

Full Length Research Paper

Safety of horticultural and livestock products in two medium-sized cities of Mali and Burkina Faso

Hamadoun Amadou^{1,3}, Christian Hülsebusch², Abou Berthe^{3,4} and Eva Schlecht^{1*}

¹Animal Husbandry in the Tropics and Subtropics, University of Kassel and Georg-August-Universität Göttingen, Steinstrasse 19, 37213 Witzenhausen, Germany.

²Deutsches Institut für Tropische und Subtropische Landwirtschaft GmbH, Steinstrasse 19, 37213 Witzenhausen, Germany.

³Institut d'Economie Rurale, B.P. 262 Bamako, Mali.

⁴Sasakawa-Global 2000, Kanu-Magnambougou Rue 6885, B.P. E3541, Bamako, Mali.

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Despite their contribution to food security, animal products and vegetables from urban and peri-urban agriculture (UPA) often raise public health and environmental concerns, given high use of agro-chemicals, organic fertilizers and wastewater. This study exemplarily investigated contamination of selected horticultural and livestock products (milk and irrigated lettuce with a potentially high microbiological contamination; and tomato and cabbage on which various pesticides were used) from Bobo Dioulasso (Burkina Faso) and Sikasso (Mali). Samples of irrigation water, organic fertilizer and lettuce were collected from six gardens; cabbage and tomato samples were collected from 15 gardens, and samples of raw and curdled milk were collected from six dairy herds in February, May, and November 2009. Thermo-tolerant coliforms and *Escherichia coli* in irrigation water significantly exceeded WHO recommendations for unrestricted irrigation of vegetables consumed raw. Microbial contamination of lettuce at garden level and market place in Bobo Dioulasso, and at garden level in Sikasso was higher than at Sikasso market ($P < 0.05$). Pesticide residues were detected in only one cabbage and one tomato sample and were below the maximum residue limit for consumption. Counts of thermo-tolerant coliforms and *E. coli* were higher in curdled than in raw milk ($P < 0.05$). Given the differences in microbial load between produce of different origin and subsequent stages along value chains, there is scope for low-cost improvement of the safety of UPA smallholders' products. However, studies of higher spatial and temporal resolution along all stages of the value chains for these products are needed in order to derive respective recommendations.

Key words: Bobo Dioulasso, *Escherichia coli*, milk, pesticide residues, thermo-tolerant coliforms, Sikasso, vegetables.

INTRODUCTION

The population of West African cities has grown at an annual rate of 5 to 7% over the past two decades, which has stimulated scientific interest in urban and peri-urban food production in this region (FAO, 2003). To satisfy the food demand of the urban population, there has been a

shift from extensive to very intensive urban and peri-urban livestock and vegetable production systems that are heavily dependent on purchased feeds and intensive use of manure, irrigation water and pesticides. Despite their contribution to food security, household income, and

*Corresponding author. E-mail: tropanimals@uni-kassel.de. Tel: +49 561 8041201. Fax: +49 5542 981230.

job creation (Cissé et al., 2005; Graefe et al. 2008; De Zeeuw et al., 2011), urban and peri-urban agriculture (UPA) practices raised concerns about public health and environmental aspects (Binns et al., 2003; Amoah et al., 2005, 2006; Ndiaye et al., 2006), due to intensive use of agro-chemicals and wastewater in vegetable production, as well as inappropriate management of feedstuffs, medicine and manure in livestock production systems. Recent work underlines the importance of livestock health care and management for the quality of animal products from UPA systems (Coulon and Priolo, 2002; Montel et al., 2003). Biological contaminants in the food chain are viruses, bacteria, fungi and parasites (Bourgeade et al., 1992; FAO/WHO, 2008), which may come from a multitude of sources. Several studies showed that fresh milk and milk products are highly contaminated with zoonotic pathogens in West African capital cities (Bonfoh et al., 2003; Pistocchini et al., 2009), and contamination of dairy products with contagious non-zoonotic pathogens was also reported (Bonfoh et al., 2003, 2005; Harouna et al., 2009; Wullschleger, 2009). While the former present a direct threat to consumer health, the latter may especially constitute a problem for the health and productivity of dairy animals (Bonfoh et al., 2003; Harouna et al., 2009). Contamination of vegetables with microbial pathogens at garden level is generally linked to the use of contaminated organic fertilizer such as human faeces (in manure or household wastes) and animal manure, and of wastewater for irrigation (Amoah et al., 2006; Qadir et al., 2010). In many cities of sub-Saharan Africa, vegetables and their irrigation water, frequently fetched from ponds, shallow wells, drains, domestic channels, and industrial wastewaters, host coliform bacteria and parasites in numbers exceeding recommended thresholds (Binns et al., 2003; Keraita et al., 2003; Amoah et al., 2005; Ndiaye et al., 2006; Chigor et al., 2010; Diogo et al., 2010). Excessive use of pesticides and high concentrations of their residues in fresh vegetables constitute another serious public health and environmental threat in West African UPA (Manirakiza et al., 2003; Rosendahl et al., 2009). Pesticide use in urban vegetable farming in the region has considerably increased over the last decade and many highly toxic pesticides are indiscriminately used without adequate control (Ntow et al., 2006; Lund et al., 2010). Studies conducted in Ghana (Amoah et al., 2006) and Benin (Assogba-Komlan et al., 2007) reported residual pesticide concentrations in vegetables above the published limits tolerable for consumption. In the cotton zone of Southern Mali and Western Burkina Faso, UPA farmers used cotton pesticides on vegetables without any consideration of food safety, public and environmental health (Cissé et al., 2005). Pesticide use and handling practices might expose farmers to chemical hazards (Dinham, 2003; Ajayi and Akinnifesi, 2007; Kedia and Palis, 2008) leading to chronic health diseases such as ophthalmic disorders (Jaga and Dharmani, 2006), myocardial infarction (Mills et al., 2009), respiratory

dysfunctions (Fieten et al., 2009), cancer and neurotoxicity (Alavanja et al., 2004; Baldi et al., 2011). Although no statistical data on illness linked to food consumption is available for West African cities, cases are repeatedly reported (Faruqui et al., 2002; Adedoyin et al., 2008). However, many lesser and least developed countries cannot assure their citizens' food security, food safety has a very low priority even if accredited control systems are already in place or being developed (Cannavan, 2004).

Against this background, the present study investigated the sources and levels of contamination from microbial pathogens in irrigation water, organic fertilizer, lettuce and milk, as well as from pesticide residues in tomato and cabbage in the UPA systems of Bobo Dioulasso (Burkina Faso) and Sikasso (Mali). These two cities were selected due to their shared agro-ecological conditions (both located in the central Soudanian zone of West Africa), and both represent the second largest city of their country, so that any health hazard would potentially concern a considerable number of consumers. While previous studies on food health hazards in West Africa focused on capital cities (Bonfoh et al., 2006; Cissé et al., 1997), the rapid population growth on the one hand and infrastructural remoteness on the other hand of secondary cities does not allow for a direct transmission of findings in capital cities to regional urban centers.

MATERIALS AND METHODS

Sampling sites

Bobo Dioulasso is situated in the south-western part of (11°16'N, 4°31'W, 460 m a.s.l.) and hosts approximately 400,000 inhabitants (Commune de Bobo Dioulasso, 2007). The climate is characterized by a rainy season (May to October) and a dry season (November to May); annual rainfall varies between 900 and 1200 mm (Millogo et al., 2008). Gardening activities are well developed along the four major rivers that cross the city. Sources of irrigation water include the rivers, smaller streams and shallow wells. Within the city, untreated sewage drains into the rivers.

Sikasso is located in the south eastern part of Mali (11°19'N, 5°40'W, 410 m a.s.l.), and harbors >135,000 inhabitants. The climate is subhumid with a dry season from November to April and a rainy season from May to October; annual rainfall varies from 900 to 1100 mm. The Lotio River that floods the city's lower lying areas (160 ha) during the rainy season leaves these with significant residual soil moisture in the first months of the dry season, enabling a well-developed production of vegetables. In addition, many shallow lakes, wells and streams in and around the city constitute major sources of irrigation water. Again, untreated sewage, stormwater, and small drains flow into the Lotio River.

Sampling of manure, irrigation water and lettuce for pathogen analysis

Based upon a baseline survey covering >100 (peri-) urban farms in the two cities (Abdulkadir et al., 2012), three representative gardens where potentially contaminated water was used to irrigate lettuce and other leafy vegetables that are consumed raw, were selected in different quarters of Sikasso (S1: Kaboila; S2: Sanoubougou; S3:

Mancourani), and of Bobo Dioulasso (B1: Dogona; B2: Bolomakote; B3: Lafiabougou). While the gardener at S2 used shallow well water for irrigation, water from the Lotio River was used to irrigate S1 and S3. Water from the Houet River was used to irrigate B1 and B2, while B3 was irrigated with water from the Kodenii River. In all cases the crops were irrigated by hand, using watering cans. Irrigation of lettuce and leafy vegetables took place twice daily: early in the morning and late in the afternoon. Gardeners at S1, S2, B1 and B3 applied only livestock manure to their lettuce crop, whereas gardeners at S3 and B2 used mineral fertilizers only. For each potentially pathogen-containing resource (manure where applicable, irrigation water and lettuce) one composite sample of five independently collected samples per garden was taken at three different periods: in February, May and November 2009. In each garden, the five independent samples of lettuce plants (200 g of fresh matter (FM) as a mix of older and younger leaves) were taken just before harvest at the 4 corners and in the centre of the two diagonals of the plot. The total area planted with lettuce ranged from 900 to 1250 m² in the three gardens in Sikasso and from 480 to 625 m² in those of Bobo Dioulasso. All six gardens had been established at least 10 years ago, and lettuce was grown on the same plots for at least three cropping cycles per year. Of the harvested lettuce plants, >80% were sold on city markets. Each lettuce harvest was therefore traced from the garden to the market place: after the harvest, all surveyed lettuce sellers in Bobo Dioulasso washed lettuce with river water (the same water used to irrigate lettuce plots), whereas in Sikasso the surveyed sellers washed lettuce with tap water or water from domestic wells. Afterwards, the sellers went straight to the market places. During display at the market, they sprinkled water on the lettuce leaves to avoid wilting. At the moment of market display, subsamples of leaves from 5 lettuce plants per garden were taken and pooled into a second sample. All samples were placed in labeled sterile plastic bags. Once per season, five independent samples (100 ml) of irrigation water were taken from where each of the 6 gardeners drew water for irrigating vegetables; these were pooled into one sample (500 ml) per garden and stored in labeled sterile glass bottles. Five manure subsamples (100 g FM) per garden were collected from the dung heap just before dung was spread on the plot. They were pooled into one sample (500 g FM) and placed in labeled sterile plastic bags. All samples were transported to the laboratory in an iced cooling box.

Sampling of milk for pathogen analysis

Close monitoring of milk production and potential hazards emerging from there was only possible in Sikasso, where, based on the initial survey (Abdulkadir et al., 2012), six typical milk producing households (M1 - M6) were selected. Households M1, M2 and M3 owned less than 20 milking cows, while M4, M5 and M6 owned more than 20 lactating cows. Four households (M3, M4, M5 and M6) were producing curdled milk and the other two (M1 and M2) raw milk. Once per sampling period, five samples of 100 ml each were taken from the milk can after morning milking (fresh milk) or the milk container (curdled milk). These were pooled into one sample per household, transferred into labeled sterile bottles and transported to the laboratory in an iced cooling box. Sampling of milk occurred in February, May and November 2009, leading to a total of 12 samples of curdled milk and 6 samples of raw milk.

Sampling of cabbage and tomato for pesticide residues

According to the baseline survey (Abdulkadir et al., 2012), farmers in both cities claimed to regularly apply chemical pesticides to cabbage and tomato plants; these vegetables were therefore selected to examine hazards from pesticide residues. In Sikasso,

cabbage was sampled from nine representative gardens, including S1, S2 and S3. In Bobo Dioulasso, tomato and cabbage were sampled from six representative gardens, including B1, B2, and B3. The area where tomato was planted varied between 680 and 1232 m² across all gardens, with about 80% of the harvested product sold. The area where cabbage was planted ranged from 900 to 5000 m² per garden with again about 80% of the harvest sold. The owners of the respective gardens confirmed that they regularly pyrethroids, organo-phosphates and organo-chlorine compounds to protect the cabbage and tomato against insect attacks. Thus, these farms were purposively selected to determine whether the respective vegetables contained pesticide residues. Ten cabbage heads and ten tomato fruits were taken along the two diagonals of each field. The ten cabbage heads were then pooled into one sample per garden and per collection period (February, May, November 2009). The ten tomato fruits were similarly pooled together to constitute one sample. Each sample was immediately placed into a labeled sterile plastic bag and transported to the laboratory in an iced cooling box.

Microbiological examination and pesticide residue analysis

Microbiological analyses were done at the Medical Biology Analysis Laboratory (EXALAB) in Bobo Dioulasso, and pesticide residue analysis was carried out at the Environmental Toxicology and Quality Control Laboratory (ETQCL) of the Central Veterinary Laboratory (LCV) in Bamako, Mali. These laboratories are reference labs in West Africa.

Subsamples of 10 g of fresh manure and lettuce, respectively, and 100 ml of irrigation water and milk were homogenized and serial dilutions were made with sterile distilled water to obtain sample dilutions ranging from 10¹ to 10⁷.

Coliform counts were performed using Violet Red Bile Agar with lactose (Speck 1976). One ml of each diluted sample was transferred to a sterile Petri dish. Ten ml of medium were added (at 48°C) to the content of each Petri dish. The inoculated solution was gently rotated by hand. After solidification, a second layer of the medium was poured on to the depth of 5 mm. The solidified solution was then incubated for 24 h at 35 and 44°C for the enumeration of total and faecal coliforms, respectively. For coliform identification, the solution was inoculated in Eosine Methylene Blue, incubated at 37°C for 24 h and the suspected colonies were identified using the micro-method identification gallery API Staph of bioMerieux® SA.

Staphylococci were cultivated on Chapman agar-agar hyperchlorinated media and their identification was based on their biochemical and bacteriological characters. *Streptococci* were identified after 24 h of incubation under CO₂ on Mueller Hinton medium used for isolation. Tryptose agar medium enriched with sheep serum was used for the isolation of *Brucella* from milk. The inoculated milk samples were incubated at 34°C in CO₂ for 48 h. For all samples, colonies were isolated and counted using the Most Probable Number technique while taking into account the dilutions.

The samples of manure, irrigation water and lettuce (garden and market level) were analyzed for helminth eggs using the concentration method (Schwartzbrod, 1998). Helminth eggs were identified using color charts for the diagnosis of intestinal parasites (WHO, 1994).

To determine pesticide residues in cabbage leaves and tomato fruits, 10 g FM of sample were homogenized with a blender, extracted with ethyl acetate, partitioned with a mixture of magnesium sulfate and sodium chloride and cleaned by dispersive solid-phase extraction (dispersive-SPE) cleanup (Food and Drug Administration, 1994). A 6890 HP chromatograph equipped with an electron capture detector and a capillary column (30 m length, 0.32 mm internal diameter) coated with HP-5 (0.25 µm film thickness) was used for the analysis of chlorpyrifos methyl, chlorpyrifos ethyl, profenofos, dimethoate, endosulfan, lambda-cyhalothrin,

Table 1. Faecal coliform contamination levels of lettuce sampled at harvest (garden level) as well as at market level in Bobo Dioulasso, Burkina Faso, and Sikasso, Mali. Values present means of three gardens per city and three harvests¹ per garden, and are expressed as log₁₀ of the geometric mean MPN² per 100 g of fresh lettuce.

Location	City	Faecal coliform counts		
		n	Geometric mean (S.D.)	Range
Garden	Bobo Dioulasso	9	5.17 (1.66)	3.74 - 7.88
	Sikasso	9	5.03 (1.66)	3.60 - 7.95
Market	Bobo Dioulasso	9	5.45 (1.43)	4.18 - 7.60
	Sikasso	9	4.31 (0.66)	3.48 - 5.60

¹ Harvest took place in February, May and November 2009; ² MPN: Most Probable Number

cypermethrin, deltamethrin, p,p'-DDT and its breakdown products, endosulfan I and II, and endosulfan sulfate.

The temperature program was as follows: 80°C held for 2 min, followed by 25°C min⁻¹ increase to 150°C, 3°C min⁻¹ increase to 200°C, 8°C min⁻¹ increase to 280°C; this temperature was kept stable for 10 min. Nitrogen was used as a carrier gas at a flow rate of 2.0 ml min⁻¹. The make-up gas was nitrogen at a flow rate of 60 ml min⁻¹. The injection volume was 1 µl in splitless mode, the injector temperature was 250°C and the detector temperature was 300°C. Chemical compounds in samples were identified by their retention times compared to the retention times of the corresponding certified pesticides standard.

Data analysis

Laboratory and survey data were analyzed using PASW 18 version (PASW Inc., 2010). Total and faecal coliform counts and *Escherichia coli* counts, expressed as Most Probable Number (MPN), were log₁₀ transformed, and one-way randomized analysis of variance (ANOVA) was used to analyze the transformed data. Independent variables considered were city (n=2), garden within city (n=3; nested), season (n=3) and their interactions; for milk the two products (raw versus curdled) were compared. Independent samples t-test and least significant difference (LSD) were used to determine differences between group means; significance was declared at *P*<0.05.

RESULTS

Microbiological contamination of lettuce at garden and market level

All of the 36 lettuce samples (18 from Bobo Dioulasso, 18 from Sikasso) contained coliforms. Across the two cities, the geometric mean (log₁₀) of thermo-tolerant coliforms ranged from 3.48 to 7.95 MPN 100 g⁻¹ FM (Table 1). No significant differences were detected between samples at the garden level (Figure 1) and at the market place (*P*>0.05). Contamination levels at garden level as well as at market place did not also differ significantly between the cool dry season (February) and the hot dry season (May). *E. coli* were found in 21 and 24% of the lettuce samples from Bobo Dioulasso and Sikasso, respectively. However, no significant difference was observed between

cities for *E. coli* contamination (log₁₀), which ranged from 3.60 to 4.68 MPN 100 g⁻¹ FM. Furthermore, *Enterobacter Klebsiella pneumonia*, *Proteus* spp., *Levinia* spp., *Morganella* spp., and *Citrobacter* spp. were found in maximum half of the samples, while *Salmonella* spp. and *Shigella* spp. were not found in any of the 36 samples. The proportion of samples from Bobo Dioulasso that contained bacteria was significantly higher than that from Sikasso (*P*<0.05).

Regardless of the city, the number of thermo-tolerant coliforms (log₁₀) in lettuce at market place ranged from 3.48 to 7.60 MPN 100 g⁻¹ FM (Table 1). Counts were significantly lower in samples from Sikasso than in those from Bobo Dioulasso, where vendors washed lettuce with (contaminated) irrigation water before selling it at different market places (Figure 2). In Sikasso, all lettuce samples that showed contamination at harvest remained contaminated until the market place. However, values of thermo-tolerant coliforms in lettuce sampled at market place were 15% lower than at the garden level (*P*<0.05), since tap water or water from residential wells was used by vendors in Sikasso to wash the lettuce. No significant difference was observed between the two sites for *E. coli* contamination.

As far as parasites are concerned, *Ankylostomes* eggs were found in both cities. *Balantidium coli*, *Entamoeba* spp., *Strongyloides* spp., *Shistosoma* spp.; *Trichocephalus* spp. were encountered on lettuce from Bobo Dioulasso, whereas *Trichomonas* spp., *Miracidium* spp., and *Anguillulea* spp. occurred on lettuce from Sikasso. While the highest number of parasites (*P*>0.05) was found in the hot dry season (May), the variety of determined species did not vary remarkably (data not shown).

Microbiological contamination of irrigation water and manure

Geometric means (log₁₀) of thermo-tolerant coliforms in irrigation water ranged from 2.00 to 7.48 MPN 100 ml⁻¹ across cities and water sources with no significant

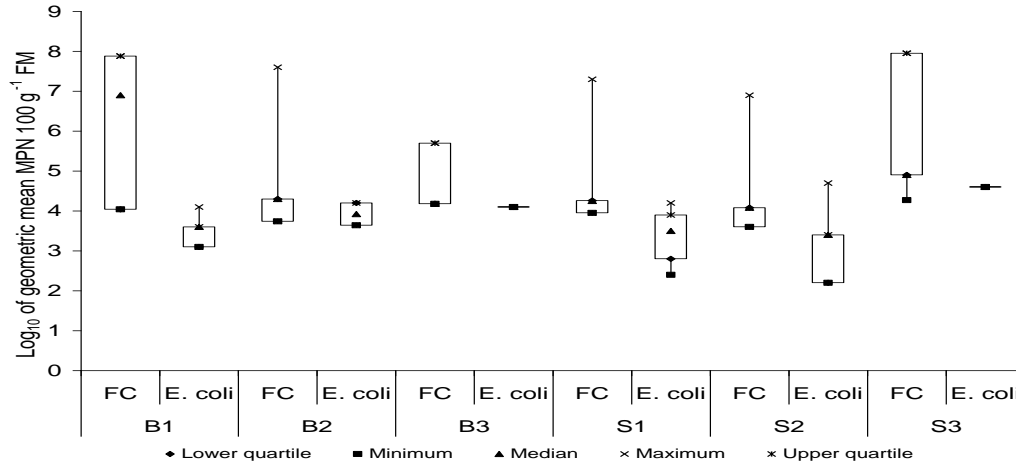


Figure 1. Average yearly variation of faecal coliform (FC) and *Escherichia coli* (*E. coli*) contamination levels of lettuce at harvest across three different garden localities in Bobo Dioulasso, Burkina Faso (B1, B2, B3) and in Sikasso, Mali (S1, S2, S3). MPN: Most Probable Number; FM: fresh matter.

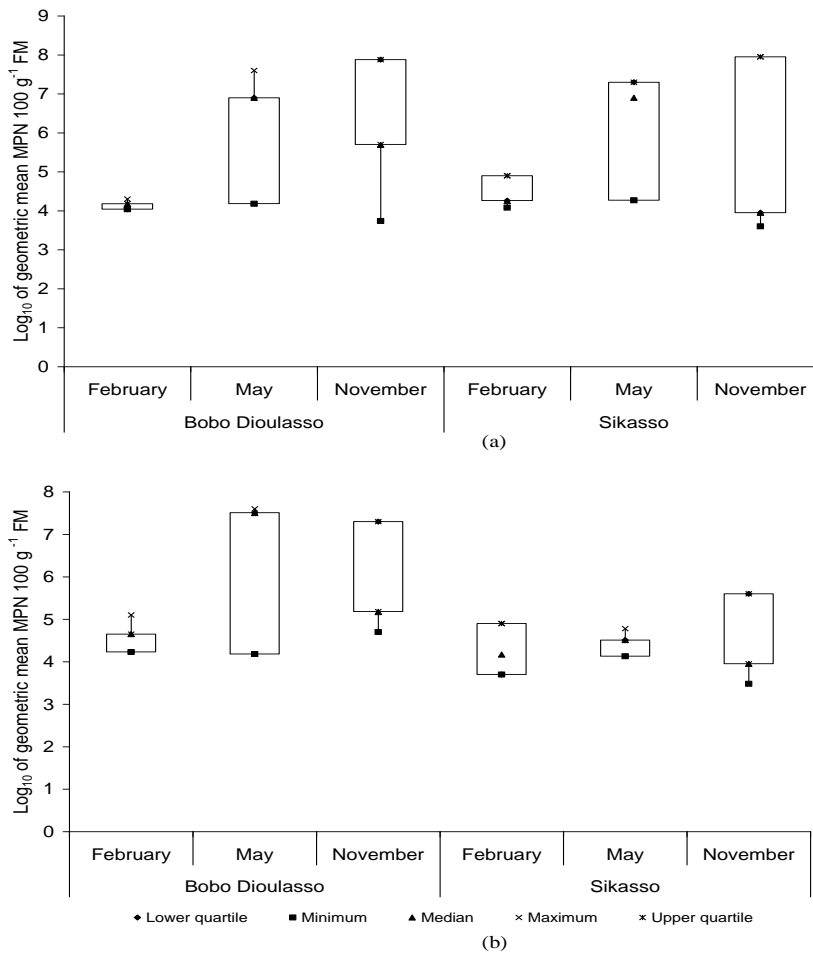


Figure 2. Changes in faecal coliform contamination levels of lettuce at garden (above) and at market level (below) in Bobo Dioulasso (Burkina Faso) and Sikasso (Mali) as determined in February, May and November 2009. MPN: Most Probable Number; FM: fresh matter.

Table 2. Faecal coliform contamination levels of irrigation water sources used in lettuce production at Bobo Dioulasso, Burkina Faso, and Sikasso, Mali. Values are presented as log₁₀ of the geometric mean MPN¹ per 100 ml of water.

Water source	City	Faecal coliform counts		
		n	Geometric mean (S.D.)	Range
Stream water	Bobo Dioulasso	9	4.35 (1.39)	3.00 - 7.48
	Sikasso	6	3.31 (1.10)	2.00 - 4.80
Shallow well water	Sikasso	3	2.88 (0.54)	2.00 - 3.30

¹MPN: Most Probable Number.

Table 3. Faecal coliform contamination levels of livestock manure used in lettuce production in Bobo Dioulasso, Burkina Faso, and Sikasso, Mali. Values are presented as log₁₀ of the geometric mean MPN¹ per 100 g of fresh manure.

City	Faecal coliform counts		
	n	Geometric mean (S.D.)	Range
Bobo Dioulasso	6	4.35 (1.81)	3.78 - 8.68
Sikasso	6	7.19 (1.14)	5.30 - 8.26

¹MPN: Most Probable Number.

difference found (Table 2). Also, no significant difference was observed between stream and well water for thermo-tolerant coliform counts in Sikasso and in Bobo Dioulasso at any of the sampling periods. Overall, *E. coli* were detected in 28% of all water samples, with a greater proportion of samples contaminated at a higher level in Bobo Dioulasso than in Sikasso. The pollution (log₁₀) ranged from 2.20 to 3.51 MPN 100 ml⁻¹. In addition, *Enterobacter Pseudomonas* sp. were determined in a few irrigation water samples from both cities during all collection periods.

There was no significant difference between the two cities in the average counts of thermo-tolerant coliforms in animal manure (Table 3). In only three samples of organic fertilizer and two samples of irrigation water, whereas in all types of samples (irrigation water, organic fertilizer and lettuce).

Microbiological contamination of milk

The most important health problems for which dairy cows were treated by their owners were trypanosomosis, bacterial diseases including pasteurellosis, and parasites. Trypanosomosis, which is the most widespread endemic disease in the zone, was targeted by 59% of the curative treatments, while parasites, pasteurellosis and other bacterial diseases, respectively, were the target of 19, 13 and 10% of all curative treatments of dairy cows. No prophylactic treatment of any of the problems was reported.

The commonly consumed dairy products in Sikasso were raw, boiled and curdled milk. Overall, six bacterial groups were identified in the milk samples, namely *E. coli*, *Streptococcus*, *Proteus Klebsiella Pseudomonas*, and *Enterobacter*. Out of the total 12 traditionally curdled and 6 raw milk samples collected during the study, only 5 and 4, respectively, were contaminated with thermo-tolerant coliform and 2 and 4, respectively, with *E. coli*. *Salmonella*, *Shigella*, *Staphylococcus*, and *Brucella* as well as parasites were not detected in any sample. *Streptococcus* was found in one curdled milk sample during the cool dry season. Ranges (log₁₀) of thermo-tolerant coliform and *E. coli* were 6.30 to 7.88 MPN 100 ml⁻¹ and 6.20 to 7.78 MPN 100 ml⁻¹, respectively, with no significant difference found between raw and curdled milk (Table 4). No bacterium was found in the curdled milk produced by two out of the six farms during any sampling period, and on one farm producing raw milk total and faecal coliform contamination levels decreased to zero from the first to the third collection period.

Pesticide contamination in cabbage and tomato

All gardeners in Bobo Dioulasso and Sikasso who grew cabbage and tomato used pesticides. In both cities Rocky 500EC, Lambda Super 2,5EC and Decis were used. In addition, Cypercal P230EC and Calfos 500EC were used in Bobo Dioulasso, while Cypalm 200EC, Conquest C176EC and Pyrical 48EC were used in Sikasso. Rocky 500EC contains endosulfan which is an organo-chlorine

Table 4. Thermo-tolerant coliform contamination levels in raw and curdled milk produced in Sikasso, Mali. Values are presented as log₁₀ of the geometric mean MPN¹ per 100 ml of produce.

Milk	Faecal coliform counts					
	Thermo-tolerant coliforms			<i>Escherichia coli</i>		
	n	Geometric mean (S.D.)	Range	n	Geometric mean (S.D.)	Range
Raw	5	4.21 (1.86)	1.88 - 6.18	2	5.99 (0.12)	5.90 - 6.08
Curdled	4	6.76 (0.74)	6.30 - 7.88	4	6.66 (0.74)	6.20 - 7.78

¹ MPN: Most Probable Number; Total sample number for curdled milk: 12; total sample number for raw milk: 6.

compound, while Calfos 500EC, Cypercal P230EC and Pyrical 480EC contain organo-phosphates. Lambda Super 2,5EC, Decis, Conquest C176EC and Cypalm 200EC are pyrethroids. A biopesticide from *Azadirachta indica* and a neonicotinoid (acetamiprid) were also used in one garden in Sikasso.

The period between the latest use (fifth or sixth application in a cropping cycle) of the pesticides and the harvest (and sampling) of cabbage and tomato varied from five to fourteen days, while the latency period for most of the used pesticides was at least two weeks. All gardeners used knapsack or hand sprayers for applying the pesticides. The persons who sprayed the pesticides were poorly protected against exposure; only few gardeners wore rubber boots and a face mask.

The total of 27 pooled samples of cabbage leaves and 9 pooled samples of tomato fruits, collected during the three sampling periods, were analyzed for residues of pesticides of the organo-phosphate group (profenofos, chlorpyrifos, chlorpyrifos methyl, dimethoate; detection limit in foodstuffs 0.006 to 0.025 ppm depending on the compound; source: Laboratoire Central Vétérinaire Bamako fact sheets – available on demand), pyrethroids (cypermethrin, deltamethrin, lambda-cyhalothrin; detection limit in foodstuffs 0.012 to 0.076 ppm depending on the compound), and organo-chlorines (endosulfan, total DDT; detection limit in foodstuffs 0.003 ppm). Residues of cypermethrin, originating from the various pyrethroids (see above) were detected in only two samples (one pooled sample of cabbage from Sikasso and another pooled sample of tomato from Bobo Dioulasso) at a concentration of 0.05 and 0.21 mg kg⁻¹ FM, respectively. No residues of other pesticides were detected.

DISCUSSION

Microbiological contamination of irrigation water and lettuce

Irrespective of period and city, irrigation water and lettuce leaves sampled during the study showed thermo-tolerant coliform levels exceeding the geometric mean count of 1×10^3 MNP 100 ml⁻¹ (water) and 1×10^3 MNP 100 g⁻¹ lettuce (wet weight) recommended by the World Health

Organization for unrestricted use of irrigation water (WHO, 1989) and lettuce, respectively (WHO, 2006). The high contamination of stream water with thermo-tolerant coliform bacteria in both cities is primarily due to direct discharge of untreated domestic sewage into these streams as they pass through residential areas. The contamination levels were similar to those previously reported from Ouagadougou, Burkina Faso (Cissé, 1997), Accra, Ghana (Amoah et al., 2005, 2006, 2007; Amponsah-Doku et al., 2010), and Niamey, Niger (Diogo et al., 2010). The high microbial contamination of lettuce in Bobo Dioulasso can be explained by the fact that out of the three gardens investigated, two irrigated with water from the Houet River all year round. Upstream, clothes were washed in the Houet and while flowing through the city the river receives solid and liquid wastes from riverside households and from the main abattoir of the city. Before reaching Dogona quarter, the water was thus highly contaminated, which explains the high coliform contamination of lettuce sampled in garden B1. The pollution of shallow wells in Sikasso, on the other hand, can be explained by surface runoff and a rising groundwater table during the rainy season. Ndiaye et al. (2006) and Amponsah-Doku et al. (2010) highlighted the role of surface runoff and the mobility of pathogens across the soil for contamination of irrigation water. In Ghana, Amoah et al. (2005) and Amponsah-Doku et al. (2010) found that runoff from residential areas and from pastures, latrines, markets, and household waste dumps contributed to coliform contamination of irrigation water. Guber et al. (2007) reported that suspended manure colloids decreased bacterial attachment to soil, clay and silt fractions, and to coated sand fractions, but did not decrease the attachment to sand fractions without coating. The low attachment of bacteria to silt and clay particles in the presence of manure colloids may lead to predominantly free-cell transport of manure-borne faecal coliforms in runoff. The high concentration of thermo-tolerant coliforms determined in organic fertilizer in both cities suggests that this material represents a major contamination source for vegetables and irrigation water. Several studies pointed to the primordial role of organic fertilizers (manure, human excreta and household waste) in the contamination processes of soils and vegetables (Amoah et al., 2005, 2006; Diogo et al., 2010). This

indicates that health risk assessments should not be limited to irrigation water, but also address alternative pathways of vegetable contamination through animal manure and soil splash. Although Unc and Goss (2004) suggested that survival conditions for enteric bacteria are unfavorable once they are excreted by the animal organism, Nicholson et al. (2005), Ferguson et al. (2007) and Guber et al. (2007) argued that some pathogens can survive for extended periods in what are considered least hospitable environments such as on fabrics and plastics (Robine et al., 2000). *E. coli* and *Enterococcus* from pig manure may survive in soil for periods of 40 to 68 days after application (Cools et al., 2001). Studies in Kumasi, Ghana, showed that the avoidance of lettuce irrigation with wastewater 6 days before harvest effectively reduced microbial contamination in the dry season (Keraita et al., 2007). However, this might adversely affect the yield and freshness of vegetables, and thus decrease farmers' profits under semi-arid growing conditions (Diogo et al., 2010).

In contrast to Sikasso, the contamination of lettuce with pathogenic micro-organisms did not significantly decrease with postharvest handling in Bobo Dioulasso. This was probably due to the fact that harvested lettuce was washed with the already contaminated river water before sale. Amoah et al. (2007) reported similar findings from Kumasi, where use of irrigation water to wash harvested lettuce before sale was a common practice by wholesalers and retailers. The significant decrease in number of coliforms from the garden to the market observed in Sikasso was mainly due to the use of clean water fetched from residential wells for washing the harvested lettuce, since no further post-harvest processing of lettuce could be observed. Nevertheless, the faecal coliform contamination level remained above the recommended WHO (2006) threshold, suggesting that washing the vegetables before marketing can decrease but not eliminate potential microbiological risk for consumer health.

Microbiological contamination of milk

Half of the milk samples from Sikasso were contaminated with faecal coliforms and *E. coli*. Treating only sick animals in dairy herds where bacterial diseases and parasites are endemic and widespread lowers milk quality (Noordhuizen and Metz, 2005). Early detection of diseases in the herd, quarantine at farm level, medication and vaccination are all extremely valuable and necessary for control of bacterial diseases in animals (Ahmad, 2005). Treatments of dairy cows only after disease manifestation might be one of the reasons for pathogen introduction into the milk (Hayes et al., 2001), others being dirty udder and teats as well as tails. None of the farmers tied the cow's tail during milking, had an appropriate milking parlor or washed hands before

milking. Milk production practices influence bacterial contamination at the barn level, and poor hygienic standards at the farm can affect the rest of the dairy production chain, as exemplified in studies from Uganda (Grimaud et al., 2007), Mali (Bonfoh et al., 2003, 2005), Ghana (Donkor et al., 2007), Kenya (Ogola et al., 2007), South Africa (Beukes et al., 2001), Niger (Harouna et al., 2009; Pistocchini et al., 2009); and Burkina Faso (Millogo et al., 2008, 2010). In addition, milk storage, transport and transformation process, can all present sources of microbial contamination (Bonfoh et al., 2005, 2006; Grimaud et al., 2007; Millogo et al., 2008). The average counts of faecal coliforms and *E. coli* determined in Sikasso were lower than those reported for raw and traditionally fermented milk from the peri-urban areas of Bamako (Bonfoh et al., 2003) and from Burkina Faso (Savadogo et al., 2004; Millogo et al., 2010), but were still above the acceptable threshold of ≤ 10 colony-forming units (cfu) per ml.

Metwally et al. (2011) reported that boiling milk for 0.5 and 1 min decreased total bacterial counts from 3.6×10^9 cfu ml⁻¹ to 6.3×10^2 and 3.2×10^2 cfu ml⁻¹. However, curdled milk produced in Sikasso is usually not boiled before processing. For two out of the six surveyed cow herds the bacterial counts were very low, pointing to cleaner milking practices, which were indeed observed.

Pesticide contamination of cabbage and tomato

The microclimate in frequently irrigated urban gardens leads to a year-round high atmospheric humidity, which favors the outbreak of insect pests and vegetables diseases that are usually controlled by the gardeners through frequent application of pesticides (Houndete et al., 2010). Given that cypermethrin residues were only detected in two vegetable samples, both at non-hazardous concentration, the results of this study do not indicate a potential consumer health hazard with regard to pesticide residues in Bobo Dioulasso and Sikasso. However, this does not mean that, had we been able to use analytical methods with a lower limit of detection, traces of pesticide residues might have been found. Since the vegetable farmers themselves reported a massive use of a wide range of pesticides during our interviews, a refined analysis of pesticide residues in vegetables grown in the two cities seems advisable. Yet, most of the surveyed farmers complained about the inefficiency and low quality of the pesticides used. This could either indicate that the pesticides used by the farmers were fake products and did not contain any active compound, or that the targeted pests had developed resistance. In fact, sucking insects such as biting aphid (*Aphis gossypii*, Glover), whitefly (*Bemisia tabaci*, Gennadius) and the bollworm (*Helicoverpa armigera*, Hübner) are common polyphagous pests in West African plantations of cotton and vegetables such

as tomato and cabbage to have developed resistance against pesticides in this region (Martin et al., 2005; Ntow et al., 2006; Houndete et al., 2010).

Attempts to obtain pesticide samples from the vendors were not successful, since the latter claimed that pesticides from the stock used for the 2009 cropping season had finished by the time we asked for samples. However, it is quite common to find inappropriate packaging in registered retailer shops: a recent study from Burkina Faso revealed that insecticides with English labels were not authorized in the "Comité Inter-état de Lutte contre la Sécheresse au Sahel (CILSS)" states and usually came from Ghana or Nigeria (Secrétariat de la Convention de Rotterdam, 2010). However, retailers declared to prefer pesticides from Ghana or Nigeria because products sold by registered plants or suppliers were far too expensive for their customers.

Although endosulfan is banned in the CILSS states, it was still found in some pesticide formulations used on vegetables in Bobo Dioulasso, such as ROCKY 500EC (endosulfan 500 g l⁻¹), ROCKY 386EC (cypermethrin 36 g l⁻¹ + endosulfan 350 g l⁻¹), CAIMAN SUPER (alpha-cypermethrin 18 g l⁻¹ + endosulfan 350 g l⁻¹) and CAIMAN ROUGE (endosulfan 250 g l⁻¹ + thiram 205 g l⁻¹). Similarly, in soil samples from Sikasso, high residue levels of six pesticides, including endosulfan (I and II), metabolite endosulfan sulfate, pp'-DDT, pp'-DDE and pp'-DDD were determined by Safiatou et al. (2007).

Consumer health hazard

The observed high levels of contamination of lettuce and milk with thermo-tolerant coliforms such as *E. coli*, *Klebsiella pneumoniae*, *Proteus Staphylococcus aureus* and *Enterobacter* clearly indicates a potential health hazard. This applied to lettuce sampled at gardens and at markets, whereby contamination at garden level seemed to be most important. It also applied to raw and curdled milk directly purchased from the farmer. Apart from *E. coli*, of which some toxin-producing strains can affect consumers (Kivaria et al., 2006), the pathogens that were identified in the milk samples hardly produce toxins, and neither do they form spores. Infection with these organisms through milk can thus be fully controlled through milk pasteurization. Toxins produced by *S. aureus* (Argudín et al., 2010), which could enter curdled milk through the udder or human skin, could also constitute a health risk (Hetzel et al., 2004), especially as it is a major cause of gastroenteritis (Le Loir et al., 2003). Yet, this pathogen was not detected in any of the samples analyzed here. In Africa many consumers fail to link food consumption to diseases. For instance, Bonfoh et al. (2003) found that 78% of people surveyed in Bamako and Mopti (Mali) were not aware of the role of food in diarrheal diseases. According to Koné (Health Reference Center Sikasso, personal communication, 18

May 2009) many people only link a disease case to food intake if it occurs immediately or only few hours after the intake of a contaminated product.

Conclusions

In Bobo Dioulasso and Sikasso, wastewater and organic fertilizers were the main sources of contamination of lettuce with coliforms far beyond food safety limits. Although post-harvest contamination of lettuce may also occur through washing with untreated wastewater, risk reduction strategies should start at the garden level and particularly target the microbial load of irrigation water and organic fertilizer. This could be achieved through the application of low cost technologies (such as aeration, solar driven thermal water treatment and composting of organic fertilizers).

Although our results suggest only very minor risks of vegetable contamination with pesticide residues in both cities, a systematic sampling of pesticides at traders' and gardeners' level and analysis of vegetable samples with a procedure allowing for a lower limit of detection are needed to conclusively answer this question.

The major factors of microbial contamination of raw and curdled milk are unhygienic milking and storing practices at the farm level. However, the fact that two out of six dairy farms had no microbial contamination in any of the sampling periods indicates that suitable milking practices are currently applied by some of the milk producers included in the study; this can be an entry point for farmer-to-farmer training programmes on dairy hygiene. Given the high consumer risk associated with microbial food contamination, in-depth investigations of higher spatial and temporal resolution along all stages of the UPA value chains for dairy and horticulture products are recommended in order to increase awareness among producers and consumers alike and to derive recommendations for increased consumer safety.

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