Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

eawag
aquatic research

Master thesis 2011

Household drinking water in rural Ethiopia - impact of defluoridation filters on microbial drinking water quality in the Rift Valley

by
Nina Küng

Supervisors:
Prof. Dr. Martin Ackermann
Dr. Rick Johnston
Dr. Frederik Hammes
Dr. Maryna Peters

Acknowledges

This thesis was carried out in Ethiopia and Switzerland, and many persons were involved in the working process. First of all, I want to thank Rick Johnston for the supervision of the thesis, as well as for his practical, technical and theoretical support.

I am very thankful to Rahel Künzle who conducted a similar research in Kenya.. We exchanged findings and experiences and she helped me a lot with statistics. Furthermore, I want to thank Frederik Hammes, Maryna Peters and Hans Ulrich Weilenmann for their assistance in the microbial lab work, as well as for inputs and helpful explanations at Eawag. Very great thanks goes to Sisay Feyera from OSHO who offered me his lab place in Modjo, who provided helpful information and discussion inputs and joined my field visits several times. Tesfaye Edosa and Feysa Lemma (both OSHO) also accompanied me to field visits and were adjuvant discussion partners. Thanks go to the rest of the OSHO crew, Gutama Kisi, Aneni Dejene, Hiwot Beyene, Esayas Samuel, Cesain, Genet, Derartu, Fikadu, Chuke, Schumi, his sheep and the other guards. I also want to thank Carina Flückiger for her helpful inputs and her offer - together with Ina Sosegno - to be human incubators for my plates. Furthermore, Martin Ackermann, Lars Osterwalder, Annette Johnson, Alexandra Huber, Tesfaye and Telahun the Bajaj-drivers, Raffael Graf and Basil for their transport between Ethiopia and Switzerland, the Daemas crew in Modjo, all my households in Wayo, Gura, Washe, Chalalaka and Sariti.

Abstract

In the Ethiopian Rift Valley, there are two main concerns about drinking water: fluoride and fecal contamination. OSHO (an Ethiopian NGO) works on a defluoridation project with household and community filters to remove fluoride from drinking water. The project area includes seven villages, with more than 300 household filters in use and one community filter. This is the first study on the impact of household defluoridation filters in rural Ethiopian microbial water quality. The visited villages use different water sources, such as surface water, boreholes, windmills or dug wells. Microbial contamination is present at the sources, and in much higher extent in the households.

Compact Dry plates (Nissui Ltd, Japan) were uses to detect E.coli, total coliforms and Enterococci as fecal indicator organisms. The method is valid for detection and enumeration of microbial water contamination even under the challenging conditions of rural Ethiopia. The bacterial growth in dependence of incubation temperature showed similar counts above 30°C, which leads to representative results even if the power supply is interrupted. It was compared to standard methods (Colilert, IDEXX, and Petrifilm, 3M) and did not show significant differences.

The household filters have an overall positive impact on fecal indicator organisms in all studied villages; nevertheless the contamination level is very high. The main contamination was taking place between the source and the inlet, respectively the jerrycan. There were no differences in drinking water quality found in a glass from household filter or community filter. The microbial inactivation of copper was tested in the lab and in the field, but no general improvement of the water quality found.

Microbial contamination levels are alarmingly high in all studied households, and measures have to be taken enhance water quality. Hygiene and sanitation are very poor or absent in the area, and diarrheal diseases widespread. Technical possibilities to improve the situation are chlorination of the filters and source water, replacement of sand filter with candle filter, installation of sanitation facilities. They will not be sustainable without awareness creation and education of the interrelation between water, sanitation, hygiene and health.

Table of Contents

Table of Figures

List of Tables

1. Introduction

Safe drinking water is indispensable for our life on this planet, and almost 1 billion of people are deficient in its access. There are around 2 millions of deaths per year related to diarrheal diseases, mostly children in developing countries, due to contaminated drinking water. By improving access to safe water, sanitation and hygiene, there can be prevented 4% of the global burden of disease worldwide [1].

Ethiopia is one of the least developed countries (Human Development Index: 157), with mostly rural population (>80%) and a life expectance of 55 years [2]. In the Ethiopian Rift Valley, there are two main concerns about drinking water: fluoride and microbial (fecal) contamination [3]. The coverage of safe water in rural Ethiopia is only 14% of the whole population, and only 8% within a walking distance of 30 minutes [4]. To have access to safe drinking water does not only imply microbial and chemically safe water, but also to have a secured supply and public access to the water sources [5]. Household treatment of water is widespread over the world, but in Ethiopia, only 5% of the population make use of it. Nevertheless, access to safe drinking water is very low [6].

The East African Rift Valley crosses Ethiopia, Kenya, Tanzania and Mozambique, where the same geogenic water related problem with fluoride occurs.
In Kenya, the Catholic Diocese of Nakuru (CDN) is working with success since more than 10 years with and on bone char technology to remove fluoride from water. In collaboration with Eawag (Swiss Institute for aquatic research), Heks (Swiss Interchurch Aid) and OSHO (Oromo Self Help Organization), this technology was introduced in Ethiopia in 2007 [7].
In Kenya, there are community filters and Household filters in different designs available, while in Ethiopia, there is only one community filter and one type of household filter [8].

The absorption process of fluoride by the filter material is investigated and improved into detail [8]. Nevertheless, until now there were no studies on the impact of the fluoride removal filters on microbial water quality.
The aim of this study is to fill this gap in the Ethiopian context, while Rahel Künzle performed a similar thesis in Kenya [9].

OSHO provided labfilters for first microbial testing on their compound in Modjo, and in five villages water quality was repeatedly tested to assess the impact of the filtering procedure on microbial water quality. Based on the data, knowledge on fecal contamination pathways are achieved and possible measures for water improvement discussed.

2. Background information

2.1 Study area

The office of the defluoridation project of OSHO is situated in Modjo, 80km south-eastern form Addis Ababa. Four villages of the project are situated around 100km south of Modjo (between Meki and Ziway) and one 230km south (between Awassa and Shashamene), see Figure 1.

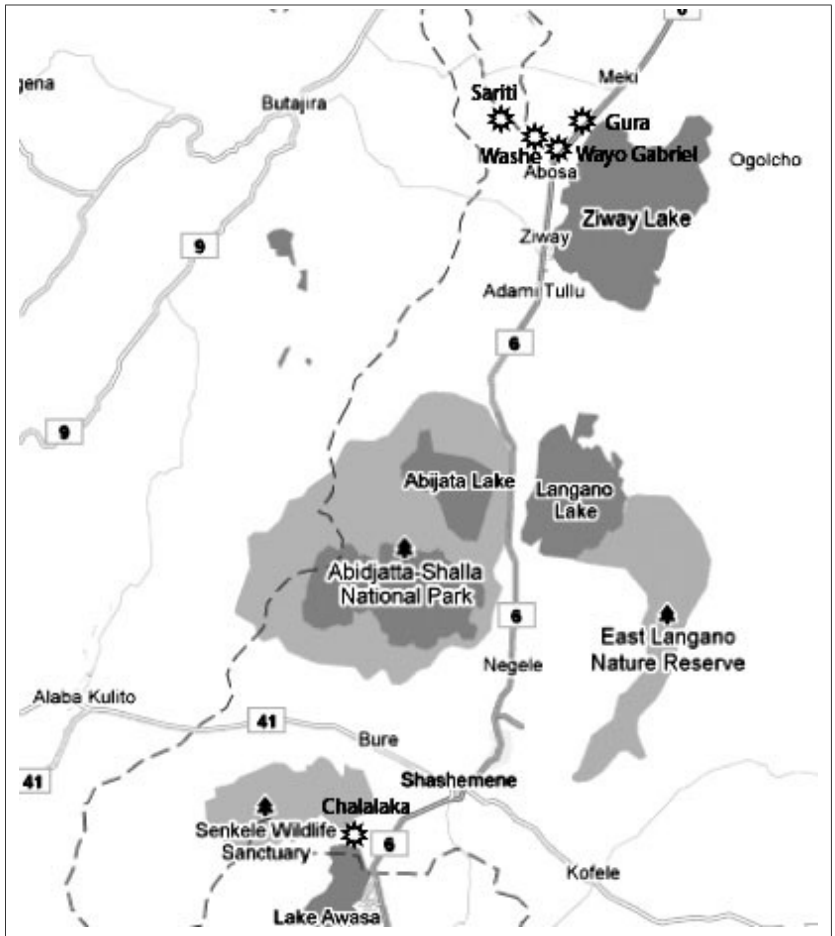

Figure 1: studied villages in the Ethiopian Rift Valley. Source: Google Maps

The defluoridation project started in 2007 with 120 household filters (HF) imported from CDN (Catholic Diocese of Nakuru, Kenya), which were subsidized by OSHO and first sold to the villagers of Chalalaka and Wayo Gabriel (see Figure 1). The filters got upgraded in 2010 with a second bucket containing a sand filter to remove turbidity while minimizing contamination of fluoride filter material through direct human touch. Today, there are 310 HF in use all with the two bucket system, and one community filter (CF), in seven villages in the Rift Valley, mainly between Meki and Ziway, with the exception of Chalalaka [10], [11].

The community filter (CF) located in Wayo Gabriel has been in operation since March 2010. It consists of two filtering units with bonechar and calcium phosphate pellets ("Nakuru Technique") and a 5000 L storage tank, and is situated next to the water point "Shibere" which sells untreated borehole water at a cost of 0.25 ETB/jerrycan. Defluoridated water is sold for 0.50 ETB/jerrycan. The fluoride concentration varies in more than one order of magnitude between the sources, with the highest content in the private dug well of Gebreyes in Wayo, and the lowest in the Shibere and Mesken Safer water points (see Table 2), though these still exceed the Ethiopian guideline for fluoride in drinking water of 1.5 mg/L [12]. In respect of microbiological contamination, all water sources are above the Ethiopian standard (zero CFU/100 mL), but it has to be noted, that the samples were taken from the taps of the water collection points, and not from the reservoir, thus contamination may occur at the taps due to contact with hands. Indicator organism counts differ in 5 orders of magnitude, with the highest counts in the lake water and the private dug well.

Table 1: studied villages in the Rift Valley with their main water source, chemical and microbiological water quality (average values) and Ethiopian Guideline values.
F: Fluoride, EC: E. coli, TC: total coliforms, ETC: Enterococci (geometric means of own data), nd: non detectable. Source: [12–14]

Village	Water source	F [mg/L]	pH	depth [m]		EC (CFU/ 100 mL)	TC (CFU/ 100 mL)	ETC (CFU/ 100 mL)
Gura	Gura windmill	11.5	8.1	36		20	200	10
	Private handpump Ayu	11.9	7.8	35		80	70	150
Wayo Gabriel	Shibere, Mesken Safer	2.8	7.6	42		9	280	40
	Schoolwindmill	10.5	8.1	73		10	270	30
	Private dugwell Gebreyes	17.7	8.3	35		1830	3000	1910
	Community filter	0.5	7.3			5	140	30
Sariti	Sariti Borehole	9.6	7.1	135		5	20	3
Chalalaka	Lake Awassa	8	7.5	22		2910	20780	6730
Ethiopian Standard		1.5	6.5-8.5			nd	nd	nd

2.2 Fluoride

The adverse health effects of excessive fluoride intake by drinking water and food are dental and skeletal fluorosis and, there is no cure existing, only prevention. The maximum permissible level of fluoride of Ethiopian Drinking Water Standard is 1.5 mg/L, based on the WHO (World Health Organization) drinking water guideline [1], [3], [12].

The origin of the fluoride in the African Rift Valley is geogenic, mainly from the groundwater and hydrothermal springs that feed subjacent water bodies that are used as drinking water [15], [16]. Alternative water sources, such as surface water, are often microbiologically contaminated and some lakes have very high fluoride concentrations besides, their access is limited [15]. Rainwater harvesting is only possible during the rainy season in summer, thus not a reliable source during the whole year in this area.

Thus, the fluoride has to be removed from the main source - the drinking and cooking water. There are different options to remove fluoride in the water, such as sorption, precipitation, physical and electrochemical processes. Physical and electrochemical defluoridation methods (i.e. reverse osmosis, ion exchange resins) are most effective but need a lot of energy and acquisition and maintenance costs are high, which makes them unsuitable for rural areas in developing countries. Fluoride sorption materials include, among others, clay minerals, activated alumina or bone char, while fluoride precipitation is done with aluminum hydroxide or calcium phosphate (contact precipitation) [10].

In Kenya, CDN improved the uptake capacity of bone char with contact precipitation, i.e. addition of calcium phosphate pellets to the filter material. This technology (the “Nakuru

technique") is more effective in fluoride removal and locally produced at low cost, thus affordable to rural people [8]. There are household filters and community filters in use with different designs for various customers (income classes) [9].

In Ethiopia, we only count with one type of HF (bonechar with pellets) and one CF (only bonechar).

2.3 Microbial water contamination

Microbiological contamination of the drinking water affects severally the health of the community, and is one of the main problems in developing countries [17]. In general, if the source water (e.g. groundwater) is microbiologically clean, it is still vulnerable to contamination on the way from the source to the drinking glass [18].
To evaluate the quality of drinking water, parameters of chemical and microbial water quality have to be assessed and guideline values fulfilled. For the microbial quality, mainly fecal indicator bacteria are used, because a lot of diseases are transmitted by feces. These indicator bacteria have to accomplish definite criteria, such as not be pathogenic but behave similarly to pathogens, be present in high numbers in feces and more numerous than pathogens, be straightforward and inexpensive to detect, and neither be present, nor multiply in natural waters [17].
The most common fecal indicator organism is *Escherichia coli* (*E.coli*), as well as thermotolerant (fecal) coliforms. Total coliforms have also been used, but as they occur in natural waters, they are not a reliable indicator of fecal contamination. As pathogens may be more resistant to chlorination and other disinfection reactions than E. coli, thus other indicators for fecal contamination were taken into account, such as intestinal Enterococci, *Clostridium perfringes* and bacteriophages [19–24]. For microbiological water quality, the Ethiopian standards (as well as WHO) says, neither E. coli and Enterococci nor total coliforms should be detectable in 100 mL of drinking water [17].
In this study, we use E.coli (EC), total coliforms (TC) and Enterococci (ETC) as fecal indicator bacteria in our experiments. The term "total coliforms" and "coliforms" were used for the same group of organisms in this study.

The range of methods and technologies to get microbiologically safe drinking water is very wide; the main issue is to prevent contamination at source and household level, thus interrupt the transmission pathway of pathogens from the fecal material (of human or animal source) to the drinking water [17]. Household treatment and storage are much more efficient to improve microbial water quality than source protection [18]. Treatment options include boiling, chemical disinfection (chlorine), filtration (sand, membrane) or irradiation (UV light, Sodis) [17], [25].

2.4 Household Filter

The OSHO household filters consist of two plastic buckets (see Figure 2): a smaller upper one with a capacity of around 15 L and a lower one with a volume capacity of about 20 L. In the upper bucket, there are 3 L of sand from lake Langano to remove turbidity, while the lower bucket consists of a mixture of 7.5 L calcium phosphate pellets (purchased from CDN) and 2.5 L of bonechar (also from CDN) and at the bottom 1 L of bonechar. The flow rate of the filter should be around 4 liters per hour and is determined by the connection-hole of both

buckets. It is made of a plastic bottle with some holes and a mesh to avoid the entrance of sand into the bottle. A small hole in the tap of the bottle controls the flow rate and is often expanded by impatient filter users and thus increased the flow rate. Most problems that are occurring with the filters are clogging of this junction. In the lower bucket, the water flows slowly through the filtering material and the fluoride gets absorbed on the media, with a minimum contact time of half an hour. The filtered and stored water can be obtained for drinking and cooking purposes through the tap. As the height of the HF is around 1 meter and space under the tap to put a recipient is needed there is a problem in filling up the HF, as a jerrycan is too heavy to pour in directly. Thus, most of the households (HH) use a device to fill up the filter, such as a plastic jar, metallic can, plastic bucket, small jerrycan, aluminum pan, plastic bowl or watering can.

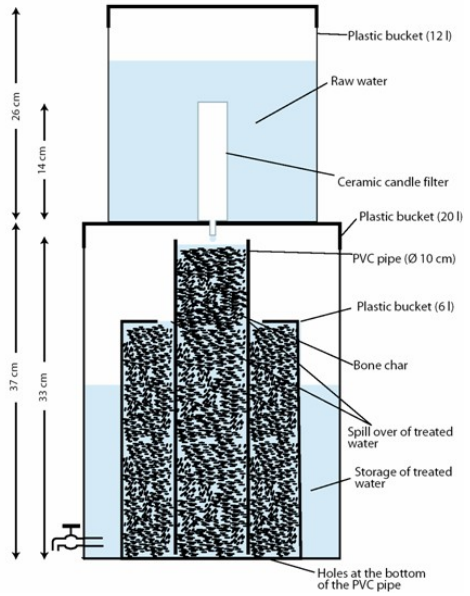

Figure 2: Filter design of household filters with bonechar. Bonechar with CaPO4-Pellets and instead of the candle filter a sand filter in the upper bucket are used (source: [10])

The water in rural communities is fetched at the source or by jerrycan or by barrel and transported home by different mechanism, such as carrying on the back or on a donkey cart.

In this thesis, the term “jerrycan” is used for the water samples taken directly from the jerrycan by canting. With the term “in” or sometimes “inlet”, the device to fill up the filters is meant. “Middle” is the outlet of the first bucket, thus a sample after the sand filter and “out” is the tap after the lower bucket (after the whole filtration process). For some households “point of consumption” samples were collected, by asking respondents for a drinking glass full of water as if they would drink it themselves.

2.5 Compact Dry Nissui plates

For defining the microbiological contamination level, Compact Dry “Nissui” Plates (Nissui Pharmaceutical Co, Ltd, Japan) were used for identification of E.coli and total coliforms (EC plates) and for Enterococci (ETC plates). These plates are originally for food industry, but can also be used for water sampling. They consist of a dry sheet medium with a cold-soluble gelling agent, chromogenic enzymes and selective agents for detection of specific bacterial strains [26].

The plates are designed for the detection above 1 bacterial colony forming unit (CFU) per mL of solution that is released in the middle of the dry medium and then incubated for 24 h at 35±2 °C. As the contamination level of drinking water is expected to not be detectable in 1 mL samples, we used membrane filtration (0.45 µm, Pall). As the growth media on the plates is dehydrated, plates can be stored over comparatively long time without refrigeration, and their handling is very easy [26–28].

Energy supply is needed for the incubation phase and not always available in the field. For this reason, different incubation temperatures and their respective growth of the bacteria were

tested, as will be explained in the method section.

In recent years, more enzyme based fluorogenic and chromogenic media to detect specific bacteria have appeared on the market. The base of the detection fluorogenic methodology is a particular substrate for a bacterial-specific enzyme and this substrate is linked to a fluorogen, normally methylumbelliferyl, which converts UV light to a visible light range. The advantage of this approach is that fluorogenic substrate is highly specific, water soluble and very sensitive. Drawbacks are the requirement of UV light, pH dependence (has to be alkaline) and high diffusion of the substrate into solid media. Chromogenic enzyme substrates use the same mechanism for a specific bacterial enzyme, but they change their color if the reaction occurs. Common substrates are indolyl based compounds since they are heat stable, water soluble and do not diffuse into the plate, i.e 5-bromo-4-chloro-3-indolyl (X), 5-bromo-6-chloro-3-indolyl (magenta), 6-chloro-3-indolyl (salmon) [29], [30].
For E.coli, the enzyme β-D-glucuronidase (GUD) is used as indicator, since it is produced by 94-96% of all E.coli strains, but also various salmonella, shigella and yersinia are generating this sugar compound, as well as some other bacteria. Various substrates can be used with GUD to detect E.coli, such as MUG (blue fluorescence, in solid and liquid media available), X-GLUC (blue-green colored colonies, TBX Agar, ISO-certified) or HQG (solid medium with ferric salt, black colonies, Uricult-Trio) [29].
The enzyme β-D-galactosidase is produced by all coliform bacteria (compare with their enzymatic definition), and can be detected chromogically (*o*-nitro-phenyl-β-D-galactopyranoside (ONPG), *p*-nitro-phenyl-β-D-galactopyranoside (PNPG), 6-bromo-3-indolyl-β-galactopyranoside (Salmon-Gal), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (XGAL), 6-bromo-2-naphthyl-β-D-galactopyranoside (BNGAL), 8-hydroxychinoline-β-D-galactoside, cyclohexenoesculetin-β-D-galactoside) or by fluorescence (4-methylumbelliferyl-β-D-galactopyranoside (MUGAL)). There are different media available that detect both coliforms and E.coli. In Table 2 commercially available media are listed which detect simultaneously E.coli and total coliforms.

There are some environmental strains that give false positives, as example Aeromonas. Also for other bacteria such as Salmonella, Staphylococcus, Closteridium and Listeria, chromogenic media are available [29]. However, these bacteria have not been of major concern to this study since we decided to work on E.coli and Enterococci.

The EC plates that were used for this study contain Magenta-GAL and X-GLUC for the detection of total coliforms and E. coli, respectively. The ETC plates contained XGLU as chromogenic substrate. The chromogenic reaction of the bacterial enzymes with the substrate produce red colored coliform colonies, blue colored E.coli colonies and blue-green colored Enterococci colonies on the plates. To avoid false-positive signals, selective agents such as antibiotics are added to the media [26–28].

Table 2: overview of media for simultaneous detection of E. coli and coliforms, as well as Enterococci which are available in the market. Adapted from [19], [27], [29]

Medium	Substrate/colour			Manufacturer
	Coliforms	*E. coli*	Enterococci	
Liquid media				
FluorocultÒ LMX broth	XGAL	MUG		Merck (Germany)
Readycult coliforms	XGAL/MUG	MUG		Merck (Germany)
ColiLert	ONPG	MUG		IDEXX (USA)
Coliquick	ONPG	MUG		Hach (USA)
Colisure	CPRG	MUG		IDEXX (USA)
Enterolert			MUD	IDEXX (USA)
Solid media				
RFluorocult agars	–	MUG		Merck (Germany)
TBX-agar	–	BCIG		Oxoid (UK), Merck (Germany)
Uricult Trio	–	HOQ		Orion (Finnland)
EMX-agar	XGAL	MUG		Biotest (Germany)
C-EC-MF-agar	XGAL	MUG		Biolife (Italy)
Chromocult	SalmonGAL	XGLUC		Merck (Germany)
Coli ID	XGAL	SalmonGLUC		bioMerieux (France)
CHROMagar ECC	SalmonGAL	XGLUC		Chromagar (France)
Rapid' E. coli 2	XGAL	SalmonGLUC		Sanofi (France)
E. coli/coliforms	SalmonGAL	XGLUC		Oxoid (UK)
ColiScan	SalmonGAL	XGLUC		MicrologyLab. (USA)
MI-agar	MUGAL	Indoxyl		Brenner et al. (1993)
HiCrome ECC	SalmonGAL	XGLUC		Union Carbide (USA)
EC plates	MagentaGal	X-GLUC		Nissui (Japan)
ETC plates			XGLU	Nissui (Japan)
MUST			MUD	Sanofi (France)
BBL mEil agar			TTC, XGLU	BD (USA)
Chromocult ETC Broth			XGLU	Merck (Germany)
Readycult ETC			XGLU	Merck (Germany)
Rapid' ETCagar			XGLU	Sanofi (France)
KF Streptococcus Agar			TTC	Kenner, Clark, Kabler (1960)
Compass ETC Agar			XGLU	Biokar (France)

Abbreviations: ETC: Enterococcus; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; Salmon-GAL, 6-bromo-3-indolyl-β-D-galactopyranoside; XGAL, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; CPRG, chlorophenol red β-galactopyranoside; XGLUC, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide; MUG, 4-methylumbelliferyl-β-D-glucuronide; TTC, triphenyl tetrazolium chloride; HOQ, hydroxyquinoline-β-D-glucuronide; XGLU: 5-bromo-4-chloro-3-indolyl-ß-D-glucopyranoside; MUD: 4-methylumbelliferyl-β-D-glucoside; TGE: Tryptone Glucose Extract

This method is not certified by ISO (International Organization for Standardization), but the EC plates are certified by AOAC (Association of Analytical Communities). Various studies confirm that Compact Dry Nissui EC plates are reliable in comparison with standardized coliform plating methods such as MPN (most probable number) or violet red bile agar (VRBA), when used for foods [31], [32].

2.6 Enterococci as fecal indicators

"Enterococci", "fecal streptococci" and "Lancefield D streptococci" are often used as synonyms in literature which leads sometimes to some confusion. Over the history of microbiology, the name and composition of this bacterial group has changed several times, starting with the discovery and nomenclature of *Enterococcus* in the end of the 19th Century by Thiercelin, according to their origin in intestines [33]. Until 1984 most Enterococcus species were named Streptococcus, for example *Streptococcus faecalis* for *Enterococcus faecalis*. Due to advances in DNA hybridization and 16S rRNA sequencing, the Enterococcus group was separated from the Streptococcus group, and most of the Lancefield D Streptococci was transferred [34]. In Figure 3, the phylogenetic dendrogram of gram positive bacteria is shown, while the position of Enterococcus in relation to others can be located, i.e Streptococcus. The terms "Lancefield D streptococci" and "fecal streptococci" seem to be exchangeable, since there is no differentiation in literature found [35–45]. Mainly two species of the Lancefield D group (*Streptococcus bovis* and *Streptococcus equinus*) remained in the Streptococcus genus, while other 17 (newer days about 33) formed the new Enterococcus group [35], [45]. Enterococcus faecalis and Enterococcus faecium are the two predominant species in the Enterococcus group that are related with human and warm blooded animal feces, as can be seen in their names [46]. According to Sherman 1937, Enterococci in general are gram-positive, catalase negative, non-spore forming bacteria that are facultative anaerobic and are present or in form of cocci or in chains [47]. They can prosper at 10 °C and 45 °C, and survive half an hour in 60 °C. Furthermore, they can grow in a 6.5% NaCl solution, until pH 9.6 and they possess the Lancefield group D antigen. However, it has been registered that not all Enterococcus species do fulfill all this criteria [34], [37], [38].

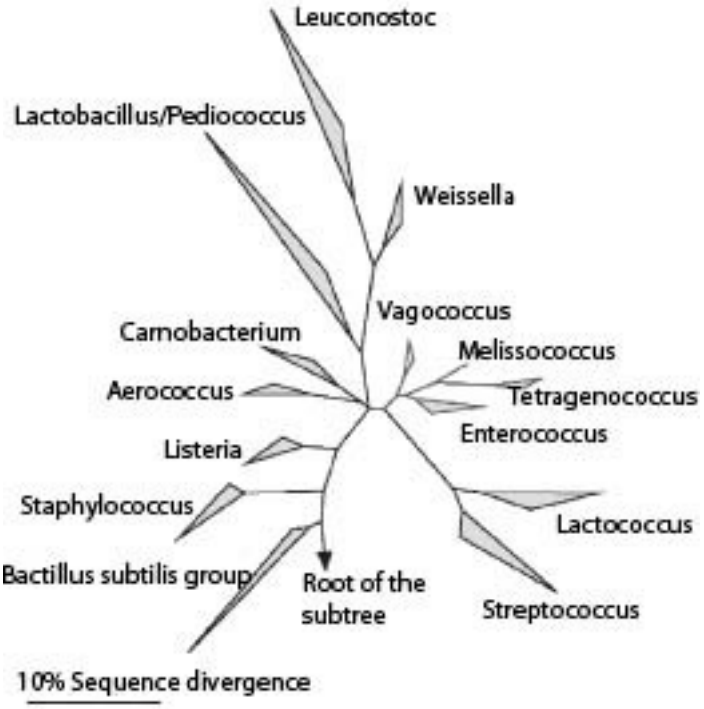

Figure 3: Dendrogram of gram positive bacteria based on 16s rRNA, where the phylogenetic position of Enterococcus can be observed; as well as the position of Streptococcus and Lactococcus, which all were classified as Streptococcus before (adapted from [79]

Enterococcus species are often mentioned to be indicators for fecal contamination of drinking waters and foods, in addition to mainly *E. Coli* [17].

The advantages of Enterococcus species to coliform species are their major resistance to chlorination, temperature and pH ranges, as well as increased survival rates in natural waters. Guidelines for Enterococci in waters are often related with recreational water quality, where Enterococci are used as indicators for gastrointestinal illnesses, transmitted through

the water. The natural habitat of *Enterococcus spp* are intestines of animals (humans included), but some species also are from plant, soil or clinical origin [33]. To avoid any inconveniences of interferences with environmental Enterococci, Maheux et al. [46], proposed an rtPCR method (real time polymerase chain reaction) to detect specifically *Ent. faecalis* and *Ent. faecium* in water which is less time consuming than cultivation methods (5h instead of 24 h) and WGA (whole genome amplification) for Enterococcus spp. With their approach, drawbacks such as decreased culturability of stressed and injured bacteria on plates can be avoided. They tested their method to detect the two mentioned Enterococcus species and Enterococcus spp against the culture based method 1600 on mEI agar, which is ISO certified. Their results are promising, most of all for rtPCR in respect of time, sensitiveness, affordability and specificity to detect only the wanted Enterococcus species. The same approach with specifically *Enterococcus faecalis* as indicator for fecal contamination in water was used by Wheeler et al. some years before (1991) [48]. His group faced the challenge to develop a specific media that does not give false positives and compared it with API 20 Strep (the official procedure to detect *Ent. faecalis*), based on ribotyping. They concluded that further investigation about the habitat of these bacteria has to be done and specific methods for a quick and specific detection have to be evaluated [48].
For the investigation to detect Enterococci in Ethiopian drinking water, ETC Dry plates from Nissui were used, but, unfortunately, there were no specific information about the components of this media. The chromogenic substrate (XGLU) and antibiotics as selective ingredients are the only declaration made by the producer [26]. There have been test series, conducted by the producer, with different bacteria strains to show the selectivity. From the 9 tested Enterococci species for the ETC plates, two *E. casseliflavus* and *E. mundtii*) are mainly associated with plants [49]. It would have been interesting, if also strains from the remaining Lancefield-D Streptococci would have been tested, and some Lactobacilli.
Also, there raises the question what the microbial testing method is looking for; if just *Enterococcus spp,* are detectable, what does it mean for the study if these bacterial group have been found. As we were looking for environmental water samples, the possibility to have environmental Enterococci is very high, thus this has not been a specific indicator for fecal contamination in our case.

2.7 Antimicrobial properties of copper

Several studies indicate that copper has antimicrobial properties, but the action mechanism is still not totally understood. Since ancient times, copper or brass vessels were used to store drinking water in the orient, also because of its microbial inactivation characteristic [50]. There is an ongoing discussion, whether copper ions are the main agent of toxic action of copper, and which influence metallic copper surfaces have on microbial activity [50], [51].
Copper is known as a pesticide, mostly branded in organic farming for vine, where it is used since long time efficiently against downy mildew (caused by *Plasmopara viticola*) [52]. The impact of copper and its alloys in comparison to other materials on microbial growth was investigated several times by various authors [50]. One publication says that dry copper surface is much faster in killing bacteria (*E.coli, Bacillus cereus, Deinococcus radiodurans*) than moist surface (minutes against hours), and give some indication on the target sites of copper toxicity (extracellular). They could show that the bacteria had injured membranes and cell envelopes which lead to cell death, and not DNA mutations are the driving toxic mechanism. Membrane proteins and lipids are the main target of copper ions from dry surfaces, and leads to cytoplasmic membrane injury and cell wall debilitation. It could not be

ascertained if the high intracellular copper concentration was the cause of mortality or if it was induced by lethal membrane damage [53]. Copper exposure may lead to the Viable-but-Nonculturable State (VBNC) of bacteria, as it was explored for the plant pathogen *Erwinia amylovora* (causal pathogen of fire blight). Due to the exposure to a higher copper concentration (0.005. 0.01 and 0.05 mM Cu^{2+}), VBNC was reached faster. Even after 9 months of VBNC state, bacteria could be resuscitated from the two lower copper concentrations due to the addition of KB broth or pear juice (two strong copper complexing agents and nutrient providers). With other copper-complexing agents such as asparagine and EDTA, resuscitation was only possible after 75 and 18 days, respectively. For the highest investigated copper concentrations, only after 2 days the cells could be resuscitated [54]. Another investigation about the mechanism of copper showed that a specific *Enterococcus hirae* mutant is more susceptible to copper than the wild type. In one experimental setting they let the two *Enterococcus hirae* strains grow on solid copper and stainless steel surface in the same media. In another experiment they used different media and copper surface for growing the Enterococcus species. The media had a remarkable influence on bacterial mortality rates due to copper exposure, which is due to their different dissolution rates of copper. The copper concentration in solution correlated with the mortality rate of the studied bacteria [55]. A comparison of *Salmonella enterica* and *Campylobacter jejuni* on copper, stainless steel and polymer surface at 10 °C and 25 °C demonstrated the antimicrobial effect of copper. Colony counts after 0, 2, 4 and 8h showed a faster decrease at 25 °C than at 10 °C on copper surfaces, while on polymer and stainless steel, colony counts slightly increased at 25 °C (8 h, 2.5 log increase) and slightly decreased a 10 °C (8h, 0.5 log reduction). After 2 h of copper exposure at 25 °C, there has been a decrease of colony counts in the range of 2 log units [56].

Also viruses get inactivated by copper surfaces, but not on stainless steel surfaces. In another experiment with *Influenza A virus* were inoculated at 22 °C on copper and stainless steel surfaces at a relative humidity of 50-60% for 24 h. After 6h on copper surface, a 4 log reduction of infectious viruses was observed, while on steel after 24 h half log reduction was obtained [57]. In a study, different metals were compared regarding their effect on *E.coli* and total coliforms (river sample) over 2.5 h of exposure at 20 °C. Surprisingly, copper and zinc had higher inactivation rates (1log reduction in 30 min) than copper and bronze (1log reduction in 60 min), followed by, brass (1log reduction in 90 min) and aluminum (less than 1 log reduction after 150 min) [58].

In India, copper pots and small copper devices were tested on their ability to inactivate bacteria (*E.coli, Salmonella Typhi, Vibrio cholerae*), and their results are promising. They could not recover any bacteria from the copper exposed water (16h of exposure time), while from the control (glass and pet bottle), all bacteria species were growing on agar plates [59]. There is a notable effect on inactivation rate of *E.coli* in copper vessels used traditionally to store water in India due to temperature (5°, 15°, 25°, 35°, pH 7) and pH (6.0,7.0,8.0,9.0, 30 °C) [60], as well as due to organic and inorganic compounds [61]. Higher temperatures lead to faster inactivation of *E.coli,* as well as extreme pH values (6.0 and 9.0). Acid conditions lead to faster inactivation than basic environments, probably due to more unbound copper ions in solution. The effect of temperature is more remarkable on inactivation rates than from the pH [60]. In respect to inorganic compounds, after 12h of exposure in a copper vessel, chloride (Cl^-) leads to an increased inactivation rate of *E.coli*, while nitrite (NO_2^-), nitrate (NO_3^-) and sulfate (SO_4^{2-}) decreased the inactivation rate. Glucose, lactose and starch addition did not show a difference in water without any additives, while adding amino acids, the inactivation rate decreased dramatically. Humic acid almost inhibited the inactivation property of copper. The amount of dissolved copper varied between 24 mg/L for amino acids and 0.4

mg/L for glucose, lactose and starch (at 30 °C, pH 7.0) [61].

There are some evidences, that copper does act in a antimicrobial way, but the mechanism behind is still not totally clear. The application of copper ions in the organic wine production is a fact that does protect the plants from fungal and bacterial attacks. The time of exposure varies notably between the experimental settings in literature, as well as the surface area and the temperature. There are some promising indications that also metallic copper does inactivate microbial activity, nevertheless reliable literature is very scare.

3. Objectives

The main objective of this study is the assessment of the impact of defluoridation filters on microbial water quality in rural Ethiopia.
This includes the detection of the fecal contamination pathway from source to drinking glass, and evaluation of measures which can be taken knowing the mechanisms.
HF contains calcium phosphate pellets which release a lot of phosphate. It is expected that - in the worst case scenario - there is an increase in microbial growth in the filtered water compared to raw water due to the high phosphate load.. Besides, it is of interest if an addition of a small amount of copper to the HF leads to a decreased microbial count since there are some indications that copper has an antimicrobial effect (see 2.7 Antimicrobial properties of copper).

In short, the hypotheses of this study are the following:
- The HF will slightly remove microbial contamination because of the sand filter, if the filter is maintained clean (as recommendations).
- Copper can improve the water quality because of its bacterial inactivation property.
- Most of the microbial contamination in the water comes through handling and not from the contaminated source.
- Compact Dry Nissui plates are a simple and reliable method for microbial testing in developing countries.
- Microbial water quality in a drinking glass is better from HF than CF, because household water treatment minimizes the recontamination of water.

4. Methods

4.1 Lab experiments

4.1.1 First Eawag experiments, February 2011

Two weeks were spent in the laboratory of environmental microbiology to get used to fecal indicator testing with the Dry Nissui plates. All experiments were performed in collaboration with Rahel Künzle who made subsequently the same fieldwork in Kenya for her Master thesis (see [9]). Technical and practical support was derived from Frederik Hammes, Maryna Peter and Hans-Ulrich Weilenmann.

First trials with Compact Dry plates under various incubation temperatures

For a first assessment of the plates a mix of primary effluent from the sewage and stream water (Chriesbach) was used in various ratios to get sufficient counts (between 20 and 200 per mL) on EC and ETC plates from the same mixture. Enterococci counts are present around 10 times less than E.coli/total coliforms counts. The primary effluent and the Chriesbach water were stored in “Schott-bottles” at 4 °C for further use. Out of it, 1 mL of different ratios and 1:10 and 1:100 dilutions were plated with filtered tap water (0.22 µm) to find a suitable range for enumeration. The plates were incubated for 24 h at 37 °C and analyzed by counting the colored colonies with a pen on the lower side of the plate. The same experiment was repeated several times to get an adequate ratio for the temperature series. Quadruplicates on EC and ETC plates were furthermore incubated, as well as single Colilert vials (1 mL sample and fill up with filtered tap water) at 11°, 20°, 30°, 37°, 42° and 45 °C, and one with variable temperature. The variable temperature was meant to mimic extreme pocket incubation (body temperature 37 °C) during day and environmental temperature during night (11 °C) in the case of lack of energy as worst case scenario for Kenya (Ethiopia has higher average temperatures in the months of study). First 7.5 h at 37 °C, then 11 °C overnight (around 13 h) and again 37 °C until counting, and then it was stored at 11 °C. To evaluate temporal differences the plates were counted after 24 h, 48 h, 96 h and 120 h at the same incubation temperature. The Colilert vials were used as a comparison to a standard method.

Impact of copper and florist wire on bacterial growth

Some literature suggests (see background information 2.7 Antimicrobial properties of copper) **t**hat copper has antimicrobial properties. To test this in a controlled experiment, florist wire was utilized as reference metal, expecting that it should not have any effect on microbial growth. The same ratio of primary effluent and Chriesbach water was used, in total 6 50 mL tubes. In two tubes, a copper wire of 6 cm length and a diameter of 0.4 mm was added (according to [59]), while two tubes were equipped with a florist wire of 6 cm (diameter 0.4 mm), and the last two were treated as control tubes. The samples were incubated at 30 °C in a shaking reactor and excluding two EC plates to see the initial concentration. After 24 h, samples were plated on EC plates, one from each type (copper, florist wire and control). After 96 h of incubation, all 6 samples were plated in different dilutions (undiluted, 1:10, 1:100) on EC and ETC plates. After 120 h of exposure to the metals, ATP was measured in all samples to estimate the amount of living organisms and the impact of copper and florist wire.

Dilution series of pure E.coli culture

To assess the viable counts on EC plates, dilution series from a pure E.coli lab-culture (stain K12) were prepared; the appropriate colony was directly taken from an agar plate. The initial concentration of living bacteria was measured by flow cytometry (10^8 counts per mL) and built the basis for the dilutions. Dilutions were made (with filtered tap water) to have 1000, 500, 100, 50, 10, 5 and 1 count per mL, duplicates were plated on EC plates and incubated for 24 h at 37 °C.

4.1.2 OSHO lab experiments, March-June 2011

The laboratory in Modjo from OSHO has basic infrastructure for chemical analysis, only the power supply was not very reliable. Distilled water was locally produced by Sisay Feyera, the incubator (Almedica Inc. Giffers, CH) used had a temperature range of 25-45 °C. To disinfect, we purchased denaturized alcohol from the pharmacy, as well as bleach (with 5% NaOCl). Disinfection of cups and other materials was done with recently boiled water (>70 °C). The residual sample water from field campaign was recycled as contaminated inlet for the lab filters. Within the sample analysis, harmful waste was produced that cannot be disposed safely under Ethiopian rural conditions. Therefore, the plates were burned on the OSHO compound, to prevent children from inadvertent exposure to pathogens cyktuvated in the plates. In regard to further studies, it is recommended to consider a more sustainable waste disposal method

Impact of HH filter on microbial water quality

4 new HH filters were installed on the compound of OSHO, to make first tests under controlled conditions about their influence on microbial contamination of inlet water (see Figure 4). They were situated in a shanty[1] behind the main building, where no direct sunlight reached them and a tap was near. Three lab filters were identical, while the fourth one had instead of the sand filter a ceramic candle, as it is sold in Kenya. Table 3 enlists the lab filters and some of their properties. It has also been of interest to study if there is a difference between the sand filter and the ceramic candle filter in respect to microbial removal. After the first problems with the operation (leaking, too fast or slow flow rate, washing the sand as indicated), the first experiment was able to be started.

Table 3: Lab filters in OSHO and their main properties. CH: Swiss, ETH: Ethiopian

Filter-No	Upper bucket	Copper	Flow rate
1	sand	-	slow
2	sand	CH	fast
3	candle	-	fast
4	sand	ETH	slow

As contaminated inlet, the local tap water from a hose that had a notable biofilm inside was used. The first samples of 100 mL were taken from “in” (water from the upper bucket), and “out” (water from the tap). For sample collection, a beaker of polypropylene (100 mL) was used and washed with medicinal alcohol after usage and rinsed with distilled water. It was filtered by membrane filtration (Millipore) and the membrane carefully placed on the EC plate that was previously moistened by 1 mL of sample water. All samples were incubated for 24 h at 35 °C.

1 A construction of corrugated iron with basement of concrete, which is used for multiple purposes.

Figure 4: Lab filters in the shanty on the compound of OSHO in Modjo. Numerated from left to the right

To asure that the outlet was realy from the contaminated water, the lower bucket was emptied before starting the experiment. Nevertheless, there was always a small amount of water staying in the lower bucket, which was mixed with the new experimental water. This may lead to mistakes in the interpretation of the results, but there was no way to avoid it. To take the "out"-sample, the tap was opened and after some time (normally half a minute) tha sample was taken.

Impact of copper to HH filters

The next step was to asses if there are any differences between Ethiopian and Swiss copper. Therefore, electric wire with copper inside was bought in Nazreth for the first trials: the copper threads had a diameter of 0.5 mm while 49 strings built one wire (1 m for 50 ETB, ZA-BVR 450/750 V 16 mm2 RoHS PANYU, yellow-green cable coating) (see Figure 5). In Switzerland a copper wire with a diameter of 0.4mm and a length of 20m was acquired (Coop, 20 m for 2.90 CHF). These two copper wires were placed into filter number 2 (Swiss copper) and 4 (Ethiopian copper) respectively, to get a total copper surface area of about 250 cm^2. In Table 3, an overview of the lab filters and the used copper, as well as the relative flow rates are specified. The wires were placed in the lower bucket of the selected lab filters and had not been touched anymore. After 23 h, the first samples from the outlet and the inlet (in filter 2 and 4 no inlet was available) were taken. 20 mL were plated on EC and ETC plates and incubated at 35 °C for 24 h. After 6 d of copper exposure, the same sampling schema was applied, as well as duplicates and blanks.

Figure 5: copper device from electricity cable, purchased from Nazreth. Left side: device with surface area of 85 cm2, right side: original cable

Comparison sand filter vs. ceramic candle filter

To observe the impact of the sand filter on microbial removal in comparison with the ceramic candle filter, a third sampling point in the middle was added. In the outlet of the first bucket, samples were taken to test for the water quality after the sand, or the ceramic candle, respectively. The sample volume was 10 mL, because very dirty water was used as inlet. For quality control, some duplicates and blanks were done.

Copper contact time in 2 L pet bottles

In literature, the only contact time indication related with copper found is one day [60], [61]. For this reason, samples of contaminated water were extracted from PET bottles after the exposure time of copper of one day. 3 different copper types found usage in the sampled households while it has been of interest if they differ in their reaction time and efficiency to inactivate bacteria. The third copper wire was purchased in Debre Zeit (1 m for 6 ETB (TSE HAR TUMKA ABLO H07V-K CE, brown cable coating, imported from Turkey). 4 PET bottles (2 L) were prepared and filled up with dirty surface water. The first sample was taken only from the dirty water and therefore served as control. The 3 copper types (2 from Ethiopia and 1 from Switzerland) were placed, each one into one bottle. After 30 min of contact time, the first samples were plated on EC plates, with a volume of 1 mL. After 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 25 h, samples and plated were taken, to see the difference over time. All samples were incubated at 35 °C for 24 h. The bottle sample after 25 h was first taken without shaking and another after shaking to see, if sedimentation has an impact. For quality control, duplicates were made in half of the samples, and blanks for every time step.

Chlorination of sand in HH filters

The last experiment with the lab filters was about chlorination of the sand. As it has been seen from formerly performed sampling sessions, sand filter hosts an elevated risk of

microbial recontamination of the water. One possibility to reduce this risk is shock chlorination [62]. Four different chlorine-based disinfectants were bought in the local market (see Table 4): Waterguard (1.25% NaOCl), Aquatabs (67 mg NaDCC per tab), bleach (5% NaOCl) and Bishan Gari (1 g $Ca(OCl)_2$ per sachet). As Bishan Gari is for turbid water, it was not included into the experiment design. In, middle and out of the lab filters were sampled before (10 mL) the chlorination and after (20 mL) 1 h of chlorine contact again, with incubation at 35 °C for 24 h as usual. In the first filter, 0.6 mL bleach was added, in the second, 1 cup of Waterguard, in the third nothing (candle filter) and in the fourth 1 Aquatab. The chlorine amount for 20 L was used; the upper bucket has contained about 15 L. In the case of Waterguard and bleach, the applied chlorine dose was arround 0.15 mg free chlorine, while in the Aquatabs the dose was higher (arround 30 mg Cl) and less available because of its chemical structure. The aim was to have residual chlorine in the sand to kill the microbes that are living there.

Table 4: Chlorination products for water in the Ethiopian market. 17 ETB = 1 USD (in June 2011)

name	content	active substance	where get	price ETB	selling unit	need per jerrycan	1 unit for x jerrycans	price per 20L jerrycan ETB
Waterguard	1.25%	NaOCl	pharmacy	4.5	150mL	2.5mL	60	0.075
Aquataps	67mg	NaDCC	pharmacy, supermarket	5	10	1 tab	10	0.5
Bleach	5%	NaOCl	supermarket	6.75	800mL	0.6mL	1333	0.005
Bishan Gari	0.1g	Ca(OCl)2	supermarket	0.09	2.5mg	1 packet	1	0.09

4.1.3 Second Eawag experiments, August 2011

As some of the experiments of the first round did not show the expected results, (i.e dilution series) a second one was carried out. Additionally, the laboratory purchased in the meantime an IDEXX Quanti Tray sealer to compare Dry Nissui plates with Colilert 2000 (most probable number). Again, Frederik Hammes and Hans Ulrich Weilenmann supported this experimental series very helpfully, and Arzu Teksoy provided valuable support.

Impact of incubation temperature and time on Dry Nissui plates, Colilert Quanti Tray 2000 (IDEXX) and 3M Petrifilm

In the first session, the recommended incubation temperature (35 °C) of the Dry plates was not assessed and the experiment was repeated and changed its design slightly. Quadruplicates of the plates with 1 mL, and one blank for each temperature were prepared. Instead of taking Colilert vials of 10 mL, quadruplicates of Colilert Quanti Tray 2000 (ISO-certified method that works with MPN, most probable number) were used and therefore the 1 mL sample were diluted with 100 mL filtered tap water. Triplicates of 3M Petrifilm for E.coli and coliforms were used for one count. Pure water of Chriesbach was used as sample, diluted with filtered tap water (0.45 µm) to get bacterial counts in the range of 20, 100 and 200 per mL. The samples were incubated at 11°, 20°, 25°, 30°, 35°, 37° and 41 °C. 500 µL samples were also plated in triplicates on standard agar plates that detect E.coli specifically. ECD-MUG agar (E.coli-Direct Agar, Biolife) shows E.coli colonies with blue fluorescence under UV light, while with TBX agar (Tryptone Bile X-Glucuronide, Bio Rad) they are indicated as blue colonies. As the agar was made before, the pouring method has not been practical and, thus 500 µL was dispersed on the surface and incubated at 37 °C. The samples were counted after 24 h, 48 h, 72 h and 120 h, to assess the impact of incubation time.

Comparison Dry Second dilution series for comparison Compact Dry plates (Nissui) with Colilert Quanti Tray 2000 (IDEXX Industries, Inc. Maine, US)

To assess the plateable counts of *E.coli* on the Dry Nissui plates in a standardized manner, it has been compared with the ISO-conform method of Colilert Quanti Tray. A pure E.coli strain from the lab (K12) that was grown over night in 10% LB-broth at 30 °C was used. For dilution, 10 mM PBS (phosphate buffered saline) buffer solution with a pH of 7.3-7.4 was taken (indicated by Frederik Hammes). To know the initial and the dilution concentration, flow cytometry was used. The dilution series started by 10^3 counts per milliliter and went town to 10^-2, with intermediate steps of 3*10^2, 1*10^2, 3*10^1, 1*10^1, 3*10-1 1*10-1 and 3*10-2. In Table 5 is shown which ranges of bacterial concentration is used for the Compact Dry EC plates and the IDEXX Quanti Tray. For the direct plating in the higher concentration range (3*10^-1 to 3*10^3), 1 mL quatruplicates were used, and duplicates for 100 mL membrane filtration (Milipore) in lower concentrations (1*10^-2 to 1*10^1), as well as for the blanks. This sample arrangement also showed the impact of filtration on E.coli growth on this medium. Quanti Tray 2000 was used in the lower concentration range (10^-2 to 3*10^1) in 100 mL duplicates and one blank. All samples were incubated at 35 °C for 24 h.

Table 5: Experimental setting for the dilution experiment with pure E.coli culture, bacterial counts were measured with flow cytometry. For the plates, 1 mL samples were plated directly and 100 mL samples filtered (membrane filtration). IDEXX was only available for 100 mL samples

Counts per mL	1 mL Dry plates	100 mL Dry plates	IDEXX 100 mL
1*10^-2		x	x
3*10^-2		x	x
1*10^-1		x	x
3*10^-1	x	x	x
1*10^0	x	x	x
3*10^0	x	x	x
1*10^1	x	x	x
3*10^1	x		x
1*10^2	x		
3*10^2	x		
1*10^3	x		

4.2 Field work in Ethiopian Rift Valley

The main part of this master thesis (4 months) was carried out in the Ethiopian Rift Valley, in the south of Modjo (see Figure 1). The field visits were accompanied by Sisay Feyera, Tesfaye Edosa or Feysa Lemma from OSHO for translation and other supports. Since a lot of unexpected happenings may occur, the planning of the field visits was normally done not

more than one week in advance. The time of conduction depended on the disposability and willingness of Sisay, Tesfaye or Feysa, transport possibilities, availability of materials and market days.

4.2.1 Sampling Design

Household selection

In total, 5 villages were visited, at least twice in the four month of study, depending on their location (Figure 1). As it can be seen from Table 1, there are different water sources available in each village. Additionally, in Wayo Gabriel, there are in total 11 private dug wells situated, and two main water points (Shibere and Mesken Safer) from the same source as well as the community filter. In Washe, there is no separate water source; the inhabitants fetch their water either from Mesken Safer, Shibere, Sariti or pound water, while all other villagers normally use the same water source. Rainwater is used temporally, depending on its availability.It might be the case that the common water source does not carry enough water because some parts of the mill are broken, or due to lack of electricity or maintenance. Thus, villagers change their sources to other font.
The plan was, to have 10 HH per village with the same source and to put a copper device in 5 of them and sample all of them sequentially to see if there is seasonal variability in the water quality. During this survey, the question about CF user rose up, and thus 10 CF users were included into the sampling. It has been a concern to test, if copper in jerrycans have an effect. The households were selected in collaboration with the social monitoring project, coordinated by Alexandra Huber, to avoid interferences and too much visits of the same households. The first visit took place in Washe, where the first three chosen HH did agree to work with us. Further three HH were added, because of absence of the inhabitants during the visiting times. In Chalalaka, the HH were chosen by visiting them, and under the advice of Feysa. In Gura, the HH were chosen form a list, but as the windmill was broken since 10 days, the HH with water in the jerrycan were sampled (only 6 HH, and in the next visit, we added 4 HH more). In Wayo, 10 HH were selected from a list, and one HH with a private dug well which uses a filter. For CF users, the caretaker, Jedeschi, recommended frequent users that are living not too far away from the CF. In Sariti, the HH were chosen by Sisay on the way walking from one house to the other until having 10 HH sampled. In the Annex_table 1 are listed all HH from this study.

As it can be noted in Figure 1, Chalakala is far away from the other villages. For this reason, they were visited only two times. Wayo Gabriel and Gura are easily accessible by public transport (minibus), while for Sariti a bike or car was needed, and Washe is theoretically reachable by feet from Wayo, but the Ethiopian translators were not willing to walk more than once this distance.

Copper device

As reference, the indication of 15cm2 copper per liter water was used [59], thus for the storage volume of about 10L in the lower bucket, around 150 cm2 was needed, thus 9 strings of 106 cm wire with the diameter of 0.5 mm each. For the first 3 HH in Washe, there was a calculation error (diameter is not equal to radius), thus only 5 strings were used and consequently only 85 cm2 copper surface was available (see Figure 5). From the second

Ethiopian copper, 16 m per bucket, while from the Swiss one 8 m was utilized.
The purpose of the copper was explained to the HH and they were asked, if they agree to put a device in their filter. Sometimes questions raised up, such as if they can use the filter if the copper is inside, or if there is anything that they have to change in their habits. After the approval by the HH, we washed the copper with filtered water and placed it in the lower bucket. The HH where we put copper in were chosen casually, sometimes under the advice of the accompanist that knew the HH better.
We also evaluated the impact of copper in jerrycans for CF users; the device was positioned into the jerrycan and sampled the next time. However, this experiment has been omitted because in most often (4/6 jerrycans), the copper device has been removed between the first and the second visit. Also because the HH change their jerrycan to fetch water form CF or Shibere, they often do not use the same for one source.

Chlorination

At the end of the stage, as there was a lot of microbial contamination found in the HH, it has been decided to test chlorination of the sand filter (as done previously in the labfilters) as well as of jerrycans in some CF users that agreed. Jerrycans were selected because of previous findings that there is one main contamination points based on our sampling, and also because of literature [63], [64]. Biofilms were present in all jerrycans from the HH (personal observation), and according to Jagals (2003, [63]), biofilms can host pathogenic bacteria and deteriorate strongly drinking water quality strongly. Steele (2008, [64]) made experiments with chlorination of jerrycans in a camp in Uganda, where the results have been promising to significantly improve the water quality, nevertheless, basic hygiene has the main impact on microbial contamination at HH level.
In Table 3 are listed chlorination products which are available in Ethiopia and their relative prices. Note that Bishan Gari does contain additionally Aluminumsulphate for removal of turbidity, for this reason, it was not tested in the present study.
We used Waterguard and Aquatabs for sand chlorination, as well as for jerrycan chlorination, depending on the circumstances in the HH. For the sand filter, in 3 HH 1 cap of Waterguard was applied, and in 2 HH one or 2 Aquatabs, depending on the amount of water present in the upper bucket. A sample from in and out was taken before chlorine addition, and 1 h after a second sample was taken (the time period was too short, but it was not possible to wait longer because of transport problems).
For the CF users, we took a sample from the jerrycan and a drinking glass before the chlorination and 1 h after. We chlorinated 2 jerrycans with Aquatabs (because they were more than half full) and 2 with Waterguard (where only small residual water was inside). 2 Aquatabs were just added into the water without any extra treatment, while 2 caps of Waterguard was added to the rest of the water and shaken strongly for 1 minute, then discharged, rinsed with CF water and then filled up at the source. In one case, the Aquatab was added like half an hour before the second sampling.

Questionnaire

Together with Rahel Künzle, we elaborated a questionnaire for the HH to have the same information from Kenya and Ethiopia. It included questions about their behavior with the filter, how often they are filling it up, from where they get the water, how often the sand is washed, how many persons live in the HH, for what purpose do they use the filtered water, and if they

like it. We asked the questions to all households, but not always at the first visit. The answers were not very meaningful; they just told us what they learned in the workshop. For this reason, this data were not used in the analysis. However, it has been of interest, from where they fetched the water in villages with different sources.

4.2.2 Sampling

Sample collection and transportation

The samples were collected with the agreement of the HH in sterile Whirl-paks (100 mL, Thiobag, Nasco) from the filter or a recipient (see Figure 6). First, only the inlet and the outlet of the filters were sampled, to see the impact of the filter on microbial water quality. The inlet means the device that is used to fill up the filter, as it is explained in the part about HH filter (see 2.1 Study area). The outlet is the tap, which previously was sterilized by flaming with a lighter. Due to misunderstanding, the sterilization of the tap was omitted at the first sampling sessions, but accomplished the following ones (starting in April).

Figure 6: sampling inlet device from one HH in Wayo with whirl-pak

The second sampling design was from source to tap, thus we took samples at every stage of water treatment, from the source to the drinking glass. This design is based on a paper from Levy et al. (2008, [65], where the pathway of contamination in rural Ecuador was investigated. In our case, these stages were the following: source, jerrycan, inlet, after the sand filter (“middle”), outlet without sterilization, drinking glass which was cleaned as the HH normally does and in the end the sterilized tap. This procedure was conducted in 4HH per village, in 2 HH with copper and 2 without.

For CF users, the samples were taken from the jerrycan and a drinking glass, which was cleaned as they normally do.

A third sampling design took place only in Wayo, to assess the microbial water quality in a drinking glass of CF users and HF users. There we took samples from in, out and glass, as well as from the jerrycan and the drinking glass (CF users).

While taking the samples, the accompanist explained to every HH the results from the previous sampling. Graphs for each HH with the appropriate data were prepared (see Annex_table 11 as example) and explained to the attendees and comments on which improvements can be done to have better water quality. They always were very interested, and asked questions.

The collected samples were transported -surrounded by aluminum foil- on a cold water bottle (purchased in the morning from a local shop) in a cooling box to the lab, generally within 6 h, in some cases within 7h. They were stored in the fridge (if power was lacking in the freezer) until plating.

Sample preparation and plating

To determine the contamination level and thus the adequate volume to filter, from the first households in Washe were filtrated 1 mL, 20 mL and 100 mL. Subsequently, the first samples were plated in 10 mL and 1 mL. In the second sampling session, the results from the first time were used to determine the adequate plating volume. This was between 1 mL (for lake water users) and 100 mL (for boreholes), mostly 10 mL for HH samples.

Figure 7: filling up the membrane filter unit with a sample from a whirl-pak

For quality control, after 10 samples one double and one blank sample from locally distilled water were prepared. The incubation takes place in a small incubator form food industry (Almedica Inc. Giffers, CH, temperature range 25-45 °C), at 35 °C for 24 h, with some power interruptions and thus longer incubation times, depending on circumstances. The plates were counted and taken photographed after 24 hours, what often was at night where light situation was not very advantageous, thus in the following morning, other pictures were taken and sometimes counted again.

We used a membrane filtration (Millipore) module for filtering samples (see Figure 7). The stand was disinfected with alcohol after every use and flushed with distilled water. The cup was reused, disinfected with alcohol and rinsed with distilled water. As blank samples were prepared with the same cup too, there has not been evidence that contamination of samples occurred due to using the same cup. As the grid lines of the locally purchased membrane filters (from Wagtech Ethiopia) were printed very dark, the filters were used upside down after discussing and testing them.

Two times, the plating was carried out in a hotel room instead of the laboratory in Modjo, because there was no time to get back to Modjo.

4.2.1 Data analysis

For analyzing the difference before and after the different steps in the filter, log reduction values (logred) were used. This means, the $\log_{10}$ (counts before/counts after), thus if the value is positive, there is a reduction in counts.
The maximum of counted colonies on the plates were 300, depending on the dilution factor, this leaded to different CFU numbers per 100 mL (maximum 30000 for 1 mL samples). If there were no counts, half detection limit was assumed, i.e. 0.5 CFU per sample volume.
The collected data was analyzed statistically with StataSE 11 (StataCorp LP, USA).

5. Results

5.1 Lab experiments

5.1.1 Eawag lab experiments

As the experiments at Eawag were performed in collaboration with Rahel Künzle, the results of the temperature experiments and the copper/florist wire exposure are very already presented in her thesis [8]. Here, the results from the second Eawag-lab-session will be presented and compared it to the results from the first session. The dilution experiment was not explained in her report, thus some details will be provided here.

Dilution series from first session

Surprisingly, there were only two colonies growing in total, one E.coli and one total coliform on the plates where 5000 were expected, although the initial concentration was detected by the flow cytometer. This may be caused by the death of the lab culture of bacteria that were exposed to filtered tap water after having been growing on the agar plate. Thus it has been decided to repeat this experiment in the next session with more adequate condition for bacterial growth.

Dilution series from second session

Flow cytometry was used to get the initial concentration of the pure E.coli culture (stain K12), and further for every dilution step (data not shown, but represented as log transformed expected value in Figure 8). The counts of E.coli on the Compact Dry plates are correlating well with the dilution concentration (R=0.999 for 1 mL, R=0.985 for 100 mL, see Figure 8). As the data indicates that filtration does not have any negative impact on bacteria, thus they grow in same numbers as without filtration. Surprisingly, on the Colilert Quanti Tray 2000, there was no growth at all, neither the yellow color, nor the blue fluorescence under UV light. Interestingly, the used E.coli strain was grown on ECD agar plates with MUG and gave blue fluorescence under UV light,

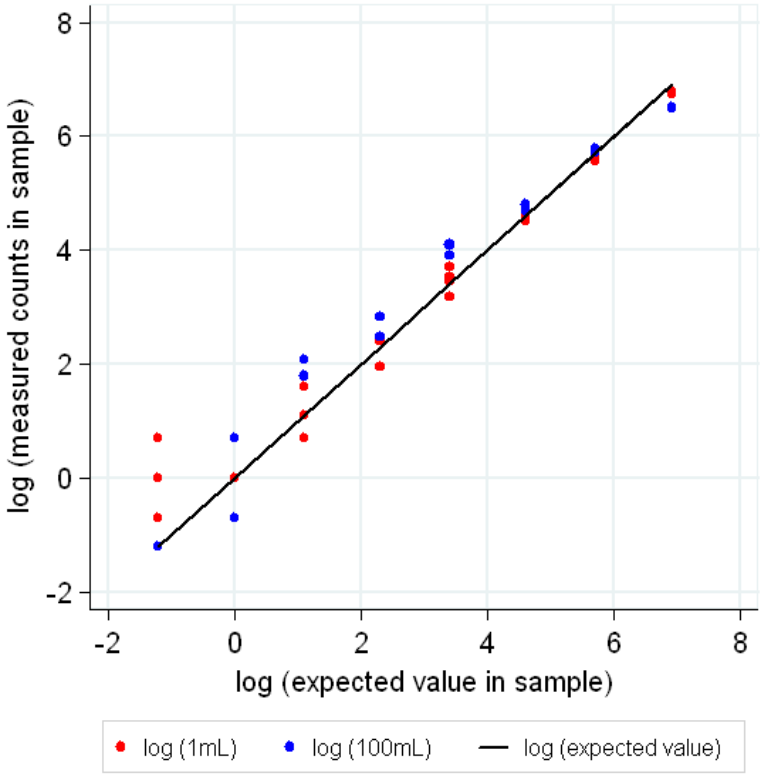

Figure 8: log-transformed dilution experiment with pure E.coli culture (strain K12) on Compact dry plates, expected counts defined by flow cytometry. 100 mL samples were filtrated by membrane filtration for low concentration (1count/100 mL until 1000 counts/100 mL), while 1 mL samples were done for higher concentrations (30 counts/100 mL until 100000 counts/100 mL).

thus it reacts with MUG (the results of this experiment is not shown). It may be that the solution was too salty for the bacteria because Colilert contains salts and the PBS solution too. To confirm the assumption that PBS has to do with the failure of this E.coli strain to grow in Colilert medium, we inoculated a high concentration of E.coli (10000 counts per mL) in PBS buffer and Colilert. A very slight yellowish coloration appeared after 24 h of incubation at 35 °C, what strongly suggests that the PBS was the problem in this assay.

Temperature experiment and incubation time

The growth of the agar plates (TBX and ECD-MUG) could not be used as reference because the colonies were growing on the borders of the plates and it was not possible to count them. The problem was that the plates contain of a solid substrate and the small amount of half a milliliter of sample did not disperse properly over the surface. It would have been more feasible to use the pouring method to get comparable results. Therefore, the results are not analyzed further.

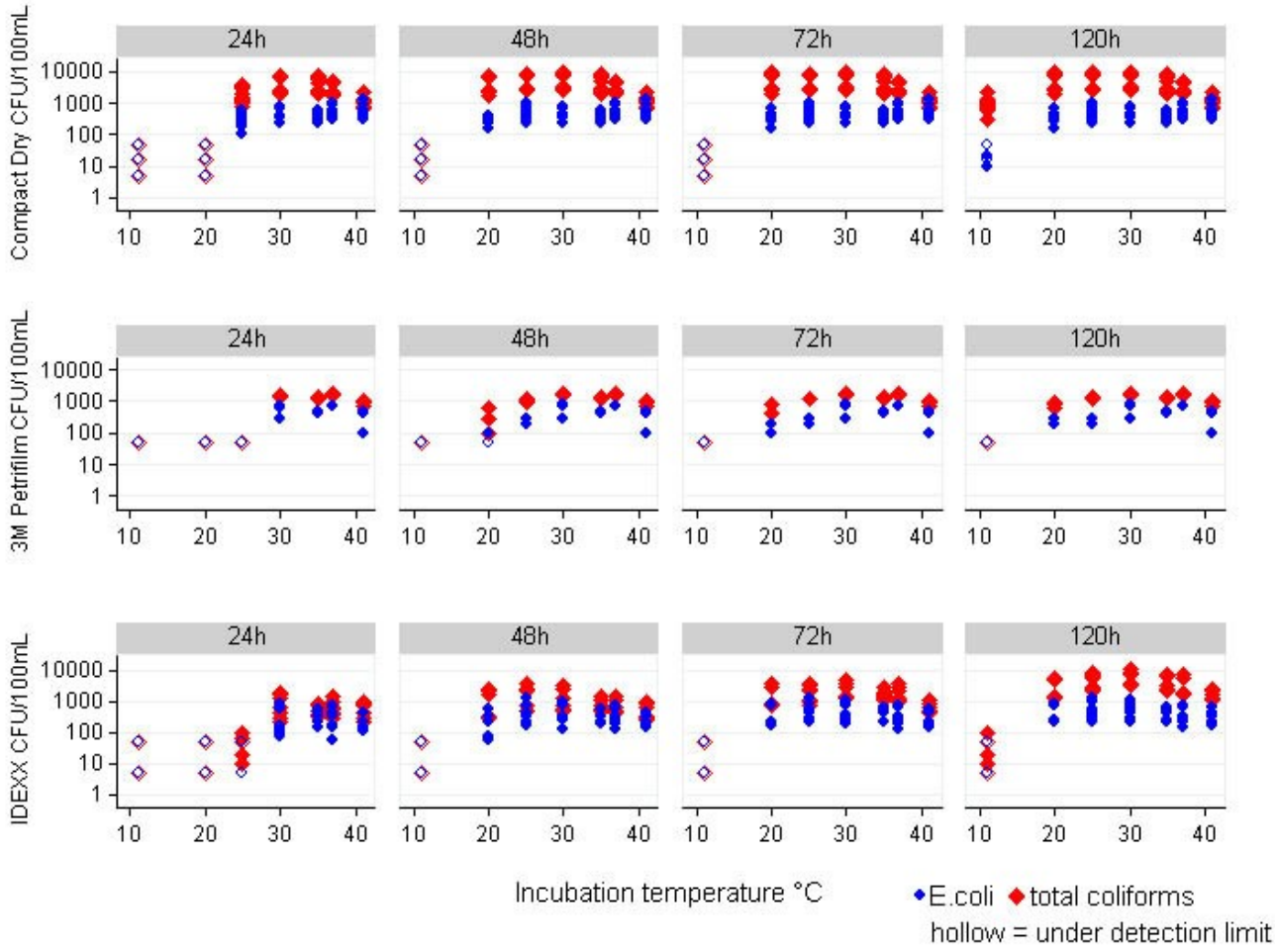

Figure 9: Impact of incubation temperature on E.coli and total coliform counts for three different methods. Upper row: Compact Dry EC plates from Nissui, 1, 3 and 10 mL samples dilution. Middle row: Petrifilm EC from 3M, 10 mL sample dilution. Lower row: Quanti Tray 2000 from IDEXX, 1 and 10 mL samples dilution. Note that due to 100 ml summation, the zero values are plotted as hollow symbols (under the detection limit, assumed half detection limit) and increased to until 50 CFU/100 mL.

In Figure 9, the results of the incubation experiment are shown, split into three rows for the three methods (Nissui Compact Dry EC plates CD, 3M Petrifilm EC and IDEXX Colilert Quanti Tray 2000). The dilution was not the same for all assays (1 mL, 3 mL and 10 mL Chriesbach water in 100 mL filtered tap water), thus the graph represents the calculated values per 100 mL of Chriesbach water. The range of total counts per 100 mL is consistent over the dilutions and the methods. It has to be noted, that if no counts were observed, half detection limit was assumed in the raw dataset. As for the present graph (Figure 9) values for 100 mL were calculated, numbers until 50 CFU/100 mL are for undetectable colonies, represented as hollow symbols (as example at 11 °C).

At 11 °C, there was no growth at all during the first 72 h, and after 120 h only on the Compact Dry plates (CD), there were some total coliforms grown what can be noted by the hollow symbols in Figure 9. At 20 °C, there was no growth after 24 h, but after 48 h E.coli and total coliforms were growing in all methods under different growth rates. In Table 6, the p-values from the two-tailed t-test for the different incubation temperatures and between the methods are listed. As indicated by the p-values, within the temperature range of 20 and 30 °C, there are significant differences of bacterial growth. For further statistical data, see Annex_table 2.

The highest E.coli counts are found at 37 °C (1300 per 100 mL on CD plates) and total coliforms at 30° and 35 °C (7300 per 100 mL on CD plates) after 24 h.
On the CD plates, the highest numbers of bacteria were growing. Comparing the methods and the incubation times, only at 25° and 41° there were significant differences between IX and CD in both indicators. Total coliforms grow significantly different in the temperature range from 25° to 41 °C, while E.coli did not show these differences. Between CD and 3M, both indicators showed significant difference in growth at 25 °C. 3M and IX did not have significant differences in the first 24 h in E.coli counts, only in total coliforms above 35 °C.

Comparing the counts after 24 h with the counts after 48 h, 72 h and 120 h, the main result is that at low temperatures (20 °C and 25 °C) there is a significant difference in both indicators over all measured times. After 120 h, total coliforms are all significantly different from those after 24 h, but this is not valid for E.coli. Thus E.coli counts are much more stable over time than total coliforms in all three studied methods.

Comparing these results with the results from the first temperature experiment with only CD plates that is discussed in the master thesis of R. Künzle, [9], the same tendency of bacterial growth can be observed. In detail, for E.coli, she found similar counts between 30° and 42 °C after 24 h that did not increase significantly at 48 h. At 20 °C, there was no growth at 24 h, while after 48 h the level was in the same range as with 37 °C. At 45 °C, growth was inhibited even for 120 h, the same can be observed for 11 °C.
There is a greater temperature dependence of total coliforms, with peak growth around 30 °C (illustrated in Figure 5.1 of [9]). With an incubation at 20° and 25 °C, coliform growth reached this peak level after 48 h, while at higher temperatures (above 37 °C), there was a significant inhibition lasting many days.

Thus at 42 °C some bacteira still grow, but at 45 °C not anymore. At temperatures between 20 °C and 30 °C they grow, but they take longer.

Table 6: p-values from the two-side t-test of incubation temperature experiment for the counts after 24 h of incubation. Significant numbers are marked as bold. CD: Compact Dry EC plates, IX: IDEXX Colilert Quanti Tray, 3M: 3M Petrifilm EC. Narrow are the counts below the detection limit (assumed to be half detection limit), that have differences because of calculations of 100 mL

	temperature	**E.coli**	**total coliforms**
all samples	11° vs 20°C	1.000	1.000
	20° vs 25°C	**<0.001**	**<0.001**
	25° vs 30°C	**<0.001**	**0.006**
	30° vs 35°C	0.211	0.748
	35° vs 37°C	**0.045**	0.516
	37° vs 41°C	0.375	**0.002**
CD vs IX	11°C	*0.719*	*0.719*
	20°C	*0.719*	*0.719*
	25°C	**<0.001**	**<0.001**
	30°C	0.453	**0.002**
	35°C	0.059	**<0.001**
	37°C	0.112	**<0.001**
	41°C	**0.014**	**0.003**
CD vs 3M	11°C	***0.046***	***0.046***
	20°C	***0.046***	***0.046***
	25°C	**0.014**	**0.012**
	30°C	0.323	0.108
	35°C	0.301	0.085
	37°C	0.263	0.253
	41°C	0.301	0.305
3M vs IX	11°C	*0.152*	*0.152*
	20°C	*0.152*	*0.152*
	25°C	*0.152*	0.940
	30°C	0.357	0.129
	35°C	0.072	**<0.001**
	37°C	0.080	**0.005**
	41°C	0.151	**0.036**

5.1.2 OSHO lab experiments

Impact of HF on microbial water quality with and without copper

The performance of E.coli, total coliforms and Enterococci in labfilters is illustrated in Figure 10. As there were very few Enterococci counts in the feed water, they have not been analyzed further. The impact of copper on microbial counts is only significant for Ethiopian copper in respect to total coliforms (p=0.016), but not for E.coli (p=0.989). Swiss copper did not show any significant impact on bacterial growth (p=0.346 for E.coli, p=0.168 for coliforms). Taking into account the log reduction values, a similar image for copper is presented: no significant impact of microbial water quality improvement.

The tendency, as it can be seen in Figure 10, is that the microbial water quality deteriorates in the sand filter but is improved in the lower bucket. In Table 7, the results of the bacterial performance in the labfilters are listed, including their significance (p-values from the two-side t-test) and in their log reduction values. It has to be noted that there were only two sampling sessions with “middle” samples (i.e. only 2 values for the candle filter), thus the statistical value is only valid to indicate a trend. For more details, see Annex_table 3.

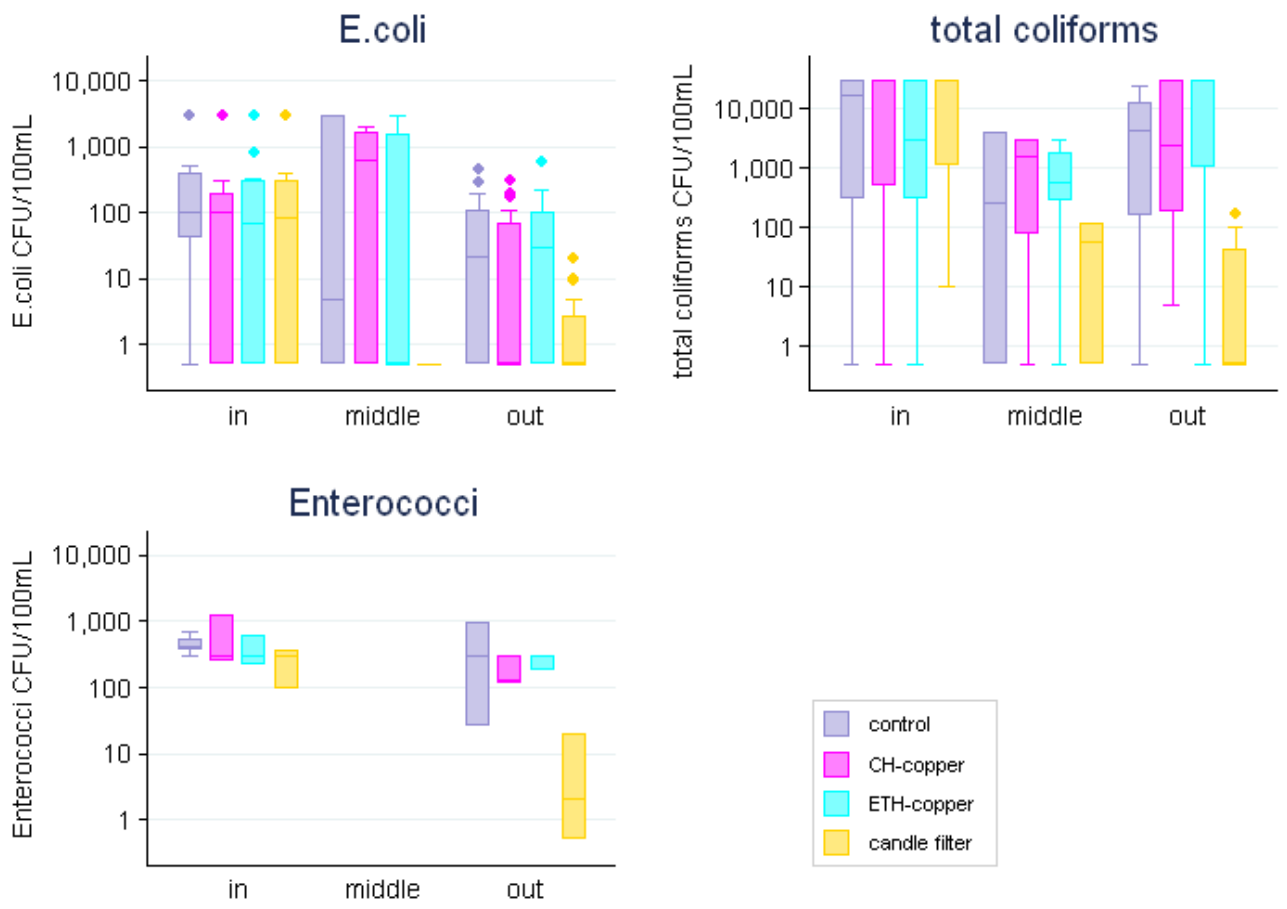

Figure 10: Indicator bacterial performance in labfilter from the inlet (in), after the sand filter, (respectively candle filter for the golden bar, middle) and the tap (out). Swiss (magenta) and Ethiopian (cyan) copper wires were added to the lower bucket of two sand filters, which were identically constructed identically to the control (lavender). A candle filter (gold) replaced the sand filter in one filter, to compare its impact on bacterial removal with the sand filter.

There is an overall significant decrease in bacterial counts in the labfilters in the order of 0.3 log units for E.coli and 0.8 log units for coliforms. In the lower bucket, the main reduction of bacterial counts has occurred, in the order of half log unit.

In the upper bucket, a remarkable difference between candle filter and sand filter can be observed (see Figure 10) which is not statistically significant because of the small sample number (n=2 for candle filter, n=8 for sand filter). The candle filter has a notable impact of more than 1 log unit reduction on the bacterial counts. Comparing the total performance of the labfilter with candle versus labfilters with sand, there is a significant difference between them ($p=0.008$ for E.coli and $p<0.001$ for coliforms for “out”-concentration, while the “in”-concentration did not show significant difference).

Table 7: p-values from two-side t-test of bacterial performance in labfilters and their log reduction values (mean with standard deviation)

p values from 2 side t-test:			
filter	position	E.coli	total coliforms
all filters	in/out	<0.001	0.002
	middle/out	<0.001	0.045
candle filter	in/out	0.065	<0.001
	in/middle	0.566	0.085
	middle/out	0.504	0.425
sand filter	in/middle	0.043	0.007
log reduction values :			
all filters	in/out	0.33 ± 0.80	0.82 ± 1.28
	middle/out	0.64 ± 1.07	0.45 ± 1.63
candle filter	in/out	0.51 ± 0.92	2.08 ± 1.51
	in/middle	1.39 ± 1.96	1 ± 0.56
	middle/out	-0.30 ± 0.43	-0.51 ± 2.20
sand filter	in/middle	-0.01 ± 0.18	-0.26 ± 0.35

Contact time of copper in bottles

Total coliforms have not been included in the analysis, because their number was too high to count (see Figure 11), thus it was focused only on E.coli for the bottle experiment. What can be observed in this picture is that the counts of total coliforms are dramatically reduced after 25 h of exposure, but still in the range above 300 CFU/plate.

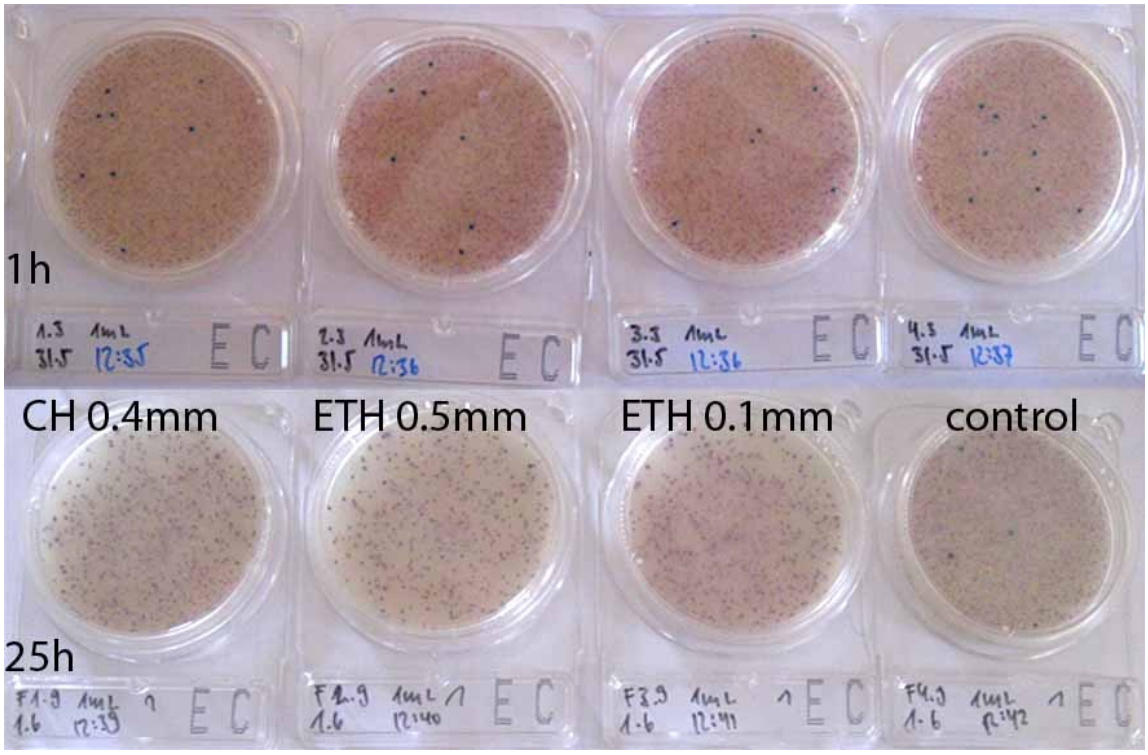

Figure 11: CD plates of copper experiment in bottles, with three different copper wires, indicated with diameter and origin (CH: Swiss, ETH: Ethiopian). Upper row: after 1 h of copper exposure, lower row: after 25 h of copper exposure.

In Figure 12, the time dependence of E.coli counts in the bottles is illustrated. The decrease in the overall E.coli counts is significant ($p<0.001$). For the Swiss copper, the mean log reduction value is 0.43±1.04, for the first Ethiopian copper (0.5mm) 0.41±0.80, for the second Ethiopian copper (0.1 mm) 0.12±0.4 and for the control 0.01±0.16. This implies, that copper from both origin reduced E.coli counts in the order of one magnitude over 25 h, with great uncertainties because of the small sample number (for further information, see Annex_table 10).
The decrease of E.coli in Figure 12 looks quite linear for copper, while the control shows a smaller decrease of counts. Between the counts of the shaken bottles after 25 h and the non-shaken bottles, there is no difference in the E. coli counts (data not shown). Visually, there was some brownish sediment after 25 h on the bottom of the bottles, which got mixed by shaking and lead to turbid water samples.

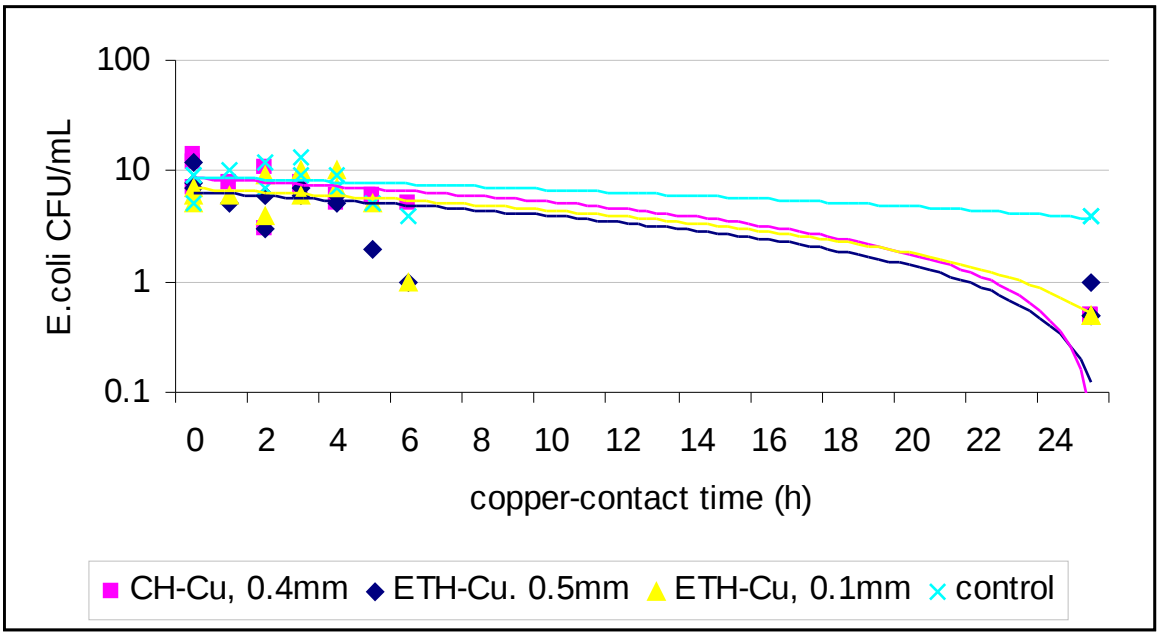

Figure 12: E.coli count in dependence of copper contact time in pet bottles with linear regression. Three different copper wires were used, Swiss copper with 0.4 mm diameter (pink circles), Ethiopian copper with 0.5 mm (dark blue diamonds) and 0.1 mm (yellow triangles) diameter, and a control sample (turquoise cross).

Shock-chlorination of sand filter

In the raw water for the chlorination experiment, there was a very small amount of E.coli present. For this reason, the focus here will be on total coliforms, because they were present in all raw waters. In Table 8, there are listed the log reduction values before and after the chlorination, subdivided into the location of the filter where the sample was taken from (further details in Annex_table 7 and Annex_table 10).

For the inlet, there is a significant reduction of 1.5 log unit in total coliform counts before and after the chlorination ($p=0.018$). After the sand filter (middle), there is still a significant decrease in coliform counts ($p=0.030$). But after the bonechar filter, in the outlet, there is a net increase of coliform counts observable. This can be explained by the short time period when

the sampling took place- the chlorine did not go through the bone char filter within a 1 h.

We could not measure chlorine concentrations, because the strips for the photometer were not available.

Table 8: Log reduction values before and after chlorination of upper buckets of three labfilters. EC: E.coli, TC: total coliforms

Location	Indicator	Mean	Standard Deviation	Minimum	Maximum
in	EC	0.00	0.00	0.00	0.00
	TC	1.58	0.20	1.38	1.78
middle	EC	-0.08	0.38	-0.60	0.30
	TC	1.79	1.13	0.32	3.06
out	EC	0.00	0.00	0.00	0.00
	TC	-0.79	1.11	-1.57	0.00

5.2 Field work in Ethiopia

A lot of field data was collected under various conditions, while sometimes it seemed difficult to compare the obtained data. Different water source quality and hygienic behavior of the users had a huge impact on the water quality on HH level. In the following, some aggregated results for the research questions will be presented

HF impact in 5 villages

In Figure 13, the impact of the HF on the level of fecal indicator bacteria in the five studied villages is displayed, together with their water source (if available). The last figure is the sum of all villages together that gives an average over the studied area. For more detailed data, see Annex_table 4and Annex_table 10.

In Chalalaka, as they use water from Lake Awassa, no source sample was taken because of huge local varieties in water appearance near the border (i.e. kids bathing, donkeys defecating, people fetching water at the same place). On the graph, there is a visible decrease in bacterial counts, which is statistically significant for E.coli (logred=0.67±1.06, p=0.031) and total coliforms (logred=0.26±0.66, p=0.043), but not for Enterococci (logred=0.38±1.75, p=0.644).

For Gura, it can be observed that microbial contamination increases from the source to the inlet, and decreases again in the outlet. Statistically, this trend has only been valid for E.coli (p=0.016) with a net decrease of 0.75 log units (logred=-0.76±0.68), and Enterococci (logred=-0.05±1.48, p=0.038) in the HF performance (in vs. out), while coliforms (logred=-0.14±0.46, p=0.428) do not show a no significant difference. For the difference between Gura windmill and the inlet, it has neither been found significance results, but an increase in 2 log units for E.coli, and 1.5 log units for Enterococci and total coliforms can be observed in the chart. Due to powers that be, only two of four visiting times, the Gura Windmill could be sampled. Once, the windmill was broken since 10 days (and as a consequence, there were few HH with inlet samples available), and another day the caretaker was on a funeral, which

lead to the same consequence. Hence very small sample size of windmill samples were available for this study, as well as inlet samples

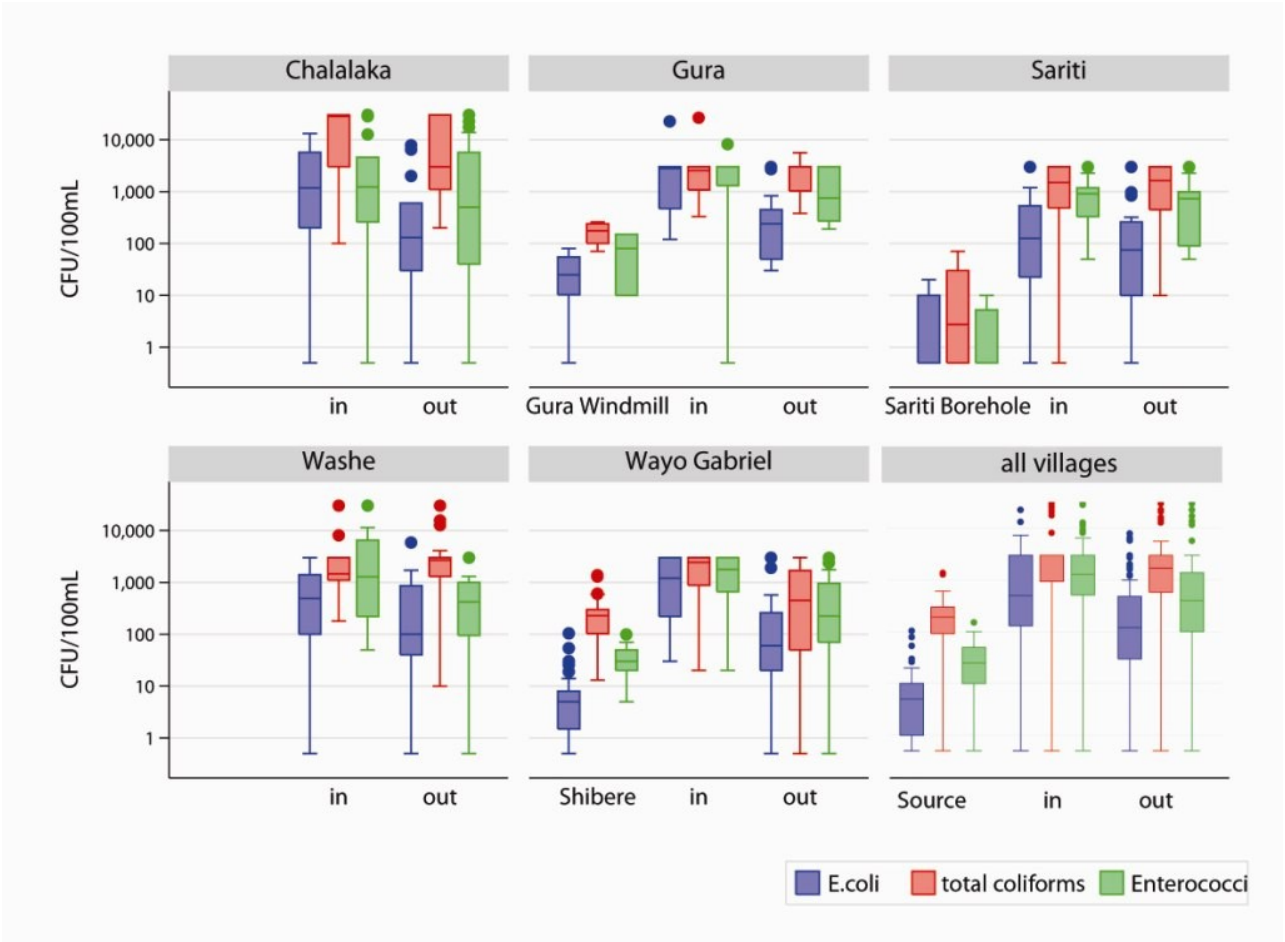

Figure 13: Impact of HF on fecal indicator contamination level in five studied villages with their water source, as well as all villages together. Only Washe has no own water source, thus the villagers get it from different sources. In Chalalaka, the inhabitants fetch water whenever they need form the lake Awassa next to the village, and due to heterogeneity of the lake, no sample was taken there.

The borehole of Sariti is the cleanest one, and the graph shows the same tendency as Gura with the highest contamination level in the inlet. The contamination from source to inlet is only significant for total coliforms ($p=0.003$) and Enterococci ($p=0.040$) and has an extension over 3 log units, while E.coli ($p=0.165$) increased but not in significant manner in 2 log units. The impact of HF on microbial counts is not significant for this village. Looking at the log reduction values of the filters, they are all near zero, very slightly positive fore E.coli (logred=0.11±1.42) and Enterococci (logred=0.52±0.96), and very slightly negative for total coliforms (logred=-0.11±1.04), thus a trend to increase in counts in the filter.

Washe differs from the other studied villages, because there is no water source nearby, and people fetch their water from a variety of different sources. Often when sampled in this village, the inlet water was not from the same source as the outlet water. This may be an explanation, why there is not a big impact of the filter on microbial counts except for Enterococci ($p=0.053$). There is a tendency for filters to improve the water moderately; the log reduction value for E.coli is 0.79 ±1.69, for total coliforms 0.11±0.96 and for Enterococci 1.06±1.87.

In Wayo Gabriel, the water source quality is in the same range as for Gura ($p=0.094/0.288$ for EC/TC), but its deterioration towards the inlet is significant ($p<0.001$ for EC/TC/ETC). The increase of bacterial counts is in the range of 2 log units for E.coli, 1 log unit for total coliforms and 2 log units for Enterococci. The same is valid for the impact of the HF on water quality in Wayo ($p<0.001$ for all indicator organisms), where the log reduction values are in the range of one log unit (logred=1.31±1.18/0.79±0.86/0.89±0.97 for EC/TC/ETC). The E.coli concentration in Wayo significantly differs from the one in Gura ($p=0.021$), while the difference in the water quality in the outlet is not significant between HH in the two villages.

In general, the tendency that can be observed in Figure 13, especially in the chart of all villages, is that the water source is relatively clean, and gets significantly deteriorated on the way to the inlet in the range of 2 log units for E.coli, 1.5 log unit for Enterococci and 1 log unit for total coliforms ($p<0.001$ for E.coli and total coliforms, and $p=0.030$ for Enterococci). The HF improves the water quality, but it does not reach the original level in any case. For E.coli, the HF impact is significant ($p<0.001$) and in the range of one log unit (0.75±1.34). In the case of total coliforms, the log reduction of the filter is much smaller (0.24±0.90) and for Enterococci, the log reduction is in the range of half log unit (0.61±1.319), but none of them is significant.

Impact of copper in HF

In Figure 14, the impact of copper on microbial water quality for each studied village is depicted. The log reduction value (inlet bacterial counts divided into outlet-counts) is shown for HF without copper and HH with copper (for data see Annex_table 5). The tendency that is observable is a slight increase of mean log reduction values, what would imply water quality improvement due to copper.

Only in two villages, there is a significant impact of copper on microbial counts. In Sariti, the increase in log reduction values due to copper addition is significant for E.coli ($p=0.002$) and coliforms ($p=0.003$) in a range of one log unit, but not for Enterococci ($p=0.460$). E.coli counts are reduced significantly in Chalalaka ($p=0.048$) in a range of 2 log units, while the other indicators are not decreasing much. The impact of the filter varies strongly between the households and the source water quality, from an improvement of 4 log units for total coliforms observed in one HH in Washe, to a deterioration of 4 log units also in total coliforms observed in a HH in Gura.

Figure 14: Log reduction values form HH in five villages with and without copper. The copper devices were submerged into the water in the lower bucket and sampled various times

Source to glass water quality

Knowledge on the contamination pathways of water from source to drinking glass is very important to evaluate where the most effective measures can be taken to improve water quality. In each village (except Washe), four HH were sampled from source to glass, and the results are presented in Figure 15 (data in Annex_table 6). The contamination levels of the HH varied considerable, thus statistical analysis gave mostly insignificant differences of the bacterial counts between the pathway steps. Only in Wayo Gabriel, the increase of contamination between the source and jerrycan is significant ($p<0.0001$ for EC, TC and ETC), as well as the decrease of counts in the lower bucket (middle/out: $p=0.0001/0.001/0.0006$ for EC/TC/ETC).

As it can be interpreted from the chart, the device to fill the filter up (“in”) is where most of the contamination takes place. The E.coli levels are much lower than the coliforms and Enterococci counts. In Chalalaka, the contamination levels are very wide and do not show a clear trend over the pathway. Coliform counts decrease in a significant manner in the lower bucket ($p=0.038$), while E.coli levels decrease notably after the filter, but not in a significant range, which is comparable with the results in Figure 13 (HF impact in 5 villages). In Gura, it can be noted a gradual deterioration of the water from source over jerrycan to inlet. The E.coli levels seems to decrease in the lower bucket and increase again in the drinking glass. For Sariti, there is a decline in water quality from the borehole to the jerrycan ($p=0.022/0.045$ for

EC/TC) and widespread in the inlet (p=0.025 for TC). A slight improvement in water quality can be observed due to the filter, however not significant.

In general, the water quality is best at the source level, and worst in the inlet device of the filter. The lower bucket meliorates the water quality and in the glass there is slightly higher bacteriological contamination.

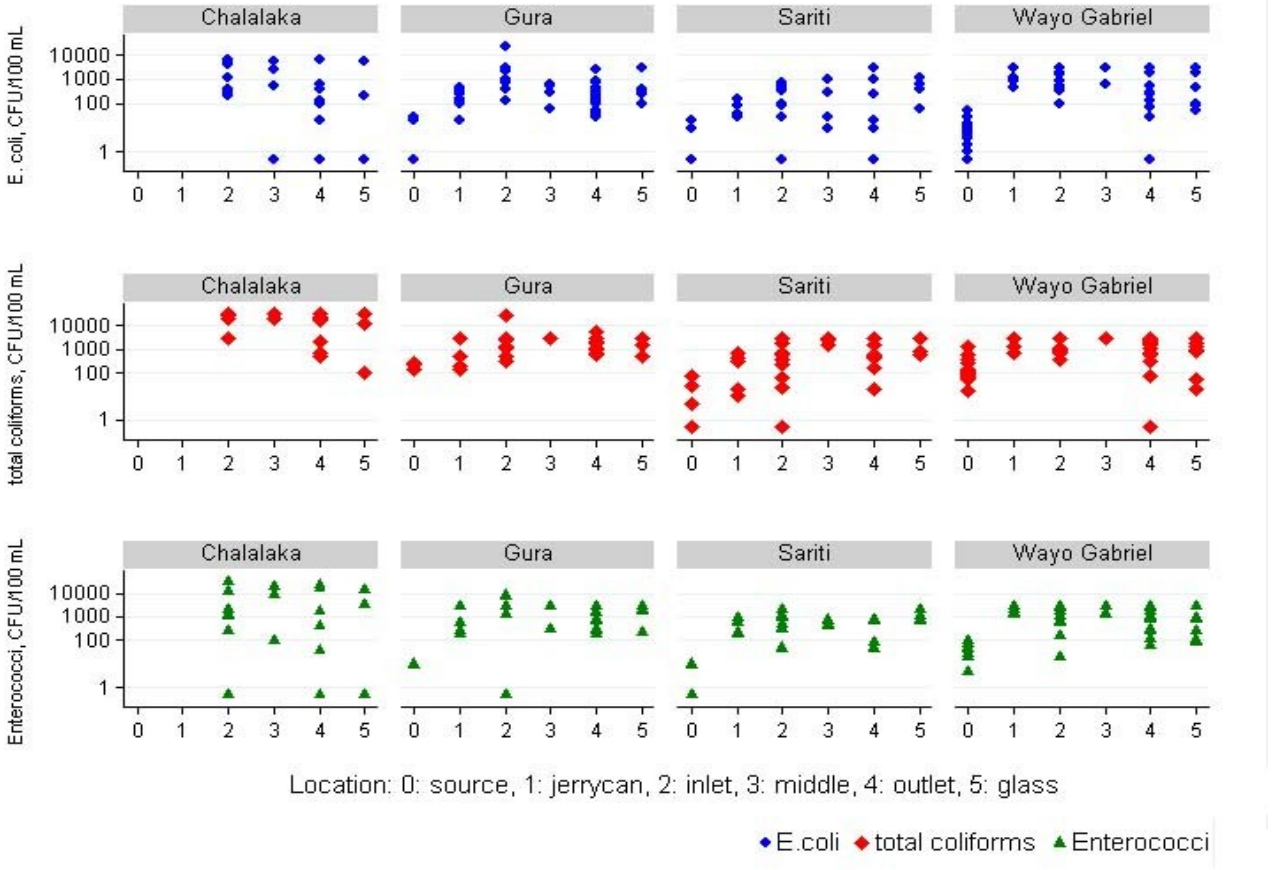

Figure 15: Fecal indicator counts over pathway of water from source to drinking glass in four villages. In Chalalaka they use lake water as source with high contamination levels, thus no differentiation between source, jerrycan and inlet was done there. The inlet is the device that is used to fill up the filter (as explained in 2.1 Household Filter).

Sterilization of taps

In Figure 16, the impact of flaming the outlet-taps of HF is depicted for all villages. It can be seen that the range of each indicator organism is very huge. Statistically, there is no significant difference (p=0.588/0.930/0.508 for EC/TC/ETC).

For the water point Shibere and the CF, further samples were taken to assess the impact of hand touching the taps while fetching water (see Annex_table 9).

There is no significant difference between the sterilized taps and the unsterilized ones of the CF (p=0.537/0.244 for EC/TC). The same situation is present at Shibere water point, with the difference, that from the six taps, two have been prolonged with a flexible tube. No statistical difference is evident between sterilized/non-sterilized taps (p=0.128/0.319 for EC/TC), neither for before/after tube (p=0.274/0.410 for EC/TC). As the flexible tube was made of plastic, no flame sterilization could be applied. The samples were taken in the morning, just after opening of the water point (no clients before).

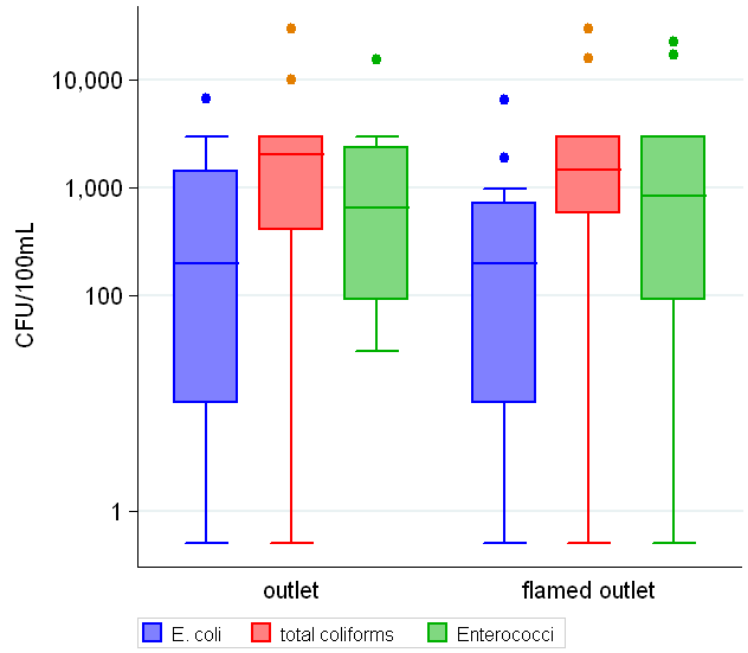

Figure 16: Impact of sterilization of the tap with a lighter on fecal indicator counts. Data from all villages.

Impact on water quality in drinking glass of HF or CF users

Source water quality in Wayo Gabriel is not significantly different between the Community Filter and Shibere water point. As shown in Figure 17 (and Annex_table 7) the water of CF users gets gradually deteriorated from the source over the jerrycan to the drinking glass, while the water of HF filter users runs through more steps until its final destination in a drinking glass (in this sampling session).

For CF users, the deterioration of water quality in the jerrycan is significant for E.coli (p=0.026) and for total coliforms (p<0.001). From the jerrycan to the drinking glass, there is no significant difference in bacterial counts.

In the case of HF users, as also indicated in previous results, the water quality gets significantly deteriorated from the source to the inlet in the range of one log unit (p=0.005 for E.coli and coliforms), and is improved by the filter in the order of two log units for E.coli (p<0.001) and one log unit for coliforms (p=0.010). The change in water quality from the tap to the glass is not significant, but in the range of one log unit for E.coli and half log unit for coliforms.

Comparing the contamination pathways, it is clearly visible, that the main contamination takes place between source and jerrycan for CF, and source and inlet for HF. Between the jerrycan

of CF users and the inlet of HF users, there is a significant difference in E.coli counts (p=0.011), but not in coliforms. In the drinking glass, there is no significant difference between CF and HF users. However, the range in HF users is much broader than for CF users, which is due to sample size. But both of them are thousandfold above the drinking water guideline of Ethiopia (<1 EC/TC in 100 mL).

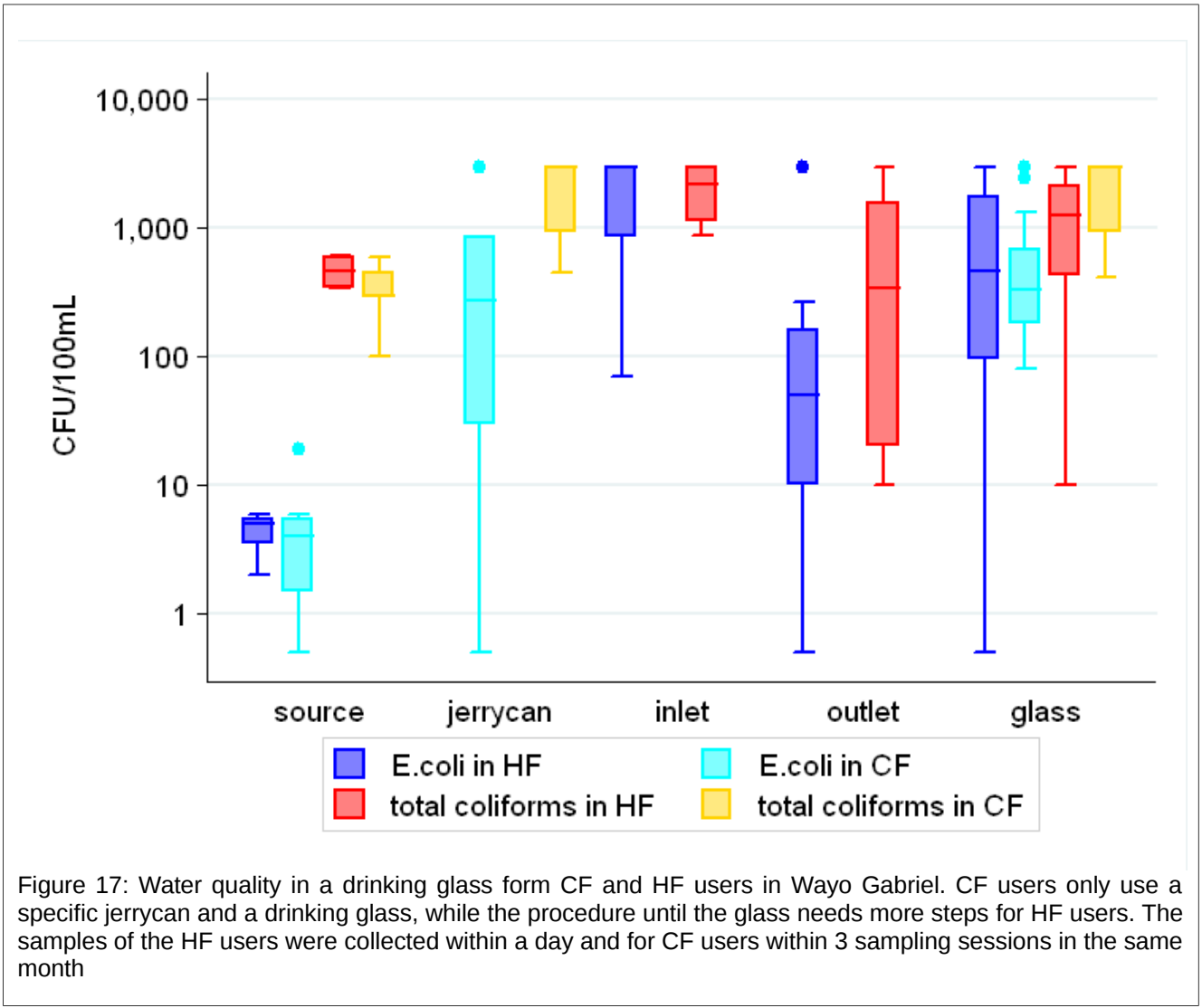

Figure 17: Water quality in a drinking glass form CF and HF users in Wayo Gabriel. CF users only use a specific jerrycan and a drinking glass, while the procedure until the glass needs more steps for HF users. The samples of the HF users were collected within a day and for CF users within 3 sampling sessions in the same month

Shock-chlorination of jerrycan

In total, 4 jerrycans were chlorinated in two different ways (Aquatabs and Waterguard) while the results are depicted in Figure 18. A reduction of almost one and a half log units in E.coli counts can be observed in the jerrycan due to chlorination, which is not significantly different (see Annex_table 9). Total coliforms get reduced by one third log units, which is significantly different (p<0.001). In the glass, a similar situation is found: almost a one log reduction in E.coli, and a reduction in coliforms of one third log units.

An interesting observation is that the contamination level between jerrycan and glass without chlorination does not change significantly; the log reduction value is in the range of zero for E.coli and of 0.3 for total coliforms. In contrary to this, with chlorination, there is a notable increase of E.coli from jerrycan to glass in a range of almost half log unit, while coliforms remain constant.

Comparing the two jerrycans that were cleaned with Waterguard with the two Aquatabs disinfected cans, we can observe a mean log reduction of 2.5 log units for E.coli, while for the Aquatabs, it is only observed a 0.3 log unit reduction for E.coli. In the drinking glass, the same tendency can be observed, but less prominent.
There is a positive impact of chlorine towards microbial killing, but in the way as it was applied in this experimental setting, it is fairly not enough to provide save drinking water.

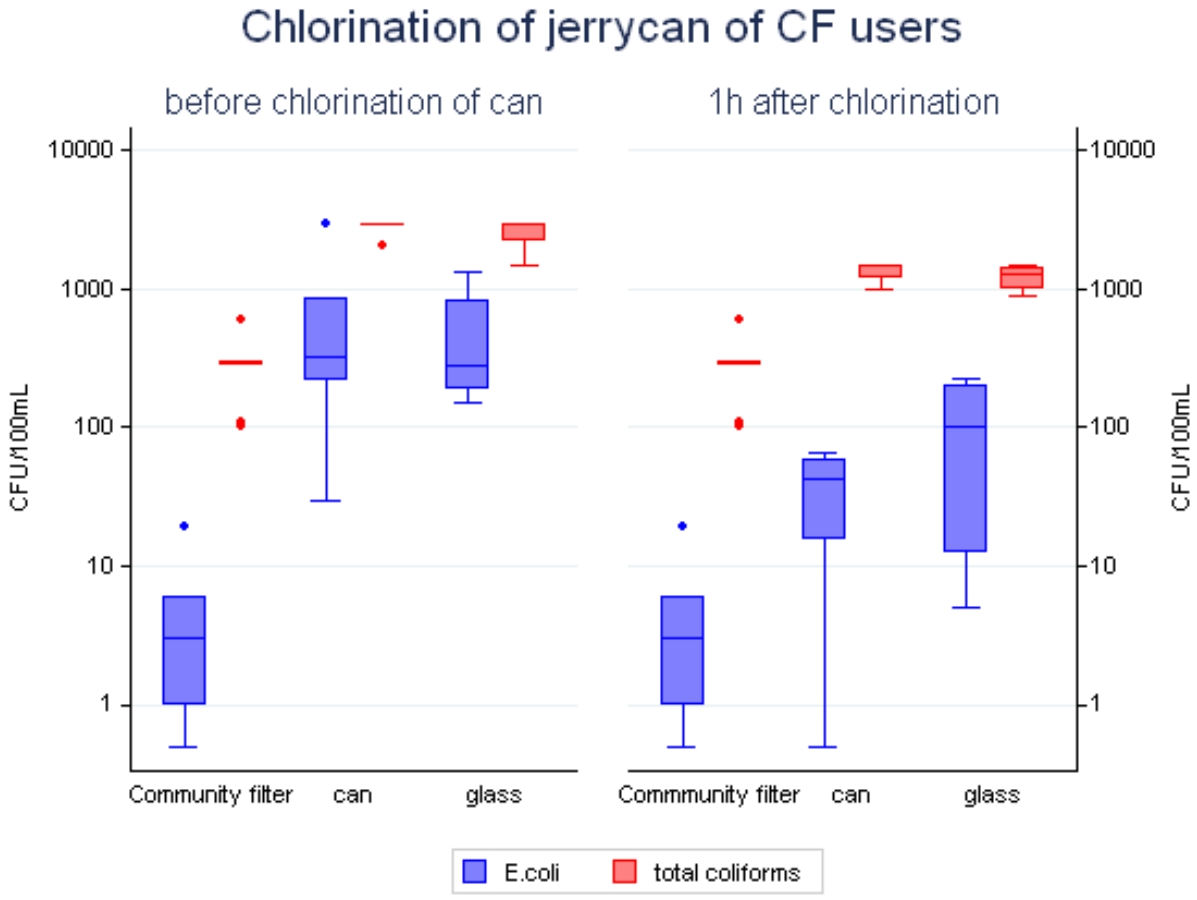

Figure 18: Chlorination of jerrycan of CF users. Samples were taken before and 1 h after chlorination from the jerrycan and drinking glass. In total 4 HH were sampled.

Chlorination of sand in HF

In three of the five HH, Waterguard was used as disinfectant while the other 2 HH used Aquatabs. In Figure 19 (and Annex_table 9), the microbial counts before and after chlorination of the sand are shown: sample results from the inlet and the outlet are enlisted. The time between chlorination and sampling was only 1 h (due to transport conditions), what is not enough to let the chlorine flow through the bonechar.
Before the application of chlorine, the filter was removing significantly more E.coli and coliforms than after, in the range of one and a half log units (p= 0.026) and nearly one log unit, respectively. With the application of chlorine, the log reduction value of the filter turned negative, what can be explained by the short contact time period (no flow through the

bonechar).
In the inlet, where the chlorine was applied, there is a significant decrease in E.coli ($p=0.002$) and total coliform ($p<0.001$) counts over 2 log units. On the other hand, in the outlet, the same order of magnitude is observable. There are no notable differences between the sand that was disinfected with Aquatabs and Waterguard.

Comparing these data with the chlorination experiment of the labfilters (assuming coliforms behave as E.coli), the log reduction due to chlorine is in the same order of magnitude. The slight increase in counts in the outlet is also present in both cases (both had 1 h of contact time with chlorine in the upper bucket).
There is a notable reduction in counts, but drinking water standards are not reached when only chlorination is applied.

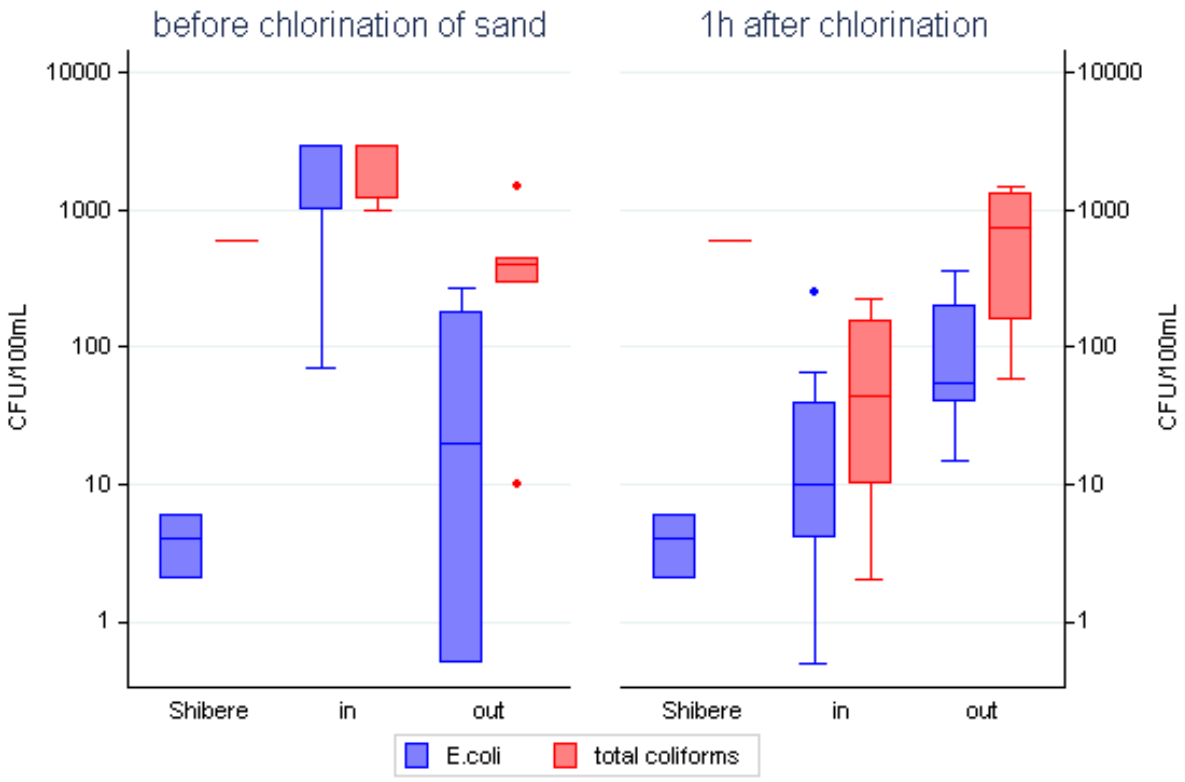

Figure 19: Chlorination of sand in HF in 5 HH in Wayo Gabriel. Sampling before and 1 h after chlorination of sand in the upper bucket.

6. Discussion

6.1 Lab experiments

6.1.1 Eawag experiments

The first dilution experiment with a pure lab culture did not work out as expected: the shock for the bacteria coming directly from the agar plate to diluted tap water could lead to a die off of them. To avoid this, in the second dilution experiment PBS buffer was used, which lead to the expected counts of the pure E.coli strain K12 on the CD plates, but not on IX. The important finding here is, that the CD plates work well with the labculture, thus they are a reliable instrument to detect E.coli.
The incubation temperature dependence of CD plates exists, but in the range of 30° to 41 °C; there are not significant differences in the counts. Thus it is a valid method for developing countries, where electricity is sometimes lacking and a stable incubation temperature cannot always be guaranteed. If the temperatures are below 30 °C, an increase in the recommended incubation time will not result in inaccurate data. As the first incubation temperature experiment was performed in February, this could have influenced the faster growth of total coliforms at 20 °C after 24 h in comparison to the second experimental session in August.
E.coli counts are much more stable over time than total coliforms in all three studied methods.
From the handling and required equipment site, of the three tested method, CD are the easiest to do if no filtration is needed. The Quanti Tray is also a simple method, but the required sealer machine limits its application. The Petrifilm is tricky to handle, to avoid the gel to leak from the film.

6.1.2 OSHO experiments

HF filters reduce fecal indicator organisms (especially E.coli) in a range of 1 log unit, while the main water improvement takes place in the lower bucket, the bonechar filter. Surprisingly, the sand filter increased the E.coli counts, but decreased total coliform counts. The candle filter showed a reduction rate of bacterial counts in the range of 1.4 log units, and has the potential to act as a valuable alternative for the sand filter. Interestingly, Künzle [9], found the adverse effect, that candle filters (from the same producer) did not improve microbial water quality in Kenya.
It has to be taken into account, that the flow rate from the upper bucket depends on the hole in the outlet, which can easily get clogged - the labfilters had not the same flow rates in the upper bucket, even though they were produced under the same conditions. Another important part is the flow rate in the lower bucket through the bonechar. We did not made any experiment to detect the time the water needs to pass through, also because there is always residual water in the lower bucket. All data from various experimental settings were aggregated to make the analysis what implies high variance of bacterial contamination, also because the raw water originated from different sources and varied significantly in its contamination level. The candle filter was previously in use as an exhibition object for visitors in the lab of OSHO, thus bonechar had a longer contact time with water than the other filters. This may have an impact on regrowth in the lower bucket of the candle filter - water was standing there for weeks without any disturbance from outside.

Due to addition of copper, there was a slight improvement of the microbial water quality but

there were nearly no differences between Ethiopian and Swiss copper in the labfilters. The natural die-off of bacteria led to the decreasing counts over time. In the contact-time experiment, taking into account normal die off rates of bacteria, the inactivation effect of copper became visible. Comparing these results with inactivation rates from literature (see 2.7 Antimicrobial properties of copper), the observed possible inactivation due to copper is negligible. It remains unclear, why the copper did not show the expected inactivation property suggested in literature.
No experiments have been performed with differing copper surface areas which may have an impact on bacterial inactivation capacity. Sudha et al, 2009, used 15 cm2 of pure copper per liter water as treatment; this volume was taken also for our experiments [59]. However, the copper wires in our experiments did not undergo a purity analysis; they were probably mixed with other metals. This could be an explanation, why results form copper experiments are not comparable with data from literature.
In respect to contact time, from the experiment in the bottles, a contact time of one day can be estimated to carry out its impact on microbial counts. This coincide with literature data on overnight copper exposure in [59] and over 24 h in [3], [4] for drinking water in developing countries.
The present findings do not support the explicit findings of other studies of microbial water improvement due to contact with metallic copper. The laboratory experiments made at Eawag and in Modjo indicate a slight inactivation process under copper exposure, but were not as univocal as literature suggests. Only the experiment from the first Eawag session after 96h exposure to copper at 30 °C (explained in [9]) showed a dramatic impact of copper on microbial growth in comparison to florist wire or the control. Due to time limitation, it has not been possible to repeat this experiment under controlled conditions.

Chlorination of sand filter could be an option for improving drinking water; we have seen that there is a significant reduction in microbial counts. In the experimental setting, time interval between the chlorine application and the second sampling has been too small, thus the chlorine could not pass through the bonechar filter. As time was lacking, a later sampling could not be conducted. Chlorine testing would have led to further details on the behavior of chlorine in the filter together with important indications for the dosing. There were not found any differences in antibacterial activity between the 3 types of chlorine applied to the sand filter (Aquatabs, bleach and Waterguard).

6.2 Field work in Ethiopia

HF filters have an overall positive impact on fecal indicator organisms in all villages; nevertheless the contamination level is very high. The main contamination was taking place between the source and the inlet, respectively the jerrycan.
In Chalalaka, the contamination level of the inlet is the highest found, what comes from the lake Awassa that they use as source. The first filters were distributed in this village at the beginning of the project (2008), and regular monitoring and visiting is stopped since one year [14]. Thus the villagers are left on their own devices, and the use and maintenance of the filters is the lowest of all studied villages. The socioeconomic level of Chalalaka is the lowest too, and the source water is very turbid, what leads to clogging of the upper bucket and the sand filter. Of all the visited HH, only 3 were using daily the filters. Nevertheless, the water after the filter is from a slightly better microbial quality- what is still thousandfold exceeded the Ethiopian drinking water standard.

The sampling in Gura was star-crossed, two times no water from the Windmill was available, and consequently very few HH had water in their jerrycans for the inlet sample. Furthermore, the notebook with all dates was lost in Gura and did not appear anymore.
In Sariti, where the source water quality was the best of the studied villages, the drinking water after the filter did not show a significant difference to the inlet.

The source water quality in Gura and Wayo Gabriel are in the same range, while Sariti has less microbial counts in the borehole. Nevertheless, only Sariti seems to have fulfilled at one sampling day the national guideline of drinking water (sampling volume 10 mL and no counts found in it).

For Ethiopia, there are few studies about microbial drinking water quality in rural areas. In Northern Gondar, microbial contamination of water sources was found in the rural areas, in a slightly smaller range of indicator organisms than we found in the Rift valley [66]. The country report from Unicef/WHO about drinking water quality found generally much less contamination in Ethiopian water sources than our study [3].
In the city of Bahir Dar, there were found coliforms in tap water and HH storage containers, the contamination was associated with poor hygiene and sanitation [67]. Contaminated water sources were even found in Addis Ababa; unsafe handling at source and in the HH deteriorates drinking water quality even in urban areas [68].

Between Community filter and Shibere, there are no significant differences in water quality. At one sampling day, there were more than 100 E.coli found after the treatment steps in the CF (sample volume 100 mL), which was probably due to contamination at the outlet. In respect to the water quality in a drinking glass from CF or HF users in Wayo Gabriel, it does not matter which defluoridation type they use, the microbial counts are not significantly different. In both cases, the contamination levels are exorbitant, and measures have to be taken to improve drinking water quality drastically.

The insignificance difference between source and inlet may be due to a very small sample size of the source water in front of larger HH samples. This would explain, why in Wayo Gabriel, there are significant differences and in other villages not. Because in Wayo, several times were sampled the water sources, and the total samples also were higher.

The copper device seems to have a slightly positive effect on microbial inactivation in the HH, as well as in the labfilters. There were no significant differences found between the three studied copper devices, neither in the laboratory experiments, nor in the field date. In all literature found about copper, there was none with field data; all experiments were conducted in a lab under controlled conditions.
The water quality varies strongly between the HH, thus the observed copper impact is not exclusive. For this reason, all HH were analyzed together for HF performance, without making difference of copper/non-copper HH.

Flaming the tap with a lighter did not showed a significant impact on the water quality. Contamination through contact between unwashed hands and filter taps were observed several times in the households, sometimes children were inserting their whole fingers into the tap. Thus contact contamination is a problem in the households, but flaming did not show the expected impact. On the one hand this may be caused by the small sample size of sterilized/unsterilized tap (n=17). On the other hand, this may be caused by the short flaming

time and that the flame did not reach the whole tap.

Looking at the contamination pathway form source to drinking glass, we can see that the main contamination takes place in the HH between the jerrycan and the device to fill up the filter. Looking at the circumstances, how the water is transported from the jerrycan to the upper bucket, this finding is not surprising. The inlet device, as explained in 2.1 Study area, was often deposited on the floor of the house and rinsed with some water before its use. The hands were not washed previously to fill up the filter, thus microbial contamination could easily pass from hands to inlet device in this step. Jerrycans were often stored without tap in the house, where children were inserting their hands into them and hence other possible contamination with microorganisms could take place. As the filter gets clogged sometimes, the HH are used to suck the outlet of the upper bucket to get rid of the blockage, or to insert a needle for wiping away. No measures are taken doing so to avoid direct touch of the filter outlet with contaminated surfaces, such as hands, needles or the face. The outlet tap is the next step of possible contamination due to touching, as discussed one paragraph on top. And the drinking glass is the last step where contamination can take place, with the same mechanisms as for the inlet device.
In literature, there are many publications based on household water treatment, due to the main contamination pathways that take place there, i.e. in the storage container and point of use [18], [64], [68–70].
Hands are the main carriers of fecal contamination in households, a high correlation between bacterial counts on hands and drinking water storage at HH level was found in Tanzania. Thus the contaminated hands are the main responsible for recontamination of drinking water at HH level and interventions of hygiene of hands could improve dramatically microbial drinking water quality. Additionally, they found a correlation between gastrointestinal and respiratory diseases and fecal bacterial counts on hands, while the counts in the stored drinking water did not correlate [71]. Hand washing was only observed before eating in the HH and soap and detergents were just used for cloth washing. Hygiene in general is very poor in all the studied HH, with local differences.
In India, they did not found significant differences of water quality at HH level due to handling and hygiene practices, what is surprising due to contrary observations and studies. Nevertheless, recontamination of drinking water was observed at HH level in high extension, where measures have to be taken to improve health of the users [72]. The drinking glass was found to have a very high risk of recontamination in Bolivia [70], the same tendency was found in our study, with a smaller sample size.
There is a tendency that microbial clean source water gets contaminated at HH level during storage, while if the watersource is bacteriolical contaminated, HH storage improves the water quality [18]. In our study, we also foundt this trend- in Chalalaka with the worst source water quality, there was an improvement due to the HF, while in the other villages, there was a deterioration in microbial water quality observed.
Interesting results were found in a study from Ecuador [65], where they could demonstrate that recontamiantion takes also place in the HH storage container if the source water was contaminated. In a study in Pakistan it did not find a correlation between microbial contamination of drinking water at the source and childhood diarrhoea. But they found incidence of diarrhoeal prevalence associated with fecal contamination in HH storage water, which is caused by recontamiantion at HH level [73].

Chlorine is the most famous water disinfectant in the world, and its bacterial killing property could be observed in this study. The two chlorination experiments in HH in Wayo Gabriel lead

to significant reductions in bacterial counts, although the contamination level remained above acceptable range.
In the case of chlorination of the sand filter, due to a high organic matter content, high bacterial load and short contact time, there was not the expected decrease until zero counts. The dosage for shock chlorination of the sand in HF was assumed on a base of water disinfection concentration, but in a porous media such as sand, additional parameters have to be taken into account.
Further investigation is needed to assess how sand filters of HF can be safely disinfected, which disinfectant is the adequate and with which frequency it has to be done. To replace the sand filters by candle filters could be an option, the labfilter results were promising in this direction. Further studies need to be performed to see the viability of candle filters for HF in Ethiopia, including availability, costs, maintenance, acceptance, microbial performance under field conditions, replacements.

The chlorination of jerrycans was more effective if performed with Waterguard in residual water under shaking, than simply addition of Aquatabs to stored water. The present biofilm in the jerrycans was not removed by any of the actions taken, and could serve as hideaway for the studied bacteria. Shaking the jerrycan with residual water and Waterguard resulted in displacement of small pieces of biofilm. Jagals et al, 2003 studied biofilms in jerrycans and their potential to deteriorate microbial water quality. They figured out that biofilm have a negative impact on microbial water quality, and potential hazardous microorganisms can host biofilms. Total coliforms was found to be part of the biofilm, while E.coli seems not to be supported by the biofilm and was introduced by handling into the jerrycan [63].
The present results go into the same direction as a study on jerrycan chlorination undertaken in Uganda by Steele et al, 2005 [64]. The used chlorine there was high strength sodium hypochlorite solution, what is comparable with Waterguard (that was used diluted in our experiment). Chlorination of the jerrycans was effective, but recontamination was taking place after few days due to unsafe water handling practices and poor hygiene in the camp environment [64]. In our case, the chlorination was not as effective, and we have got contaminated source water, but the findings are comparable. The problem of recontamination is very high in all studied villages due to poor hygiene practices and lack of awareness by the HH.

7. Conclusion and outlook

The microbial water quality in the studied villages at HH level is alarming, waterborne diseases are common in the visited villages. In seven of the 47 HH that were visited during the four month of study, a resident had severe diarrhea. Sanitary infrastructure is lacking in the studied villages, and hand washing only observed before eating by adults. Domestic animals were inside the house, i.e. chickens and cats, and contribute to the high microbial contamination level inside the HH.
Looking at the Disease Risk Index of Trevett and Carter (2008) [74], the studied villagers are all at high risk and immediate measures have to be taken to improve the situation.

HF and CF are designed to remove fluoride form drinking and cooking water to improve the health of the users. They are effective to remove fluoride and have a broad acceptance in the population of the affected villages [8]. Unfortunately, fluoride is not the only health problem that we can observe in the studied villages- diarrheal diseases are very common and can directly be associated to poor microbial water quality at the point of use [12], [13]. The HF improves slightly the water quality, but the contamination level is still thousandfold exceeding drinking water guideline values.

There are different approaches to improve drinking water quality at point of use in the villages [74], [75]. They will be presented in the following; they are not exclusive and can be combined, and make no claim to be complete.

- Technology improvement:
The replacement of the sand filter by candle filters; as laboratory experiments indicate, candle filters remove microorganisms significantly. This implicates further investigation on Ethiopian market for ceramic candle filters, where (if) they are provided, what is their life time and their cost. Further the acceptance by the villagers has to be examined, as well as maintenance and replacement of them.
There is a promising approach form India to remove fluoride, arsenic and coliform bacteria all in one in an enhanced household sand filter [76]. Looking into detain of the functioning of this sand filter, probably there could be a joint venture to build something similar based on bonechar. Fortunately, in Ethiopia there is not a concern with arsenic, thus an adaption of the technology to make the filter simpler could be an option.

- Disinfection
In the HF, the sand was discovered to be a source of recontamination of the drinking water, also because indicated sand washing was not performed by the HH. Regular chlorination of the sand is a valid option to kill microorganisms, dosage and application needs more investigation, as well as social acceptance (taste of residual chlorine). The experiments done showed a positive impact on decreasing bacterial counts in the sand filter due to chlorination, independently which chlorine was used.
Another possibility to disinfect the sand is with boiling water, this would not have an impact on the water taste. An important aspect of this type of sand disinfection is enough contact time by high temperatures in the sand to kill all the microorganisms. The plastic of the upper bucket should resist without problems to elevated temperatures, the mesh may be damaged by contact with boiling water. Further experiments have to be conducted to check the

feasibility of this disinfection method in the HH.
Microbial contamination takes place in the jerrycan and in the inlet device, before it reaches the sand filter. Shock-chlorination did show a positive impact on microbial water quality in the small experimental setting on jerrycans. Regular chlorination and removal of biofilm in the jerrycan would improve the water quality of entering water the HH. Adding concentrated chlorine to residual water and shake it showed better bacterial killing rates than addition of chlorine tabs to full jerrycan. Careful instruction and handling of concentrated chlorine such as bleach or Waterguard need to be given to the users to avoid accidents.
Chlorination of the CF would provide microbial clean source water with residual chlorine to protect the water from recontamination. The dosing of chlorine in the CF is easier to control and monitor than on HH level. It has to be investigated, what the consumers think about chlorine taste of water, if they are willing to buy it and use it.

- Hygiene promotion
The main part of fecal contamination found in drinking water in the rural HH comes from unsafe handling practices, directly related with poor hygiene and sanitation. Behavior change in personal hygiene is basic to improve health of the dwellers and avoid contamination of drinking water. Educational programs for awareness creation of the relation hygiene-sanitation-water-health should be implemented in all villages. Collaboration with health workers and schools for coordination of activities in the villages is recommended. As an example, there is a training program for a workshop about water/sanitation/hygiene made by Inter Aide Ethiopia for rural villages [77].
Impulses to create awareness of the importance of safe sanitation facilities are needed in the community as well as support to realize sanitary constructions for HH and schools.

- Interventions at HH
Building racks to store dishes and other utensils out of reach of children and domestic animals to avoid contamination through contact with fecal matter. Additionally, the covering of the utensils lead to minimize contact with dust and flies. Using taps for jerrycans, which have to be maintained clean and out of touch for children, leads to safer storage of water at home. Ways to get domestic animals out of the house have to be searched in collaboration with the HH, with the aim of minimizing deposition of feces inside the house.

- Market
If there is no affordable soap available in the village, the best hand washing promotion will not be effective. The same is valid for chlorination, or jerrycans with a tap that can be hanged on the wall to facilitate hand washing. Local providers of needed goods have to be included in promotion planning, as well as the small shops in the villages.

Under all these aspects, it is important to address all the villagers, not only the ones that actually do use defluoridated water. In a study in Central Ethiopia about portable drinking water device (Life straw) and the frequency of real use of it, they found out that only 30% of the HH were using the device frequently, and only 10% used it consistently [76]. This implies that even knowing the improvement of water due to a new technology, behavior change needs a lot of time and conviction. What the people say and how they behave is not always the same in respect of use a new technology [78].

The project on defluoridation filters is a first step to address water quality problems in Ethiopian HH in the Rift Valley. To further improve quality of life in the project region, the focus

should be on education, hygiene and sanitation. These aspects imply the most cost effective measures to improve living conditions of villagers in long and short term [75].

Bibliography

[1] WHO, “Facts and Figures on Water Quality,” *Water Sanitation and Health*, 2011. [Online]. Available: http://www.who.int/water_sanitation_health/facts_figures/en/index.html. [Accessed: 19-Sep-2011].

[2] UN, “Ethiopia: Data of UN,” *Country profile*, 2011. [Online]. Available: http://data.un.org/CountryProfile-aspx?crname=Ethiopia. [Accessed: 19-Sep-2011].

[3] O. S. Dagnew Tadesse, Assefa Desta, Aberra Geyid, Woldemariam girma, Solomon Fisseha, “Rapid assessment of drinking- water quality in the Federal Democratic Republic of Ethiopia, Country Report,” Geneva, 2010.

[4] A. Devi and K. Bostoen, “Extending the critical aspects of the water access indicator using East Africa as an example.,” *International journal of environmental health research*, vol. 19, no. 5, pp. 329-41, Oct. 2009.

[5] P. Jagals, “Does improved access to water supply by rural households enhance the concept of safe water at the point of use? A case study from deep rural South Africa,” *Water Science & Technology*, vol. 54, no. 3, p. 9, Aug. 2006.

[6] G. Rosa and T. Clasen, “Estimating the scope of household water treatment in low- and medium-income countries.,” *The American journal of tropical medicine and hygiene*, vol. 82, no. 2, pp. 289-300, Feb. 2010.

[7] S. Esayas, M. J. Mattle, and L. Feyisa, “Household water treatment : Defluoridation of drinking water by using bone char technology in Ethiopia,” in *34th Annual WEDEC Conference*, 2009.

[8] H. Korir et al., “The development of bone char based filters for the removal of fluoride from drinking water,” in *34th WEDC International Conference*, 2009, p. 8.

[9] R. Künzle, “Household drinking-water quality in the Kenyan Rift Valley,” ETH Zurich, Switzerland, 2011.

[10] F. Kage and K. Müller, “Fluoride removal in low and middle-income countries,” *PEAK B12/08*. Eawag, Dübendorf, Switzerland, pp. 1-51, 2008.

[11] S. Feyera, “Defluoridation project,” 2010.

[12] Ministry of Water Resources, “Ethiopian water quality standard,” *Ethiopian Guidelines, Specification for drinking water quality*, 2002. [Online]. Available: www.eah.org.et/docs/Ethiopian water quality standard.doc.

[13] OSHO, “Possible field sites for RP and IP: Results od the field trip in November 2009.” Modjo, Ethiopia, pp. 1-28, 2009.

[14] S. Feyera, “Personal communication.” Modjo, Ethiopia, 2011.

[15] H. Kloos and R. T. Haimanot, “Distribution of fluoride and fluorosis in Ethiopia and prospects for control.,” *Tropical medicine & international health : TM & IH*, vol. 4, no. 5, pp. 355-64, May 1999.

[16] T. Ayenew, “The distribution and hydrogeological controls of fluoride in the groundwater of central Ethiopian rift and adjacent highlands,” *Environmental Geology*, vol. 54, no. 6, pp. 1313-1324, Jul. 2008.

[17] WHO, “WHO guidelines for drinking-water quality.,” Geneva, Jan. 2008.

[18] J. Wright, S. Gundry, and R. Conroy, “Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use.,” *Tropical medicine & international health : TM & IH*, vol. 9, no. 1, pp. 106-17, Jan. 2004.

[19] HACH, “The Use of Indicator Organisms to Assess Public Water Safety Technical Information Series- Booklet No.13,” *World Health*, no. 13. HACH Company, pp. 1-48, 2000.

[20] a H. Farnleitner et al., “Escherichia coli and enterococci are sensitive and reliable indicators for human, livestock and wildlife faecal pollution in alpine mountainous water resources.,” *Journal of applied microbiology*, vol. 109, no. 5, pp. 1599-608, Nov. 2010.

[21] G. Bitton, “Microbial Indicators of Fecal Contamination: Application to Microbail Source Tracking,” Florida, 2005.

[22] Nicholas Ashbolt et al., “Pathogens, Pathogen indicators, and indicators of fecal Contamination,” in *Report of the Experts Scientific Workshop on Critical Research Needs for the Development of New for the Revised Recreational Water Quality Criteria*, Warrenton, Virginia: , 2007, pp. 35-56.

[23] D. C. Melira Stevens, Nicholas Ashbolt, “Review of Coliforms, as Microbial Indicators of Drinking Water Quality,” 2003.

[24] P. Tallon, B. Magajna, C. Lofranco, and K. T. Leung, “Microbial Indicators of Faecal Contamination in Water: A current Perspective,” *Water, Air, and Soil Pollution*, vol. 166, pp. 139-166, 2005.

[25] M. M. Stevenson, “Monitoring Effective Use of Household Water Treatment and Safe Storage Technologies in Ethiopia and Ghana,” Massachusetts institute of Technology, 2008.

[26] Hyserve, “Compact Dry, Easy test method for counting micro-organisms.” p. 12.

[27] Nissui Pharmaceuticals Co. Ltd, “Compact Dry ‘ Nissui ’ EC For Coliform and E.coli, User’s manual.”pp. 1-4, 2009.

[28] Nissui Pharmaceuticals Co. Ltd, “Compact Dry ‘Nissui’ simple and Easy Dry Media for Microbial Count and Detection,” *Yeast*. pp. 1-5, 2007.

[29] M. Manafi, “New developments in chromogenic and fluorogenic culture media.,” *International journal of food microbiology*, vol. 60, no. 2-3, pp. 205-18, Sep. 2000.

[30] P. Conda Laboratorios, “Chromogenic media for Microbiology,” *Microbiology*. p. 12.

[31] S. Hosokawa and H. Kodaka, “Efficacy of Compact Dry EC for Coliform Detection in Seafood,” *Japanese Journal of Food Microbiology*, vol. 27, no. 2, pp. 80-85, 2010.

[32] H. Kodaka, S. Mizuochi, H. Teramura, and T. Nirazuka, “Comparison of the compact dry EC with the most probable number method (AOAC official method 966.24) for enumeration of Escherichia coli and coliform bacteria in raw meats. Performance-Tested Method 110402.,” *Journal of AOAC International*, vol. 89, no. 1, pp. 100-14, 2006.

[33] G. Giraffa, “Enterococcus,” in *Encyclopedia of Food Microbiology*, 1st ed., R. K. Robinson, C. A. Batt, and P. Tortorello, Eds. Academic Press, 1999, pp. 617-624.

[34] K. Fisher and C. Phillips, “The ecology, epidemiology and virulence of Enterococcus.,” *Microbiology*, vol. 155, no. 6, pp. 1749-57, Jun. 2009.

[35] W. Köhler, “The present state of species within the genera Streptococcus and Enterococcus.,” *International journal of medical microbiology : IJMM*, vol. 297, no. 3, pp. 133-50, Jun. 2007.

[36] R. Facklam, “What Happened to the Streptococci : Overview of Taxonomic and Nomenclature Changes,” *Clinical Microbiology Reviws*, vol. 15, no. 4, pp. 613-630, 2002.

[37] C. for I. Standards Unit, Evaluations and Standards Laboratory, “Identification of Streptococcus Species, Enterococcus Species and Morphologically similar Organisms,” *Health (San Francisco)*. pp. 1-21.

[38] H. Leclerc, L. a Devriese, and D. a Mossel, “Taxonomical changes in intestinal (faecal) enterococci and streptococci: consequences on their use as indicators of faecal contamination in drinking water.,” *The Journal of applied bacteriology*, vol. 81, no. 5, pp. 459-66, Nov. 1996.

[39] Lawrence W. Slanetz and Clara H. Bartley, “Detection and sanitary significance of fecal streptococci in water,” *American Journal of Public Health*, vol. 54, no. 4, pp. 609-614, 1964.

[40] P. A. Hartman, G. W. Reinbold, and D. S. Saraswat’, “Indicator Organisms- A Review. I. Taxonomy of the Fecal Streptococci,” *International journal of systematic bacteriology*, vol. 16, no. 2, pp. 197-221, 1966.

[41] B. A. Kenner, H. F. Clark, and Paul W. Kabler, “Fecal Streptococci Cultivation and Enumeration of Streptococci in Surface Waters,” vol. 9, pp. 15-20, 1961.

[42] K. J. Littel and P. a Hartman, “Fluorogenic selective and differential medium for isolation of fecal streptococci.,” *Applied and environmental microbiology*, vol. 45, no. 2, pp. 622-7, Feb. 1983.

[43] B. Pinto, R. Pierotti, G. Canale, and D. Reali, “Characterization of ‘faecal streptococci’ as indicators of faecal pollution and distribution in the environment.,” *Letters in applied microbiology*, vol. 29, no. 4, pp. 258-63, Oct. 1999.

[44] S. D. Lin, “Evaluation of Methods for Detecting Coliforms and Fecal Streptococci in Chlorinated Sewage Effluents,” Urbana, 1974.

[45] K. H. Schleifer and R. Kilpper-Balz, “Transfer of Streptococcus faecalis and Streptococcus faecium to the Genus Enterococcus nom. rev. as Enterococcus faecalis comb. nov. and Enterococcus faecium comb. nov.,” *International Journal of Systematic Bacteriology*, vol. 34, no. 1, pp. 31-34, Jan. 1984.

[46] A. F. Maheux et al., “Method for rapid and sensitive detection of Enterococcus sp. and Enterococcus faecalis/faecium cells in potable water samples.,” *Water research*, vol. 45, no. 6, pp. 2342-54, Mar. 2011.

[47] S. Morrison, Donald (Scottish MRSA Reference Laboratory, Glasgow, “The Enterococci,” no. 1991, 2001, pp. 921-936.

[48] A. L. Wheeler, P. G. Hartel, D. G. Godfrey, J. L. Hill, and W. I. Segars, “Potential of Enterococcus faecalis as a human fecal indicator for microbial source tracking.,” *Journal of environmental quality*, vol. 31, no. 4, pp. 1286-93, 1991.

[49] Stephan Speidel (HyServe), “personal communication.” 2011.

[50] G. Borkow and J. Gabbay, “Copper as a biocidal tool.,” *Current medicinal chemistry*, vol. 12, no. 18, pp. 2163-75, Jan. 2005.

[51] G. Borkow and J. Gabbay, “Putting copper into action: copper-impregnated products with potent biocidal activities.,” *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, vol. 18, no. 14, pp. 1728-30, Nov. 2004.

[52] A. Pellegrini, D. Prodorutti, A. Frizzi, C. Gessler, and I. Pertot, “Development and Evaluation of a Warning Model for the Optimal Use of Copper in Organic Viticulture,” *Journal of Plant Pathology*, vol. 92, no. 1, pp. 43-55, 2010.

[53] C. Espírito Santo et al., “Bacterial killing by dry metallic copper surfaces.,” *Applied and environmental microbiology*, vol. 77, no. 3, pp. 794-802, Feb. 2011.

[54] M. Ordax, E. Marco-Noales, M. M. Lopez, and E. G. Biosca, “Survival Strategy of Erwinia amylovora against Copper : Induction of the Viable-but-Nonculturable State,” *Applied and environmental microbiology*, vol. 72, no. 5, pp. 3482-3488, 2006.

[55] C. Molteni, H. K. Abicht, and M. Solioz, “Killing of bacteria by copper surfaces involves dissolved copper.,” *Applied and environmental microbiology*, vol. 76, no. 12, pp. 4099-101, Jun. 2010.

[56] G. Faúndez, M. Troncoso, P. Navarrete, and G. Figueroa, “Antimicrobial activity of copper surfaces against suspensions of Salmonella enterica and Campylobacter jejuni.,” *BMC microbiology*, vol. 4, p. 19, Apr. 2004.

[57] J. O. Noyce, H. Michels, and C. W. Keevil, “Inactivation of influenza A virus on copper versus stainless steel surfaces.,” *Applied and environmental microbiology*, vol. 73, no. 8, pp. 2748-50, Apr. 2007.

[58] A. J. Varkey, “Antibacterial properties of some metals and alloys in combating coliforms in contaminated water,” *Scientific Research and Essays*, vol. 5, no. 24, pp. 3834-3839, 2010.

[59] V. B. P. Sudha, K. O. Singh, S. R. Prasad, and P. Venkatasubramanian, “Killing of enteric bacteria in drinking water by a copper device for use in the home: laboratory evidence.,” *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 103, no. 8, pp. 819-22, Aug. 2009.

[60] R. Sharan, S. Chhibber, S. Attri, and R. H. Reed, “Inactivation and injury of Escherichia coli in a copper water storage vessel: effects of temperature and pH.,” *Antonie van Leeuwenhoek*, vol. 97, no. 1, pp. 91-7, Jan. 2010.

[61] R. Sharan, S. Chhibber, S. Attri, and R. H. Reed, “Inactivation and sub-lethal injury of Escherichia coli in a copper water storage vessel: effect of inorganic and organic constituents.,” *Antonie van Leeuwenhoek*, vol. 98, no. 1, pp. 103-15, Jun. 2010.

[62] G. M. Powell and D. Rogers, “Shock Chlorination for Private Water Systems,” *Small*. Kansas State University, pp. 1-4, 2005.

[63] P. Jagals, C. Jagals, and T. C. Bokako, “The effect of container-biofilm on the microbiological quality of water used from plastic household containers.,” *Journal of water and health*, vol. 1, no. 3, pp. 101-8, Sep. 2003.

[64] A. Steele, B. Clarke, and O. Watkins, “Impact of jerry can disinfection in a camp

environment - experiences in an IDP camp in Northern Uganda.," *Journal of water and health*, vol. 6, no. 4, pp. 559-64, Dec. 2008.

[65] K. Levy, K. L. Nelson, A. Hubbard, and J. N. S. Eisenberg, "Following the water: a controlled study of drinking water storage in northern coastal Ecuador.," *Environmental health perspectives*, vol. 116, no. 11, pp. 1533-40, Nov. 2008.

[66] M. Admassu, M. Wubshet, and B. Gelaw, "A survey of bacteriological quality of drinking water in North Gondar," *Ethiop.J.Health Dev.*, vol. 2, no. 18, pp. 112-115, 2004.

[67] B. A. Milikyas Tabor, Mulugeta Kibret, "Bacteriological and physochemical quality of drinking water and hygiene- Sanitation practices of the consumers in Bahir Dar City, Ethiopia," *Ethiopian Journal of health Sciences*, vol. 22, no. 1, pp. 19-26, 2011.

[68] J. Crampton, "Maintaining clean water : contamination during water collection and storage in Addis Ababa, Microbiological comparison of water quality at source and point-of-use," Addis Ababa, 2005.

[69] T. F. Clasen and A. Bastable, "Faecal contamination of drinking water during collection and household storage: the need to extend protection to the point of use.," *Journal of water and health*, vol. 1, no. 3, pp. 109-15, Sep. 2003.

[70] S. Rufener, D. Mäusezahl, H.-J. Mosler, and R. Weingartner, "Quality of drinking-water at source and point-of-consumption--drinking cup as a high potential recontamination risk: a field study in Bolivia.," *Journal of health, population, and nutrition*, vol. 28, no. 1, pp. 34-41, Feb. 2010.

[71] A. J. Pickering et al., "Hands, water, and health: fecal contamination in Tanzanian communities with improved, non-networked water supplies.," *Environmental science & technology*, vol. 44, no. 9, pp. 3267-72, May 2010.

[72] J. Eshcol, P. Mahapatra, and S. Keshapagu, "Is fecal contamination of drinking water after collection associated with household water handling and hygiene practices? A study of urban slum households in Hyderabad, India.," *Journal of water and health*, vol. 7, no. 1, pp. 145-54, Mar. 2009.

[73] P. K. Jensen, G. Jayasinghe, W. van der Hoek, S. Cairncross, and a Dalsgaard, "Is there an association between bacteriological drinking water quality and childhood diarrhoea in developing countries?," *Tropical medicine & international health : TM & IH*, vol. 9, no. 11, pp. 1210-5, Nov. 2004.

[74] A. F. Trevett and R. C. Carter, "Targeting appropriate interventions to minimize deterioration of drinking-water quality in developing countries.," *Journal of health, population, and nutrition*, vol. 26, no. 2, pp. 125-38, Jun. 2008.

[75] L. Haller, G. Hutton, and J. Bartram, "Estimating the costs and health benefits of water and sanitation improvements at global level.," *Journal of water and health*, vol. 5, no. 4, pp. 467-80, Dec. 2007.

[76] R. Devi, E. Alemayehu, V. Singh, A. Kumar, and E. Mengistie, "Removal of fluoride, arsenic and coliform bacteria by modified homemade filter media from drinking water.," *Bioresource technology*, vol. 99, no. 7, pp. 2269-74, May 2008.

[77] P. Network, "Hygiene and sanitation communities training Inter Aide Ethiopia," *Network*, no. December. p. 16, 2007.

[78] P. M. Mutheki et al., "Comparative performance of bone char-based filters for the removal

of fluoride from drinking water," in *35th WEDC International Conference*, 2011, pp. 1-4.

[79] G. Klein, "Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract," *International Journal of Food Microbiology*, vol. 88, no. 2-3, pp. 123-131, Dec. 2003.

9. Annex

Annex_table 1: Households participating in the study

ID-No	Filter	Name	Village	Water source	Cu	Sampling dates	# persons
60	1	Alemu Kuma	Wayo Gabriel	Shibere	8.4	8.4, 18.5, 15.6	4
76	8	Abayineh Tamire	Wayo Gabriel	Shibere	8.4	8.4, 18.5	8
70	12	Tesfaye Kebede	Wayo Gabriel	Shibere	8.4	8.4, 18.5, 15.6, 27.6	8
69	16	Merkoriwos Gizaw	Wayo Gabriel	Shibere	-	8.4, 18.5, 15.6	9
62	18	AlemuTadesse	Wayo Gabriel	Shibere	-	8.4, 4.5, 18.5, 15.6, 27.6	3
61	20	Bayisa Midhaksa	Wayo Gabriel	Shibere	-	8.4, 4.5, 18.5, 15.6	8
82	27	Minda Teshome	Wayo Gabriel	Shibere	8.4	8.4, 4.5, 27.6	4
24	49	Tufa Tafa	Wayo Gabriel	Shibere	8.4	8.4, 18.5, 15.6	2
101	50	Demissie Degefe	Wayo Gabriel	Shibere	-	8.4, 18.5, 15.6	6
25	77	Tafa Kufa	Wayo Gabriel	Shibere	-	8.4, 4.5, 15.6, 27.6	2
53	52	Gebreyes Birhane	Wayo Gabriel	Handdugwell	-	8.4, 18.5, 15.6, 27.6	9
	128	Mulgeta Korme	Washe	Shibere/Sariti	17.3	17.3, 23.3, 19.4, 3.6	6
204	97	Korme Gebaba	Washe	Shibere/Sariti	17.3	17.3, 23.3, 19.4, 3.6	10
	123	Ayano Karu	Washe	Shibere/pound	17.3	17.3, 3.6	6
	116	Beci Tucufa	Washe	Shibere	-	23.3, 3.6	3
	125	Ayi Tufa	Washe	Shibere/Ziway	-	23.3, 19.4, 3.6	4
	121	Bijo Karu	Washe	Shibere	-	19.4, 3.6	
428	6	Malafiya Soressa	Gura	Gura Windmill	13.4	13.4, 29.4, 1.6, 14.6	5
412	10	Abe Gudeta	Gura	Gura Windmill	-	13.4	6
435	13	Robe Ba'aj	Gura	Gura Windmill	13.4	13.4, 29.4, 1.6	6
409	27	Jelde Degaga	Gura	Gura Windmill	-	13.4, 29.4, 1.6, 14.6	4
401	18	Ayu Abe	Gura	Gura Windmill	-	29.4, 1.6, 14.6	8
410	26	Kushu Asefa	Gura	Gura Windmill	1.6	1.6, 14.6	
402	22	Alami Ido	Gura	Gura Windmill	1.6	1.6, 14.6	
434	1	Bobaso Edo	Gura	Gura Windmill	1.6	1.6, 14.6	
406	9	Damamo Abebe	Gura	Gura Windmill	-	1.6	
324	278	Kibe Geleta	Sariti	Sariti Borehole	-	6.4, 11.5, 3.6	3
323	259	Zubeyda Wabela	Sariti	Sariti Borehole	6.4	6.4, 11.5, 3.6	5
343	251	Machal Ahimadin	Sariti	Sariti Borehole	-	6.4, 11.5, 3.6	6
336	239	Abebe Hasen	Sariti	Sariti Borehole	6.4	6.4, 11.5, 3.6	4
334	253	Fikire Fikade	Sariti	Sariti Borehole	6.4	6.4, 11.5, 3.6	7
306	244	Bareda Dabebe	Sariti	Sariti Borehole	6.4	6.4, 11.5, 3.6	7
329	252	Eshete H/Yohanis	Sariti	Sariti Borehole	6.4	6.4, 3.6	
351	257	Biru Degarege	Sariti	Sariti Borehole	-	6.4, 11.5, 3.6	8
339	265	Abebe Gebre	Sariti	Sariti Borehole	-	6.4, 11.5, 3.6	
349	269	Tekele Araga	Sariti	Sariti Borehole	-	6.4, 11.5, 3.6	7
(14)		Bati Tufa	Chalalaka	Lake Awassa	-	24.3, 21.5	12
(22)		PA Office	Chalalaka	Lake Awassa	-	24.3, 21.5	3
(20)		Anota Tashite	Chalalaka	Lake Awassa	-	24.3, 21.5	9
(12)		Minishir Dhekama	Chalalaka	Lake Awassa	23.3	24.3, 21.5	1
(21)		Tesfaye Wako	Chalalaka	Lake Awassa	23.3	24.3, 21.5	5
(4)		Tesfaye Lochore	Chalalaka	Lake Awassa	23.3	24.3, 21.5	9
(1)		Niguse Bati	Chalalaka	Lake Awassa	-	24.3, 21.5	6
(5)		Zawuge Lochore	Chalalaka	Lake Awassa	23.3	24.3, 21.5	9
(8)		Sabare Masele	Chalalaka	Lake Awassa	23.3	24.3, 21.5	4
(9)		Desta Tuke	Chalalaka	Lake Awassa	-	24.3, 21.5	6
		Asnaku Feysa	Wayo Gabriel	Community filter	25.5	18.5, 25.5, 15.6, 27.6	

		Badegech Taye	Wayo Gabriel	Community filter	25.5	18.5, 25.5, 15.6	
		Gelane Siko	Wayo Gabriel	Community filter	25.5	18.5, 25.5, 15.6, 27.6	
		Zenebech Debela	Wayo Gabriel	Community filter	25.5	18.5, 25.5, 15.6, 27.6	
		Firehiwot Abeyo	Wayo Gabriel	Community filter	-	18.5	
		Nunush Tsegaye	Wayo Gabriel	Community filter	25.5	18.5, 25.5	
		Shura Asheme	Wayo Gabriel	Community filter	-	18.5, 15.6	
		Nunush Teshome	Wayo Gabriel	Community filter	25.5	18.5, 25.5, 15.6	
		Fire Degaga	Wayo Gabriel	Community filter	-	25.5, 15.6, 27.6	
		Abebe Fitamu	Wayo Gabriel	Community filter	-	25.5, 15.6	

Annex_table 2: p-values of two-side t-test of incubation temperature experiment

time and method	temperature	EC	TC
CD vs 3M 24h	11°C	**0.0458**	**0.0458**
	20°C	**0.0458**	**0.0458**
	25°C	**0.0138**	**0.0119**
	30°C	0.3231	0.1083
	35°C	0.3009	0.0847
	37°C	0.2626	0.2527
	41°C	0.3011	0.3046
3M vs IX 24h	11°C	0.1516	0.1516
	20°C	0.1516	0.1516
	25°C	0.1516	0.9396
	30°C	0.3570	0.1295
	35°C	0.0720	**0.0005**
	37°C	0.0801	**0.0053**
	41°C	0.1509	**0.0365**
all 24h vs 48h	11°C	1.0000	1.0000
	20°C	**0.0000**	**0.0000**
	25°C	**0.0009**	**0.0009**
	30°C	0.3882	0.2666
	35°C	0.7326	0.6167
	37°C	0.8169	0.5684
	41°C	0.8843	0.7604
all 24h vs 72h	11°C	1.0000	1.0000
	20°C	**0.0000**	**0.0000**
	25°C	**0.0002**	**0.0004**
	30°C	0.2897	0.1367
	35°C	0.5419	0.3529
	37°C	0.7055	0.1383
	41°C	0.7651	0.5314
all 24h vs 120h	11°C	0.7760	**0.0002**
	20°C	**0.0000**	**0.0000**
	25°C	**0.0002**	**0.0000**
	30°C	0.1927	**0.0037**
	35°C	0.4360	**0.0203**
	37°C	0.6732	**0.0063**
	41°C	0.5781	**0.0102**

Annex_table 3: p-values from two-side t-test and logred values of labfilter experiments

	Variables	EC	TC
labfilters all ttest	**in vs out**	**0.0007**	**0.0016**
	middle/out	**0.0000**	**0.0445**
candlefilter ttest (middle only 2 data points)	in/middle	0.5662	0.0850
	middle/out	0.5040	0.4250
	in/out	0.0645	**0.0000**
sandfilters ttest	in/middle	**0.0430**	**0.0071**
logred of labfilters all	in/out	0.33±0.80	0.82±1.28
	middle/out	0.64±1.07	0.45±1.63
logred candle	in/middle	1.39±1.96	1±0.56
	middle/out	-0.30±0.43	-0.51±2.20
	in/out	0.51±0.92	2.08±1.51
logred sand	in/middle	-0.01±0.18	-0.26±0.35
ttest sand/candle	in	0.7802	0.4934
	middle	0.2800	0.1685
	out	0.0078	0.0000
labfilter chlorine	**before/after in**	-	**0.0181**
	before/after middle	0.4366	**0.0304**
	before/after out	0.2666	0.7248
bottle exp	time 0/25	**0.0000**	-

Annex_table 4: p-values form two-side t-test of HF impact in 5 villages

site	Variables	EC	TC	ETC
chalalaka	in/out	**0.0311**	**0.0431**	0.6443
gura	in/out	**0.0156**	0.4280	**0.0381**
	source/in	0.2517	0.2978	0.1035
washe	in/out	0.9779	0.7285	**0.0536**
wayo	**in/out**	**0.0001**	**0.0009**	**0.0004**
	source/in	**0.0000**	**0.0000**	**0.0000**
sariti	in/out	0.7029	0.7646	0.7071
	source/in	0.1649	**0.0035**	**0.0395**
wayo/gura	shibere/gura source	0.3343	0.3343	
	wayo/gura in	**0.0208**	0.8947	0.7195
	wayo/gura out	0.8781	0.7079	0.1112
cf vs shibere	shibere vs cf	0.0940	0.2875	0.4049

Annex_table 5: p-values from two-side t-test of logred values from copper in villages

village	**EC**	TC	ETC
chalakala	**0.0478**	0.1185	0.5184
gura	0.4009	0.5820	0.6108
sariti	**0.0021**	**0.0031**	0.4605
washe	0.6914	0.7419	0.1019
wayo	0.7955	0.0559	0.9503

Annex_table 6: p-values from two-side t-test of source to galss sampling in the villages

village	variable	EC	TC	ETC
chalalaka	in/middle	0.4245	0.4245	0.6891
	middle/out	0.3406	0.0654	0.4098
	out/glass	0.6966	0.5957	0.8510
gura	source/jerrycan	0.0788	0.2011	-
	jerry/in	0.1525	0.3546	0.0944
	in/middle	0.3049	0.8059	0.5572
	middle/out	0.8868	0.2680	0.2317
	out/glass	0.4402	0.8804	0.5524
sariti	**source/jerrycan**	**0.0219**	**0.0449**	**0.0116**
	jerry/in	0.1408	0.2089	0.2867
	in/middle	0.5614	**0.0491**	0.3741
	middle/out	0.8434	0.1137	0.6892
	out/glass	0.5968	0.2652	0.0874
wayo	**source/jerrycan**	**0.0000**	**0.0000**	**0.0000**
	jerry/in	0.5976	0.8849	0.8654
	in/middle	0.5283	0.0786	0.3457
	middle/out	**0.0222**	**0.0161**	**0.0463**
	out/glass	0.2962	0.5212	0.9994

Annex_table 7: p-values from two-side t-test of drinking glass quality between CF/HF users

variable	EC	TC
cf/shibere	0.9363	0.1734
glass cf/hf	0.6667	0.1924
jery/in	**0.0114**	0.8260
in/out (hf)	**0.0004**	**0.0104**
source/can (cf)	**0.0259**	**0.0000**
source/in (hf)	**0.0052**	**0.0048**
can/glass (cf)	0.8225	0.7428
out/glass (hf)	0.2016	0.3952

Annex_table 8: p-values from two-side t-test of tap sterilization of HF and CF, and CF treatment steps

	variable	EC	TC
shibere sterilization	out/tap	0.1280	0.3182
	out/tubo	0.2741	0.4095
	tubo/tap	0.1098	0.3696
	out/tap&tubo, what people fetches	0.1778	0.5892
cf	1/2 (treatment steps)	0.9348	0.0745
	2/3 "	sample error (0.4342)	**0.0347**
	3/outlets	(0.0761) sample error	0.0761
	out/sterilized	0.5368	0.2439
all taps from HH		0.5877	0.9296

Annex_table 9: p-values from two-side t-test and logred values from chlorination in field

	Variables	EC	TC
CF p-values	**cf vs jerry**	**0.0335**	**0.0000**
	jerry vs glass	0.5875	0.6481
	jerry vs Cl-jerry	0.2128	**0.0005**
	glass vs Cl-glass	0.2014	**0.0131**
	Cl-jerry vs Cl-glass	0.2665	0.5289
logred	jerry/glass before Cl	0.01±0.77	0.34±0.19
	jerry/glass after Cl	-0.47±0.64	0.04±0.05
	jerry before/after Cl	1.42±1.52	0.31±0.13
	glass before/after Cl	0.89±1.08	0.32±0.21
HF p-values	source vs in	0.1084	0.1546
	in vs out	**0.0265**	**0.0574**
	in vs Cl in	**0.0025**	**0.0009**
	out vs Cl out	0.6941	0.9395
	Cl in vs Cl out	0.1931	**0.0583**
log red	in/out before Cl	1.65±1.45	0.95±0.77
	in/out after Cl	-0.43±0.91	-0.65±0.84
	in before/after Cl	2.10±1.31	1.72±0.88
	out before/after Cl	-0.80±1.37	-0.25±0.47

Annex_table 10: log reduction values and t-tests in general

	variable	observations	mean	Std.Dev	Min	Max
bottle experiment 0/25h B1	EC logred	8	.4308948	1.040513.	-.0377886	3
B2	EC logred	9	.4090268	.8010199	-.1760913	2.477121
B3	EC logred	8	.129178	.4000915	-.2787536	1
B4	EC logred	7	.0138443	.1631434	-.2552725	.2552725
all labfilters	EC logred	28	.4960083	.548417	0	2.60206
	TC logred	33	.8325489	1.429767	-2.477121	4.778151
labfilters before Cl inlet	EC logred	3	0	0	0	0
	TC logred	3	.5391907	.8694115	-.2397603	1.477121
middle	EC logred	3	-.1003433	.1737998	-.30103	0
	TC logred	3	-.4694803	.413593	-.7800519	0
out	EC logred	3	.1003433	.1737998	0	.30103
	TC logred	3	1.008671	.4684148	.5402915	1.477121
after chlorination in	EC logred	3	-.2006867	.3475995	-.60206	0
	TC logred	3	-1.173385	1.34755	- 2.025306	.3802112
middle	EC logred	3	-.1003433	.1737998	-.30103	0
	TC logred	3	-.209463	.3628005	-.6283889	0
out	EC logred	3	.1003433	.4598309	-.60206	.30103
	TC logred	5	-.4432556	1.426685	-2.025306	1.0086
logred in before/after Cl	EC logred	3	0	0	0	0
	TC logred	3	1.579382	.1989703	1.380211	1.778151
middle	EC logred	4	-.0752575	.3787878	-.60206	.30103
	TC logred	4	1.790668	1.12948	.3171168	3.058805
out	EC logred	2	0	0	0	0
	TC logred	2	-.7870156	1.113008	-1.574031	0
labfilters in/middle log reduction values	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 2 | 6 -.0066791 .0726749 .1780164 -.1934958 .1801376 3 | 6 .9545622 .4269683 1.045855 -.1429948 2.052119 ---+--- combined | 12 .4739415 .2522544 .8738349 -.0812667 1.02915 ---+--- diff | -.9612413 .4331092 -1.926269 .0037862 --- diff = mean(2) - mean(3) t = -2.2194 Ho: diff = 0 degrees of freedom = 10 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0254 Pr(|T| > |t|) = 0.0507 Pr(T > t) = 0.9746					
labfilters in/middle for candle	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]					

filter. ttest eclogred if flag==2| flag==3, by(flag)	---------+--------- 2 | 2 1.389076 1.389076 1.96445 -16.2608 19.03896 3 | 2 -.30103 .30103 .4257207 -4.125979 3.523919 ---------+--------- combined | 4 .5440228 .7581094 1.516219 -1.868619 2.956665 ---------+--------- diff | 1.690106 1.42132 -4.42534 7.805551 --- diff = mean(2) - mean(3) t = 1.1891 Ho: diff = 0 degrees of freedom = 2 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.8218 Pr(|T| > |t|) = 0.3564 Pr(T > t) = 0.1782
ec labfitlers in/middle and middle/out for candle filters . ttest tclogred if flag==2| flag==3, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---------+--------- 2 | 2 1 .39794 .5627722 -4.056307 6.056307 3 | 4 -.5192599 1.101213 2.202426 -4.023811 2.985291 ---------+--------- combined | 6 -.0128399 .77344 1.894533 -2.001031 1.975351 ---------+--------- diff | 1.51926 1.669698 -3.116564 6.155084 --- diff = mean(2) - mean(3) t = 0.9099 Ho: diff = 0 degrees of freedom = 4 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.7928 Pr(|T| > |t|) = 0.4143 Pr(T > t) = 0.2072
tc labfilters middle/in and middle/out log red values . ttest tclogred if flag==2| flag==3, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---------+--------- 2 | 6 -.2553821 .1446113 .3542239 -.6271172 .1163531 3 | 6 1.10264 .3174033 .7774762 .2867286 1.918551 ---------+--------- combined | 12 .4236288 .263749 .9136534 -.1568789 1.004137 ---------+--------- diff | -1.358022 .348794 -2.135183 -.5808603 --- diff = mean(2) - mean(3) t = -3.8935 Ho: diff = 0 degrees of freedom = 10 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0015 Pr(|T| > |t|) = 0.0030 Pr(T > t) = 0.99
ec labfilters candle middle/in and middle/out log red values . ttest	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---------+--------- 2 | 2 1.389076 1.389076 1.96445 -16.2608 19.03896 3 | 2 -.30103 .30103 .4257207 -4.125979 3.523919 ---------+--------- combined | 4 .5440228 .7581094 1.516219 -1.868619 2.956665

eclogred if flag==2| flag==3, by(flag)

```
---------+--------------------------------------------------------------------
    diff |     1.690106    1.42132               -4.42534    7.805551
------------------------------------------------------------------------------
    diff = mean(2) - mean(3)                                  t =   1.1891
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8218         Pr(|T| > |t|) = 0.3564          Pr(T > t) = 0.1782
```

tc labfilters candle middle/in and middle/out log red values

. ttest tclogred if flag==2| flag==3, by(flag)

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       2 |       2           1      .39794    .5627722   -4.056307    6.056307
       3 |       4   -.5192599    1.101213    2.202426   -4.023811    2.985291
---------+--------------------------------------------------------------------
combined |       6   -.0128399     .77344    1.894533   -2.001031    1.975351
---------+--------------------------------------------------------------------
    diff |            1.51926    1.669698               -3.116564    6.155084
------------------------------------------------------------------------------
    diff = mean(2) - mean(3)                                  t =   0.9099
Ho: diff = 0                     degrees of freedom =        4

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7928         Pr(|T| > |t|) = 0.4143          Pr(T > t) = 0.2072
```

chalalaka HF in/out	EC logred	20	.6721622	1.059614	-.7781513	3.342423
	TC logred	20	.2605508	.6629369	-1.041393	1.951823
	ETC logred	17	.3818466	1.749582	-2.30103	3.643453.
Gura in/out	EC logred	18	.7550624	.6798555	-.8050234	1.875061
	TC logred	18	-.1419012	.4588711	-.9586073	.6717677
	ETC logred	10	-.0522793	1.483492	-3.778151	1.436693

keep if village=="Gura" source/inlet as log values => calculation of log red.

. ttest logec if flag==1| flag==2, by(flag)

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       3    3.592985    .4115175    .7127693    1.822368    5.363602
       2 |      19    7.290435    .2936534    1.280006    6.673492    7.907378
---------+--------------------------------------------------------------------
combined |      22    6.786238    .3777589    1.771846    6.000645     7.57183
---------+--------------------------------------------------------------------
    diff |           -3.69745    .7672952               -5.298     -2.0969
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                  t =  -4.8188
Ho: diff = 0                     degrees of freedom =       20

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0001         Pr(|T| > |t|) = 0.0001          Pr(T > t) = 0.9999
```

Gura: TC ttest logtc if flag==1| flag==2, by(flag)

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       4    5.017585     .295857    .5917141    4.076036    5.959134
       2 |      19    7.503558    .2317059    1.009983    7.016762    7.990354
---------+--------------------------------------------------------------------
combined |      23    7.071215    .2805719    1.345576    6.489344    7.653085
---------+--------------------------------------------------------------------
```

	diff | -2.485973 .5289048 -3.585891 -1.386055 --- diff = mean(1) - mean(2) t = -4.7002 Ho: diff = 0 degrees of freedom = 21 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0001 Pr(|T| > |t|) = 0.0001 Pr(T > t) = 0.9999
Gura: ETC . ttest logetc if flag==1| flag==2, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 1 | 2 3.65661 1.354025 1.914881 -13.54791 20.86113 2 | 10 7.857222 .2172037 .6868583 7.365873 8.348571 ---+--- combined | 12 7.15712 .5317303 1.841968 5.986789 8.327451 ---+--- diff | -4.200612 .6890313 -5.735869 -2.665354 --- diff = mean(1) - mean(2) t = -6.0964 Ho: diff = 0 degrees of freedom = 10 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0001 Pr(|T| > |t|) = 0.0001 Pr(T > t) = 0.9999

Sariti in/out	EC logred	29	-.0589604	1.289013	-3.30103	1.939519
	TC logred	29	-.2123667	1.018464	-3.477121	2.477121
	ETC logred	19	.4475053	.9512333	-1.077272	3

.in/out of saiti, flag =3 is where in/out is mentioned . keep if village=="Sariti" . . ttest eclog if flag==2| flag==3, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 2 | 4 -.8013312 .2362054 .4724107 -1.553042 -.0496203 3 | 30 .1074285 .2594032 1.42081 -.4231107 .6379677 ---+--- combined | 34 .0005156 .2353111 1.372088 -.4782284 .4792595 ---+--- diff | -.9087596 .724066 -2.383634 .5661146 --- diff = mean(2) - mean(3) t = -1.2551 Ho: diff = 0 degrees of freedom = 32 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.1093 Pr(|T| > |t|) = 0.2185 Pr(T > t) = 0.8907
sariti TC . ttest tclog if flag==2| flag==3, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 2 | 4 -.6055932 .453947 .9078939 -2.050255 .8390686 3 | 30 -.1126313 .1891649 1.036099 -.499517 .2742543 ---+--- combined | 34 -.1706269 .1752564 1.021912 -.5271887 .185935 ---+--- diff | -.4929619 .5454707 -1.604049 .6181257 --- diff = mean(2) - mean(3) t = -0.9037 Ho: diff = 0 degrees of freedom = 32

	Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.1864 Pr(|T| > |t|) = 0.3729 Pr(T > t) = 0.8136
sariti ETC . ttest etclog if flag==2| flag==3, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 2 | 4 -.3109409 .1708031 .3416061 -.8545124 .2326307 3 | 20 .5248771 .214629 .9598502 .0756534 .9741008 ---+--- combined | 24 .3855741 .1912187 .9367766 -.0099919 .7811402 ---+--- diff | -.835818 .4934344 -1.859138 .1875024 --- diff = mean(2) - mean(3) t = -1.6939 Ho: diff = 0 degrees of freedom = 22 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0522 Pr(|T| > |t|) = 0.1044 Pr(T > t) = 0.9478

Washe	EC logred	17	.798573	1.686276	-2.30103	3.778151
	TC logred	17	.1146958	.9613104	-1.290035	2.477121
	ETC logred	11	1.064375	1.870596	-1.041393	4.778151

for wayo filter impact in/out . keep if village=='' Wayo Gabriel'' . . ttest eclog if flag==2| flag==3, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 2 | 9 .5657498 .346726 1.040178 -.2338018 1.365302 3 | 28 1.318109 .2240011 1.185303 .8584963 1.777721 ---+--- combined | 37 1.135103 .1946052 1.183737 .7404248 1.52978 ---+--- diff | -.7523589 .4420885 -1.649846 .1451285 --- diff = mean(2) - mean(3) t = -1.7018 Ho: diff = 0 degrees of freedom = 35 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0488 Pr(|T| > |t|) = 0.0977 Pr(T > t) = 0.9512
wayo TC . ttest tclog if flag==2| flag==3, by(flag) // these too	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 2 | 9 .4061577 .289213 .8676391 -.2607688 1.073084 3 | 28 .7897174 .1617362 .8558273 .4578622 1.121573 ---+--- combined | 37 .6964191 .1418461 .8628162 .4087418 .9840963 ---+--- diff | -.3835598 .3289742 -1.051413 .2842934 --- diff = mean(2) - mean(3) t = -1.1659 Ho: diff = 0 degrees of freedom = 35 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.1258 Pr(|T| > |t|) = 0.2515 Pr(T > t) = 0.8742

wayo etc
. ttest etclog if flag==2| flag==3, by(flag

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
2	9	.453873	.354854	1.064562	-.3644218	1.272168
3	23	.8901261	.2018524	.9680501	.4715098	1.308742
combined	32	.7674299	.1765316	.9986138	.4073912	1.127469
diff		-.436253	.3910958		-1.234977	.362471

diff = mean(2) - mean(3) t = -1.1155
Ho: diff = 0 degrees of freedom = 30

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.1368 Pr(|T| > |t|) = 0.2735 Pr(T > t) = 0.8632

glass compariso: logred in/tap	EC logred	12	1.504727	1.352761	-.30103	3.778151
	TC logred	12	.9776504	.8229377	0	2.176091
	ETC logred	8	.8420803	1.214448	-1.146128	2.50515
glass comparsion out/glass	EC logred	8	-.5620206	1.843277	-3.778151	2.079181
	TC logred	8	-.1546651	.4280473	-1.068716	.3532696
	ETC logred	8	-.0415386	.1772901	-.3222193	.2108534

sio EC if source/inlet are different in all villages

.
. ttest ecper if flag==1| flag==2, by(flag)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
1	58	10.41379	2.44102	18.59026	5.525734	15.30185
2	136	1603.074	224.428	2617.258	1159.224	2046.923
combined	194	1126.918	165.6908	2307.805	800.1204	1453.715
diff		-1592.66	344.1794		-2271.518	-913.8015

diff = mean(1) - mean(2) t = -4.6274
Ho: diff = 0 degrees of freedom = 192

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 1.0000

sio TC
. ttest tcper if flag==1| flag==2, by(flag)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
1	58	243.6121	34.15838	260.1425	175.2111	312.013
2	136	5508.643	789.205	9203.633	3947.839	7069.448
combined	194	3934.562	579.3298	8069.13	2791.931	5077.192
diff		-5265.031	1210.504		-7652.625	-2877.438

diff = mean(1) - mean(2) t = -4.3495
Ho: diff = 0 degrees of freedom = 192

	Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 1.0000					
sio etc . ttest etcper if flag==1| flag==2, by(flag)	Two-sample t test with equal variances --- Group | Obs Mean Std. Err. Std. Dev. [95% Conf. Interval] ---+--- 1 | 19 35.07895 8.868044 38.65491 16.44788 53.71002 2 | 101 2993.827 582.6471 5855.531 1837.871 4149.782 ---+--- combined | 120 2525.358 499.9121 5476.263 1535.482 3515.234 ---+--- diff | -2958.748 1347.97 -5628.095 -289.4009 --- diff = mean(1) - mean(2) t = -2.1950 Ho: diff = 0 degrees of freedom = 118 Ha: diff < 0 Ha: diff != 0 Ha: diff > 0 Pr(T < t) = 0.0151 Pr(|T| > |t|) = 0.0301 Pr(T > t) = 0.9849						
sio (source/in/out) log red values all	EC logred	118	.7500593	1.366182	-3.30103	4.093421
	TC logred	118	.2452331	.9045188	-3.477121	3.181844
	ETC logred	86	.6136026	1.306584	-3.778151	4.778151

Annex_table 11: Example of graphs to explain results to HH