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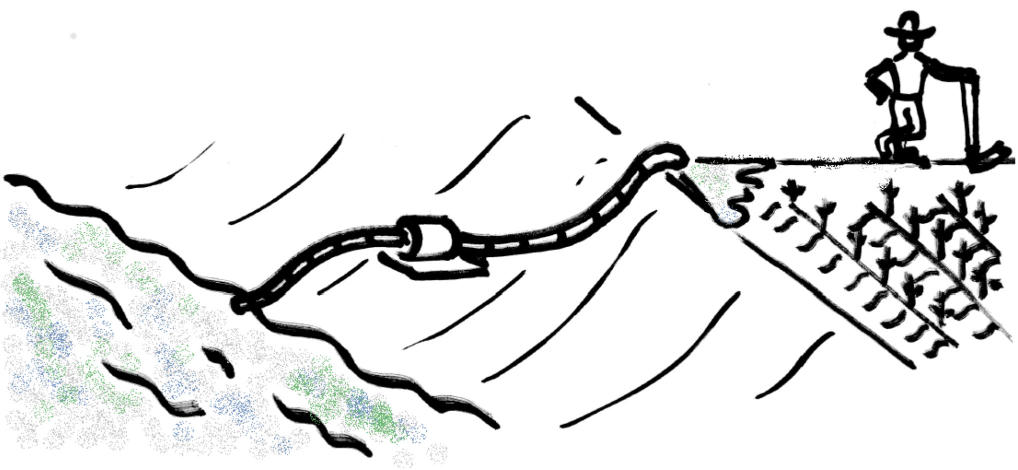
Farm-based technologies for management of risks from irrigation with wastewater-polluted sources in Cochabamba, Bolivia

Luis Fernando Perez Mercado



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Abstract

Wastewater irrigation is a global practice that allows reusing water and nutrients in agriculture, but also poses risks of introducing pathogens/pollutants into agricultural systems and food. In order to manage these risks, on-farm measures can be implemented as barriers along the pathway that pathogens/pollutants must follow to reach the population/place at risk, in cases where treatment plants are not a viable option. The aim of this thesis was to evaluate several on-farm measures in terms of i) reduction in health/environmental risks, and ii) feasibility of implementation in the context of an agricultural system producing lettuce with wastewater-polluted irrigation source (river water) in a semi-arid area of Bolivia.

The microorganisms assessed for health risks from consumption of lettuce from the studied system were *Ascaris lumbricoides*, enterotoxigenic *Escherichia coli* (ETEC) and rotavirus, while the environmental risks assessed were nitrogen excess in soil under high and low irrigation efficiencies. The risks were assessed in four scenarios: 1) direct use of river water (baseline scenario), 2) baseline scenario with biochar filtration, 3) baseline scenario with riverbank filtration, and 4) baseline scenario with water-source substitution (the river water) two weeks before harvest. Water quality and performance data of tested on-farm measures were collected in field studies and laboratory experiments and used as input for risk assessments.

Health risks were above WHO recommended health targets in the baseline scenario, while the nitrogen input to soil was at least two-fold the lettuce requirement. The health target was achieved by riverbank filtration for *A. lumbricoides* and ETEC, and by on-farm filtration for *A. lumbricoides*. Only on-farm biochar filters reduced the estimates of nitrogen accumulation near the equilibrium point (0 kg ha^{-1}) for high efficiency irrigation. No reduction in risk was found for wastewater substitution in this study.

The implementation of riverbank filtration was found to be highly dependent on local context (soil properties), while implementation of biochar filters were constrained by the high surface area required.

This research contributed to the body of knowledge by testing on-farm measures not previously investigated and by identifying bottlenecks that affect the feasibility/reliability of the studied on-farm measures for risk management.

Keywords: Pathogens, nutrient recycling, farm-based measures, health, ecotechnology, irrigation scheduling, biochar filtration, riverbank filtration, risk assessment

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Gårdsbaserade åtgärder för hantering av risker från bevattning med avloppsförorenat vatten i Cochabamba, Bolivia

Sammanfattning

Att använda avloppsvatten för bevattning är ett tillvägagångssätt som används i större delen av världen som främjar återanvänds av vatten och växtnäringsämnen i jordbruket, men även öppnar upp för risken att introducera patogener/föroreningar i jordbruk-, och livsmedelssystem. Gårdsbaserade åtgärden kan användas som barriärer längst vägen patogenerna/föroreningarna måste ta för att komma i kontakt med befolkningen/platsen som är utsatta för riskerna, på ställen där avloppsreningsverk inte är möjliga. Målet med denna avhandling var att utvärder flera gårsbaserade åtgärden med avseende på i) minskning i hälso-, och miljörisker , och ii) genomförbarheten att i det implementera i det studerade kontexten av ett jordbrukssystem som producerar sallad med avloppsförorenad bevattningskälla (flodvatten) i ett medeltorrt område i Boliva.

Mikroorganimserna som utvärderades för hälsoriskerna som konsumtion av sallad från det studerade jordburkssystemet medför var Ascari lumbricoides, enterotoxigenisk Escherichia coli (ETEC) och rotavirus, medan de utvärderade miljöriskerna var kväveöverflöd i jord under hög-, samt låg bevattningseffektivitet. Riskerna i fyra olika scenarier utvärderades: 1) direkt användning utav flodvatten (baslinje), och de övriga tre var baslinjesystemet och antingen 2) filtrering med biokolfilter, 3) filtrering genom flodbank, eller 4) ersättning av vattenkällan, av flodvattnet. Data för vattenkvalitet och effektivitet av utvärderade gårdsbaserade åtgärden samplades i fält-, och laboratorieexperiment och användes i riskvärderingen.

Hälsoriskerna var över Världshälsoorganisationens gränsvärden i baslinjesystemet, medan kvävetillförseln var som minst två gånger så stor som salladsbehovet. Hälsoriskerna var under gränsvärdet med flodbanksfiltrering för A. lumbricoides och ETEC, och med biokolsfiltrering för A. lumbricoides. Endast biokolsfiltrering minskade den beräknade kväveackumuleringen till jämviktspunkten (0 kg ha-1) vid hög bevattningseffektivitet. I denna studie ledde ersättning av bevattningskälla inte till någon minskning i risk.

Implementeringen av flodbanksfiltrering visade sig i hög grad bero på lokalt kontext (jordegenskaper), medan implementeringen av biokolfilter begränsades av den stora ytan som krävdes. Gårdsbaserade åtgärden har tidigare inte studerats och denna forskning bidrar således till kunskapsbanken genom utvärdering, samt identifikationen av genomförbarheten/pålitligheten, av dessa system.

Nyckelord: Patogener, näringskretslopp, gårdsbaserade åtgärder, hälsa, ekoteknologi, bevattningsschema, biokolfiltrering, flodbanksfiltrering, riskvärdering

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Tecnologías en parcela para el manejo de riesgos en producción agrícola bajo riego con aguas residuales en Cochabamba, Bolivia

Resumen

El riego con aguas residuales permite reutilizar agua y nutrientes en agricultura, pero también implica riesgos de contaminación de sistemas agrícolas y alimentos. Cuando las plantas de tratamiento no son una opción viable, los riesgos pueden ser manejados mediante tecnologías en parcela que actúan como barreras en el camino que los patógenos/contaminantes siguen hasta que constituyen un riesgo. El objetivo de esta tesis fue evaluar varias tecnologías en parcela, en términos de i) reducción de riesgos para la salud/medio ambiente, y ii) factibilidad de implementación en sistemas agrícolas que usan aguas residuales para regar lechuga en zonas semiáridas de Bolivia.

Los microorganismos escogidos para evaluar los riesgos de infección por consumo de lechuga fueron *Ascaris lumbricoides*, *Escherichia coli* enterotoxigénica (ETEC) y rotavirus, mientras que los riesgos ambientales evaluados fueron el exceso de nitrógeno en el suelo con altas y bajas eficiencias de riego. Se definieron cuatro escenarios: 1) uso del agua de un río que recibe agua residual doméstica (escenario base), y los tres restantes formados por el escenario base agregando 2) filtración con biochar, 3) filtración mediante el lecho del río, o 4) sustitución del agua del río como fuente de riego dos semanas antes de la cosecha. Los datos de calidad del agua y rendimiento de las tecnologías evaluadas fueron recopilados mediante estudios de campo y laboratorio, y se utilizaron como insumo para las evaluaciones de riesgos.

Los riesgos de infección en el escenario base excedieron el valor recomendado por la OMS, mientras que el aporte de nitrógeno al suelo sería el doble del requerimiento del cultivo de lechuga. El valor recomendado por la OMS fue logrado mediante filtración de lecho del río para *A. lumbricoides* y ETEC, y mediante filtración con biochar para *A. lumbricoides*. Sólo la filtración con biochar redujo la acumulación estimada de nitrógeno casi hasta el punto de equilibrio (0 kg ha^{-1}) con altas eficiencias de riego. Sustituir el agua del río como fuente de riego no reduciría ninguno de los riesgos considerados. La aplicación masiva de la filtración mediante lecho de río se vería fuertemente condicionada por el contexto local (propiedades del suelo). Por su parte, la implementación de filtros de biochar sería limitada por la superficie requerida.

Esta investigación puso a prueba varias tecnologías en parcela que no fueron investigadas previamente, y permitió identificar cuellos de botella que afectan su viabilidad/confiabilidad para el manejo de riesgos.

Palabras clave: Patógenos, reciclaje de nutrientes, tecnologías en parcela, salud, planificación del riego, filtración con biochar, filtración con lecho de río

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Dedication

To Queti, Yetita y Wawata. You were here all the time.

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., Vinnerås, B. (2018). Pathogens in crop production systems irrigated with low-quality water in Bolivia. *Journal of water and health*, 16 (6), pp. 980-990.

II Cossio, C.*, Perez-Mercado, L.F., Norrman, J., Dalahmeh, S., Vinnerås, B., Mercado, A., McConville, J. (2019). Impact of treatment plant management on human health and ecological risks from wastewater irrigation in developing countries–case studies from Cochabamba, Bolivia. *International journal of environmental health research*, DOI: 10.1080/09603123.2019.1657075

III Perez-Mercado, L.F.*, Lalander, C., Joel, A., Ottoson, J., Dalahmeh, S., Vinnerås, B. (2019). Biochar filters as an on-farm treatment to reduce pathogens when irrigating with wastewater-polluted sources. *Journal of environmental management*, 248, 109295.

IV Perez-Mercado, L.F., Lalander, C., Berger, C., Dalahmeh, S.* (2018). Potential of Biochar Filters for Onsite Wastewater Treatment: Effects of Biochar Type, Physical Properties and Operating Conditions. *Water*, 10 (12), 1835

V Perez-Mercado, L.F.*, Lalander, C., Joel, A., Ottoson, J., Vinnerås, B. Managing microbial risks from informal wastewater-irrigated agriculture by wastewater substitution (manuscript)

Papers I-IV are reproduced with the permission of the publishers.

* Corresponding author.

The contribution of Luis Fernando 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 paper, with revisions by the co-authors.

II Cossio and Perez-Mercado planned the study, performed the fieldwork and wrote the paper with revisions by the co-authors. Perez-Mercado performed the quantitative risk assessments, with revisions by Dalahmeh and Norrman.

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

IV Dalahmeh and Perez-Mercado planned the study and performed the laboratory work. Dalahmeh and Perez-Mercado wrote the paper, with revisions by the co-authors.

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

Abbreviations

BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DALY	Disability Adjusted Life Years
ETEC	Enterotoxigenic *Escherichia coli*
HLR	Hydraulic loading rate
NH_4-N	Ammonium nitrogen
NO_3-N	Nitrate nitrogen
QMRA	Quantitative microbial risk assessment
RBF	Riverbank filtration
TON	Total organic nitrogen

1 Introduction

Agriculture for food production demands large volumes of water (70% of global freshwater use according to Faour-Klingbeil and Todd (2018)) and increasing amounts of water and nutrients to match the needs of the growing global population. Domestic wastewater is a reliable source of water and nutrients that can be reused in agriculture (Connor *et al.*, 2017). As such, it has great potential to reduce pressure on other water sources and decrease the need for chemical fertilisers (Keuckelaere *et al.*, 2015; Hamilton *et al.*, 2007; Toze, 2006). However, wastewater reuse in agriculture also poses several hazards for human health, environmental quality and food safety, because of potential introduction of pathogens and other pollutants (*e.g.* salts, metals, pharmaceutical residues, *etc*.) into agricultural production systems (World Health Organization, 2006; Scott *et al.*, 2004). The most common way to date to deal with these risks in reusing wastewater has been implementing wastewater treatment plants to reduce the concentrations of pollutants to safe levels. This approach has been reported as successful in several countries (*e.g.* Israel, Australia, USA, Mediterranean countries) where domestic wastewater is treated and safely used for agricultural production.

However, such an approach has been unsuccessful in a global perspective, since the area of land irrigated with unsafe wastewater world-wide is estimated to be 10-fold larger than the area irrigated using treated wastewater (Drechsel & Evans, 2010). This is strongly linked to the income level of the countries, because only 28% and 8% of the wastewater is treated in lower-middle and low-income-countries, respectively, and most is used in agriculture either directly or indirectly after dilution in water streams (O'Connor *et al.*, 2017; Keraita *et al.*, 2010a). The situation is challenging even when treatment infrastructure exists, because lack of financing and weak technical/institutional capacity often lead to low treatment performance (Cossio *et al.*, 2017; Qadir *et al.*, 2010).

Unlike high income countries, where contact between contaminated water and food is uncommon and microbiological risks are under control (*e.g.* disease burden from foodborne pathogens is 35-52 and 140-1276 DALYs per 100,000 inhabitants for high and low-middle income regions, respectively), high loads of pathogens are common in wastewater in low and middle income countries (Havelaar *et al.*, 2015; Keraita *et al.*, 2015). The link between human diseases and food-water-environment has not been thoroughly explored in low income countries, but a high disease burden due to ingestion of pathogens can be expected where wastewater is widely used for irrigation (Faour-Klingbeil & Todd, 2018; Havelaar *et al.*, 2015). The lack of wastewater treatment also causes degradation of water quality by eutrophication (Connor *et al.*, 2017). This has a severe impact on aquatic ecosystems, contributing to water scarcity, which is especially critical in semi-arid/arid zones.

The Stockholm Framework has been promoted world-wide by the World Health Organization (2006) to address the microbiological risks associated with reuse of wastewater. This framework proposes that such risks can be managed not only in the treatment plant, but at multiple points along the pathway pathogens cross when infecting humans (*e.g*. on-farm, after harvesting of produce, at market level, *etc*.), and that the same applies to other types of pollutants, and not only to health risks (World Health Organization, 2006). Various on-farm measures (*e.g*. die-off until harvest, river bank filtration) are reported to be efficient in reducing microbial contamination of produce (Verbyla *et al.*, 2016; Huibers *et al.*, 2004). Success in implementing on-farm measures also depends on adaptation to local characteristics and practices in field conditions, such as plot size, irrigation method, water quality, vegetables grown *etc*. (Keraita *et al.*, 2014a). However, most previous studies about on-farm measures in field conditions have focused on agricultural production systems located in Africa (Mayilla *et al.*, 2016; Drechsel & Keraita, 2014; Keraita *et al.*, 2014a), limiting their applicability in other contexts (*e.g.* on-farm sand filters described in Keraita *et al.* (2014a) treat water in volumes appropriate for watering-can irrigation, but too small for flood irrigation). Therefore this thesis sought to assess several on-farm measures -not previously investigated- in terms of: i) reduction in health and environmental risks, and ii) feasibility of implementation, in field conditions of an agricultural system with wastewater irrigation by furrow.

2 Objectives and structure of the thesis

The general aim of the thesis was to evaluate the potential of filtration and water substitution for on-farm management of microbial and nitrogen excess risks in an agricultural system that uses wastewater-polluted sources for irrigation of lettuce. Specific objectives were to:

- Quantitatively assess the baseline risks in terms of disease burden from lettuce consumption and nitrogen excess in soil for the agricultural system studied (Papers I & II)
- Evaluate how implementing biochar filtration, improved riverbank filtration and substitution of irrigation water as on-farm measures affect the baseline risks, and discuss the suitability of implementation for the agricultural system studied (Papers III, IV & V)

2.1 Structure of the thesis

This thesis comprised two parts: 1) description and assessment of baseline risks in the current system of lettuce production with wastewater-polluted sources for irrigation (*i.e.* the baseline scenario), and 2) evaluation of on-farm measures as alternatives for risk management in the baseline system (*i.e.* each on-farm measure as scenario). Evaluation in all cases was based on scenario analysis comprising risk assessments and feasibility evaluations for implementation of on-farm measure(s) in the baseline system.

Part 1 (red box in *Figure 1*) is based on Papers I & II. Paper I characterised the system studied and determined the prevalence of faecal microbes within the system. Paper II complemented the baseline by determining the prevalence of faecal microbes and concentrations of nutrients in untreated and partially treated wastewater from several contexts similar to the baseline.

The on-farm measures evaluated in Part 2 (green boxes in *Figure 1*) were:

- Biochar filtration, based on Papers III & IV. Paper III assessed the reduction in faecal microbes and Paper IV the reduction in nitrogen forms by biochar filters. Both were carried out at laboratory scale.
- Riverbank filtration, based on data in Paper I which, besides the baseline, determined the reduction in faecal microbes in local riverbank filtration systems.
- Water-source substitution, based on Paper V, where the concentrations of faecal microbes on lettuce irrigated with different water sources in experimental plots were determined.

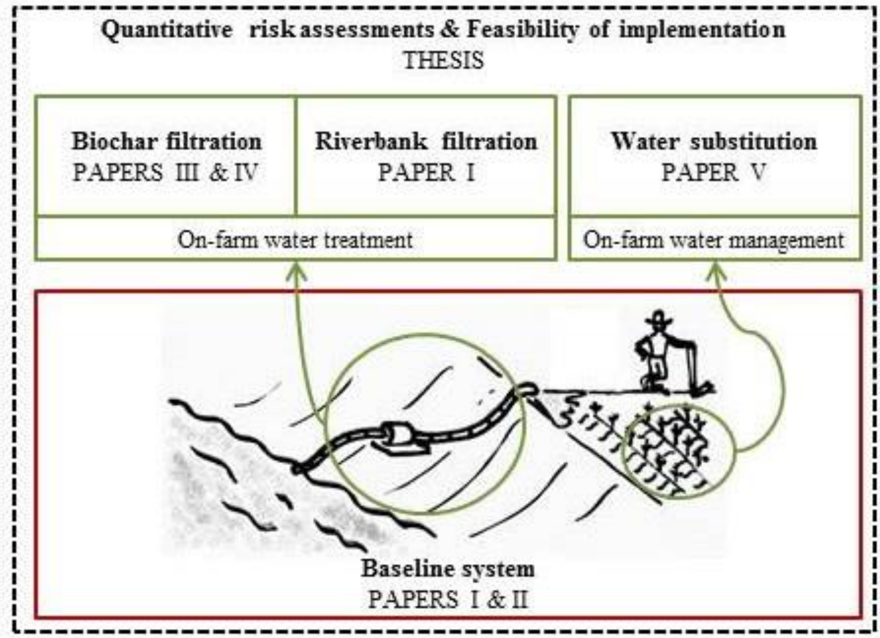

Figure 1. Schematic diagram showing the work presented in this thesis.

3 Background

3.1 Reuse of water and nutrients through wastewater irrigation

Domestic wastewater is basically comprised of water, plant nutrients and organic matter, which are valuable inputs for crop production. Water comes mostly from kitchen and sanitary facilities. Most of the nutrients come from human excreta (nitrogen (N) comes mainly from urine, while phosphorus (P) comes from urine, faeces and detergents) and their concentrations in water depend on the sanitation system, water use habits of the population and rainfall entry into sewage (Tchobanoglous *et al.*, 2014). Assuming a medium strength concentration of wastewater, it has been estimated that, if all the municipal wastewater produced globally were used for irrigation, it would provide around 8000 m^3 ha^{-1} water, 322 kg N ha^{-1} and 64 kg P ha^{-1} per year to ~40 million hectares (Mateo-Sagasta *et al.*, 2015). Such numbers are compatible with the requirements of several crops, which for many range between 3000-13 000 m^3 water ha^{-1}, 10-250 kg N and 3-50 kg P ha^{-1} per crop campaign (Critchley *et al.*, 2013; Scaife & Bar-Yosef, 1995). Thus, domestic wastewater can be considered a ready-to-use source of water and nutrients for crop production.

Furthermore, wastewater is a reliable source of water and nutrients, usually at no cost (Mateo-Sagasta *et al.*, 2015). This is especially valuable in regions where water is scarce or farmers cannot afford mineral fertilisers. Even more importantly, by replacing mineral fertilisers, the environmental impacts from fertiliser production can potentially be lowered (Connor *et al.*, 2017). Wastewater irrigation could thus support transition to a circular economy by reducing water withdrawals, shortening the cycle of nutrients and contributing to environmental sustainability (Connor *et al.*, 2017).

3.2 Constraints on using wastewater for vegetable production

Domestic wastewater contains different types and levels of undesirable constituents, which pose microbial and chemical risks to farmers, consumers and ecosystems when it is used for irrigation (Qadir *et al.*, 2015). Microbial risks come from viruses, bacteria, protozoa and intestinal worms, while agents for chemical risks are commonly grouped as metals and metalloids, nutrients, salts and ions, and micropollutants, *e.g.* pesticides and pharmaceuticals (Connor *et al.*, 2017). Risks to farmers and nearby communities come mainly from the increased probabilities of direct contact with wastewater during irrigation events (*i.e.* water splashes, water ingestion and water sprayed in the air), while risks to consumers come mostly from ingestion of contaminated produce (World Health Organization, 2006). Although risks to farmers are higher (World Health Organization, 2006), risks to consumers could affect a larger proportion of the population, especially in settings where wastewater irrigation is informal and widely practised. Risk to ecosystems come from release of compounds that could have a negative impact on the environment in concentrations exceeding the carrying capacity of the ecosystem receiving the pollution load (Qadir *et al.*, 2015). The major ecological risks arise from excess nutrients (*i.e.* nitrogen and phosphorus), as they have already changed the chemistry of many aquatic systems globally, leading to eutrophication and groundwater contamination (Connor *et al.*, 2017; Glibert, 2017; Jaramillo & Restrepo, 2017).

Implementing treatment plants to reduce and dilute the polluting compounds in wastewater before its use is the conventional way of managing such risks. This approach has not been effective in low-middle income countries, due to financial and technical limitations (Cossio *et al.*, 2017; Qadir *et al.*, 2010; World Health Organization, 2006). Consequently, large volumes of untreated/insufficiently treated wastewater are discharged to the environment and used for irrigation either directly or after dilution with surface water. Around 30 million of hectares are irrigated globally with water from streams comprising 20-100% of untreated/insufficiently treated wastewater (Thebo *et al.*, 2017). Wastewater irrigation can be linked to the disease burden of unsafe water, sanitation and hygiene (*i.e.* diarrhoeal disease among consumers and farmers), but also to the burden of malnutrition (*i.e.* either from reduced consumption of fresh produce if contamination is suspected, or from malabsorption of nutrients due to continuous ingestion of faecal microorganisms (Humphrey, 2009; Suárez & Bradford, 1993)). Together, unsafe water, sanitation and hygiene, and malnutrition represent ~13% of the overall disease burden in low-middle income countries (Lopez *et al.*, 2006).

3.3 The Stockholm Framework

The financial and technical requirements for successfully managing risks only through establishment of treatment plants are not likely to be achieved in low-middle income settings in the coming years (Connor *et al.*, 2017; World Health Organization, 2006). In order to address the health risks from reusing wastewater, a framework for risk management/assessment has been proposed as guidelines by the World Health Organization (2006) in order to set realistic health targets. The framework aims to support decisions about management of risks from wastewater irrigation by: i) assessing microbial risks considering the whole pathway (*i.e.* exposure route) that pathogens must follow to reach the population at risk, and ii) identifying the most effective barrier(s)[1] along the pathway to reduce the exposure and analysing the feasibility of implementing these measures in the given context (Olivieri *et al.*, 2014; World Health Organization, 2006). The efficiency of each barrier and the cumulative effect of these barriers in terms of pathogen reduction are considered. In this way, the guidelines aim at evidence-based decision making, more flexibility and enabling more contextualised risk management (Keuckelaere *et al.*, 2015). The framework is already being implemented in some countries. For example, in Jordan, the guidelines have been included in the 2016-2025 National Water Strategy (Connor *et al.*, 2017).

Although the guidelines published by World Health Organization (2006) emphasise microbial risks, they can also be applied to chemical health risks and environmental/ecological risks. However, such risks have received relatively little attention within the field of wastewater irrigation, especially in areas where microbial contamination is typically high, and even less attention in combination with pathogenic risks (Dickin *et al.*, 2016; Keuckelaere *et al.*, 2015; Simmons *et al.*, 2010).

3.4 Farm-based management of risks

Farms are considered suitable/realistic locations along the exposure route to implement barriers for risk reduction in wastewater irrigation (Keraita *et al.*, 2010b). These on-farm measures can be divided into: i) on-farm water treatments and ii) water handling measures (also known as “no-treatment”

1 Barriers can be defined as measures aiming to prevent transmission, reduce infectivity or decrease pathogens concentration (Nordin, 2007)

measures) (World Health Organization, 2006). On-farm treatments are based on the processes used in conventional treatments, although their features, *i.e.* values of design parameters and pollutant removal capacity, differ widely (Keraita *et al.*, 2014a). This means that the removal capacity of on-farm measures is very often lower than that of the same processes in treatment plants. Some examples of on-farm water treatments are on-farm ponds (*e.g.* dugouts, drums, concrete tanks, adapted irrigation infrastructure) and on-farm filtration systems with different filter media (organic, sand, gravel, soil filters) (Keraita *et al.*, 2014a). Examples of water handling measures for reducing health risks are irrigation methods that minimise contact between wastewater and crops, and extending the time from the last irrigation to harvest to allow die-off of pathogens (Adegoke *et al.*, 2018; Amoah *et al.*, 2011). To my knowledge, no water handling measures have been reported for nutrients.

The pollutant removal capacity of on-farm measures depends strongly on the context. For instance, depending on the filter material used and its uniformity, organic filters can remove 1 to 4 $\log_{10}$ for coliform bacteria (Keraita *et al.*, 2014a), while on-farm ponds with biomass can re-release the nutrients captured if not periodically harvested (Simmons *et al.*, 2010).

3.5 Riverbank filtration

Riverbank filtration (RBF) is a technology based on the filtering effect of the soil. It treats water through physicochemical and biological processes that occur as water passes through riverbank soil. In a simplified manner, a riverbank filtration system consists of wells for water extraction located close to a river and recharged by water from the river (Verbyla *et al.*, 2016). When a stream is polluted with wastewater, riverbank filtration can be implemented on-farm by digging shallow wells and using the 'treated' water collected from the wells for irrigation. It is considered a robust contaminant removal system, as it has been demonstrated to remove pathogens, nutrients, organic matter and several micropollutants (Pan *et al.*, 2018; Sharma & Kennedy, 2017).

Since riverbank filtration relies on soil for water treatment, the characteristics of the particular soil play a major role in its removal efficiency (Tufenkji *et al.*, 2002). A high proportion of sand in the treatment zone is favourable to achieve adequate levels of pollutant removal and permeability (Sprenger *et al.*, 2014; Medema *et al.*, 2003). The presence of these materials is typical in alluvial valley aquifers, although the degree of fluvial action also affects the composition of soils, resulting in heterogeneity in the soil material (Tufenkji *et al.*, 2002). A heterogeneous soil type can lead to preferential flow through larger pores, reducing or nullifying treatment efficacy. The travel time of water (*i.e.* the distance from the river to the extraction well) also plays a role in treatment efficiency, as a longer distance will mean longer contact time between river water and filter (*i.e.* the soil), and therefore higher removal of pollutants (Tufenkji *et al.*, 2002). However, the presence of appropriate material (sand) in the filtering soil is more important than the travel time of water, as most water treatment may occur in the first few metres if sand dominates in the soil (Sprenger *et al.*, 2014).

The collection wells in riverbank filtration can vary from fairly simple structures to highly complex installations at a depth of several hundred metres (Freitas *et al.*, 2017; Verbyla *et al.*, 2016; Levantesi *et al.*, 2010). Wells can also differ in their infrastructure. Most wells described in published literature about riverbank filtration have walls lined with concrete rings and lids covering the top (Freitas *et al.*, 2017; Levantesi *et al.*, 2010; Tufenkji *et al.*, 2002). These wells are commonly surrounded by a layer of gravel/sand to facilitate drainage of water towards the well. This type of well is referred to as a 'protected well' in this thesis. Other wells identified in this thesis consisted of excavations with no protection against external factors (*e.g.* animals, surface runoff) or erosion (*i.e.* no lining on the walls or cover), resulting in wellhead diameter >5 m (Paper I). Such wells are referred to as 'unprotected wells' in this thesis.

3.6 Biochar filtration

Biochar (non-activated charcoal) is a suitable material for filtration systems as it has demonstrated microbial removal rates comparable to sand (a proven material for pathogen removal), but with larger particle diameter than sand (Keraita *et al.*, 2014a; Molaei, 2014; Sidibe, 2014). This is due to some physical properties whose values are more suitable for filtration in biochar than in sand, *e.g.* specific surface area of biochar ≥170 $m^2\ g^{-1}$ and porosity ≥60%, compared with 0.15 $m^2\ g^{-1}$ and 34%, respectively, for sand (Dalahmeh, 2016). An additional advantage is that grain diameter of biochar can be selected from a wider range than sand and therefore clogging risks can be minimised. Research has recently started on use of biochar as a filter medium for domestic wastewater treatment, but not yet for on-farm wastewater treatment.

Filters are rather complex systems in which several mechanisms and interactions take place. Although these mechanisms differ depending on the flow type (*i.e.* saturated or intermittent), pathogens in filters are reduced basically through the same steps: retention and elimination (Keraita *et al.*, 2014a; Kadlec & Wallace, 2008; Stevik *et al.*, 2004). 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[2]. As regards biofilters with intermittent flow (which were studied in this thesis), nitrogen is removed mostly by nitrification-denitrification, biofilm assimilation and adsorption (Saeed & Sun, 2012). Reported removal rates for biochar filters are 1.6 to 4.5 $\log_{10}$ for bacteria, 1 to 2.3 $\log_{10}$ for viruses and ~50% for total nitrogen, under wastewater treatment plant conditions (*i.e.* hydraulic loading rates between 32 and 37 L m^2 d^{-1} treating sieved municipal wastewater) (Dalahmeh, 2016; Molaei, 2014; Sidibe, 2014).

3.7 Cessation of irrigation

One of the most highly recommended on-farm measures to reduce pathogens is cessation of irrigation a few days before crops are harvested (Keraita *et al.*, 2010b). This practice provides time for natural pathogen die-off on crops and its effectiveness depends on environmental factors (*i.e.* inactivation is favoured by hot, sunny weather) (World Health Organization, 2006). It is considered a reliable mechanism for pathogen reduction and, according to World Health Organization (2006), a reduction of 0.5 to 2 $\log_{10}$ day^{-1} for viruses and bacteria can be expected. A major constraint in implementing cessation of irrigation is its effect on physical quality and yield of vegetables, which reduces its acceptability among farmers (Mayilla *et al.*, 2016; Amoah *et al.*, 2011). For instance, high yield losses (~1.4 ton ha^{-1}) in lettuce were attributed to cessation of irrigation in a study carried out in Ghana (Keraita *et al.*, 2010b).

2. Biotic and abiotic factors affect survival of pathogens once retained in filters, according to Stevik *et al.* (2004). The biotic factors are linked to survival ability of each specific pathogen (*e.g.* helminths survive longer than bacteria) and to presence/absence of other microorganisms which can harm the retained pathogens (*e.g.* by predation or by secreting inhibitory substances). The abiotic factors are linked to the environmental conditions determining survival of the pathogens (*i.e.* moisture content, pH, temperature and organic matter content).

4 Methodological approach

In this thesis, an adaption of the Stockholm Framework described by the World Health Organization (2006) was employed, since several on-farm measures were tested as barriers against pathogens and nitrogen within an agricultural system irrigated with polluted sources. In terms of methodology, the work comprised three major components: the production system, quantitative risk assessments and evaluation of feasibility for implementation in the system (*Figure 1*). The production system was the unit on which the different scenarios were built, while the risk assessments and feasibility evaluation were applied to all scenarios, enabling comparisons. Sections 4.1-4.5 provide information common to all scenarios tested. Information about inputs specific to each scenario are provided in subsequent chapters. Specifically, the information presented in Chapter 4 covers:

- The agricultural system in terms of boundaries, components and processes
- The methodology followed to apply the quantitative risk assessments
- The criteria used to evaluate the feasibility for implementation of on-farm measures.

4.1 The agricultural system

Irrigation of vegetables with water from polluted streams or even partially treated effluents is a common scenario in arid/semi-arid peri-urban zones of Bolivia (Ministerio de Medio Ambiente y Agua, 2013). Despite some differences specific to context, these agricultural systems have many features in common (*e.g.* irrigation by flooding with frequency 2-4 times week^{-1}, intensive production of vegetables). For this thesis, the agricultural system for lettuce production located next to the river Rocha (significantly impacted by partially treated and untreated domestic wastewater from human settlements in the Municipality of Sacaba, Cochabamba, Bolivia) was studied (Paper I) and used as a baseline scenario.

In brief, the system is located in a semi-arid area of the Bolivian highlands (~2600 m.a.s.l.) and is characterised by intensive production of vegetables, which is only possible through irrigation during the drier months (typically March-November). Lettuce is the main crop in terms of crop rotation, and it has been observed that some farmers grow only lettuce throughout the year (Paper I). The length of one lettuce crop season[3] ranges between 7 and 9 weeks, depending on the temperature, and furrow irrigation is performed 2-3 times per week. Manure (from dairy cattle and poultry) is applied some days before transplantation once every second crop season of lettuce and two chemical fertilisers (NPK and urea) are applied, one week and one month after transplantation, respectively. Water for irrigation in the zone is pumped from the polluted river in most cases. Some farmers have constructed riverbank filtration wells and use them for irrigation (Verbyla *et al.*, 2016), a practice which is assessed separately in Chapter 7 of this thesis.

Contaminated river water and manure are sources of pathogens and nitrogen in the agricultural system. Chemical fertilisers are also a source of nitrogen. The flows of pathogens and nitrogen from their sources until contact with the product and with the soil are shown in *Figure 2*.

As can be seen from the diagram, the system includes two inputs for pathogens (composted/long-term stored manure and river water) and three for nitrogen (composted/long-term stored manure, river water and chemical fertilisers). In the risk assessments, the amount of pathogens from manure was considered negligible because manure is only applied once every second crop season, compared with a minimum of 32 irrigations with wastewater-polluted sources during the same period (Paper I). Nitrogen deriving from manure and chemical fertilisers was also excluded from the risk assessments, in order to estimate whether nitrogen from wastewater is sufficient for lettuce requirements. The processes affecting the fate of pathogens and nitrogen within the agricultural system are described in sections 4.2.2 and 4.3.2, respectively. The system outputs were the amount of pathogens on harvested lettuce and the amount of nitrogen accumulated in soil.

[3]The period between transplantation and harvest.

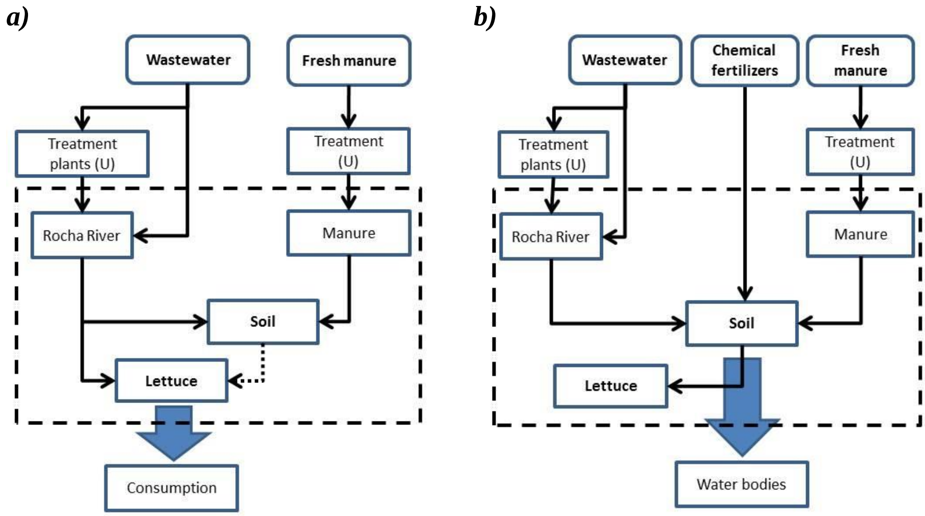

Figure 2. Flows of (a) pathogens and (b) nitrogen in agricultural systems that irrigate lettuce with wastewater-polluted water from the river Rocha, Bolivia. Solid arrows indicate likely flows, while dotted arrows show possible flows. The part of the diagrams inside the dotted square represents the agricultural system. Large blue arrows indicate flow to points where presence of pathogens and nitrogen poses risks. (U) indicates that actual levels of treatment are uncertain.

4.2 Quantitative risk assessment for risk management

The risks assessments were based on the methodology described by Haas *et al.* (1999) for microbial risks. This methodology consists of the following steps: hazard characterisation, definition of exposure and dose-response models, and risk quantification. The hazard characterisation step involves identifying the harmful agent and the spectrum of consequences associated with it. It is typically carried out through literature and database research. In the case of pathogens, it includes information about prevalence, previous outbreaks and corrective actions taken, besides parameters associated with the pathogen such as case:fatality ratios, transmission routes, disease burden, *etc*. Similarly, in the case of environmental risks, information should be gathered about previous occurrence of events associated with the specific pollutant (*e.g*. release of pollutant to streams, algal blooms (for nutrients) *etc*.) and pollutant pathways. In the exposure assessment, a scenario of environmental transport/fate of the harmful agent is determined and expressed as a mathematical model, which is used to calculate the dose (*i.e*. the amount of harmful agent reaching the location where it causes an adverse effect). The dose-response analysis complements the exposure assessment, as it sets a model to estimate the probability of occurrence of adverse effects based on the doses calculated in the exposure assessment. The risk quantification integrates both models to estimate the magnitude, uncertainty and variability of the risk. As many of the model inputs are expressed as ranges of values, the output from risk quantification is typically another range of values. In this thesis, the risk quantification involved Monte Carlo simulation in order to provide the models with values for 10 000 simulations for each risk. This procedure was performed with @Risk software (Palisade Corp., Ithaca, NY).

4.3 Microbial risk assessment for lettuce consumption

4.3.1 Hazard characterisation

Three pathogens were investigated to assess the risks from consumption of lettuce irrigated with wastewater-polluted sources: group A human rotavirus (RV), enterotoxigenic *E. coli* (ETEC) and *Ascaris lumbricoides*. They were chosen in order to represent the variety of wastewater-borne pathogenic diseases and the marked differences in survival and infectivity of such pathogens. Although zoonotic pathogens can enter the system through manure, in risk assessments their concentrations were considered negligible compared with wastewater-borne pathogens (see section 5.1).

Rotavirus group A is endemic world-wide and is the leading cause of severe diarrhoea in children aged ≤5 years, accounting for half of all cases requiring hospitalisation (Haas *et al.*, 2014). In typical cases, following an incubation period of 1-3 days after ingestion, the symptoms of disease manifest abruptly, with fever, vomiting and watery diarrhoea. These symptoms normally disappear within 3-7 days, although fatalities may occur, mainly in children <1 year old (World Health Organization, 2013). During infection, rotaviruses are shed in high concentrations (>10^{12} particles g^{-1}) in the stools and vomit of infected individuals and transmission occurs by the faecal-oral route, either from person to person or via contaminated fomites such as soils and crops (Sánchez & Bosch, 2016; Haas *et al.*, 2014; World Health Organization, 2013). In Bolivia, although the annual number of rotavirus-related gastroenteritis cases has decreased since rotavirus vaccination started in 2008, the disease is still prevalent as it accounts annually for about 3% of deaths and 25% of hospitalisations, both related to acute gastroenteritis in children <5 years old (Inchauste *et al.*, 2017). Rotavirus epidemiology in Bolivia is characterised by one period of more intense rotavirus circulation during winter (*i.e*. June-July) in a background of year-round transmission (Inchauste *et al.*, 2017).

Escherichia coli typically colonises the gastrointestinal tract of infants within a few hours after birth and most strains rarely cause disease in healthy individuals (Kaper *et al.*, 2004). The *E. coli* types causing diarrhoeal diseases differ regarding their preferred colonisation sites, virulence mechanisms and clinical symptoms, but all are spread by the faecal-oral route of transmission or via contaminated fomites (Gomes *et al.*, 2016). Enterotoxigenic *E. coli* (ETEC) is one such diarrheagenic *E. coli* type, and its strains are important causes of diarrhoea in infants and children, exceeding 200 million cases per year and causing about 75 000 deaths, mainly in areas with poor sanitary conditions in low and middle income countries (Gomes *et al.*, 2016; Haas *et al.*, 2014). The world-wide incidence of ETEC is difficult to determine, because the causative agents of diarrhoeic infections are usually not identified (Clements *et al.*, 2012). The ETEC incubation period is 10-72 h, symptoms include cramping, vomiting, diarrhoea and prostration, and the illness lasts 3-5 days (Haas *et al.*, 2014). In Bolivia, the prevalence of ETEC has been estimated to be ~6% in children with and without diarrhoea, with its infection peak occurring between April and September (Gonzales *et al.*, 2013).

The roundworm *Ascaris lumbricoides* is found world-wide, although infection occurs with the greatest frequency in areas with inadequate sanitation and mostly in children 3-8 years old, although the whole population under 15 years old is considered the most vulnerable (Navarro *et al.*, 2009). Up to 10% of the population in low and middle income countries are infected with intestinal worms, a large percentage of which is caused by *Ascaris lumbricoides*. The incubation period is variable, with 4-8 weeks being required after ingestion of eggs for worms to reach the intestines (World Health Organization, 2001). The pathology is predominantly chronic and the symptoms are correlated with worm load; light loads are asymptomatic but heavier loads cause abdominal symptoms, diarrhoea, malnutrition and, in the worst case, constipation (Walker *et al.*, 2013). The eggs are excreted in the faeces of the infected host in concentrations of 10^4-10^5 eggs g^{-1} and spread by wastewater or soil to food (Haas *et al.*, 2014). In Bolivia, the prevalence of *A. lumbricoides* infection is estimated to range between <5% in urban areas and >50% in some rural areas (Chammartin *et al.*, 2013), although it could be lower due to a recently implemented national deworming programme (Spinicci *et al.*, 2018).

4.3.2 Exposure model

The three enteric pathogens described above are persistent in the environment and can be spread through wastewater. In this thesis, exposure to pathogens through ingestion of lettuce irrigated by furrow with wastewater-polluted sources was studied for several exposure scenarios. Contamination of lettuce crops during cultivation can occur through contact between produce and a contaminated matrix, and through internalisation of pathogens into the lettuce. A range of activities and events, including irrigation, rainfall and agricultural practices, can lead to contact between produce and a contaminated matrix, *i.e.* soil, manure or water (Alegbeleye *et al.*, 2018; World Health Organization, 2006). Once contact occurs, pathogens can attach to the surface of produce and persist from the time of contamination to harvesting (Alegbeleye *et al.*, 2018; Li *et al.*, 2015; Tomass & Kidane, 2012). Attached viruses and bacteria can also enter edible parts of crops through the stomata or wounds in vegetal tissue (Alegbeleye *et al.*, 2018; Li & Uyttendaele, 2018). Internalisation of pathogenic viruses and bacteria from contaminated soil/water can also occur by root uptake in leafy crops, although extremely unusual concentrations in irrigation water would be required to obtain bacterial concentrations in leaves comparable to surface contamination (~8 log_{10} CFU mL^{-1} of bacteria in soil solution to detect *Salmonella* spp. in 30% of basil samples, compared with ~7 log_{10} CFU mL^{-1} in irrigation water to obtain 3-4 log_{10} CFU g^{-1} in 100% of basil samples by direct contact between water and leaves) (Jechalke *et al.*, 2019; Li & Uyttendaele, 2018; Wright *et al.*, 2017).

The exposure scenarios were defined based on implementation of the selected on-farm measures in the model agricultural system (*Figure 1*). These included no implementation (baseline scenario, Chapter 5), biochar filtration (Chapter 6), riverbank filtration (Chapter 7) and irrigation water substitution (Chapter 8). For all scenarios, exposure to pathogens was estimated from the microbial load on lettuce and rates of lettuce consumption in the model area (Cochabamba, Bolivia). Microbial load on lettuce was determined through calculations or by direct measurements on lettuce. When calculations were used, they were based on microbial load in irrigation water, estimated volume of irrigation water retained on lettuce and assumptions about survival/decay of microbes on produce until harvest. When possible, concentrations of microbes were also determined on lettuce samples. Since microbial loads of water and lettuce were determined based on microbial indicators (Papers I-V), indicator:pathogen ratios were calculated based on median values in previously- published[4] studies that included the same indicators and pathogens as were studied in this thesis, in order to calculate the concentration of pathogens. All sources of information/data for each scenario are detailed in tables presented in the respective chapters.

4. Published by others. All microbial data for this thesis were determined as indicators.

No reduction in pathogens between harvest and consumption of lettuce was assumed, because harvested lettuce is quickly transported to city markets (*i.e*. 2 hours or less until reaching the market), where it is immediately offered for sale. As regards consumption, it was assumed that three portions of raw unwashed lettuce were ingested weekly and that the three portions came from the same head. Consumption of unwashed lettuce was assumed as a worst case, as there is no information about practices for washing/disinfection of lettuce during its preparation for consumption. Rates of lettuce consumption in Cochabamba were calculated from data reported by Verbyla *et al.* (2016). In total, 43 weeks year^{-1} (irrigation period between March-December) were considered for exposure, resulting in 129 ingestions (Paper II). The input data and assumptions in the exposure assessment for quantitative microbial risk assessments (QMRA) are summarised in Table *1*. It should be noted that decay of *A. lumbricoides* was considered negligible and, consequently, no decay parameters for *A. lumbricoides* are presented.

Table 1. *Exposure assessment parameters and assumptions used for all quantitative microbial risk assessments in this thesis*

Input variable	Units	Value or distribution	References
Indicator:pathogen ratio			
Thermotolerant coliforms:rotavirus		1 : 1.2x10^{-5}	Toranzos *et al.* (1988)
E. coli:ETEC		1 : 6.6x10^{-2}	Gonzales *et al.* (2013)
Helminths:*A. lumbricoides*		1 : 3.7x10^{-1}	PAPER I
Volume of irrigation water captured by lettuce	mL g^{-1}	Uniform (0.089; 0.1275)	Lim and Jiang (2013); Shuval *et al.* (1997)
Rotavirus loss of infectivity on lettuce due to time (k)	day^{-1}	Constant (0.063)	Leblanc *et al.* (2019)
ETEC decay parameters (r) on lettuce			
r due to solar radiation (r_1)	m^2 kW^{-1} h	Constant (0.037)	Ottoson *et al.* (2011)
solar radiation (Sr)	kW m^{-2} h^{-1}	Uniform (4.8; 5.2)	SolarGis (2016)
sunny hours per day (Sh)	h day^{-1}	Uniform (6.1; 7.5)	SolarGis (2013)
r in absence of solar radiation (r_2)	h^{-1}	Constant (0.018)	Ottoson *et al.* (2011)
Time between irrigation and ingestion (T-hold)	day	Uniform (1; 3)	PAPER I
Lettuce consumption			
consumption per capita (I)	g serving^{-1}	Exponential (33.5) truncated at 5 g	Verbyla *et al.* (2016)
number of servings per week	serving	Constant (3)	Verbyla *et al.* (2016)

Uniform distributions are defined by minimum and maximum values (min; max)
Exponential distributions are defined only by their mean (μ)

4.3.3 Dose-response models

For the pathogens considered in this thesis, the full and the approximate Beta-Poisson models were used. The full Beta-Poisson model predicts the probability of infection (P_{inf}) as:

$$p_{inf} = 1 - {}_1F_1(\alpha, \alpha + \beta, -d), \quad (1)$$

where ${}_1F_1$ is the Kummer confluent hypergeometric function, *d* is the dose of the selected pathogen consumed and α and β are fit parameters. This model can be replaced by a simplified approximation for some pathogens. The approximate Beta-Poisson model predicts the probability of infection as

$$p_{inf} = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}. \quad (2)$$

Teunis and Havelaar (2000) demonstrated that the simplified model produces significant overestimation of the risks at low doses, and underlined that the simplified model should be considered valid only when $\beta >> 1$ and $\alpha << \beta$. In this thesis, the full Beta-Poisson model was applied only to rotavirus, because ingestion doses in the studied scenarios can be low according to data obtained in Papers I, II & V. Although parameters to apply the full Beta-Poisson to pathogenic *E. coli* are available, the strain studied to obtain these parameters is particularly virulent (Teunis *et al.*, 2004), which could lead to overestimation of the risks at low doses. The models, values of fit parameters and assumptions of the dose-response used in this thesis are summarised in Table *2*.

Since it was assumed that a person would eat lettuce from the same head three times in one week, it was assumed that the three daily probabilities of infection during that week were equal. These weekly probabilities of infection (P_{week}) were calculated as:

$$P_{week} = 1 - \prod_{k=1}^{3} \left(1 - p_{inf-k}\right) \quad (3)$$

where p_{inf-k} is the daily infection probability for the *k*th iteration of 129 daily exposure events in the *j*th of 10 000 simulations (section 4.2), where events are assumed to be equal throughout the week (3 exposures per week).

In turn, weekly probabilities were used as inputs for calculation of annual probability of illness per person (P_{ill}), calculated as:

$$P_{ill} = 1 - \left(1 - \left(P_{week} \times P_{ill:inf}\right)\right)^{w} \quad (4)$$

where $P_{ill:inf}$ is the probability of illness per infection case and w is the number of weeks considered for exposure (*i.e.* 43 weeks; see section 4.2.2).

The annual disease burden was calculated using the disability-adjusted life-year (DALY) metric, expressed as the number of years lost due to illness, disability or premature death. The annual disease burden (A) was estimated as:

$$\mathrm{A} = \mathrm{P}_{ill} \times \mathrm{D} \times \mathrm{S}_F \tag{5}$$

where D is the disease burden (DALYs per case of illness) and $\mathrm{S_F}$ is the proportion of the population susceptible to every specific disease. The disease burdens from each pathogen were compared with two target values: the pre-existing disease burden in Bolivia and the maximum additional disease burden threshold recommended for wastewater reuse in developing countries.

Table 2. *Dose-response parameters and assumptions*

Parameter	Units	Value or distribution	References
Dose-response			
Rotavirus [a]	$P_{inf\text{-}R}$ day^{-1}	α=0.167, β= 0.191	Teunis and Havelaar (2000)
ETEC [b]	$P_{inf\text{-}ET}$ day^{-1}	α=0.087, β=71.087	Enger (2015?)
Ascaris lumbricoides [b]	$P_{inf\text{-}A}$ day^{-1}	α=0.104, β= 1.096	Navarro *et al.* (2009)
Probability illness:infection	Proportion		
Rotavirus		Uniform (0.35; 0.90)	Verbyla *et al.* (2016)
ETEC		Constant 1.0 [c]	Enger (2015?)
Ascaris lumbricoides		Uniform (0.121; 0.228)	Barker *et al.* (2014)
Disease burden per illness case	DALYs		
Rotavirus		Uniform (0.015; 0.026)	Verbyla *et al.* (2016)
ETEC		Uniform (0.002; 0.01)	Havelaar *et al.* (2015)
Ascaris lumbricoides		Uniform (0.04; 0.07)	Havelaar *et al.* (2015)

[a]Full Beta-Poisson model; [b]simplified Beta-Poisson model; [c]assumed to be 1.0, because the dose-response model is based on presence/absence of symptoms.

4.4 Assessment of nitrogen excess risks from irrigation

4.4.1 Hazard characterisation

In this thesis, nitrogen was investigated for excessive application risks when irrigating lettuce with wastewater-polluted sources.

Domestic wastewater contains valuable plant nutrients, such as nitrogen and phosphorus. Nitrogen is an essential constituent of all proteins, constituting 2-4% of plant dry matter, and contributes to growth, leaf production and size enlargement in plants (Roy *et al.*, 2006). Although availability of nutrients is considered to be a driver for wastewater use in agriculture, the nutrient concentrations vary significantly and can reach levels which are excessive

(Qadir *et al.*, 2015). Excessive nitrogen can be accumulated in soil and later washed off to groundwater or surface water bodies, causing eutrophication or toxicity (Elgallal *et al.*, 2016). Several cases of eutrophication have been identified in water bodies of Bolivia, and linked to wastewater-polluted streams used for irrigation (Archundia *et al.*, 2017; Morales *et al.*, 2017; Acosta & Ayala, 2009). However, most farmers have not yet adapted their fertilisation practices to the nutrient content in such streams (Paper I), leading to potential nutrient excesses.

The situation for phosphorus is similar to that for nitrogen, with excess leading to eutrophication of surface water bodies. Phosphorus has also been connected to the eutrophication detected in Bolivia (Archundia *et al.*, 2017; Morales *et al.*, 2017; Acosta & Ayala, 2009). However, the data necessary to assess the risks of excess phosphorus from irrigation are available only for one water source (raw wastewater), due to laboratory limitations, and therefore phosphorus is excluded from further discussion in this thesis.

4.4.2 Models of nitrogen flow and fate

Different forms of nitrogen are present in domestic wastewater and can be made available to plants by irrigation. Nitrogen is present in both organic and inorganic forms in wastewater. However, it is only available for plant uptake in some inorganic ionic forms (*i.e.* as ammonium (NH_4^+) and nitrate (NO_3^-)), with ammonium concentrations normally exceeding nitrate concentrations in wastewater (Roy *et al.*, 2006). During irrigation, a portion of the ammonium from wastewater is quickly converted to nitrate via nitrification and then lost to the atmosphere via denitrification (Elgallal *et al.*, 2016; Barton *et al.*, 1999). The organic nitrogen and remaining ammonium (*i.e.* the portion that was not quickly nitrified) from wastewater usually bind to soil particles, while nitrate stays dissolved in the soil solution and can easily move with water flow (*i.e.* leaching). Once in soil, soil bacteria mineralise some forms of organic nitrogen, making it plant-available, and easily convert bound ammonium to nitrate. Among all the forms of nitrogen accumulated in soil, nitrate is the most relevant in terms of environmental water contamination risks, because it is highly mobile and can reach surface water bodies via runoff and subsurface flow, or groundwater via leaching (Elgallal *et al.*, 2016).

The scenarios evaluated for nitrogen excess were the same as for microbial risks (see section 4.2.2), except those involving riverbank filtration, where evaluation was not performed due to lack of data. For all scenarios, the amount of available nitrogen accumulation/excess in soil (N_{soil}) was estimated by comparing the amount of nitrogen added to soil via irrigation with wastewater and the nitrogen uptake rates of lettuce, as:

$$\mathrm{N}_{soil} = N_{dose} - N_{req} \quad (6)$$

where N_{dose} is the dose of available nitrogen applied during one lettuce cropping campaign (24 irrigations) and N_{req} is the requirement of available nitrogen during one lettuce cropping campaign. For each scenario, two evaluations were performed based on irrigation efficiency during application: one assuming high efficiency and the other assuming low efficiency. It is important to highlight that the risks of accumulation/excess in soil were estimated without taking into account soil characteristics/properties. Soil properties greatly affect the fate of nutrients (Qadir *et al.*, 2015). However, investigating the impact of soil properties on nutrient leaching was beyond the scope of this thesis, where the aim was to investigate whether on-farm water treatment/management measures are feasible alternatives to manage nutrient risks.

Amounts of nitrogen added to soil were calculated for every irrigation event from loads in irrigation water, estimation of water volume applied and loss of nitrogen from soil during/immediately after irrigation (*i.e.* nitrification), as:

$$\textstyle\sum_{i-n} N_{dose} = v \times (1 - N_{den}) \times (\mathrm{NH_4\text{-}N} + \mathrm{NO_3\text{-}N} + \mathrm{N}_{min}) \quad (7)$$

where v is the volume of water applied in the n_{th} irrigation event, N_{den} is the proportion of available nitrogen lost by denitrification, and the other parameters are concentration of ammonium-nitrogen, nitrate-nitrogen and mineralisable nitrogen (N_{min}) in irrigation water. Nitrate-nitrogen (NO_3-N) was measured directly for each water source. N_{min} was calculated based on the amount of organic nitrogen and the proportion of biodegradable organic matter in the water source (Paper II), as:

$$\mathrm{N}_{min} = (\mathrm{TON} - \mathrm{NH_4\text{-}N}) \times (BOD_5/COD) \quad (8)$$

where TON is total organic nitrogen determined by the Kjeldahl method, BOD_5 is 5-day biochemical oxygen demand and COD is chemical oxygen demand. These three parameters were determined in Papers I-V in this thesis. Ammonium-nitrogen was calculated as:

$$\mathrm{NH_4\text{-}N} = \mathrm{TON} \times (\mathrm{NH_4\text{-}N}/\mathrm{TON}) \quad (9)$$

The basic input data and assumptions of the model are summarised in Table *3*. Further information relevant to every particular scenario is provided in the corresponding chapter (Chapters 5-8).

Table 3. *Excess nitrogen risk parameters and assumptions*

Parameter	Units	Value or distribution	References
Requirement of available nitrogen from one lettuce cropping season	kg ha^{-1}	Constant (110)	Scaife and Bar-Yosef (1995)
Volume of water applied per irrigation			
1st month after lettuce transplantation	m^3 ha^{-1}	Uniform (120; 200)	Tarqui Delgado *et al.* (2017) and PAPER V
2nd month after lettuce transplantation	m^3 ha^{-1}	Uniform (160; 240)	
High efficiency of furrow irrigation		Constant (0.3)	Maldonado (2001)
Low efficiency of furrow irrigation		Constant (0.7)	
Proportion of available nitrogen lost by denitrification		Uniform (0.13; 0.29)	Ryden and Lund (1980)

4.5 Criteria to evaluate feasibility of on-farm measures

The feasibility of on-farm alternatives for risk management was evaluated based on: i) determining which alternative(s) can reduce the evaluated risks to acceptable levels and ii) their implementation requirements for these levels of risk.

5 Baseline system: Lettuce irrigated with wastewater-polluted sources

5.1 System description

Risks from irrigation with five water sources were assessed for the baseline scenario. The five water sources assessed were raw wastewater, settled wastewater, river water (polluted Rocha river, see section 4.1), spring water and unpolluted river (Rocha river in the high part of the basin, where the river begins and has not been impacted by any human activity).

To my knowledge, irrigation of vegetables with raw wastewater has not been reported in Bolivia, but was included in the assessment as reference, as a worst case scenario. Likewise, spring water and unpolluted river water were included as reference for risks with ‘clean’ sources. Settled wastewater and river water were included to represent irrigation with partially treated and polluted streams, respectively, which are common scenarios reported in Bolivia (Ministerio de Medio Ambiente y Agua (2013) and world-wide (Thebo *et al.*, 2017). Concentration of pollutants and assumptions in the risk assessments performed are shown in Table *4*.

5.2 Microbial risks

Consumption of lettuce irrigated with both wastewater types and river water had higher estimated risks than lettuce irrigated with spring water (*Figure 3*). Most risks with the wastewaters and river water were lower than the pre-existing disease burden in Bolivia and higher than the maximum additional disease burden, while the risks with spring water were consistently lower than the threshold for all pathogens.

Table 4. *Parameters and assumptions used in risk assessments, with sources, for baseline scenarios (raw wastewater, settled wastewater, polluted river and spring water). Concentrations of pathogens are shown in log10 eggs/cfu/pfu mL*$^{-1}$ *and concentrations of chemical parameters are in mg L*$^{-1}$. *μ = mean, σ = standard deviation*

Water sources	*A. lumbricoides* Lognormal (μ; σ)	ETEC Lognormal (μ; σ)	Rotavirus Lognormal (μ; σ)		Sources
Raw wastewater	-1.86; -1.33	4.05; 3.78	0.54; 0.26		PAPER II
Settled wastewater	-2.23; -1.78	1.94; 1.95	-1.58; -1.57		PAPER V
Polluted river	-2.91; -3.21	3.17; 4.04	-0.34; 0.52		PAPERS I & V
Spring water	-3.64; -3.88	-0.11; 0.38	-3.63; -3.14		PAPER V
	Total organic N (TON) Lognormal (μ; σ)	Nitrates Lognormal (μ; σ)	Ratio NH_4-N/TON PERT (min; likely; max)	Ratio BOD_5/COD Normal (μ; σ)	
Raw wastewater	91.87; 73.1	0.08; 0.06	0.52; 0.92; 1	0.61; 0.14	PAPER II
Settled wastewater	86.89; 15.8	0.26; 3.84	0.63; 0.94; 1	0.64; 0.06	PAPER V
Polluted river	90.59; 15.5	3.42; 3.11	0.90; 0.06[a]	0.70; 0.05	Direccion de Medio Ambiente de Sacaba (2014); Contraloria General del Estado (2011); PAPER V
Spring water	NA	22.76; 8.79	NA	NA	PAPER V
Unpolluted river	NA	3.73; 22.32	NA	NA	Direccion de Medio Ambiente de Sacaba (2014)

[a]The ratio NH_4-N/TON in polluted river water was found to follow a normal distribution.

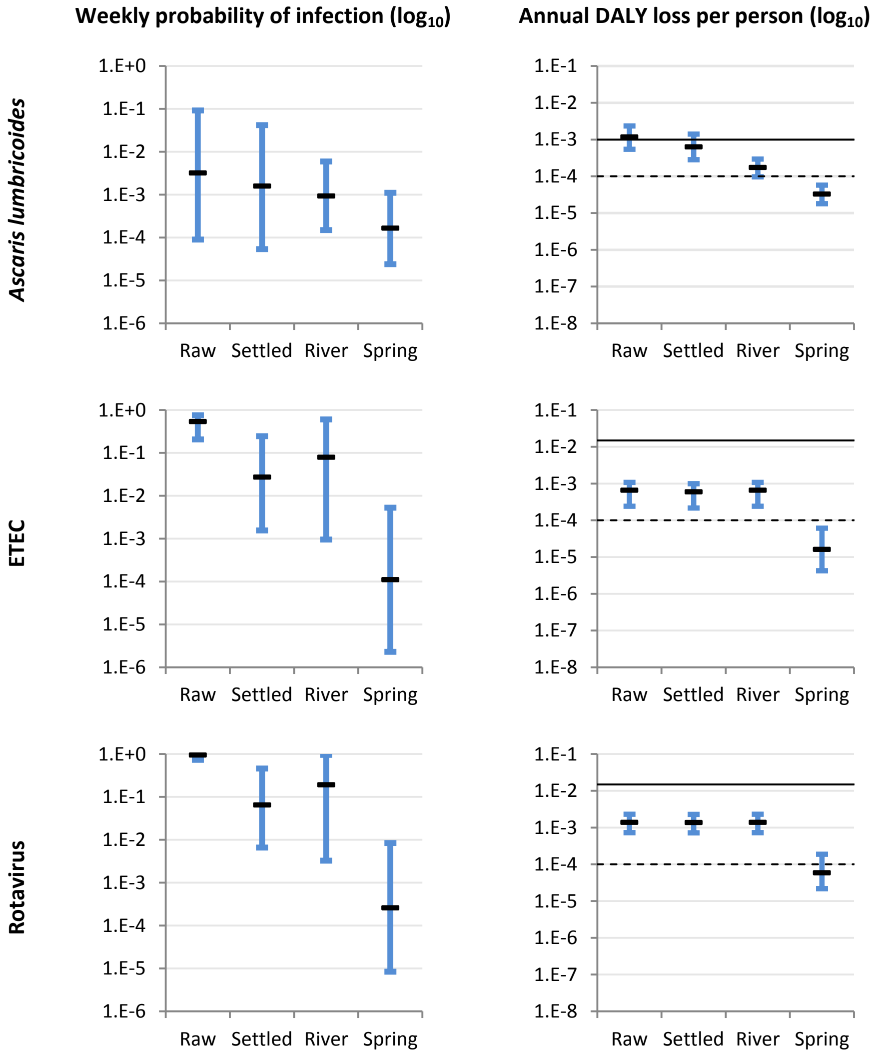

Figure 3. Comparison of health risks from *Ascaris lumbricoides*, enterotoxigenic *Escherichia coli* (ETEC) and rotavirus as weekly probability of infection and annual disease burden per person per year for consumption of lettuce irrigated with raw wastewater (Raw), settled wastewater (Settled), wastewater-polluted river water (River) and spring water (Spring). The black markers and error bars represent the 50th percentile and 95% confidence interval, respectively. Solid lines indicate the pre-existing disease burden of intestinal nematodes (for *Ascaris lumbricoides*) and diarrhoeal diseases (for ETEC and rotavirus) in Bolivia (Pruss-Ustun *et al.*, 2008). Dashed lines indicate the maximum additional disease burden threshold recommended for wastewater reuse in developing countries (Mara & Sleigh, 2010).

Despite ETEC and rotavirus having higher weekly probabilities of infection (0.05 to 1) than *A. lumbricoides* (0.001 to >0.01), only risks from *A.*

lumbricoides with raw and settled wastewater increased the DALY loss (*i.e.* disease burden) above the pre-existing level. This finding indicates that irrigation of lettuce or other leafy vegetables with partially treated wastewater can be the major contributor (up to 100%) to the disease burden of intestinal nematodes in Bolivia. The ETEC and rotavirus risks from consumption of lettuce irrigated with wastewater or river water could contribute up to ~10% to the pre-existing burden of diarrhoeal diseases in Bolivia. This percentage is a major concern, because it reflects that these two pathogens (among many possible diarrheagenic pathogens in faeces) are being transmitted specifically by wastewater irrigation (transmission through other contaminated fomites is also possible) and together contribute to ~20% of the pre-existing burden of diarrhoeal diseases in the whole country (World Health Organization, 2013; Clements *et al.*, 2012).

Weekly probabilities of infection with raw wastewater were higher than with settled wastewater and river water in all cases. Comparing the latter, lower probabilities of infection with river water than with settled wastewater were found for *A. lumbricoides*. This is likely due to some dilution effect in the river or ascaris eggs settling off in the river bottom. Dilution seems unlikely, since most/all of the flow in the river is comprised of wastewater discharge during the dry season (Huibers *et al.*, 2004). Whether from dilution or settlement, this effect(s) can be considered significant because it would result in a lower disease burden (~1 $\log_{10}$) than with raw wastewater. Unlike *A. lumbricoides*, the probabilities of ETEC and rotavirus infection with river water were slightly higher (0.5-1 $\log_{10}$) than with settled wastewater. Higher concentrations of rotavirus in river water than settled wastewater might be explained by highly concentrated raw wastewater being discharged to the river, since rotavirus cannot increase in numbers in the environment. However, the most likely explanation is faecal bacterial growth in river water, because both ETEC and rotavirus concentrations in water sources were calculated from bacterial indicators (generic *E. coli* and thermotolerant coliforms; see Table *1*).

5.3 Nitrogen excess risks in soil

The estimated amounts of nitrogen from irrigation water accumulated in soil followed the pattern raw wastewater = settled wastewater = river water > spring water > unpolluted river water (*Figure 4*). The large surplus amounts in raw and settled wastewater and river water (220-1000 kg ha^{-1} for one season) are partly a consequence of high concentrations of ammonium nitrogen found in the sources studied in this thesis (*i.e.* strongly concentrated wastewaters typically have ~40 mg L^{-1} (Tchobanoglous *et al.*, 2014), while the concentrations were 50-90 mg L^{-1} in the raw and settled wastewaters and river water studied here, see Table *4*). Similar concentrations have been reported for municipal wastewater in Bolivia (60 mg L^{-1} for raw wastewater from El Alto was reported by PNUMA-Titicaca (2011) and 100 mg L^{-1} for raw water from Cochabamba by Durán *et al.* (2003)). This is likely related to limited access to water or specific practices in Bolivian households.

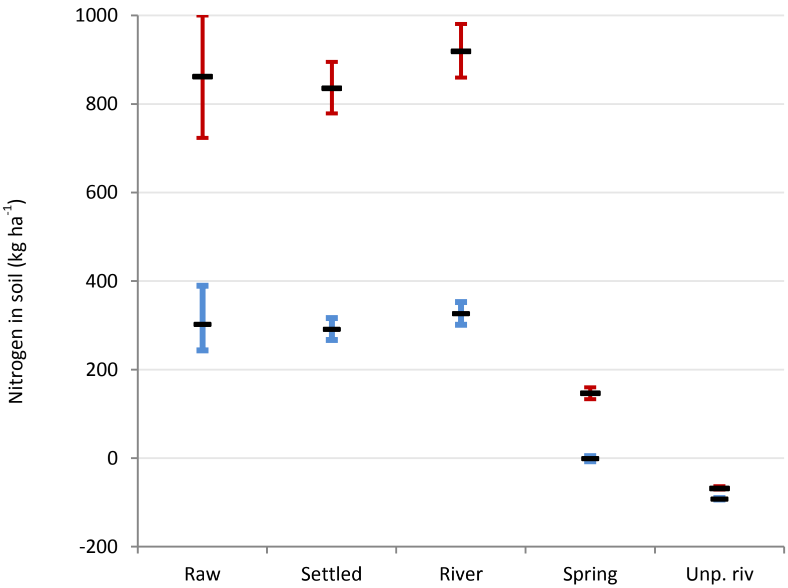

Figure 4. Calculated amounts of available forms of nitrogen accumulated in soil after one crop season of lettuce under scenarios of low (red) and high (blue) efficiency of furrow irrigation with different water sources. The black markers and error bars represent the 50th percentile and 95% confidence interval, respectively. Irrigation water sources were raw wastewater (Raw), settled wastewater (Settled), wastewater-polluted river water (River), spring water (Spring) and unpolluted river water (Unp. riv). Negative values indicate deficit of nitrogen.

As regards irrigation with unpolluted river water, addition of fertilisers would be required to cultivate lettuce in both irrigation efficiency scenarios studied (high and low, see section 4.4.2), as the accumulated nitrogen concentrations were below zero (-50 to -100 kg ha^{-1}). Conversely, spring water was found to potentially accumulate nitrogen in soil. This is explained by the higher concentrations of nitrates in spring water (one order of magnitude higher than in unpolluted river water, see Table *4*). These are likely due to processes of soil contamination upstream, as spring water seems to originate from infiltration into the soil in the high part of the basin during the rainy season (Paper V) and this water flows through peri-urban zones before being discharged in the spring.

Estimated amounts of nitrogen supplied with river water were 30 and 90 kg ha^{-1} higher than with settled wastewater for the scenarios of high and low irrigation efficiency, respectively. This difference was caused by higher concentrations of nitrates and ammonium in river water (Table *4*) and it is likely an expression of the different ages of both sources. The settled wastewater monitored in this thesis can be considered younger than river water, because it flows immediately to the treatment plant and stays for a few hours in an anaerobic sludge reactor. Young wastewater is high in organic nitrogen and ammonium, and low in oxidised forms like nitrates due to anaerobic conditions (Sedlak, 2018). On the other hand, the river receives and transports domestic wastewater along several kilometres, where it is exposed to oxygen transference either from the air or from dilution water. In the presence of oxygen, organic nitrogen is converted to ammonium and ammonia is oxidised into nitrates, which explains their higher contents in river water. Either way, the difference (*i.e.* higher amounts of nitrogen in river water than settled wastewater) was considered not to be significant from a risk management perspective, because the lower values of confidence interval with river water were similar to the higher values with settled wastewater.

As expected, all amounts of nitrogen in soil under low irrigation efficiency were higher than under high irrigation efficiency for the same water source. However, the differences in nitrogen in soil between high and low irrigation efficiency were ~30, ~150 and ~600 kg ha^{-1} for unpolluted river water, spring water and the three wastewater sources (raw, settled, river), respectively. The impact of concentration of available nitrogen in water on this difference is evident on comparing unpolluted river water with spring water (3.7 and 22.8 g N m^{-3} in water resulted in differences of ~30 and ~150 kg ha^{-1}, respectively, in soil). The large amounts and high uncertainty associated with this difference (~600 kg ha^{-1}) are in line with Qadir *et al.* (2015) and pose a major challenge in on-farm management of nutrients. Although these results should not be considered absolute due to limitations inherent to the methodology (*e.g.* soil characteristics were not considered, results were not validated in field, denitrification values can be higher with water sources high in organic matter), they reveal the potential of increasing the efficiency of furrow irrigation to manage nitrogen excess risks from wastewater.

5.4 Need for on-farm risk management

Most microbial and nitrogen risks from irrigation with settled wastewater and river water in the context studied were similar to risks from using raw wastewater for irrigation of lettuce. In the case of nitrogen, a need for risk management is evident, since surplus amounts (220-1000 kg) from one season can be enough for 2-8 more crop seasons of lettuce (requiring 110 kg N per crop season of lettuce; see *Table 3*).

In the case of microbial risks, all can be considered unacceptable because they contribute significantly (*i.e.* by ~10-100%) to the pre-existing disease burden in Bolivia, following the approach of Mara and Sleigh (2010). It should be noted, however, that some of these metrics are outdated. For example, the pre-existing disease burdens used as reference are based on data from 2002 (Pruss-Ustun *et al.*, 2008), and the burdens have likely decreased due to the recent national rotavirus vaccination campaign (Inchauste *et al.*, 2017) and deworming programme (started in 2017) carried out in Bolivia. Although the data sample is small, the finding that helminth egg concentrations in river water samples taken in 2017-2018 were ~2 $\log_{10}$ lower than in 2014-2015 (Papers I & V) supports this assumption. If the pre-existing disease burden was actually lower and pathogen-specific (*i.e.* instead of generic diarrhoeal disease burden and intestinal nematodes), the microbial risks identified from irrigation would represent a higher proportion than reported here.

On the other hand, the indicator:pathogen ratios assumed for concentrations of *A. lumbricoides* and rotavirus in water are based on data collected before the vaccination and deworming campaigns (2014-2015 in Paper I for *A. lumbricoides* and 1986 in Toranzos *et al.* (1988) for rotavirus), and therefore they have likely decreased. If the indicator:pathogen ratios were lower, concentrations of pathogens in water, and consequently the risks, would be lower than calculated. Thus, the effects of updating the metrics used to assess risks from *A. lumbricoides* and rotavirus would be contradictory and remain unclear. It is unlikely that risks from ETEC are lower than calculated in this thesis, because no campaign focusing on this pathogen has been performed (*i.e.* the ratio *E.coli*:ETEC would not change). In contrast, if the pre-existing disease burden of diarrhoeal diseases were lower, ETEC risks would represent a higher proportion than calculated.

Implementing wastewater treatment plants before water is discharged to the river is an alternative for risk management in the context studied here. However, some context-related constraints are likely to hamper the effect of such implementation on risks. Most common technologies for wastewater treatment in Bolivia (*i.e.* Imhoff tanks, septic tanks, anaerobic filters) do not aim for significant removal of either nutrients or pathogens (Paper II;

(Ministerio de Medio Ambiente y Agua, 2013). Although pond systems are common in Bolivia (~45% of treatment plants) and can remove nutrients and pathogens, they require large areas, which are not available in the model area (*i.e*. peri-urban zones of cities in low-middle income countries). In addition, wastewater treatment plants in Bolivia very often (~90% of cases) suffer management problems related to lack of technical expertise and financial resources (Cossio *et al.*, 2017; Ministerio de Medio Ambiente y Agua, 2013), significantly impacting their performance and their potential for management of risks from water reuse (Paper II).

On-farm risk management in the studied context could involve: i) building/replacing wastewater treatment plants, and ii) complementing the incomplete treatment provided in treatment plants that do not aim to remove nutrients/pathogens, or achieve only low removal efficiencies due to inadequate management.

6 On-farm intermittent biochar filtration

6.1 System description

Risks from irrigation with three water sources were compared in order to evaluate the potential of intermittent biochar filtration for on-farm management of risks. These three water sources were polluted Rocha river water (River); river water treated with on-farm biochar intermittent filtration (On-farm biochar filtration); and river water treated with biochar intermittent filtration under treatment plant conditions (Treatment plant biochar filtration). Irrigation with polluted river water was included as a baseline scenario (*i.e.* no modifications to the current situation). The On-farm biochar filtration scenario was the same as the baseline, but with river water biochar-filtered on-farm at 400 L m^{-2} d^{-1} (*i.e.* 12-fold the design hydraulic loading rate (HLR); Paper III) prior to irrigation. Treatment plant biochar filtration was included as a best-case scenario in which wastewater was biochar-filtered in treatment plant conditions, *i.e.* at HLR according to design criteria (34 L m^{-2} d^{-1}). In order to calculate the amount of pollutants (either pathogens or nitrogen) in water after On-farm biochar filtration and Treatment plant biochar filtration, their corresponding removal efficiencies reported in Papers II & III were applied to river water concentrations. Concentrations of pollutants and removal efficiencies are shown in Table *5*. Characteristics of the systems were as described in section 5.1, except for the water source.

Table 5. *Parameters and assumptions used in risk assessments for polluted river water and biochar-filtered river water under on-farm and treatment plant conditions. Concentrations of pathogens are shown in log_{10} eggs/cfu/pfu mL^{-1} and concentrations of chemical parameters in mg L^{-1}, while the removal rates by biochar filters are shown in log_{10} for pathogens and % for chemical parameters.* μ *= mean,* σ *= standard deviation*

	A. lumbricoides	ETEC	Rotavirus			Source
Concentrations	Lognormal (μ; σ)	Lognormal (μ; σ)	Lognormal (μ; σ)			
- River	-2.91; -3.21	3.17; 4.04	-0.34; 0.52			PAPERS I & V
Removal rates	Normal (μ; σ)	Normal (μ; σ)	Normal (μ; σ)			
- On-Farm	1.94 0.37	1.17 0.31	0.86 0.39			PAPER III
- Treatment plant	NA	4.52 1.39	2.27 0.65			PAPER III
	Total organic N (TON)	Nitrates	Ratio NH_4-N/TON	Ratio BOD_5/COD		
Concentrations	Lognormal (μ; σ)	Lognormal (μ; σ)	Normal (μ; σ)	Normal (μ; σ)		
- River	90.59; 15.5	3.42; 3.11	0.90; 0.06	0.70; 0.05		Direccion de Medio Ambiente de Sacaba (2014); Contraloria General del Estado (2011); PAPER V
	TON	Nitrates	NH_4-N	BOD_5	COD	
Removal rates	Normal (μ; σ)	Normal (μ; σ)	Normal (μ; σ)	Normal (μ; σ)	Normal (μ; σ)	
- On-Farm	0.69 0.16	0.16 0.14	0.98 0.01	0.97 0.01	0.96 0.03	PAPER III (Unpublished data)
- Treatment plant	0.95 0.05	0.31 0.38	0.92 0.10	0.98 0.01	0.98 0.01	PAPER IV

6.2 Microbial risks

Weekly probabilities of infection followed the pattern River > On-farm biochar filtration for all pathogens studied, and On-farm biochar filtration > Treatment plant biochar filtration for rotavirus and ETEC (no surrogate for *A. lumbricoides* was tested for Treatment plant biochar filtration) *(Figure 5)*. There was clear agreement between the removal rates achieved by both types of biochar filtration (Table *5*) and the differences in weekly infection probabilities (*i.e*. pathogen removal rates in $\log_{10}$ were approximately equal to their respective reduction of infection probabilities in $\log_{10}$).

The reductions in weekly infection probabilities achieved by On-farm biochar filtration were sufficient to lower the annual disease burden from *A. lumbricoides* to acceptable levels. When using Treatment plant biochar filtration for irrigation, risks from lettuce consumption for both *A. lumbricoides* (assuming that *A. lumbricoides* removal rate with Treatment plant biochar filtration ≥ On-farm biochar filtration) and ETEC were acceptable. This difference between On-farm biochar filtration and Treatment plant biochar filtration in ETEC removal derives from the effect of high HLR on mechanisms of microbial removal, possible reasons for which are thoroughly discussed in Paper III. In brief, thickness/formation of the biofilm layer on the surface of filter media seems to be hampered at high HLR (≥200 L m^{-2} d^{-1}). This decreases contact opportunities between filter media and microbes in water, affecting in particular removal of smaller pathogens such as bacteria. Rotavirus removal in On-farm biochar filtration or Treatment plant biochar filtration would be not enough to reach the target value of disease burden. This can be explained by the reduced contact opportunities between filter media and virus due to its small size (≤0.03 µm), regardless of HLR.

Since pathogen size is strongly linked to removal rate by On-farm biochar filtration, implementing such filtration units in the system studied would be effective if *A. lumbricoides*, and perhaps other slightly smaller microbes (*i.e*. ≥8 µm, Paper III), were the target pathogens. If ETEC and rotavirus were targeted, additional measures (~3 $\log_{10}$ units) to On-farm biochar filtration would be required.

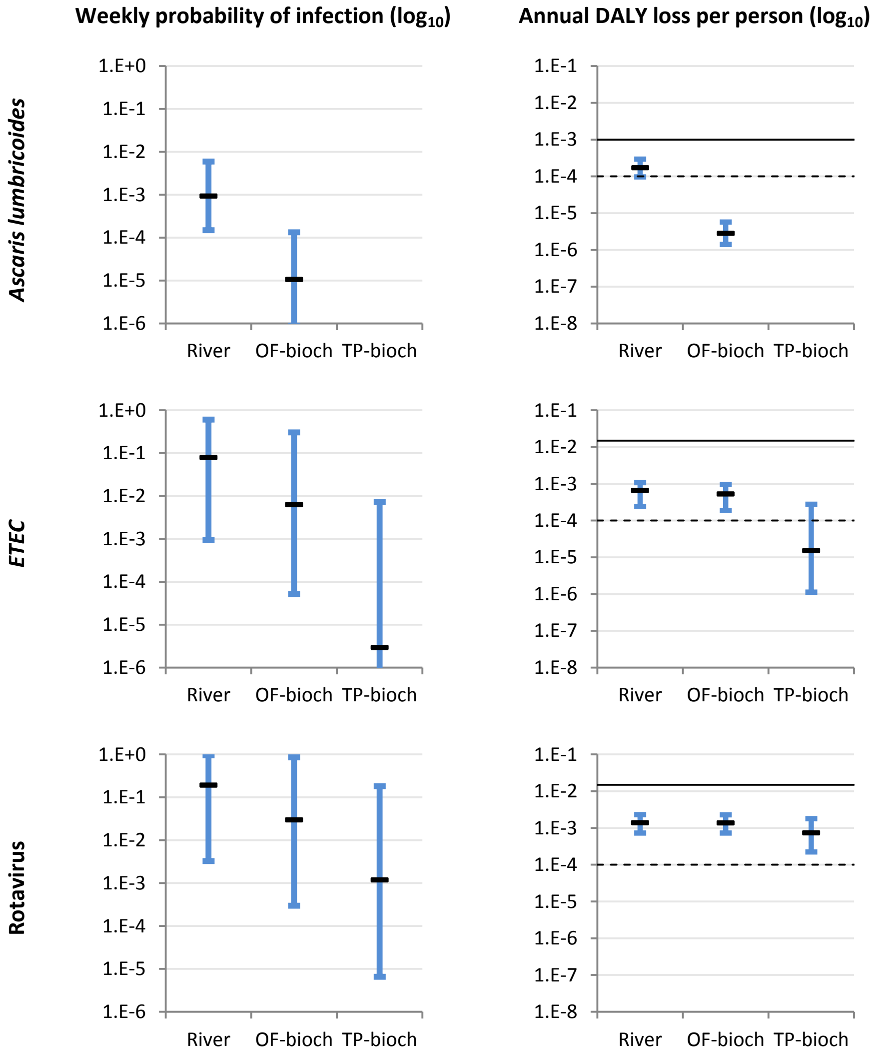

Figure 5. Comparison of health risks from *Ascaris lumbricoides*, enterotoxigenic *Escherichia coli* (ETEC) and rotavirus as weekly probability of infection and annual disease burden per person per year for consumption of lettuce irrigated with wastewater-polluted river water left untreated (River) or treated with biochar intermittent filtration under on-farm (OF-bioch) or treatment plant (TP-bioch) conditions. The black markers and error bars represent the 50th percentile and 95% confidence interval, respectively. Solid lines indicate the pre-existing disease burden of intestinal nematodes (for *Ascaris lumbricoides*) and diarrhoeal diseases (for ETEC and rotavirus) in Bolivia (Pruss-Ustun *et al.*, 2008). Dashed lines indicate the maximum additional disease burden threshold recommended for wastewater reuse in developing countries (Mara & Sleigh, 2010).

It should be highlighted that the uncertainty was high (≥2 $\log_{10}$ units) for weekly infection risks from ETEC and rotavirus. This could have led to overestimation of their respective annual disease burdens, as each person was exposed for 43 weeks. It is possible that such uncertainty was caused by using two different bacteria (*i.e*. *E. coli* and *Enterococcus* spp.) and two different viruses (bacteriophages MS2 and ϕX174) to estimate removal rates for ETECand rotavirus estimated in this thesis should be regarded as approximate, and actual removal of the pathogens by biochar filters should be determined in future studies.

6.3 Risk of nitrogen excess in soil

The estimated amounts of nitrogen from irrigation water accumulated in soil followed the pattern River > On-farm biochar filtration ≥ Treatment plant biochar filtration (*Figure 6*). Both scenarios of irrigation efficiency with Treatment plant biochar filtration were close to the equilibrium point (-50 and 25 kg ha^{-1}). Using Treatment plant biochar filtration can be considered an optimal scenario, because irrigation water would match the nitrogen requirement of lettuce and the supply could potentially be optimised through management of irrigation efficiency.

Despite the significant reduction in nitrogen amount accumulated in soil with On-farm biochar filtration compared with untreated river water (*i.e*. ~700 and ~300 kg ha^{-1} lower under low and high irrigation efficiency, respectively), irrigation following On-farm biochar filtration still resulted in nitrogen excess under both irrigation efficiency scenarios. However, the excess amount with On-farm biochar filtration under high efficiency was close to the equilibrium point (*i.e*. ~40 kg ha^{-1}), and similar to that for Treatment plant biochar filtration under low efficiency. This implies that On-farm biochar filtration combined with high irrigation efficiency can be an effective alternative for on-farm management of nitrogen excess risks in the system studied.

Removal of nitrogen in biochar filters is carried out mostly by the biofilm, as discussed in depth in Paper IV. In brief, it is achieved through bacterial nitrification-denitrification and, to a lesser extent, through biological assimilation of ammonium- and nitrate-nitrogen in the biofilm. These processes seemed to be affected by high HLR (400 L m^{-2} d^{-1}) in on-farm conditions, since the higher the HLR, the thinner the biofilm (Paper III) and the shorter the hydraulic residence time. This likely decreases the capacity for nitrogen-removing processes, as indicated by the removal rates of total organic nitrogen and nitrate-nitrogen (Table *5*).

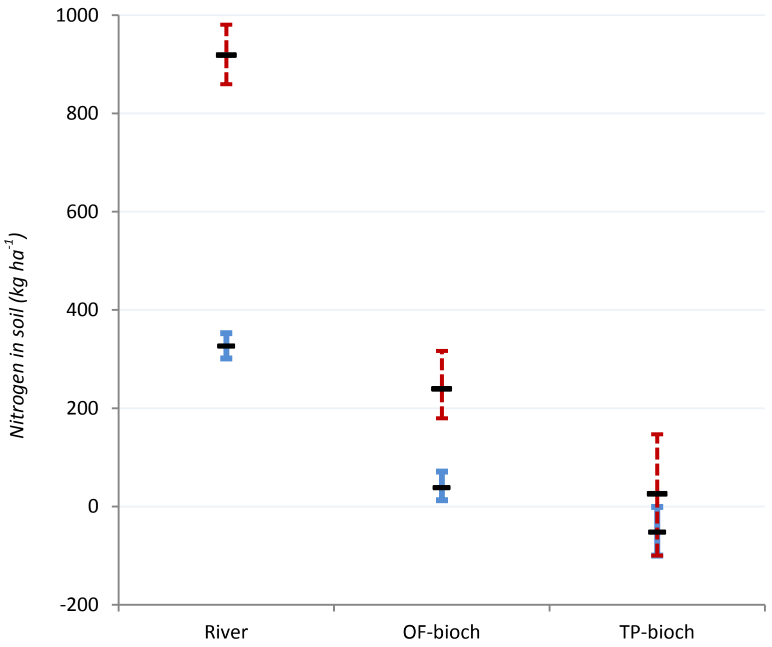

Figure 6. Calculated amounts of available forms of nitrogen accumulated in soil after one crop season of lettuce under scenarios of low (red) and high (blue) efficiencies of furrow irrigation with different water sources. The black markers and error bars represent the 50th percentile and 95% confidence interval, respectively. Irrigation water sources were wastewater-polluted river water left untreated (River) or treated with biochar intermittent filtration under on-farm (OF-bioch) or treatment plant (TP-bioch) conditions. Negative values indicate nitrogen deficit.

6.4 Feasibility of implementation

Under the study conditions, implementing On-farm biochar filtration could not be considered feasible. From a purely risk reduction point of view, it was found to be an effective on-farm measure to manage risks from helminths, nitrogen and likely protozoa (*i.e.* 1-2 $\log_{10}$ reduction in *Cryptosporidium* spp. surrogate, which could be considered significant in the multibarrier approach; Paper III). Nevertheless, where risks from helminths/protozoa in wastewater are a concern, it is likely that bacteria and viruses are also a concern. Additional barriers would then be needed in any case. In addition, implementation of On-farm biochar filtration would require an area of 25 m^2 to irrigate a lettuce plot of 500 m^2. As discussed in Paper III, available land is scarce in peri-urban areas in Bolivia, posing a major constraint to implementation.

The data obtained indicate that microbial removal is higher with smaller effective diameter of biochar (*i.e.* 1.4 mm) under on-farm conditions, most likely because the proportion of micropores in the filter medium is higher (Paper III). Biochar filters with even smaller effective diameter (*i.e.* 0.7 mm) were found to remove nitrogen amounts similar to 1.4 mm biochar, with no evidence of clogging in treatment plant conditions (Paper IV). It can then be speculated that using On-farm biochar filtration with smaller effective diameter of biochar would enhance removal of smaller pathogens, without affecting nitrogen removal and with no clogging. Another alternative could be to use biochar with significantly larger surface area, which could be done by regulating the temperature and availability of oxygen during pyrolysis and by selecting appropriate feedstock for biochar production (Shaaban *et al.*, 2018). These alternatives have not yet been studied for On-farm biochar filtration, and are therefore potential research objects for future studies in order to enhance On-farm biochar filtration implementation.

Beyond these alternatives to enable On-farm biochar filtration, the potential of Treatment plant biochar filtration should be highlighted. Unlike the most common technologies for wastewater treatment, it can significantly reduce risks from wastewater irrigation for nitrogen and most pathogens (Papers III & IV). This implies benefits in terms of risk management because wider protection of the environment (*i.e.* not only farm produce and fields as with on-farm biochar filtration) would be enabled. In addition, the evidence suggests that implementing Treatment plant biochar filtration would require a smaller area than currently used (Paper III). Although On-farm and Treatment plant implementations are not directly comparable because the conditions are not the same (section 3.4), the potential of biochar filters for risk management is better exploited under treatment plant conditions, making it a strong alternative for decentralised treatment of wastewater in peri-urban zones.

7 Improved riverbank filtration

7.1 System description

Microbial risks from irrigation with four water sources were compared in order to evaluate the potential of protected riverbank filtration for on-farm management of risks. Nitrogen was not evaluated, due to insufficient data. The four water sources were polluted Rocha river water (River), and river water treated with unprotected riverbank filtration (Unprotected-RBF), protected riverbank filtration with a gravel layer (Gravel-RBF) and protected riverbank filtration with a biochar layer (Biochar-RBF). Irrigation with river water was the baseline scenario and Unprotected-RBF was considered a reference scenario, since riverbank filtration has already been proven to be an efficient on-farm alternative for wastewater treatment (Keraita *et al.*, 2014a). As pathogen removal depends on distance from stream and soil texture, removal efficiencies for Unprotected-RBF and Gravel-RBF were estimated for 10 m by means of logarithmic regressions with data from Paper I. Removal efficiencies for viruses and bacteria in Biochar-RBF were obtained by adding the removal rates for continuous flow biochar filtration to Unprotected-RBF. Since water flow in riverbank filtration is transient (*i.e*. continuous but unsteady; Sprenger *et al.* (2014)), it was considered that continuous flow filtration in biochar would represent this better than intermittent filtration. Removal rates of continuous flow filtration were determined in experiments carried out by Choque (2018). Concentrations of pathogens, removal efficiencies and parameters of the regressions are shown in *Table 6.*

Table 6. *Regression parameters, concentrations (log_{10} eggs/cfu/pfu mL^{-1}), removal rates and assumptions for microbial risk assessments with polluted river water and river water treated with unprotected riverbank filtration (Unp-RBF), RBF improved with gravel (Grav-RBF) and RBF improved with biochar (Bioch-RBF). μ= mean, σ = standard deviation*

	A. lumbricoides			ETEC			Rotavirus			Source
Concentrations	Lognormal (μ; σ)			Lognormal (μ; σ)			Lognormal (μ; σ)			
- Polluted river	-2.91;	-3.21		3.17;	4.04		-0.34;	0.52		PAPERS I & V
Removal rates	Normal (μ; σ)			Normal (μ; σ)			Normal (μ; σ)			
- Unp-RBF	0.28	0.10		4.05	0.51		1.89	0.84		PAPER I
- Grav-RBF	0.67	0.24		4.80	0.54		2.32	1.11		PAPER I
- Bioch-RBF	NA			4.51	0.53		2.30	1.06		PAPER I & Choque (2018)
Regression parameters										
*y= a · Ln(x) + b	a	b	R^2	a	b	R^2	a	b	R^2	
- Unp-RBF	0.03	0.21	0.73	0.44	3.04	0.97	0.20	1.42	0.72	PAPER I
- Grav-RBF	0.07	0.51	0.69	0.54	3.76	0.98	0.25	1.74	0.76	PAPER I

* y = reduction in log_{10} units; x = distance from stream in m.

7.2 Microbial risks

Weekly probabilities of infection followed the pattern River > Unprotected-RBF = Gravel-RBF = Biochar-RBF for all pathogens studied (*Figure 7*). There was clear agreement between the removal rates and the differences in weekly probabilities of infection.

The reduction in weekly infection probabilities achieved by the three riverbank filtration well types was sufficient to lower the annual disease burden to acceptable levels for *A. lumbricoides* and ETEC, but not for rotavirus. This difference between pathogens can be expected due to their different sizes, as larger pathogens are more effectively removed by filtration (Stevik *et al.*, 2004). Bacterial removal was similar to values found in previous studies of riverbank filtration wells under optimal conditions (3-4 log_{10} at 30-40 m from the water stream in soils with a high proportion of sand in the treatment zone according to Gutiérrez *et al.* (2017) and Weiss *et al.* (2005)). In contrast, removal of viruses in this thesis (2-3 log_{10} at 30-40 m) was lower than with riverbank filtration under optimal conditions (4-6 log_{10} at 30 m according to Sprenger *et al.* (2014) and Tufenkji *et al.* (2002)). This suggests that the subsoil in the study area has a texture/structure that is coarser than the optimum, retaining larger pathogens but allowing passage of smaller pathogens (Schijven & Hassanizadeh, 2000). However, no soil texture studies were performed prior to implementation of riverbank filtration wells in the zone.

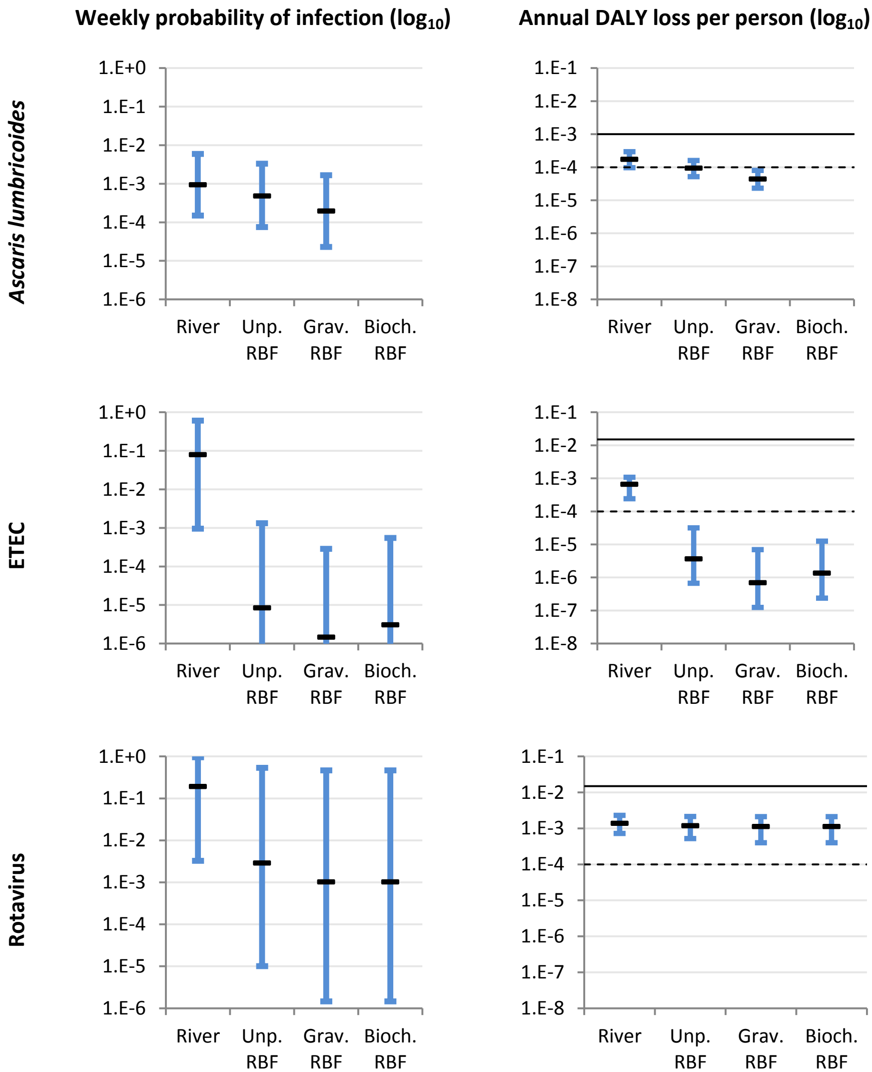

Figure 7. Comparison of health risks from *Ascaris lumbricoides*, enterotoxigenic *Escherichia coli* (ETEC) and rotavirus as weekly probability of infection and annual disease burden per person per year for consumption of lettuce irrigated with wastewater-polluted river water left untreated (River) or treated with unprotected riverbank filtration (Unp. RBF), RBF improved with gravel (Grav. RBF) or RBF improved with biochar (Bioch. RBF). The black markers and error bars represent the 50th percentile and 95% confidence interval respectively. Solid lines indicate the pre-existing disease burden of intestinal nematodes (for *Ascaris lumbricoides*) and diarrhoeal diseases (for ETEC and rotavirus) in Bolivia (Pruss-Ustun *et al.*, 2008). Dashed lines indicate the maximum additional disease burden threshold recommended for wastewater reuse in developing countries (Mara & Sleigh, 2010).

As regards improved riverbank filtration (*i.e*. Gravel-RBF and Biochar-RBF), although it reduced the weekly probability of infection compared with Unprotected-RBF for all pathogens studied (*Figure 7*), this reduction was only significant (*i.e*. 1 $\log_{10}$) for the annual disease burden from ETEC. It is likely that the difference in microbial removal between Unprotected-RBF and Grav-RBF is due to the filtering effect of the gravel layer and lining (Paper I). There was no significant difference between risks with Gravel-RBF and Biochar-RBF, because the microbial removal rates with biochar were found to be similar to gravel under continuous flow (*Table 6*). This means that the effect of favourable properties of biochar as a filter medium (*i.e*. larger surface area, larger proportion of micropores than sand; Paper IV) is somehow limited under continuous flow. It can be speculated that intermittent vertical flow stimulates flow of water out of micropores (making them available for incoming water) more effectively than horizontal continuous flow, due to gravity and the fringe where air and water occupy the voids between/within particles. In horizontal continuous flow, micropores can become ‘dead zones’ where water moves significantly more slowly or does not move at all, and therefore this space is non-available for treatment processes. To my knowledge, there are no published studies about horizontal continuous flow biochar filters for domestic wastewater treatments and therefore future studies are required to confirm/understand this type of filter.

The findings above are in line with expectations for riverbank filtration, but cannot explain why reduction rates of *A. lumbricoides* were lower than rates for smaller microbes. As discussed in Paper I, this seems to be related to runoff transporting microbes from agricultural soil irrigated with wastewater to the riverbank filtration wells. In that case, lining of the walls and smaller diameter of the wellhead in protected wells would reduce the contamination through runoff.

As with On-farm intermittent biochar filtration (Chapter 6), the uncertainty for weekly infection risks from ETEC and rotavirus was high ($\geq$2 $\log_{10}$ units), which could have led to overestimation of their annual disease burdens (see section 6.2). Therefore, burden of disease estimated for ETEC and rotavirus in the present chapter should be also regarded as approximate, and actual removal of the pathogens (ETEC and rotavirus) by riverbank filtration wells should be determined in future studies.

7.3 Feasibility of implementation

Riverbank filtration has already been implemented in a specific area in the study area. The riverbank filtration wells were introduced in 2006-2007 in light of growing contamination of the Rocha river and pressure from the authorities to prohibit vegetables being irrigated with water from the river. Farmers along the river were then put under pressure and improvised different strategies, including unplanned riverbank filtration[5]. Based on that experience, it is possible to identify two factors (soil heterogeneity, subsurface flow) that could affect its implementation in other zones.

Heterogeneity in soil material was not studied before the farmers' wells were established and remains unknown. Riverbank filtration wells studied in this thesis (Paper I) did not remove viruses as efficiently as expected (see section 7.2), indicating that the soil has a suboptimal structure for riverbank filtration. Since soil structure could have been unsuitable for removal of any microbe, implementing the riverbank filtration wells in the study area without prior soil studies was a risky measure.

Subsurface water flow was also not studied before implementation of farmers' wells. During field work for Paper I, it was observed that the velocity of recharge of the riverbank filtration wells sometimes does not match the frequency of irrigation required by farmers, encouraging a continuous search for alternative water sources. This has led to occasional use of lower-quality water from other sources (*i.e.* other riverbank filtration wells and Rocha river water), most likely resulting in increased risks of produce contamination (Paper I). Therefore, it can be concluded that preliminary studies of soil structure and recharge velocity should be undertaken before implementing riverbank filtration wells, in order to ensure the sustainability of the measure. Despite its high potential, this on-farm measure is highly dependent on local conditions, which limits its applicability.

[5]Information about implementation of wells was collected through informal interviews during fieldwork for Paper I.

8 Wastewater substitution

8.1 System description

Risks from irrigation with four water sources were compared in order to evaluate the potential of wastewater substitution for on-farm management of risks. The four water sources compared were settled wastewater (Settled), polluted Rocha river water (River), river water later substituted with spring water (River + Spring) and spring water (Spring) (*Figure 8*). As in the baseline system (Chapter 5), settled wastewater, river water and spring water were included as worst case, baseline and better case scenarios, respectively. The River + Spring scenario was the same as the River scenario, but with river water being replaced by spring water around 11-14 days before harvest. Although spring water is not commonly available in the study context (see section 4.1), it was used to represent substitution of wastewater with any other water source of higher quality. Specifically, it was assumed that farmers could treat and store river water on-farm during the first weeks of lettuce cultivation, and use this stored water for irrigation around two weeks before harvest. Thus, spring water was intended to be a surrogate for river water treated on-farm.

In previous chapters, microbial risks were calculated based on pathogen concentrations on lettuce, which were estimated from microbial concentrations in water samples. For the on-farm measure studied in this chapter (*i.e.* water substitution), actual microbial concentrations on lettuce and in water samples were determined in experimental plots (Paper V). The microbial concentrations (*i.e.* on lettuce and in water samples) were used separately to estimate pathogen concentrations on lettuce, which in turn were used to calculate microbial risks in this chapter. Concentrations of microbes and parameters used to model nitrogen risks are shown in *Table 7*. Characteristics of the systems were as described in section 5.1, except for the water source.

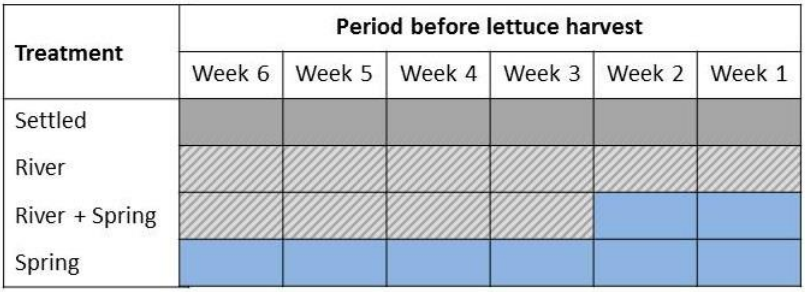

Figure 8. Structure of the four treatments applied to investigate the effect on risks from substituting wastewater with a cleaner source for irrigation of lettuce in experimental plots. The water source used for irrigation of lettuce is shown as a function of time before harvest. Weekly use of settled wastewater (Settled), wastewater-polluted river water (River) and spring water (Spring) is represented with solid grey, grey with pattern and solid blue respectively.

8.2 Microbial risks

In general, weekly probabilities of infection from lettuce consumption followed the pattern Settled = River = River + Spring > Spring for all pathogens studied. As in previous chapters, there was clear agreement between microbial concentrations (in both soil and water, see Table *7*) and weekly infection probabilities.

Reductions in weekly infection probabilities achieved by wastewater substitution (River + Spring in *Figure 9*) were not sufficient to significantly lower annual disease burden for any of the pathogens studied. This small reduction in microbial risks was unexpected (*i.e.* cessation of irrigation is credited with several $\log_{10}$ reductions per day for viruses and bacteria on vegetables, see section 3.7) and it is likely linked to several factors relevant to contamination/survival of microbes on lettuce leaves and the variability in field conditions (Paper V). In brief, river water substitution influenced the microbial concentrations on lettuce, but its effect was counteracted by weather conditions in winter, variations in soil and daily temperature/sunlight regime (both for bacteria), and concentrations of helminths in soil from previous campaigns.

It remains unclear exactly how these factors counteracted reductions in microbial concentrations on lettuce, because they are associated with different mechanisms. For instance, lower temperatures in winter result in: i) longer survival of pathogens on lettuce and ii) longer cultivation period. Both these effects of winter could have affected microbial concentrations on lettuce (*e.g.* the longer the cultivation period, the higher the number of irrigation events per crop season, resulting in higher contamination than for lettuce cultivated in autumn/spring), but it is not clear whether one or both did so, or to what extent.

Table 7. *Parameters and assumptions used in risk assessments with settled wastewater, polluted river water, spring water and polluted river water substituted with spring water 11-14 days before harvest (River + Spring). The concentrations of pathogens are shown in log_{10} eggs/cfu/pfu mL^{-1} or g^{-1} and the concentrations of chemical parameters are in mg L^{-1}. μ = mean, σ = standard deviation*

	A. lumbricoides	ETEC	Rotavirus		Sources
	Lognormal (μ; σ)	Lognormal (μ; σ)	Lognormal (μ; σ)		
Water samples					
Settled wastewater	-2.23; -1.78	1.94; 1.95	-1.58; -1.57		PAPER V
Polluted river	-2.91; -3.21	3.17; 4.04	-0.34; 0.52		PAPERS I & V
Spring water	-3.64; -3.88	-0.11; 0.38	-3.63; -3.14		PAPER V
Lettuce samples					
Settled wastewater	-0.07; -0.14	0.65; 1.01	-2.87; -2.51		PAPER V
Polluted river	0.24; 0.77	0.40; 0.82	-3.12; -2.69		PAPER V
River + spring	-0.51; -0.41	0.25; 0.48	-3.28; -3.03		PAPER V
Spring water	0; -2.13; -2.13 [a]	-0.75; -0.47	-4.28; -3.99		PAPER V
	Total organic N (TON)	Nitrates	Ratio NH_4-N/TON	Ratio BOD_5/COD	
	Lognormal (μ; σ)	Lognormal (μ; σ)	PERT (min; likely; max)	Normal (μ; σ)	
Water samples					
Settled wastewater	86.89; 15.8	0.26; 3.84	0.63; 0.94; 1	0.64; 0.06	PAPER V
Polluted river	90.59; 15.5	3.42; 3.11	0.90; 0.06 [b]	0.70; 0.05	Direccion de Medio Ambiente de Sacaba (2014); Contraloria General del Estado (2011); PAPER V
Spring water	NA	22.76; 8.79	NA	NA	PAPER V

[a]The concentration of *A. lumbricoides* in spring water was assumed to follow a triangular distribution.
[b]The ratio NH_4-N/TON in polluted river water was found to follow a normal distribution.

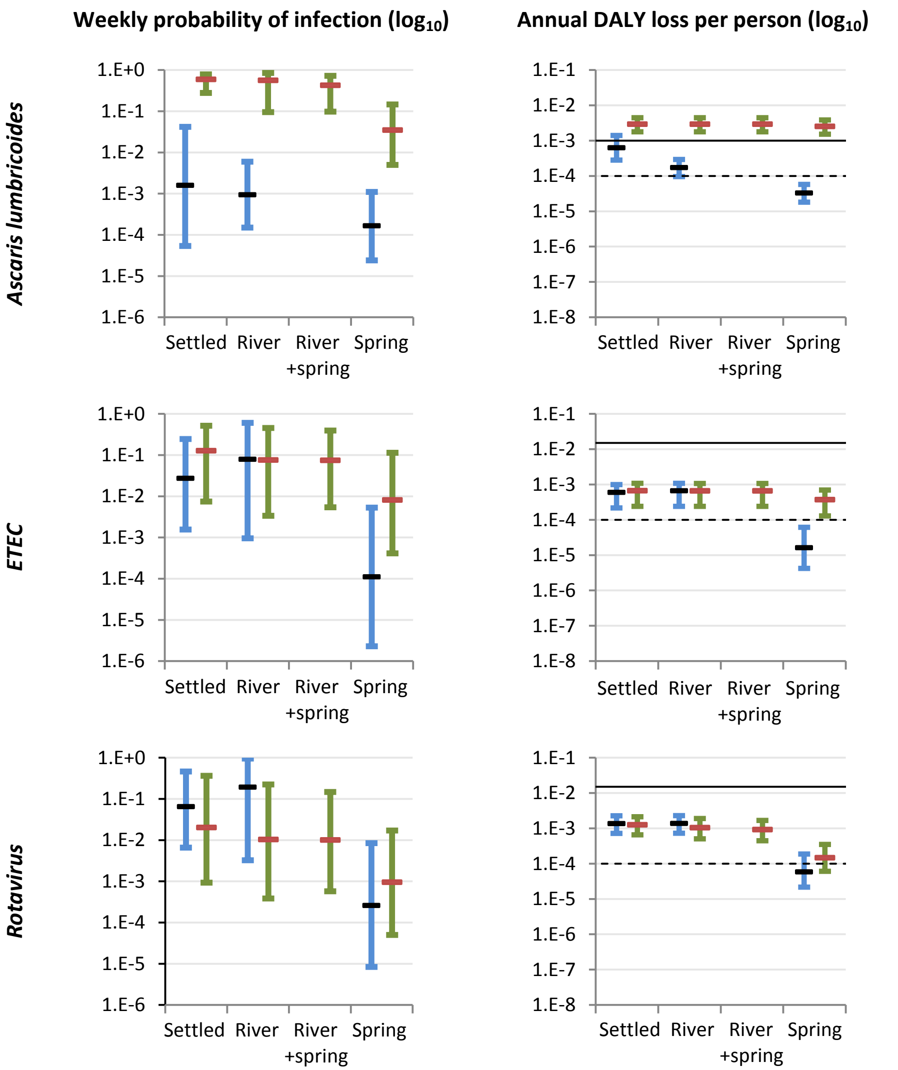

Figure 9. Comparison of health risks from *Ascaris lumbricoides*, enterotoxigenic *Escherichia coli* (ETEC) and rotavirus as weekly probability of infection and annual disease burden per person per year for consumption of lettuce irrigated with settled wastewater (Settled), wastewater-polluted river water (River), wastewater-polluted river water later substituted with spring water (River+spring) and spring water (Spring). The black markers/blue error bars represent the 50th percentile/95% confidence interval of risks calculated from water samples, while the red markers/green error bars represent the 50th percentile/95% confidence interval of risks calculated from lettuce samples. Solid lines indicate the pre-existing disease burden of intestinal nematodes (for *Ascaris lumbricoides*) and diarrhoeal diseases (for ETEC and rotavirus) in Bolivia (Pruss-Ustun *et al.*, 2008). Dashed lines indicate the maximum additional disease burden threshold recommended for wastewater reuse in developing countries (Mara & Sleigh, 2010).

In addition, the concentrations of microbes on lettuce showed high variability and only ~40% could be explained by the factors considered in the experiment (Paper V). Such variance is likely to have originated from variations in several parameters relevant to microbes on lettuce, due to the field conditions. For example, daily temperatures in winter range from ~3 to ~26 °C, while in autumn/spring they range from ~8 to 29 °C. It is unknown how such variation impacts microbial survival, as it is not considered in the reduction rates given by World Health Organization (2006) or in the reduction rates reported by studies on microbial survival (Paper V).

Differences between risks calculated from water samples and from lettuce samples for *Ascaris lumbricoides* and ETEC were identified (*Figure 9*). These could be explained by the uncertainty from the aforementioned factors counteracting die-off and the variability in field conditions. As is common practice in microbial risk assessment assessments (Keuckelaere *et al.*, 2015), the models for risk based on water samples in this thesis simulated the concentration of pathogens on lettuce considering die-off in steady conditions of temperature and sunlight (see section 4.3.2). Such models do not account for the effect of factors counteracting die-off or for the variability of temperature and sunlight in field conditions. For instance, extended survival of a small proportion of microbial populations (Seidu *et al.*, 2013) could determine their significant accumulation on lettuce after many irrigation events, but this is commonly not considered in models for risk assessment. Regardless of the causes of this limitation in the models (*e.g.* lack of data, limited available knowledge, specificities of the context, as described by World Health Organization (2006)), it seems clear that they have led to significant differences, especially for ascaris, between health risks based on water samples and water samples.

As regards rotavirus, its concentrations on lettuce and water were calculated from bacterial indicators, but the survival was modelled with viral die-off kinetics (Table *1*). Therefore, the differences found between concentrations of rotavirus on lettuce from water and lettuce samples (*i.e.* risks from lettuce samples higher than with water samples for spring water) were as expected.

8.3 Risks of nitrogen excess in soil

The estimated amounts of nitrogen from irrigation water accumulated in soil followed the pattern Settled = River = River + Spring > Spring (*Figure 10*). Despite some reduction in nitrogen accumulated in soil compared with Settled and River water (*i.e.* around 150-200 and 50 kg ha^{-1} lower under low and high irrigation efficiency, respectively), irrigation with River + Spring resulted in nitrogen excess under both irrigation efficiency scenarios.

Lower accumulation of nitrogen in soil irrigated with River + Spring than with Settled and River was expected, due to lower addition of nutrients from spring water. However, no significant differences were found between River + Spring and the other water sources (Settled and River) due to the high concentrations of nitrates measured in spring water. Nitrates in spring water most likely originated from urban contamination processes (see section 5.3). The quality of substitute water in terms of nitrogen concentration is then an important factor to consider in order to reduce probabilities of nitrogen excess in soil.

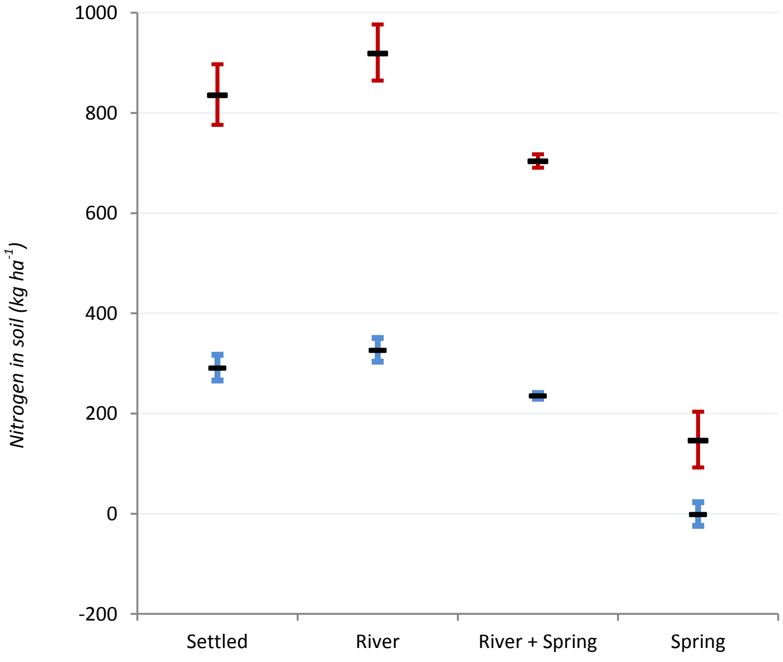

Figure 10. Calculated amounts of available forms of nitrogen accumulated in soil after one crop season of lettuce under scenarios of low (red) and high (blue) efficiency of furrow irrigation with different water sources. The black markers and error bars represent the 50th percentile and 95% confidence interval, respectively. Irrigation water sources were settled wastewater (Settled), wastewater-polluted river water (River), wastewater-polluted river water substituted with spring water (River + Spring) and spring water (Spring). Values below zero indicate nitrogen deficit.

8.4 Feasibility of implementation

Wastewater substitution for irrigation of lettuce was not effective to significantly reduce any of the risks studied. Thus, its implementation would not add any benefit as part of the multibarrier approach in the study context.

The results suggest that reduction of pathogens on lettuce by wastewater substitution could be optimised if it is carried out in autumn/spring (*i.e.* winter was found to counteract microbial die-off, see section 8.2 and Paper V). However, it should be considered that such optimisation might not significantly reduce the levels of excess nitrogen in soil. On the one hand, the total amount of nitrogen applied through wastewater (*i.e.* before substitution) is likely to be higher than the total requirement from lettuce. On the other hand, water substitution could imply stopping addition of nutrients during the last weeks (unless the nitrate content in the replacement source is high, as in this thesis), which might affect the yield of lettuce. If that is the case, nitrogen addition through fertilisers could be required. Finally, a longer period of cessation of irrigation could be an option to be investigated as a barrier in the context studied here.

9 General discussion

9.1 Findings of this thesis in perspective

In this section, the findings presented in previous chapters are used to position the measures studied within the 'global map' of available on-farm measures in terms of risk reduction and implementation.

9.1.1 Baseline system: Furrow irrigation

According to World Health Organization (2006), in terms of microbial risks furrow irrigation contaminates leaf crops less than overhead methods, but more than drip irrigation (World Health Organization, 2006). This seems logical, as water is spread directly on leaves in overhead irrigation and on the soil surface/ sub-surface in drip irrigation. This has been confirmed in studies by Bastos and Mara (1995) and Song *et al.* (2006), where lettuce contamination with furrow irrigation was reported to be slightly higher (~1 $\log_{10}$) than with drip irrigation. These findings may have led to a smaller inclination to study the microbial risks associated with furrow irrigation since the publication of World Health Organization (2006) guidelines (*i.e.* only Woldetsadik *et al.* (2017), to the best of my knowledge, reported microbial contamination of produce linked to furrow irrigation with wastewater), focusing rather on risks from irrigation with watering cans, a method widely practiced in low-income settings in West Africa (Keraita *et al.*, 2014a). From that point of view, furrow irrigation could be considered an already applied on-farm measure in the system studied in this thesis. However, data collected in the thesis suggest that the risk of microbial contamination of lettuce with furrow irrigation can be similar to that with use of a watering can. For example, in Paper V a concentration of 0.2 $\log_{10}$ ascaris eggs g^{-1} was recorded on lettuce following furrow irrigation with 0.1 $\log_{10}$ eggs

L^{-1} (Table 7), while Seidu *et al.* (2008) reported 1.8 $\log_{10}$ g^{-1} on lettuce from watering can irrigation with 0.6 $\log_{10}$ L^{-1}. The data in Paper V also showed large variation in microbial contamination of lettuce (2-3 $\log_{10}$ g^{-1}), and therefore the level of pathogen transference to lettuce by furrow irrigation could not be determined with accuracy. Thus, more studies are needed to clarify the actual capability of furrow irrigation as an on-farm measure.

As regards nitrogen excess risks, large amounts of nutrients are applied to soil through flood irrigation techniques (including furrow irrigation). Although the need for optimising the amounts of water and nutrients in wastewater irrigation is recognised, no specific on-farm measures have been proposed (Qadir *et al.*, 2015). Such optimisation is possible in furrow irrigation through simple practices such as variable (surge) flows or irrigation during hours of low atmospheric water demand (Scott *et al.*, 2014). The risk assessments in this thesis showed that optimising the efficiency of furrow irrigation could significantly contribute to regulating the nitrogen input from wastewater to the agricultural system, although more thorough studies should be carried out to confirm this potential in wastewater irrigation.

9.1.2 Baseline system: Cessation of irrigation

Cessation of irrigation is credited by World Health Organization (2006) as contributing several (0.5-2) $\log_{10}$ units of microbial reduction per day. These reduction levels have been confirmed in studies by *e.g.* Keraita *et al.* (2007), where average reductions of 0.6 $\log_{10}$ cfu faecal coliforms per day were achieved on lettuce. In this thesis, it was found that cessation of irrigation at least one day before harvest is already practised, with no enforcement, as a side-effect of the irrigation technique used in the study area, since it is very difficult to walk on flooded soil immediately after furrow irrigation (Paper I). Adoption of cessation of irrigation in other contexts has been unsuccessful, mostly due to benefits of irrigation immediately before harvest, *e.g.* Amponsah *et al.* (2016) reported that farmers in Ghana prefer irrigating their vegetables just before harvest because the vegetables look fresh and humid soil facilitates harvesting. However, in this study, cessation of irrigation between the last irrigation event and harvest had no significant effect on microbial concentrations on lettuce (PAPER V). This likely has to do with several factors counteracting microbial die-off, as discussed in PAPER V (Section **Error! Reference source not found.**). It should be highlighted that we did not aim to assess the die-off between the last irrigation and harvest in the experiment in PAPER V (*i.e.* microbial concentrations on lettuce were not measured before harvest). So, we cannot assure that such factor (cessation of irrigation last irrigation-harvest) affects the microbial concentrations on lettuce and, thus, its effect in terms of risk reduction remains unknown

9.1.3 On-farm wastewater treatment: Biochar filtration

On-farm filtration has largely been proposed as an alternative for on-farm risk reduction (Keraita *et al.*, 2010b; World Health Organization, 2006). In Keraita *et al.* (2014a), different filtration types and filter materials for potential on-farm implementation are presented. However, at the time of collecting data for this thesis, only anaerobic filtration had been tested in on-farm conditions, in two studies. In one of these, Keraita *et al.* (2008) reported reductions of ~1.6 $\log_{10}$ and >1 $\log_{10}$ for bacterial indicators and helminth eggs with slow sand filters at ~3 m^3 m^2 d^{-1}. In the other, Kaetzl *et al.* (2019) reported ~2.3 $\log_{10}$ bacterial reductions with slow biochar filters at ~1.2 m^3 m^2 d^{-1} complemented with previous anaerobic filtration. The data for intermittent biochar filtration in this thesis (Paper III) showed removal rates similar to sand filtration (~1.3 $\log_{10}$ of bacterial indicators and >1 $\log_{10}$ of helminth eggs; Table *5*), but at a lower hydraulic loading rate (~0.4 m^3 m^2 d^{-1}) and, unlike sand and biochar anaerobic filters, with no signs of clogging. Consequently, the surface required for implementation of slow sand, slow biochar and intermittent biochar filters would be ~3, ~9 and ~25 m^2, respectively, to irrigate a lettuce plot of 500 m^2 (Paper III).

Slow biochar filters seem the best on-farm filtration option, because they can achieve >2 $\log_{10}$ reductions in bacteria (~1 $\log_{10}$ reduction in bacteria is not significant in terms of reducing microbial risks, see section 6.2) and because the area required is small (~2% of the plot). However, slow biochar filters require an additional (currently unknown) area for pre-treatment with anaerobic filters and periodic replacement of the upper layer, which might challenge its acceptability in contexts similar to that studied in this thesis, as discussed in section 6.4 and Paper III. In addition, slow biochar filters do not remove nutrients (Kaetzl *et al.*, 2019). Although preserving nutrients in wastewater for irrigation is considered beneficial because it reduces the need for mineral fertilisers in food production (Mateo-Sagasta *et al.*, 2015), the assessments in this thesis showed that some nutrient reduction is needed to limit the risk of large volumes of nitrogen accumulating in soil (section 6.3). Thus, the intermittent biochar filters tested in this thesis can still be a feasible option in contexts where nitrogen removal is required, as long as size and microbial reduction are optimised.

9.1.4 On-farm wastewater treatment: Riverbank filtration

Riverbank filtration has been largely studied for drinking water purposes and has been shown to be efficient in removing many contaminants such as organic matter, pesticides, microbes from stream water (Dash *et al.*, 2010). Implementing riverbank filtration for irrigation can be very simple (digging shallow wells next to a stream) and this is likely the reason for its popularity for irrigation next to polluted streams in West Africa (Keraita *et al.*, 2014a). In informal irrigation in low-middle income settings, farmers seldom implement riverbank filtration aiming to reduce water pollution, but rather to reduce the distance between the water source and the irrigated plot (Ganso *et al.*, 2014). Even in those cases, a reduction of at least 1-2 $\log_{10}$ and higher can be expected for bacteria and helminths, respectively, in the water collected from wells (Keraita *et al.*, 2014b). These reductions were largely met in the riverbank wells measured in this thesis (~3 $\log_{10}$ reduction for *A. lumbricoides* and ETEC) (Paper I, Table *6*). To the best of my knowledge, re-contamination of riverbank filtration wells had not been considered prior to this thesis. This process is most likely specific to settings where wastewater irrigation is performed by flooding techniques or where rainfall events are very intense, both favouring run-off towards wells. The risk assessment showed that, if confirmed, re-contamination of wells might increase the annual disease burden by ~1 $\log_{10}$ DALYs for ETEC and *A. lumbricoides*, which could be relevant where disease burdens are high.

9.1.5 On-farm water management: Water substitution

Substituting wastewater with a cleaner irrigation source in order to prolong the time between the last irrigation with wastewater and harvest has been suggested as a potential alternative to reduce microbial risks by World Health Organization (2006). This recommendation is based on two studies, by Shuval (1978) and Vaz da Costa-Vargas *et al*. (1996), that have been cited repeatedly (Tripathi *et al.*, 2014; Keraita *et al.*, 2010b; World Health Organization, 2006). The recommendation has remained unchallenged despite contradictory evidence about microbial die-off on vegetables under controlled conditions (see section 8.2). To my knowledge, this thesis is the first work to test water substitution under field conditions since the publication of those two studies. The results showed that water substitution might not be a reliable on-farm measure to reduce microbial risks or nitrogen excess risks, at least in uncontrolled field conditions as tested in Paper V. Further studies are required to clarify whether/how this measure can be optimised for risk management.

9.2 Scope of risk assessment outcomes

Methodological choices can be crucial for the outcome of risk assessments. In this section, assumptions and uncertainties that could have an impact on the risk assessments are discussed/acknowledged.

9.2.1 Microbial risks

Ratios indicator:pathogen

The ratios used to assess microbial risks in this thesis were all based on data from Bolivia. That for *Ascaris lumbricoides* was based on the number of *Ascaris* spp. and total helminth eggs found together in the Rocha river during 2014-15 (Paper I); that for ETEC was based on the actual *E. coli*:ETEC ratio in stool samples from children under 5 years with and without diarrhoea in Cochabamba and La Paz (another Bolivian city) (Gonzales *et al.*, 2013); and that for rotavirus was based on the number of rotavirus and total coliforms in raw sewage and Rocha river water during 1987 (Toranzos *et al.*, 1988). The method used to detect *Ascaris* spp. eggs was based on microscopic examination and did not include determination of the viability (Paper I). Furthermore, concentrations of rotavirus and ascaris in wastewater could be lower than the assumed ratios, due to national campaigns to control both pathogens since the sampling dates (see section 5.4). As regards ETEC, concentrations in wastewater could be lower than assumed because sewage represents excreta from the whole population (not only children >5), and the presence of ETEC in stool samples has been shown to decrease with increasing age of the individual (Gonzales *et al.*, 2013). Thus, concentrations of the microorganisms studied, and the viability of *Ascaris lumbricoides,* could have been overestimated, leading to overestimation of the calculated baseline risks.

The indicators used in Paper III to assess bacteria and virus removal in biochar filters in on-farm conditions were generic *E. coli* and *Enterococcus* spp. for ETEC, and phages MS2 and ϕX174 for rotavirus. The removal rates from both bacterial indicators were combined into one removal rate, which was used to estimate removal of ETEC (see Table 5). An analogous procedure was followed for rotavirus. However, removal rates were 0.2-0.6 $\log_{10}$ higher for the virus and bacteria with the highest isoelectric point (phage ϕX174 and *E. coli*, with 6.7 and 5.6, respectively, while phage MS2 and *Enterococcus* spp. have 3.5 and <4.0, respectively) and the larger bacteria (generic *E. coli*, rod-shaped and up to 6 µm) (Topcu & Bulat, 2010; Gallardo-Moreno *et al.*, 2004; Schijven & Hassanizadeh, 2000; Sherbet & Lakshmi, 1973; Harden & Harris, 1953). The pathogens studied here (ETEC and rotavirus) would likely be removed at the highest rates measured for the indicators in Paper III, as they share relevant features with such indicators (*i.e*. ETEC is a rod-shaped *E. coli* and rotavirus has an isoelectric point of 8 (Michen & Graule, 2010)). Thus, using two rates per organism could have led to overestimation of the actual risks for ETEC and rotavirus.

Models for microbial transfer and decay/survival on lettuce

Pathogen concentrations on lettuce in this thesis were modelled based on the volume of water captured by lettuce in the study by Shuval *et al.* (1997), where whole lettuce heads were immersed in water and the volume of water caught on produce was measured. Such data are commonly used in risk assessments to fill the information gap regarding transfer of pathogens to agricultural produce through wastewater irrigation (Keuckelaere *et al.*, 2015). However, it is unlikely that a lettuce head under furrow irrigation would retain as much water as a totally immersed head. It could thus be the case that pathogen concentrations on lettuce calculated in this thesis were overestimated.

Another source of uncertainty is that the model used in this thesis only accounts for the last single irrigation event and does not include transfer of pathogens from soil. Significant accumulation of pathogens on leaf crops could have occurred from previous irrigation events and by splashing of pathogens from soil (Allende *et al.*, 2017). These factors were not included in the model due to lack of data for the conditions studied in this thesis, but they could lead to higher concentrations of pathogens on produce than estimated.

The data used to model decay of pathogens on lettuce were obtained from different studies under different conditions. No decay was assumed for *A. lumbricoides* (section 4.3.2) because the published decay rates on lettuce were obtained in environmental conditions widely different from those in this thesis (*i.e.* mean temperature of 30°C by Seidu *et al.* (2013)). The ETEC decay rate on lettuce was calculated using data from Ottoson *et al.* (2011), where decay of *E. coli* O157 on lettuce was significantly affected by temperature and light intensities. Thus, it was possible to model the decay of ETEC based on the daily variation in sunlight/darkness. However, temperature was not included, due to lack of data on die-off at the daily temperature variations in conditions in the study area used in this thesis. Rotavirus decay was based on Leblanc *et al.* (2019), where the effect of temperature was studied and found to be significant for decay of bovine rotavirus on blueberries. As this rate did not consider either daily sunlight/darkness or survival on lettuce, and viruses may survive differently on different vegetable surfaces (Deng & Gibson, 2017), actual decay rate of rotavirus on lettuce could be different than modelled in this thesis.

Consumption of wastewater-irrigated lettuce

In this thesis, it was assumed that the whole population would consume wastewater-irrigated lettuce. This is not true, as availability of lettuce not irrigated with wastewater has been reported for Bolivian markets and as at least 30% of the area devoted to production of lettuce uses freshwater sources for irrigation (Diez de Medina *et al.*, 2013; Ministerio de Medio Ambiente y Agua, 2013). Although cross-contamination has been reported in markets because lettuce is commonly handled/washed together (*i.e.* wastewater-irrigated and non-wastewater-irrigated) by the sellers (Rodríguez *et al.*, 2015; Ministerio de Medio Ambiente y Agua, 2013), it is unlikely that all the lettuce available in markets is contaminated at the same levels found in plots for this thesis. Thus, actual consumption of wastewater-irrigated lettuce could have been overestimated and, consequently, the calculated disease burden for Bolivia.

9.2.2 Nitrogen excess risks

This thesis did not intend to provide a tool to model the fate of nitrogen from wastewater when applied through irrigation. The dynamics of nitrogen in agricultural systems are complex and depend on factors such as temperature, availability of organic carbon, volume of water applied, soil properties and others (Elgallal *et al.*, 2016; Saeed & Sun, 2012). The intention was rather to compare different sources/on-farm strategies for wastewater irrigation in a simplified manner, but including the variability in nitrogen concentrations, irrigation technique and crop requirement. Thus, it is not possible to ascertain whether the surplus amounts of nitrogen in soil estimated in this thesis are accurate.

The concentration of nitrogen in domestic wastewater applied by flood irrigation has been shown to be sufficient to meet the requirements of different crops. Hernández-Martínez *et al.* (2018) reported inputs from wastewater of 30-50 mg NH_4-N L^{-1}, which exceeded by >2-fold the nitrogen requirements of maize and fodder oats (180 and 250 kg ha^{-1}, respectively). The requirement of lettuce is lower (110 kg ha^{-1} according to Scaife and Bar-Yosef (1995)) and the concentrations in the water sources studied in this thesis were higher (50-90 mg NH_4-N L^{-1}; Table *4*) than in Hernández-Martínez *et al.* (2018). Therefore, it is likely that the nitrogen input from wastewater is enough or even excessive to fertilise lettuce crops in the conditions studied in this thesis. Consequently, the risks of nitrogen excess in soil are high because, besides wastewater, cattle/poultry manure and chemical fertilisers are supplied during cultivation (Papers I & V).

10 Conclusions

Regarding the baseline risks:

- Health risk assessments showed that, for the three pathogens studied, disease burdens from lettuce consumption exceeded the $\leq 10^{-4}$ DALY health target in the baseline agricultural system. The assessments also demonstrated that consumption of lettuce irrigated with sources commonly used for irrigation of vegetables in the study area in Bolivia (settled wastewater and wastewater-polluted river) posed similar risks to consumption of lettuce irrigated with a worst-case source (raw wastewater).
- Nitrogen excess risk assessments showed that the available forms of nitrogen accumulated in soil after one crop season of lettuce would be enough to fertilise at least one additional crop season. The assessments also showed that the amounts of nitrogen accumulated in soil when lettuce was irrigated with sources commonly used for irrigation of vegetables (settled wastewater and wastewater-polluted river water) were similar to when lettuce was irrigated with a worst-case source (raw wastewater). According to the risk assessments, increasing the efficiency of furrow irrigation (*e.g.* by irrigating with variable flows or irrigating during hours of low atmospheric water demand) would halve the amounts of nitrogen accumulated in soil when irrigating lettuce with wastewater and wastewater-polluted river water.

Regarding biochar filtration as an on-farm measure:

- Health risk assessments showed that only the disease burden from *Ascaris lumbricoides* would be reduced below the $\leq 10^{-4}$ DALY health target if biochar filters were implemented on-farm, while removal of ETEC and rotavirus was found to be non-significant in terms of risk reduction. Besides low pathogen removal rates, a major constraint to use of biochar filters as an on-farm measure is the area they require for implementation.
- Nitrogen excess risk assessments demonstrated significant reductions in nitrogen accumulated in soil with on-farm biochar filters. However, the

amount of nitrogen accumulated only approached the equilibrium point (0 kg ha^{-1}) when furrow irrigation was applied with high efficiency.

Regarding riverbank filtration as an on-farm measure:

- Health risk assessments demonstrated that only the disease burden from rotavirus exceeded the $\leq 10^{-4}$ DALY health target when wastewater-polluted stream was treated by riverbank filtration. The assessments also showed that contamination of collection wells for irrigation water might threaten the risk reduction achieved. Despite its high potential, riverbank filtration is highly dependent on local conditions, limiting its applicability.

Regarding substitution of irrigation water as an on-farm measure:

- Health risks assessment showed that substituting wastewater for irrigation with a cleaner source would not be enough to lower the disease burden from lettuce consumption below the $\leq 10^{-4}$ DALY health target for any of the pathogens studied.
- Nitrogen excess risk assessments revealed that the amounts of nitrogen accumulated in soil would be similar whether or not wastewater was substituted by a cleaner source for irrigation.

11 Future research and development

Regarding on-farm measures for systems with wastewater irrigation:

- Transfer of pathogens to lettuce in systems irrigated by furrow with wastewater depends on concentrations of pathogens in water and soil, but the mechanisms for such transfer are not clear. Re-suspension of pathogens in soil to water and water/soil spills to lettuce should be evaluated, especially for highly persistent pathogens.
- Daily variations in sunlight and temperature and how these relate to reduction/growth/survival of pathogens on vegetable surfaces should be analysed.
- Pathogen transfer to lettuce and reduction/growth on lettuce should be incorporated into the exposure model on an irrigation-event basis. The modelled concentrations of pathogens on lettuce should be validated.
- The smallest effective diameter tested in biochar filters for on-farm treatment of wastewater did not show any signs of clogging after four months of continuous operation. Longer-term studies with different organic loading rates should be carried out to confirm the robustness of biochar filters in terms of clogging. Smaller effective diameters and their efficiency in terms of pollutants reduction should also be tested.
- Input of available nitrogen into the agricultural system was found to be excessive, particularly if application of chemical fertilisers and manure would be accounted for. Nitrogen flows in the system should be studied considering all inputs and outputs identified.
- Optimising the efficiency of water application in furrow irrigation was identified as a possible on-farm measure to reduce the risks of excessive supplying of nitrogen. The effectiveness of this measure in terms or risk management and feasibility of implementation should be studied.
- Wastewater irrigation entails more risks than studied here. Phosphorus flows should be studied because, like nitrogen, phosphorus poses environmental risks if applied in excess. Pharmaceutical and personal care

products (PPCPs) can accumulate in soils, with unknown effects, and thus flows of PPCPs in the agricultural system and how they are affected by on-farm measures should also be investigated.

Topics specific to Bolivia:

- The available national disease burden data are not pathogen-specific and are likely outdated due to the effect of recent national campaigns to control rotavirus and ascaris. Likewise, the area cultivated with vegetable crops and irrigated with wastewater is unknown. These might lead to wrong decisions about health and environmental management in Bolivia. Studies should be carried out to update the disease burden figures and to quantify the area and the crops irrigated with wastewater or polluted streams.
- There is no information about practices, technologies or risks linked to manure management. This can be critical, as manure can introduce contaminants and pathogens to the agricultural system. Performance of manure treatment (if any) in terms of pathogens, nutrients and relevant pollutants should be investigated.

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Popular science summary

The principal motivation for recycling wastewater in agricultural systems is water scarcity. This is especially the case in arid and semiarid areas of the world, such as in Bolivia, where irrigation with wastewater allows water reclamation and supplies plant nutrients. Wastewater irrigation has immense potential to improve food security and sustainability of communities, yet also entails health and environmental risks. However, as proposed by the World Health Organisation, these risks can be safely managed by identifying the risk pathway and by placing multiple barriers along the pathway to complement or substitute the work of a wastewater treatment plant. This doctoral thesis focused on developing and testing on-farm measures that could act as safety barriers to reduce risks, and analysed their suitability for implementation in the context of wastewater-irrigated agriculture in Bolivia.

The overall aim was to quantify the risks associated with production of lettuce irrigated with wastewater-polluted water sources and to test whether three farm-based measures could help reduce these risks. The focus was on microbial risks from the consumption of lettuce and on environmental risks from excessive amounts of nitrogen entering the soil from the irrigation water. Four scenarios were assessed, a baseline system and three scenarios including different on-farm measures. The baseline system i) was direct use of wastewater polluted river water, while the three on-farm measures explored were ii) filtration of wastewater before irrigation using biochar as filtration media, iii) improved wells for collection of soil-filtered wastewater before irrigation, and iv) substituting wastewater with a cleaner water source two weeks before harvest. The work included collection and analysis of samples from plots managed by farmers, laboratory experiments and experimental plots. The microbial risks were evaluated for a virus, a bacteria and an intestinal worm and were considered high if they exceeded the values recommended by the World Health Organization. Nitrogen excess risks were considered high if nitrogen applied to soil was twice the lettuce requirement.

Results showed that both microbial and nitrogen excess risks were high in the baseline system, clearly demonstrating the need for implementing farm-based measures. Yet, none of the on-farm measures tested would reduce all of the risks investigated. Riverbank filtration worked best to reduce microbial risks, reducing two (bacterial and worm) out of three risks. It was followed by biochar filtration, which reduced only risks from worms. Wastewater substitution did not reduce any microbial risks. With regards to nitrogen excess, only biochar filtration could reduce risks to almost zero accumulation, as long as irrigation efficiency was improved.

In spite of the small reduction of risks, it was possible to identify some key aspects that could increase the performance of each studied measure. For instance, biochar filtration could be improved by reducing the size of the biochar particles, and nitrogen excess could be reduced by optimizing the amount of water applied during irrigation. Although the on-farm measures evaluated in this work did not reduce all the risks sufficiently, the multi-barrier approach should not be discarded, as other measures along the risk pathway can be explored to reduce the risks further.

Populärvetenskaplig sammanfattning

Den främsta orsaken för återvinning av avloppsvatten i jordbrukssystem är vattenbrist, särskilt i torra och medeltorra områden av världen, till exempel i Bolivia. Bevattning med avloppsvatten leder till återvinning av vatten samtidigt tillgodoses växtnäringsämnen och har en enorm potential att öka livsmedelssäkerheten och hållbarheten i samhällen, men det medför också hälso-, och miljörisker. Som föreslagits av Världshälsoorganisationen kan emellertid dessa risker hanteras på ett säkert sätt genom att identifiera riskvägen och placera barriärer längs vägen, för att komplettera eller ersätta avloppsreningsverk. Fokus för denna doktorsavhandling var att utveckla och utvärdera gårdsbaserade åtgärden som kan fungera som barriärer för att minska riskerna, samt att analysera möjligheten att implementera på jordbruk som använder sig av avloppsbevattning. Det övergripande syftet var att kvantifiera riskerna förknippade med produktion av sallad bevattnad med avloppsförorenat flodvatten och testa tre gårdsbaserade åtgärder som skulle kunna minska dessa risker. Fokus var på mikrobiella risker vid förtäring av sallad odlad med avloppsförorenat flodvatten, samt på miljörisker kopplat till kväveöverflöd från bevattningskällan. Fyra scenarier utvärderades, ett baslinjesystem och tre scenarier som innefattade olika gårdsbaserade åtgärder. Baslinjesystemet i) var bevattning med avloppsförorenat flodvatten och de tre utvärderade gårdsbaserade åtgärderna var ii) biokolfiltrering eller iii) flodbanksfiltrering av det avloppsförorenade flodvattnet före bevattning, samt iv) ersättning av avloppsförorenade flodvattnet med en renare vattenkälla två veckor före skörden. Arbetet inkluderade insamling och analys av prover från odlingslotter som hanterades av jordbrukare, laboratorieexperiment och experimentella odlingslotter. De mikrobiella riskerna utvärderades med avseende på ett virus, en bakterie och en inälvsmask och ansågs höga om de överskred Världshälsoorganisationens gränsvärden. Riskerna förknippade med kväveöverskott ansågs höga om kvävemängden som tillfördes var två gånger salladens kvävebehov.

De mikrobiell riskerna, såväl som kväveöverskottet, var höga i bassystemet, vilket tydligt visade behovet av att införa gårdsbaserade åtgärder. Ingen av de studerade gårdsbaserade åtgärderna minskade alla de risker som utvärderades. Flodbanksfiltrering minskade de mikrobiella riskerna mest, där två (bakterie- och inälvsmask) av tre risker minskades. Därefter följde biofiltrering, vilket endast minskade riskerna för inälvsmaskar. Vattenersättning minskade inga mikrobiella risker. Vad gäller kväveöverskott kunde endast biokolfiltrering minska riskerna till jämviktslänge (då det tillfördes lika mycket som behövdes av salladen), så länge bevattningseffektiviteten var hög.

Trots de små minskningen av riskerna i de utvärderade gårdsbaserade åtgärden var det möjligt att identifiera några viktiga aspekter som kunde öka effektivteten av varje utvärderad åtgärd. Exempelvis kan biokolfiltreringen förbättras genom att använda mindre biokolspartiklar i filtrerna, och kväveöverskottet kan reduceras genom att optimera mängden vatten som tillförs under bevattning. Även om de gårdsbaserade åtgärderna utvärderade i denna studie inte minskade alla riskerna tillräckligt bör multibarriärmetoden inte slopas, utan andra åtgärder längs riskvägen bör undersökas för att ytterligare minska riskerna.

Resumen de ciencia popular

La principal motivación para reusar aguas residuales en agricultura es la escasez de agua. Éste es el caso especialmente en zonas áridas y semiáridas del mundo, donde el riego con aguas residuales además proporciona nutrientes a las plantas. El riego con aguas residuales tiene un inmenso potencial para mejorar la seguridad alimentaria y la sostenibilidad de las comunidades, pero también conlleva riesgos para la salud y el medio ambiente debido a la contaminación. No obstante, de acuerdo a lo propuesto por la Organización Mundial de la Salud, tales riesgos pueden ser manejados identificando la ruta de los contaminantes y colocando múltiples barreras a lo largo de dicha ruta para complementar o sustituir el trabajo de una planta de tratamiento de aguas residuales. Esta tesis doctoral se enfocó en desarrollar y probar medidas que podrían actuar como barreras de seguridad en la parcela agrícola con el fin de reducir riesgos. Además, en esta tesis se analizó la idoneidad de dichas medidas para su implementación en el contexto de producción agrícola con aguas residuales en Bolivia.

El objetivo general fue cuantificar los riesgos asociados con la producción de lechuga regada con fuentes de agua contaminadas con aguas residuales, y probar si tres medidas aplicadas antes/durante el riego podrían ayudar a reducir estos riesgos. El estudio se centró en los riesgos microbianos del consumo de lechuga y en los riesgos ambientales provenientes de las cantidades excesivas de nitrógeno que el agua residual aporta al suelo. En total se evaluaron cuatro escenarios: uno de referencia y tres en los que se simuló la aplicación de diferentes medidas en parcela. El escenario de referencia consistió en i) el uso directo del agua de los ríos contaminados con aguas residuales, mientras que las tres medidas en parcela exploradas fueron ii) filtración de aguas residuales antes del riego utilizando biochar como medio de filtración, iii) pozos mejorados para la recolección de aguas residuales filtradas por el suelo antes del riego, y iv) sustituir las aguas residuales con una fuente de agua más limpia dos semanas antes de la cosecha. El trabajo incluyó la recolección y análisis de muestras de parcelas gestionadas por agricultores, experimentos de laboratorio y parcelas experimentales. Los riesgos microbianos fueron evaluados en base a un virus, una bacteria y un gusano intestinal, y se consideraron altos si superaban los valores recomendados por la Organización Mundial de la Salud. Los riesgos de exceso de nitrógeno se consideraron altos si el nitrógeno aplicado al suelo era el doble o más que el requerimiento del cultivo de lechuga.

Los resultados mostraron que tanto los riesgos de exceso de nitrógeno como los microbianos eran altos en el escenario de referencia, lo que demuestra claramente la necesidad de implementar medidas basadas en parcela. Sin embargo, ninguna de las medidas probadas reduciría todos los riesgos considerados. La filtración de ribera de río demostró capacidad para reducir los riesgos microbianos, reduciendo dos (bacterias y gusanos) de tres riesgos. En cambio, la filtración con biochar redujo solo los riesgos de los gusanos, y la sustitución de aguas residuales no redujo ningún riesgo microbiano. Respecto al exceso de nitrógeno, solo la filtración con biochar podría reducir los riesgos de modo que la acumulación sea casi nula, siempre que se mejorara la eficiencia del riego.

A pesar de la baja reducción de riesgos, fue posible identificar algunos aspectos clave que podrían servir para aumentar el rendimiento de cada medida estudiada. Por ejemplo, la capacidad de los filtros con biochar podría aumentarse reduciendo el tamaño de las partículas de biochar, mientras que el exceso de nitrógeno podría reducirse optimizando la cantidad de agua aplicada durante el riego. Aunque las medidas en parcela aquí evaluadas no redujeron suficientemente todos los riesgos, el enfoque de barreras múltiples no debe descartarse, ya que se pueden explorar otras medidas a lo largo de la ruta de los contaminantes para reducir aún más los riesgos.

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Re-use of wastewater for irrigation can contribute to close the loop of water and nutrients. Major limitations of wastewater irrigation are the risks of pathogenic contamination of produce and environmental contamination due to excessive application of nutrients. As part of an innovative approach, three on-farm measures for reduction of these risks were investigated. This study has shown that these measures are constrained by the context and that additional measures are required to manage the risks here studied.

Luis Fernando Perez Mercado received his graduate education from the Department of Energy and Technology at the Swedish University of Agricultural Sciences (SLU). His undergraduate degree was obtained from the Universidad Mayor de San Simon, in Bolivia.

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