus* spp. can be considered to be representative of a wide range of enterobacteria.

Saccharomyces ceverisiae was used as a model organism for the pathogenic protozoan *Cryptosporidium parvum* because it is non-pathogenic, widely available and has already been considered an appropriate surrogate for *Cryptosporidium parvum* oocyst transportation (Davies et al., 2008). *Cryptosporidium parvum* has been reported to have isoelectric point and size values similar to *S. cerevisiae*, with between 2.2 to 3.9 as the isoelectric point, zeta potential of between -38 and -40 and a size ranging between 3 and 5 µm (Ongerth and Stibbs, 1987, Hsu and Huang, 2002, Helmi et al., 2008). Furthermore, given its size (*Table 1*), it is understood that if *Saccharomyces cerevisiae* are removed, larger parasites such helminth eggs are also removed at at least a similar rate.

5 Results and discussion

5.1 Microbial concentrations in the agricultural system (PAPER I)

5.1.1 Lettuce

The median coliphage concentration in lettuce (5 pfu g^{-1}) final (*Figure 2*) (PAPER I) was similar to the concentrations (2 pfu g^{-1}) found by both Verbyla et al. (2016) in a previous study performed at the same location, and Song et al. (2006) who found about 10 pfu g^{-1} in lettuce irrigated with microbial-inoculated water. The median of *E. coli* (1.9 x 10^3 cfu g^{-1}) (*Figure 2*) (PAPER I) was similar to that obtained by Mhongole et al. (2016), who found an average of 1.3 x 10^4 cfu g^{-1} on Chinese cabbage that had been surface irrigated with water from a highly polluted river. With regard to helminth eggs, the median concentrations found in the present study were 1 *Ascaris* spp. egg g^{-1} (100 % samples above detection limit) and 0.5 *Taenia* spp. egg g^{-1} (8 % samples below detection limit) (*Figure 2*) (PAPER I). The median of *Trichuris* spp. egg concentration was 0, but this was because 54 % of the samples had values below the detection limit (*i.e.* <0.1 eggs g^{-1}) (PAPER I). The average *Trichuris* spp. concentration in produce samples was 0.1 eggs g^{-1} (*Figure 2*).

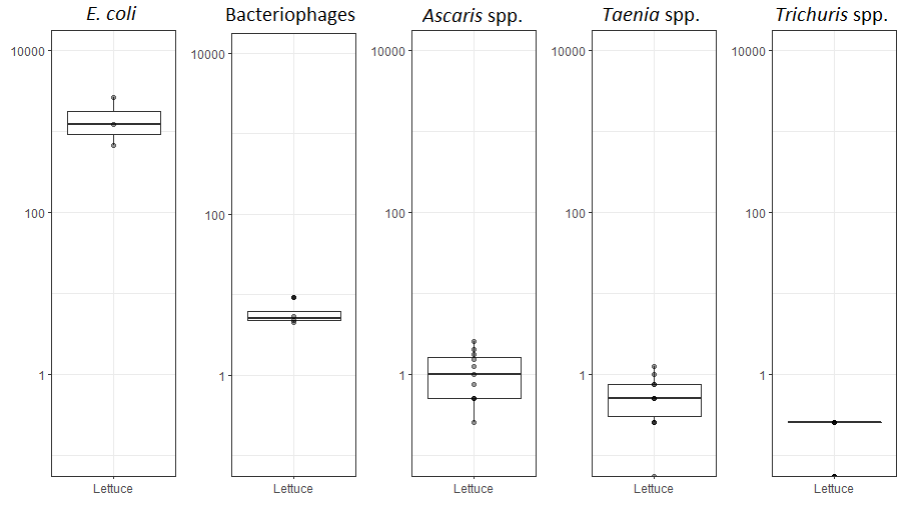

Figure 2. Occurrence of monitored microorganisms (fcu,pfu,cysts,eggs) per gram in samples of harvested lettuce.

All the lettuce samples analysed were above the detection limits for bacterial and viral indicators (PAPER I). For helminth eggs, *Ascaris* spp. had the highest prevalence in the analysed lettuce samples, followed by *Taenia* spp. and *Trichuris* spp. (PAPER I). This finding is consistent with studies by Gupta et al. (2009) and Woldetsadik et al. (2017), who found that *Ascaris* spp. prevalence in wastewater-irrigated lettuce is the highest compared to other helminth eggs and is supported by the high resistance of *Ascaris* spp. eggs in the environment (Feachem et al., 1983). The prevalence found in the present study (Paper I), *i.e.* 100 % *Ascaris* spp., 92 % *Taenia* spp. and 46 % *Trichuris* spp., was among the highest reported by studies carried out on wastewater-irrigated lettuce crops managed by farmers, such as Gupta et al. (2009), who found 36 % *Ascaris* spp. and 2 % *Trichuris* spp.

The concentrations of helminth eggs and bacterial and viral indicators in lettuce established in the present study were similar to or higher than those from previous studies carried out with wastewater irrigation. Furthermore, the presented results demonstrated a consistent prevalence (above 90 %) of almost all the types of microbes studied – except *Trichuris* spp. at 46 % – in most of the vegetable samples. According to Xu and Warriner (2005), this is indicative of high probabilities of faecal contamination of produce.

It was only possible to perform comparisons for *Ascaris* spp. and *Taenia* spp. when comparing the effect on lettuce of water quality in unprotected and protected riverbank wells due to the limited number of samples of the remaining organisms (*i.e. E. coli*, Bacteriophages and *Trichuris* spp.). Concentrations of *Ascaris* spp. and *Taenia* spp. in lettuce were significantly

higher when the crop was irrigated with water from unprotected riverbank wells (*i.e.* medians of 1.9 *Ascaris* eggs g^{-1} and 0.8 *Taenia* cysts g^{-1}) than with water from protected wells (*i.e.* medians of 0.5 *Ascaris* spp. eggs g^{-1} and 0.3 *Taenia* spp. cysts g^{-1}) (PAPER I). Although mechanisms of crop contamination with pathogens are not fully understood, it has been widely demonstrated that different water qualities used for irrigation lead to different propensities of microbiological contamination of the crops, as reviewed by Keuckelaere et al. (2015). Considering the levels of the microbes found on lettuce and the minimal infective doses of human-pathogenic *Ascaris* spp (*i.e.* 1-10 eggs) reported, consumption of the studied lettuce would result in a high risk of infection irrespective of the water source (Bitton, 2005, World Health Organization, 2006) (PAPER I).

Apart from water sources, there are other factors that can explain the microbial concentrations on produce identified in the agricultural system. The irrigation is performed by furrowing the plot, which (together with flooding) has been widely proven to be a risk factor in the transference of pathogens from wastewater to leaf vegetables, as reviewed by Uyttendaele et al. (2015). The time between final irrigation and harvest varies between 1 and 6 days, depending on several factors related to the trade of the produce and the availability of labourers. Significant die-off of some pathogens on crops might occur depending on the time between watering and harvest (World Health Organization, 2006).

5.1.2 Soil

Median concentrations of the studied microbes in soil samples were 1.6e+04 *E. coli* cfu gr^{-1}, 64 coliphage pfu gr^{-1}, 2.5 *Giardia* spp. cysts gr^{-1}, 1.8 *Cryptosporidium* spp. oocysts gr^{-1}, 6.6 *Ascaris* spp. eggs gr^{-1}, 4.4 *Taenia* spp. eggs gr^{-1} and 2.9 *Trichuris* spp. eggs gr^{-1}. Compared with the US-EPA classification system of biosolids from 2002, the obtained results correspond to class B biosolids (*i.e.* less than 10^6 thermotolerant coliforms CFU gr^{-1}, and the presence of *E. coli*, *Cryptosporidium* spp., *Giardia* spp. and enteroviruses), which restricts its use to application on forest land that has limited public or livestock access (Santamaria and Toranzos, 2003). This would mean that the studied soils have concentrations of microbes at levels similar to treated sewage sludge. This also may be understood as a warning of significant health risks if such soils are used to crop leaf vegetables, supported by some evidence about the contamination of vegetables with pathogens from soil. Jimenez et al. (2006) found concentrations of between 2 to 4 log10 *Salmonella* cfu g^{-1} as well as 1-14 helminths eggs g^{-1} in spinach leaves where soils had been fertilised

with sludge from Ecosan toilets. It therefore seems evident that concentrations of microbes found in the studied soils indicated high probabilities of faecal contamination in the agricultural system. Nevertheless, it should be noted that a more realistic evaluation of the risks associated with pathogen content in soil can be obtained by performing microbial risk assessments in different exposure scenarios (Gerba and Smith, 2005).

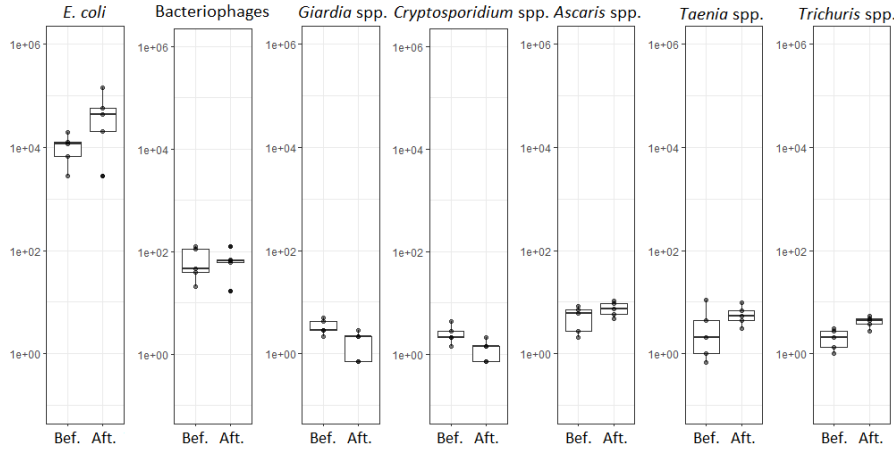

Figure 3. Occurrence of studied microorganisms (cfu, pfu, cysts, eggs) per gram in soil samples; "Bef": soil samples before the transplantation; "Aft": soil samples at harvest

No clear pattern was identified when comparing microbial concentrations in soil at planting with those at harvesting (PAPER I) (*Figure 3*). This could be explained by the confluence of several factors that influence the fate of microorganisms in soils. First, the long-term survival of some of the studied microbes in soil (*i.e.* several months for helminth eggs, up to 12 weeks for protozoa, and between 11 and 170 days for enteric viruses) hinders the interpretation of data from only one lettuce cropping season (60 days on plot) (PAPER I) (Guan and Holley, 2003, Santamaria and Toranzos, 2003, Rzeżutka and Cook, 2004). Furthermore, a number of environmental factors such as soil moisture, soil saturation state, texture of soil, soil pH, cation concentrations, soluble organic compounds, temperature, exposure to sunlight and rainfall characteristics impact the microbial fate in soil (Santamaria and Toranzos, 2003). The studied plots were continuously (every 2-3 days) provided with different amounts of water, thus continually affecting the total amount of organic matter and pathogens applied per plot. Moreover, different amounts and types of manure had also been applied for many years prior to the study (PAPER I). The level of manure treatment is uncertain, and it is mostly bought from farms where cattle are fed with raw wastewater-irrigated fodder and

consequently may ingest high numbers of helminth eggs (Huibers et al., 2004, World Health Organization, 2006).

Several factors have an impact on the fate of pathogens in the soil: i) protection against UV radiation in the soil, which increases when vegetables grow, but does not occur throughout the cropping season; ii) soils in the study zone having at least 20 % clay and 20 % sand (Metternicht and Fermont, 1998), with the clay content favouring retention of microbes, while sand favours microbial washing out (*i.e.* leakage); iii) a soil washing effect during the rainy season; iv) constant agricultural labour (*e.g.* tillage, weeding and earthing up), which can increase the exposition of microbes in soil to adverse environmental factors such as UV radiation or drier environments in topsoil (PAPER I). Both the variability and temporal scale on which such factors act in soil hamper understanding of the dynamics of pathogens in soil based on only the short (7-8 weeks) crop season studied.

No significant difference was found between microbial concentrations in soils irrigated with water from unprotected wells compared with soils irrigated with water from protected wells (PAPER I), which is in contrast to the significant difference found between microbial concentrations in lettuce irrigated with different water qualities (*i.e.* protected and unprotected wells) (PAPER I). This can be explained by i) the greater complexity of the soil than of produce as environments for pathogens (explained above), ii) the time during which soils have been receiving pathogenic loads, and iii) cross-contamination from the use of lower-quality water sources (*i.e.* other wells or the river). Soils act as a pathogen recipient over several years (Santamaria and Toranzos, 2003) as they are fed with pathogens at each irrigation and also preserve pathogens from earlier irrigations or even several cropping seasons before. This may lead to an established background content of pathogens in soil at every cropping season. In contrast, due to its short lifecycle (6-8 weeks), lettuce would mainly act as a recipient of microbes from wastewater. In terms of cross-contamination, it has been noted that the volume required for irrigation sometimes exceeds the recharge flow of both type of wells (*i.e.* the driest months of July-October), which could lead to irrigation with river water of even lower quality than the riverbank wells (PAPER I) (*Figure 3*). Therefore, it seems that in the short term (*i.e.* one lettuce cropping campaign), water quality is the main driver of the hygienic quality of lettuce. However, a longer-term monitoring of microbes in soil could clarify the hygienic impact on the agricultural system.

5.1.3 Water sources

The concentrations of studied microbes from the two types of wells mostly exceeded the limits set by several national regulations for irrigation of crops eaten raw (*i.e.* 10^3 *E. coli* L^{-1} and less than 1 nematode egg L^{-1}), according to the report by Uyttendaele et al. (2015) (PAPER I). The exceptions were mostly water samples from unprotected wells (*Figure 4*) for *E. coli*, although none of them were below the limit for helminth eggs.

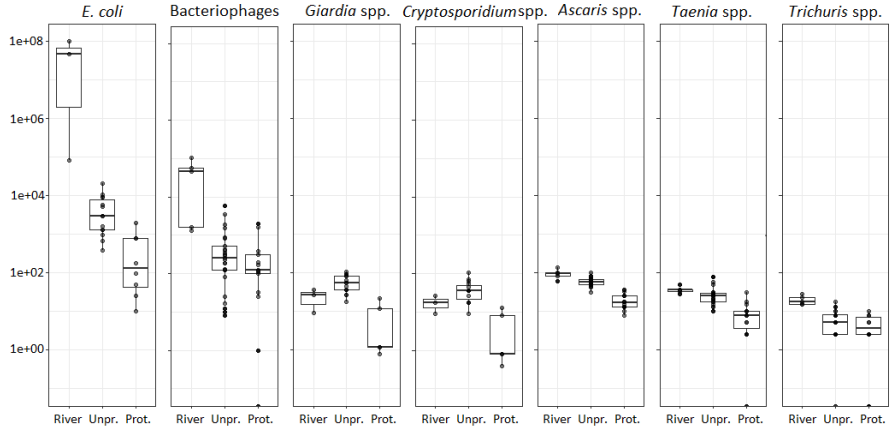

Figure 4. Occurrence of studied microorganisms (cfu, pfu, cysts, eggs) per liter in water samples; "Unpr.": water samples from unprotected wells; "Prot.": water samples from protected wells

The concentrations of the studied microbes were significantly higher in water from the unprotected wells than from the protected wells, except for coliphages (PAPER I) (*Figure 4*). Indeed, median concentrations in unprotected wells were $5.6x10^1$ *Ascaris* spp. eggs L^{-1}, $2.6\ x10^1$ *Taenia* spp. eggs L^{-1}, 5 *Trichuris* spp. eggs L^{-1}, $5.4x10^1$ *Giardia* spp. cysts L^{-1}, $3.6x10^1$ *Cryptosporidium* spp. cysts L^{-1}, $2.9x10^3$ *E. coli* fcu L^{-1} and $2.5x10^2$ coliphages pfu L^{-1} (PAPER I) (*Figure 4*). All the analysed water samples from the unprotected wells were above the detection limit of all the microbes being evaluated. Besides the significant difference in medians, several samples from protected wells were below detection limits (BDL): $1.7x10^1$ *Ascaris* spp. eggs L^{-1}, 6 *Taenia* spp. eggs L^{-1} (17 % BDL), 3 *Trichuris* spp. eggs L^{-1} (44 % BDL), 1 *Giardia* spp. cysts L^{-1}, 1 *Cryptosporidium* spp. cysts L^{-1} (20 % BDL), $1.4x10^2$ *E. coli* fcu L^{-1} and $1.1x10^2$ coliphages pfu L^{-1} (18 % BDL) (PAPER I) (*Figure 4*). This difference could be attributed to the protection provided by the cover and concrete lining, and enhanced filtration mechanisms provided by the layer of sand in protected wells. The cover and lining limit transportation of microbes from soil by surface runoff, which is a major contamination

mechanism of well water with parasites (PAPER I). Sand forms an homogenous layer of filtration media that may counteract preferential flows of subsurface water due to soil heterogeneity (macropores) as well. However, virus removal through sand filtration is not significant at short retention times (less than 1 $\log_{10}$ unit at HRT shorter than 30 m) due to the small size of viruses (Aronino et al., 2009) (*Table 1*). This may explain why only virus concentrations were not significantly different when comparing water from protected and unprotected wells.

Furthermore, some other characteristics of unprotected wells could explain the differences in microbial concentrations. The wellheads of unprotected wells are at least five metres in diameter, which means a larger surface than the protected ones, increasing the risk of the intrusion of surface water. Unlike protected wells, vegetation grows inland of the unprotected wells (bushes, trees), which represents a permanent source of both organic matter and shade (protection against UV radiation) for the water, potentially providing protection against UV radiation to the microbes and as, a consequence, leading to their increased survival. Due to its configuration, people and animals can easily access the shore, which could contaminate the well water. Despite these issues, RBF appear to be a feasible alternative as a barrier to improve the quality of polluted water (compared to untreated river water), while protected wells offer even greater protection.

5.1.4 Summary of the hygienic state of the agricultural system

Evidence of faecal contamination has been found throughout the system studied (PAPER I). With regard to the elements in the system:

- treated manure was not tested for microbes, and it remains a possible input of pathogens into the system when incorporated as a soil amendment (Huibers et al., 2004, Jimenez et al., 2006)
- the two types of riverbank wells studied have statistically different hygienic qualities. Nevertheless, both wells were considered a major source of pathogens into the system because their hygienic quality has a direct effect on microbial prevalence in lettuce and also due to the high intensity and type (every 2-3 days by furrow) of irrigation (PAPER I). The wells were also a likely reservoir of pathogens washed out of the soil
- soil was considered the major reservoir of pathogens in the system. The prevalence and variety of microbes found and the characteristics (intensity, high volumes, presence of associated organic matter) of irrigation lead to the belief that soil provides pathogens with a long-term buffer against

environmental factors (PAPER I). As a reservoir, it may contribute to microbial concentrations in wells through run-off and could also re-contaminate water during irrigation. Although soil-to-crop contamination is theoretically possible (Jimenez et al., 2006), the results showed that the impact of soil as a source of pathogens in lettuce is not as significant as the impact of water

- lettuce was the main output of pathogen flows in this study. The levels of produce contamination are significantly related to water quality, and might be related to soil in the long run. The levels and variety of microbes found were evidence of faecal contamination of the produce, which implies increased health risks to consumers.

These findings have two implications for management. First, the risk to consumers could be managed in the short term by improving water quality. Second, if water quality were improved, full cleaning of the system would not be possible in the short run, due to soil's role as a reservoir for the system. This would have implications for the health of farmers, given their increased exposure to water and soil while performing manual agricultural tasks in the studied system.

5.2 Microbial removal with filtration (PAPERS I & II)

5.2.1 Bacterial reduction

Bacterial removal from riverbank filtration was about 4 $\log_{10}$ based on the difference between medians of *E.coli* from the river and unprotected wells (*Figure 5*). Similar *E.coli* removal has been obtained in RBF wells located 30-37 m from different rivers monitored for a year (Weiss et al., 2005), and slightly less (*i.e.* 3 log *E. coli*) in wells 26 m away (Gutiérrez et al., 2017). The cited obtained levels are equivalent to some wastewater treatment options, and could become a significant barrier for pathogens (World Health Organization, 2006). However, variability in data was also observed in the bacterial concentrations (*Figure 5*). Variability can be due to several different factors, such as soil texture variations, different distances to the river and environmental changes (Sprenger et al., 2011, Keraita et al., 2014). Therefore, these results need to be confirmed in more detailed studies and longer-term monitoring.

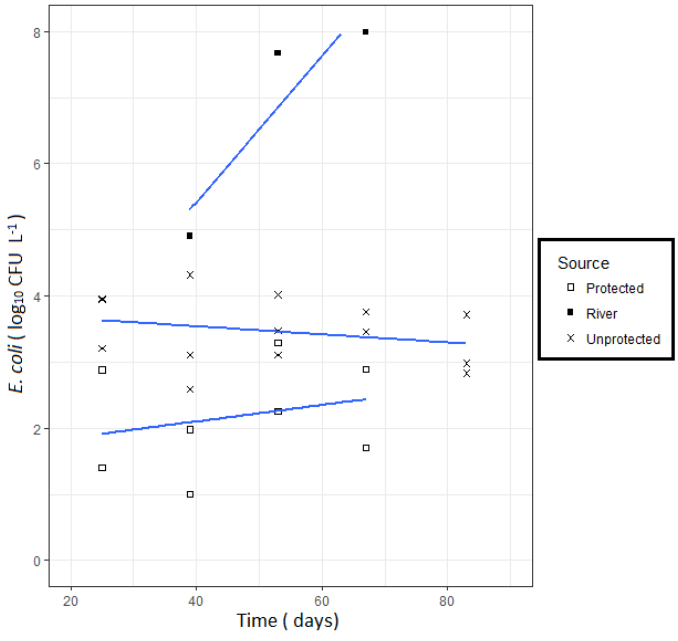

Figure 5. Concentrations of *E. coli* found in the three studied water sources: river, unprotected wells and protected wells over the time of the studies

Protected wells consistently reported lower *E. coli* concentrations than unprotected wells, resulting in *circa* one $\log_{10}$ unit of difference compared to the unprotected wells (*Figure 5*) (PAPER I) at 130 L m^{-2} d^{-1}. These results were similar to those obtained by Jiménez-Cisneros et al. (2001) who reported a reduction of 1 $\log_{10}$ unit in both *Salmonella* spp. and faecal coliforms in rapid sand filters at 300 m^3 m^{-2} d^{-1}. The sand/filter layer in protected wells was considered comparable to rapid sand filtration because both operate at high HLRs treating wastewater as it passes through. The difference in bacterial removal between protected and unprotected wells could at least partly be due to the improved filtering effect of the sand/gravel layer and lining (PAPER I).

Similar to the difference between unprotected and protected wells, reductions in both *E. coli* and *Enterococcus* spp. were about 1 $\log_{10}$ unit in intermittent biochar filters at high HLRs (200 and 400 L $m^{-2}d^{-1}$) (*Figure 6*). However, only filters with biochar d_{10} of 1.4 mm reached such reduction levels (*Figure 6*) (PAPER II). As reviewed by Stevik et al. (2004), it has been widely demonstrated that the size of filtering media is often directly related in terms of pore size and surface area. Smaller grain diameters provide more sites for bacterial adsorption but also enhance straining of bacteria by providing more pore sizes within the range of bacterial cells (Stevik et al., 2004). With regard to biochar filters, Dalahmeh (2016) reported significantly higher hydraulic

retention times with d_{10} 1.4 mm than d_{10} 2.8 mm, which suggests a higher proportion of smaller pores in d_{10} 1.4 mm biochar. This is in good agreement with the inverse relationship found between grain diameter and removal of both the bacteria studied (*Figure 6*), and would explain why only filters with the smallest grain diameter removed bacteria significantly.

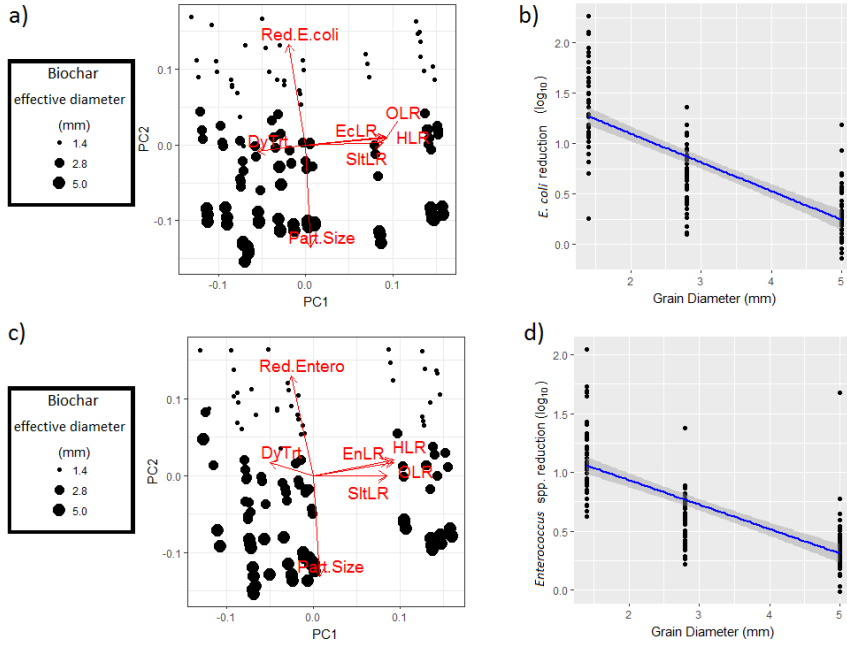

Figure 6. Graphical representation of principal component analysis performed for a) *E. coli* and c) *Enterococcus* spp. respectively at high HLRs (200 and 400 $Lm^{-2}d^{-1}$); linear regression model fit for reduction of b) *E. coli* and d) *Enterococcus* spp. respectively, according to grain diameter of the filtering media. "Red.E.coli": *E. coli* reduction; "Red.Entero": *Enterococcus* spp. reduction; "EcLR": *E. coli* loading rate: "EnLR": *Enterococcus* spp. loading rate; "OLR": organic loading rate; "HLR": hydraulic loading rate; "Part.Size": Effective diameter of biochar; "DyTrt": Time of every loading regime; "SltLR": Inflowing electric conductivity

The results of the PCAs performed also showed the minor role played by the other analysed factors (*Figure 6*). The contribution made by all the analysed factors did not increase the variance explanation in a linear regression model with only grain diameter to explain bacterial removal (*i.e.* 58 % for E. *coli* and 52 % for *Enterococcus* spp.) (PAPER II). A number of studies reviewed by Stevik et al. (2004) have demonstrated that high hydraulic loading rates result in higher flow and flow velocity through larger pores. This leads to a decrease in bacterial straining, reduced contact opportunities between bacteria and adsorption sites, and thinner biofilm layers (Liu and Tay, 2002,

Stevik et al., 2004). Since the influences of organic loading rate and microbial loading rate in bacterial removal are mainly adsorption-mediated, a decreased role of the analysed factors was expected. This decreased role does not mean that such factors do not have an impact at high HLRs (Kadlec and Wallace, 2008) (PAPER II), but does indicate the increasing relative importance of grain diameter.

The difference found between *E. coli* and *Enterococcus* spp. in variance explanation from grain diameter (PAPER II) can be explained by the difference in cell shape. *Enterococcus* spp. are mostly spherical and *E. coli* strains are predominantly long and thin, which would make *E. coli* more easily strained (Weiss et al., 1995, Willey et al., 2009). This might imply that straining is a reduction mechanism that continues to act at high HLRs. The findings on bacterial reduction by filtration (PAPERS I & II) and the studies mentioned above indicate that grain diameter of filtering media is a major factor in bacterial reduction by filtration at high HLRs (above 130 L m^{-2} d^{-1}), and that an effective grain diameter of 1.4 mm in biochar filters would remove 1 $\log_{10}$ bacteria at such HLRs.

5.2.2 Bacteriophages reduction

The reduction in coliphages (*i.e.* somatic and FRNA) from riverbank filtration was *circa* 2 $\log_{10}$ when compared with the medians from the river and unprotected wells (PAPER I) (*Figure 7*). Such a reduction is lower than the reduction of 5 $\log_{10}$ measured at 4 metres off a heavily polluted river in Delhi, India (Sprenger et al., 2014), and 5-6 $\log_{10}$ measured at 25 m off the Meuse river in the Netherlands (Tufenkji et al., 2002). However, both studies were performed in extraordinarily appropriate places for RBF, given the estimated travel time of 6 days through a 96 % sandy soil (Sprenger et al., 2014) and the sand filters implemented for aquifer recharge (Tufenkji et al., 2002). Since viruses are small (20-95 nm for most groups of indicator bacteriophages), nano and micropores are necessary for their removal (Leclerc et al., 2000, Schijven and Hassanizadeh, 2000). A likely explanation for the level of bacteriophage reduction found in this study is a soil texture with a higher proportion of larger pores compared with the cited reports. Nonetheless, the obtained levels could become a significant barrier for viral pathogens.

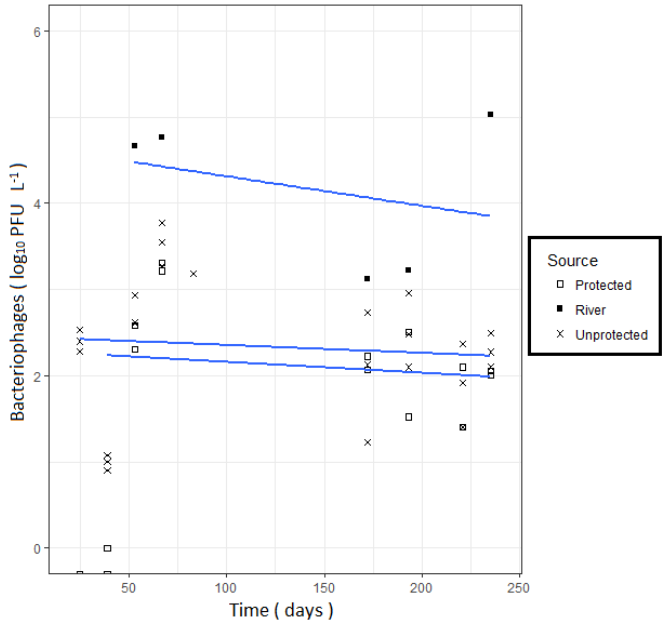

Figure 7. Concentrations of coliphages (pfu L^{-1}) found in the three studied water sources: river, unprotected wells and protected wells over the time of the studies

Although concentrations of bacteriophages in protected wells were generally lower than in unprotected wells, the difference was not statistically significant (*Figure 7*). (PAPER I). This indicates a minor filtering effect on the virus from the sand/gravel layer and lining in protected wells. Such minor effects contrast with Sprenger et al. (2014), who reported an average reduction of 4 $\log_{10}$ in coliphage concentration through 1 m of 96 % sandy soil. An explanation for this might be the coarser texture of sand/gravel mixture compared with the 96 % sandy soil in the study of Sprenger et al. (2014) (PAPER I). A coarser texture has a higher proportion of macropores, which is critical for viral removal through filtration media because of their size (Schijven and Hassanizadeh, 2000).

Similarly to monitored bacteriophages in the RBF system, the reduction of phage MS2 was less than 1 $\log_{10}$ unit in the studied biochar filters at high HLRs (200 and 400 L $m^{-2}d^{-1}$) (*Figure 8*) (PAPER II). These results are in good agreement with the ones reported by Young-Rojanschi and Madramootoo (2014) who found an average removal of 0.85 $\log_{10}$ MS2 in intermittent biosand filters with d_{10} of 0.17 mm at 256 L $m^{-2}d^{-1}$. However, the reduction of phage ϕX174 was 0.9-1.4 $\log_{10}$ for filters with biochar d_{10} of 1.4 mm; filters with a larger grain diameter removed less than 1 $\log_{10}$ (PAPER II). Heistad et al. (2009) obtained a similar reduction in phage ϕX174 (around 1.6 $\log_{10}$) in

intermittent filters with expanded clay aggregate d_{10} of 2-4 mm (Filtralite™) as filtering media, at 132-254 L $m^{-2}d^{-1}$. The explanation for this slight difference (*i.e.* 0.9-1.4 $\log_{10}$ biochar against 1.6 $\log_{10}$ Filtralite™) could be the higher porosity, higher surface area and different pH of Filtralite™ compared to biochar, which provides improved adsorption conditions. The differences in removal between the viruses were similar to the findings of Sidibe (2014) for biochar filters at HLR of 34 L $m^{-2}d^{-1}$, and could be explained by marked differences in isoelectric points and hydrophobicity (Schijven and Hassanizadeh, 2000)(PAPER II). Regardless of the surface properties, an inverse relationship was found between grain diameter and removal of both model viruses (PAPER II). This is in line with Dalahmeh et al. (2011), who reported a significantly higher reduction of MS2 in biochar filters with d_{10} of 1.4 mm compared to d_{10} of 2.8 mm at 34 L $m^{-2}d^{-1}$. This difference was attributed to a higher straining effect from smaller grain diameters.

The PCA of the data indicated that virus removal was affected differently by the evaluated factors for the two model viruses (*Figure 8*) (PAPER II). Similarly to what was found for the bacterial reduction, the contribution of all the analysed factors did not significantly increase the variance explanation (46 %) compared to that (45 %) of a linear regression model with only grain diameter in phage ϕX174 removal (PAPER II). As with bacteria, increased HLR induces higher flow through larger pores, reducing contact opportunities between the virus and adsorption sites, which thus reduces the influence of organic loading rate and phage ϕX174 loading rate (Schijven and Hassanizadeh, 2000, Stevik et al., 2004) Furthermore, Lalander et al. (2013) reported that OLR did not have any influence on ϕX174 removal in activated charcoal filters at low HLR, which was explained by the need for OLRs higher than 70 g BOD_5 m^{-2} day^{-1} to compete with phages for adsorption sites.

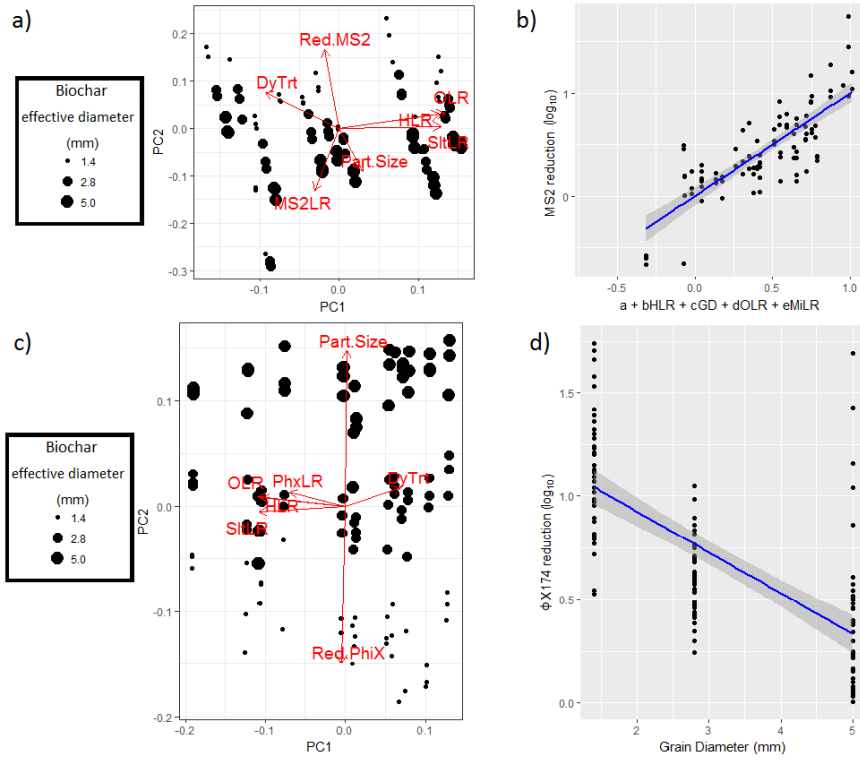

Figure 8. Graphical representation of principal components analysis performed for bacteriophages a) MS2 and c) ϕX174, at high HLRs (200 and 400 $Lm^{-2}d^{-1}$); and of linear regressions model fit for reduction of b) MS2 and d) ϕX174, respectively. "Red.MS2": phages MS2 reduction; "Red.PhiX": ϕX174 phages reduction; "MS2LR": MS2 phages loading rate; "PhxLR": ϕX174 phages loading rate; "OLR": organic loading rate; "HLR": hydraulic loading rate; "Part.Size": Effective diameter of biochar; "DyTrt": Time of every loading regime; "SltLR": Inflowing electric conductivity

In contrast to what was found for ϕX174 and bacteria, bacteriophage MS2 reduction was affected by several factors at high HLRs (*Figure 8*). Besides grain diameter of the filtering media, principal component analysis showed a direct relationship between MS2 reduction and time, and an inverse relationship with MS2 loading rate. Increased bacteriophage MS2 reduction with time has been reported by Sidibe (2014) for biochar filters at low HLRs. Increased removal with time is normally related to the development of biofilm. Since such a development seems to be limited by high HLRs, the direct relationship found here could be expressing a different factor that was not included in this study. Increasing the MS2 loading rate expresses a higher number of bacteriophages passing through the filtering media per time unit. A higher number of MS2 bacteriophages means higher competence for

adsorption sites. In addition, if adsorption occurs, MS2 creates weaker bonds than other viruses and bacteria at neutral pHs, due to its low isoelectric point. This was demonstrated by Bales et al. (1991), who reported significantly higher MS2 detachment from sand when raising the pH from 5, compared to bacteriophage PRD-1. Therefore, MS2 is considered a worst-case virus model, and a better understanding of virus behaviour in filters is obtained when another virus model is also studied (Schijven and Hassanizadeh, 2000). The findings on microbial reduction by filtration (PAPERS I & II) and the studies mentioned above indicate that the grain diameter of filtering media is a major factor in the reduction of some viruses by filtration at high HLRs (>130 L m^{-2} d^{-1}), and that an effective grain diameter of 1.4 mm in biochar filters would remove 0.5-1.3 $\log_{10}$ virus pfu at such HLRs.

5.2.3 Helminth eggs

Medians of helminth egg concentrations were similar in the river and unprotected wells (94 A*scaris* spp. and 34 *Taenia* spp. eggs L^{-1} in river and 56 *Ascaris* spp. and 25 *Taenia* spp. eggs L^{-1} in unprotected wells), although the river had slightly higher values (PAPER I) (*Figure 9*). Furthermore, protected well concentrations (17 *Ascaris* spp. and 6 *Taenia* spp. eggs L^{-1}) were found to be significantly lower than the concentration of helminth eggs in unprotected wells (PAPER I). The concentrations were far higher than the 0.05 helminth eggs L^{-1} reported by Levantesi et al. (2010) for an aquifer-recharging well, and the absence of helminth eggs reported by Freitas et al. (2017) for a drinking water well receiving water from a polluted river. These low levels can be explained by some characteristics of the studied wells: the well cited by Levantesi et al. (2010) is 350 m away from the injection point of water, and the well studied by Freitas et al. (2017) is 15 cm in diameter, with a gravel filter around it and a cement seal from the wellhead to 3 m deep. The similar levels of helminth concentrations found in the river and the unprotected wells suggest preferential flows through macropores or even cracks in the subsoil, given the size of helminth eggs. The difference in helminth concentration between protected and unprotected wells may be due at least partly to the improved filtering effect of the sand/gravel layer and lining.

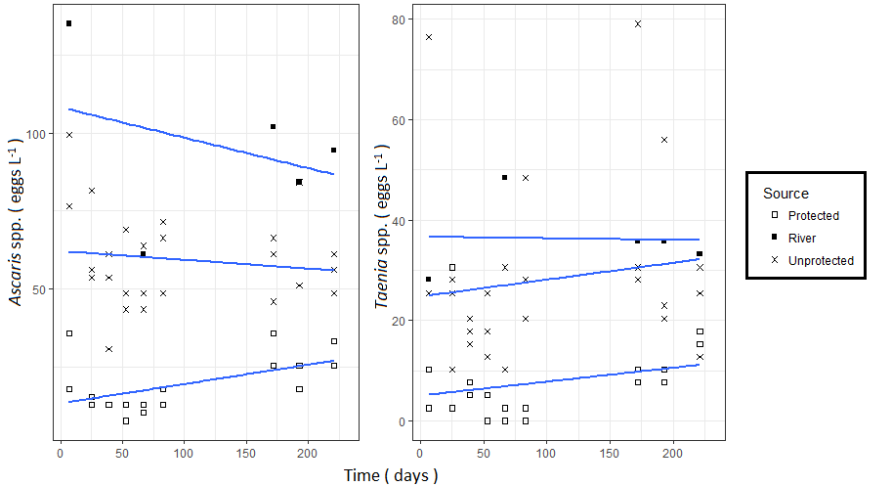

Figure 9. Graphical representation of the concentrations of a) Ascaris spp. (eggs L^{-1}) and b) Taenia spp. (eggs L^{-1}) found in the three studied water sources: river, unprotected wells and protected wells over the time of the studies

5.2.4 Protozoan cysts/oocysts

Concentrations of both *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts found in the river were consistently lower than those found in the unprotected wells, but higher than in the protected wells (*Figure 10*). Medians of both protozoa concentrations from unprotected wells (54 *Giardia* spp. cysts L^{-1} and 36 *Cryptosporidium* spp. oocysts L^{-1}) were double the respective medians from the river (27 *Giardia* spp. cysts L^{-1} and 18 *Cryptosporidium* spp. oocyst L^{-1}), although it was not possible to perform a statistical comparison (PAPER I). In contrast, medians from unprotected wells were significantly higher than medians in protected wells, which were all less than 10 cysts/oocysts L^{-1}. Protozoan reduction from protected wells was in good agreement with the 1 $\log_{10}$ unit reduction of *Giardia* spp. and *Cryptosporidium* spp. oocysts reported in three riverbank wells that receive water from a polluted river located 30 m away (Weiss et al., 2005). The similar values found for unprotected wells and the river can partly be explained by the protective effect of trees and floating *Lemna* spp. against UV inactivation in water in unprotected wells (PAPER I), but also by the heterogeneity of the filtering media (*i.e.* soil). Removal of *Cryptosporidium* spp. oocysts through filtration occurs by physical straining and sedimentation (Tufenkji et al., 2004). Straining might be reduced by preferential flows to macropores in filtering material, as demonstrated by Darnault et al. (2004) in sandy and silt loam soils. Regarding sedimentation, Tufenkji and Elimelech (2005) have demonstrated

that oocyst sedimentation is more accurately modelled when considering the existence of both repelling and attracting electrical charges in the filtering media: repelling zones are linked to slower rates of sedimentation. Heterogeneity of filtering material might result in both zones with different electrical charges, therefore with different sedimentation rates of oocysts and macropores. Both could explain the reduced removal rate of protozoa in unprotected wells. With regard to protected wells, the filtering effect from the additional sand/gravel layer and lining might also provide a more homogenous filtering media, enhancing straining and sedimentation of oocysts.

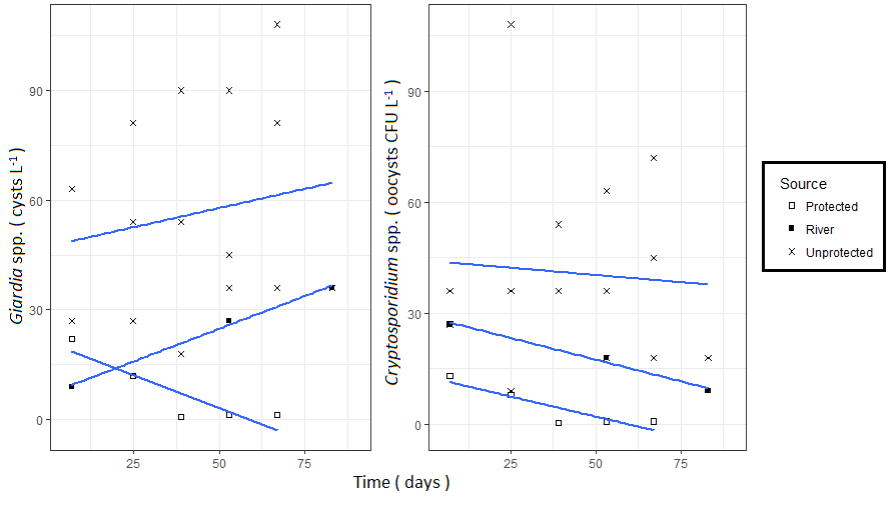

Figure 10. Concentrations of a) Giardia spp. (cysts L^{-1}) and b) Cryptosporidium spp. (cysts L^{-1}) found in the three studied water sources: river, unprotected wells and protected wells over the time of the studies

The results of the biochar filters experiment showed a reduction of 1-1.7 $\log_{10}$ *S. cerevisiae* at high HLRs (200 and 400 L $m^{-2}d^{-1}$) in filters with biochar d_{10} of 2.8 mm and smaller (*Figure 11*) (PAPER II). Such results are lower than 3-4 $\log_{10}$ *Cryptosporidium* spp. removal with intermittent sand filters d_{10} of 0.16 - 0.9 mm at 40 - 100 L m^{-2} d^{-1} reported by Logan et al. (2001). They also identified a significant inverse relationship between *Cryptosporidium* spp. reduction and both HLR and grain diameter, which is in line with the result of Kim et al. (2010) for *Cryptosporidium parvum* removal in porous media. This is in good agreement with the explanations given in Section 5.2.1. Smaller protozoan reductions could be expected in sand filters at 200-400 L m^{-2} d^{-1} and larger grain diameters, resulting in reductions similar to those obtained in the present study (PAPER II).

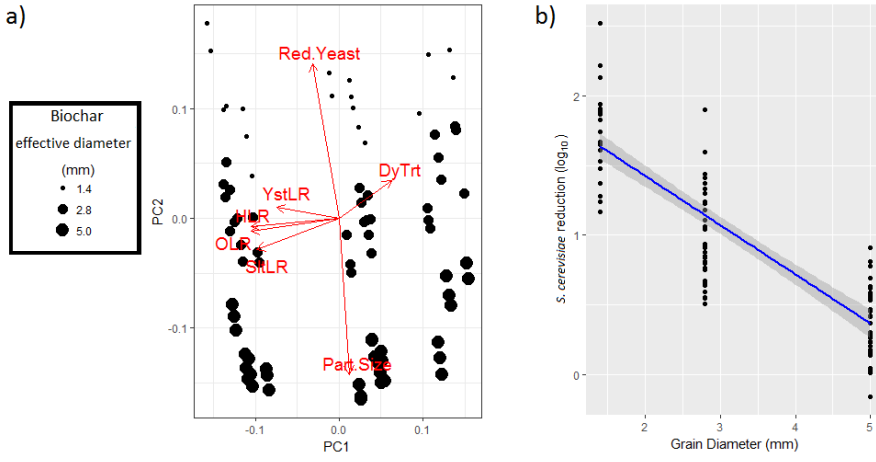

Figure 11. Graphical representation of a) principal components analysis performed for *Saccharomyces cerevisiae* at high HLRs (200 and 400 L m^{-2} d^{-1} ; b) linear regression model fit for reduction of *S. cerevisiae* over the grain diameter of the filtering media. "Red.Yeast": *S. cerevisiae* reduction; "YstLR": *S. cerevisae* loading rate; "OLR": organic loading rate; "HLR": hydraulic loading rate; "Part.Size": Effective diameter of biochar; "DyTrt": Time of every loading regime; "SltLR": Inflowing electric conductivity

Similarly to bacteria, the results of the PCAs suggested that inclusion of all the analysed factors in *S. cerevisiae* increased the variance explanation of the linear regression model to 78 % compared to the 72 % explanation provided when including grain diameter alone (*Figure 11*) (PAPER II). Similar explanations to those discussed for bacteria (Section 5.2.1) are likely to be the explanation for this finding. Consequently, the increased relative importance of grain diameter for *S. cerevisiae* (72 %) removal compared to bacterial removal (52-58 %) is likely to be due to both the larger cell size of *S. cerevisiae*, and the reduced influence of biofilm-mediated (organic loading rate and *S. cerevisiae* loading rate) factors. Tufenkji et al. (2006) reported the results of several studies that found an inverse relationship between biofilm presence and *Cryptosporidium* spp. removal. Although the causes of such phenomena remain unclear and the filter study was performed with *S. cerevisiae*, this could explain the low influence of organic loading rate and microbial loading rate in *S. cerevisiae*.

The findings on microbial reduction by filtration (PAPERS I & II) and the studies mentioned above indicate that the grain diameter of filtering media is a major factor in the reduction of protozoa – and probably helminth eggs as well – by filtration at high HLRs (>130 L m^{-2} d^{-1}), and that an effective grain diameter of 2.8 mm or smaller in biochar filters would remove at least 1 $\log_{10}$ *Cryptosporidium* spp. oocysts at such HLRs. The downside with using a small

grain size in the filtration is the increased retention time in the filter, and consequently the space requirement for the filter to accommodate high hydraulic loading rates.

5.3 On-farm barriers to disease transmission

Found microbial removal rates from both riverbank filtration and biochar filtration can be complemented with other on-farm barriers. Considering the studied context, two barriers seem to be appropriate as complements. Biochar filtration implementation most probably would need a reservoir to store the filtered water until irrigation; storage of water can be another barrier for pathogens. On the other hand, the high levels of UV radiation from sunlight and the aridity of the climate in the zone might provide significant levels of pathogenic die-off.

5.3.1 Water filtration and storage

Filters remove pathogenic microorganisms from water by retaining them in the filtration media first before they are inactivated (Stevik et al., 2004). Retention times in on-farm implementations are often shorter than in treatment plants (Keraita et al., 2014). However, these filters are able to remove significant numbers of helminth eggs and protozoa (Keraita et al., 2010) and have the potential to significantly decrease the microbial risk, given the low minimum infective dose and long-term survival in the environment of such organisms when using untreated water.

Additional pathogen removal can be achieved with storage. During storage, pathogens tend to settle and be exposed to UV radiation from sunlight. The level of UV radiation in the Bolivian highlands reaches values that are sufficiently intense to inactivate significant amounts of pathogens, with up to 720 mJ cm^{-1} in one hour being reported in Cochabamba by Fisher et al. (2012). One day of undisturbed settling of wastewater can remove 1 log unit of different pathogens (Keraita et al., 2010).

5.3.2 Die-off before harvest

Depending on climatic conditions, pathogens are reduced in the environment by approximately 1 log unit per day after irrigation (World Health Organization, 2006). Reduction is more rapid in hot and dry weather, but slower in wet weather (World Health Organization, 2006). However, some vegetables require constant water application even close to harvesting,

providing a wet environment that could increase the pathogens' survival. Given the number of factors involved (*i.e.* water requirement of crops and the need for specific environmental conditions), the effectiveness of die-off should be assessed according to the implementation context.

5.4 On-farm filtration

According to the results of this study, on-farm filtration – both riverbank filtration and biochar intermittent filtration – has been shown to be an effective barrier against most pathogens. However, the suitability of on-farm measurements is also related to the requirements for their implementation in relation to the context. Consequently, the suitability of riverbank filtration was analysed according to implications in two scenarios: i) new implementations in the basin as an alternative given the low quality of river water, and ii) implementation of protected wells. Moreover, the suitability of biochar filters was analysed according to implications of full-scale on-farm implementation in the basin as an alternative to irrigation. Three main aspects were analysed for such filters: i) the feasibility of optimal grain diameter at full scale, ii) the on-farm surface requirement and iii) the required water management changes on farms.

5.4.1 Suitability of riverbank filtration

New implementations in the study basin

As mentioned above, particle distribution and the pollution level of the river are major factors in the effectiveness of riverbank filtration as a treatment. Regarding soil texture, heterogeneity in soil material was not studied before the wells were established and remains unknown. However, concentrations of larger microbes (*i.e.* protozoan cysts, oocysts and helminth eggs) found in the wells seem to indicate preferential flows in the riverbank soils of the studied zone (PAPER I). The river in the basin has determined the formation of alluvial terraces in the study basin, which is considered appropriate for RBF (Tufenkji et al., 2002). However, the surrounding mountain chains are located at variable distances from the river (Metternicht and Fermont, 1998). Since geological material is also influenced by the distance from the mountains, riverbanks in the zone could differ in soil composition, ultimately resulting in a suboptimal texture for RBF implementation.

However, pollution levels in the river have generated a number of sections along the stream where dissolved oxygen in the water is depleted (Contraloria

General del Estado, 2011). Consequently, the formation of anoxic zones in soil is likely in these sections, which might result in inappropriate conditions for RBF implementation (Tufenkji et al., 2002).

In terms of agricultural use the velocity of recharge of the studied RBF wells was sometimes found not to match the frequency of irrigation required by farmers, encouraging a continuous search for alternative water sources (PAPER I). This has led to the eventual use of lower-quality water sources, resulting in increased risks of faecal contamination of produce. To determine the feasibility of new implementations of RBF wells in the basin, further studies on basin geology and recharge flow dynamics in the basin are required.

Implementation of protected wells

Protection for wells has been widely implemented for drinking water (Levantesi et al., 2010, Freitas et al., 2017). It has a dual function: protection against contamination and enhancing water flow to wells. The results from the present study on microbial removal suggest a significant effect of additional protection on water microbiological quality (PAPER I). The additional sand/gravel layer provides improved – in terms of grain diameter and uniformity – filtration media surrounding the wells. Such media can potentially reduce the effect of preferential flows, thus increasing microbial removal.

From the agricultural perspective of the systems described in the present study, a major advantage of protected wells is the area required: at 1 m in diameter it is less than 0.9 m^2 including walls. Such a reduced area is highly valuable in periurban contexts, where the available farming area is limited. However the ready-to-pump water volume in protected wells (around 7 m^3 every 4 days) is a far lower irrigation volume compared to the unprotected wells (at least four times larger), and allows for the irrigation of just one 100 m^2 plot every third day during the driest months of the year (October-November). Restriction due to volume could be overcome if farmers changed irrigation technique, although such major changes in production practices might hamper its acceptability among farmers (Keraita et al., 2010). Implementation of improved wells is considered to be a promising barrier for wastewater irrigation management, but it requires additional site-specific studies for implementation, mainly to ensure whether the flow meets the farmer's requirements.

5.4.2 Suitability of on-farm charcoal filters

Optimal grain diameter of charcoal

The results of the present study demonstrated the importance of the grain diameter of the filter media for pathogen removal at high HLRs, mainly for bacteria and viruses (PAPER II). However, these results are applicable to defined effective diameters and uniformity distribution of the material. Full-scale implementation of filters implies large volumes of filtering material with certain physical properties that are not always achieved (Keraita et al., 2014). Deviations to larger particle sizes in production of filter material could significantly reduce pathogen removal under high HLRs, mainly for bacterial and virus reduction (PAPER II).

However, grain diameters of 1.4 and 2.8 mm both exhibited significant removal of *S. cerevisiae*, and therefore similar removal of oocysts might be expected (PAPER II). The grain diameter of biochar in full-scale implementations on farms (*i.e.* at high HLRs) could be implemented with certain flexibility between such grain diameters if the main objective is protozoan and helminth removal.

Required surface area

The required surface area for a full-scale implementation of charcoal filters is linked to water requirements. This, in turn, is linked to several characteristics of the farming systems, such as irrigation frequency and volume required. The average plot area is 500 m2 with a range of 100-1000 m2. Lettuce is the most water-demanding crop cultivated in the zone (onion, potato and cabbage are also cropped). Irrigation is performed along furrows every second or third day. It has been estimated that an average 5 cm water layer is required per irrigation event. Under these conditions, 5 m3 of water are required for irrigation of 100 m2 per irrigation event. However, implementation of a biochar filter has been assumed with the highest tested HLR (i.e. 400 L m-2 d-1). A biochar filter with an area of 25 m2 (5 m x 5 m) would be able to treat 10 m3 per day, which would be enough for irrigation of 200 m2 every second day. Since water would be treated for several days, implementation of reservoirs would probably be needed. Although reservoirs might provide an additional barrier for pathogen reduction, their implementation would require a greater surface area.

Water management

Keraita et al. (2014) suggest that on-farm implementations for risk management should not require activities that are much different from current practices as a way of facilitating their adoption. However, new practices often require a certain degree of management change, which must be adequately communicated. Two major water management changes have been identified as associated with intermittent biochar filters.

The first is intermittent loading of the filter. Farmers have expertise in pumping water for irrigation, but at a single event. After starting to pump water, they move to the plot to irrigate. The efficiency of the treatment has been tested under intermittent flow because it increases the removal of other pollutants (*e.g.* BOD_5) and does not require backwashing. This issue could be resolved by adding a timer to automate the intermittent feeding. However, the implications of such changes should be discussed with farmers prior to implementation.

Another issue that might favour adoption of biochar filters is reducing the dependence on subsurface flows. Currently, irrigation is dependent on, and sometimes limited by, subsurface flow for well recharge. Indeed, if filters and reservoirs have enough capacity, sufficient volumes of water could be pumped in advance.

It can be concluded that, despite the significant removal of parasites, the introduction of intermittent biochar filters as an on-farm wastewater treatment is not a feasible option due to the requirement for both a large area and new water management practices.

6 Conclusions

Evidence of faecal contamination was found throughout the system studied. The two types of riverbank wells studied had statistically different hygienic qualities, although both were found to be a major source of pathogens in the system. Soil was considered the major reservoir of pathogens in the system. The prevalence and variety of the microbes found and the characteristics of irrigation lead to the belief that soil provides pathogens with a long-term buffer against environmental factors. Levels of produce contamination were significantly related to water quality. Moreover, the levels and variety of microbes identified in lettuce were considered evidence of faecal contamination.

It was found that filtration can effectively reduce the pathogen loads from wastewater under the on-farm conditions studied. However, the grain diameter of filtration media proved to be a key factor in microbial removal, especially for viruses and bacteria. The present results indicated that microbial reduction rates decrease significantly with grain diameters of 2.8 mm (or larger) and suboptimal uniformity of filtration media.

Moreover, it was found that the protected riverbank filtration studied was an effective barrier against all the microbes studied. They were able to provide average reductions of at least 60 % of helminth eggs, 1 $\log_{10}$ unit of protozoa, 2 $\log_{10}$ of viruses and about 5 $\log_{10}$ of bacteria. Furthermore, they removed about 1 $\log_{10}$ more than unprotected wells of all studied microbes except viruses, which did not show any difference in reduction in either well type.

Microbial removal with biochar filters at hydraulic loading rates ≥ 200 L m^{-2} d^{-1} was at least 1 $\log_{10}$ bacteria and protozoa oocysts, but only with effective biochar grain diameters of about 1.4 mm. Only protozoa oocysts could be removed by at least 1 $\log_{10}$ with grain effective diameters of about 2.8 mm. Neither the electrical conductivity of the inflowing water nor the applied organic loading rate had any effect on microbial removal at ≤ 1000 µS cm^{-1}

and ≤ 15 g BOD_5 m^{-2} d^{-1}, respectively, when hydraulic loading rates ≥ 200 L m^{-2} d^{-1}.

This study has demonstrated the high probabilities of faecal contamination of the agricultural system studied that used wastewater for irrigation. It also demonstrated that the feasibility of filtration as an on-farm water treatment is extremely context dependent. Riverbank filtration proved to be an effective and upgradeable on-farm barrier against pathogens provided that the requirements of soil type are met. Intermittent biochar filtration proved to be effective at removing protozoan oocysts under the on-farm conditions studied, although it would imply a requirement for large surfaces and changes in the usual on-farm water management, which might reduce its acceptability by farmers.

7 Future research

Since this study was based on only a few monitored wells, it is still to be confirmed whether higher pathogen reduction showed by the protected wells is due to the additional layer. More detailed studies are necessary to establish and confirm the characteristics of such removal.

Current knowledge is limited about soil's role as a reservoir of pathogens from wastewater in the long term and the feasibility of produce contamination from soil. Its role as a reservoir should be examined for the long-term survival pathogens during more than one crop season.

Biochar as a filtration medium in continuous and batch flows for wastewater treatment has not yet been studied. Knowledge of pathogen reduction under such conditions might lead to its use in both on-farm filtration and the upgrading of riverbank filtration wells.

Although implementation of on-farm biochar filtration is not considered feasible on its own, it could still be applied as part of a multi-barrier approach if jointly applied with other measures. Other on-farm measures, such as die-off before harvest, should then be investigated in the studied context.

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