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Production in Aquatic Peri-Urban Systems in Southeast Asia

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1. KVL & NIHE PARTNER FOOD QUALITY REPORT FOR HANOI AND PHNOM PENH MICROBIOLOGICAL PART

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1.1 Abstract

Aquatic vegetables were sampled at two field sites in peri-urban Hanoi during April to June and November to December 2005 in order to asses their microbiological quality and to identify possible sources of contamination during the 'field to market' chain. Vegetables were analysed for thermotolerant coliform bacteria, the protozoan parasites *Cryptosporidium*, *Giardia* and *Cyclospora* and for helminth eggs. The field sites included one peri-urban commune in the south of Hanoi where untreated wastewater was used for irrigating and fertilising aquatic vegetables ('wastewater site') and one other peri-urban commune in the eastern part of Hanoi where rainwater and pig manure was used to irrigate and fertilise aquatic vegetables ('Non-wastewater site'). The main vegetable produced at both sites was water spinach ('morning glory') - a standard ingredient in the Vietnamese cuisine and very commonly consumed by the population. Samples were taken before and after washing the vegetables post-harvest and upon arrival to the market. In addition, a market study was carried out at one larger urban market in Hanoi and one smaller peri-urban market in the southern part of Hanoi. In Cambodia, near Phnom Penh, morning glory growing near two inlets and one outlet from the Boeng Cheung Ek wetlands were analyzed for levels of faecal pollution.

In Hanoi, thermotolerant coliforms were present at relatively low levels on water spinach ($<10^4$ cfu/g), although slightly higher levels were found at markets. There were no apparent differences between seasons. Protozoan parasites were found throughout the 'farm to fork chain' with highest prevalences in the field at the wastewater-site in Hoang Liet commune. *Giardia* was the most prevalent protozoan, particularly at the wastewater-site, whereas *Cryptosporidium* was most prevalent during the dry season at the wastewater site. *Cyclospora* was detected at both sites and at markets but was less prevalent than *Giardia* and *Cryptosporidium.* It is concluded that protozoan parasites are present in Vietnam in the farm to fork chain and may pose a threat to occupational health as well as to consumers' health. Helminth eggs were present at very low levels in vegetable samples from fields and markets and do not appear to pose a food safety problem in the current setting.

In Phnom Penh, relatively high levels of *E. coli* and thermotolerant coliforms were detected on water spinach, indicating a high level of faecal contamination and there was a positive correlation between microbiological water quality and microbiological vegetable quality. Protozoan parasites were detected at both inlets and outlet to the lakes, with Gia*rdia* being more prevalent than *Cryptosporidium* and *Cyclospora.* There was a positive correlation between levels of faecal contamination (*E. coli*) and prevalences of *Giardia* at one of the inlets, indicating faecal contamination as a risk factor for protozoan parasites being present on vegetables. Helminth eggs

were found in low levels both at inlets and outlets. The groups of parasites identified may pose an occupational health risk for farm workers as well as a health problem for consumers.

1.2 Introduction

Wastewater used for irrigation of aquatic vegetables and as water source for fish cultivation in Thanh Tri district a peri-urban commune in the South of Hanoi, is of both domestic and industrial origin. In Bang B village in the Thanh Tri district where the water analysis was carried out, about 50 % of the households cultivate aquatic vegetables. Untreated wastewater from the To Lich river is pumped into the fields near the village to irrigate and fertilize mainly morning glory crops but also water dropwort, water cress and water mimosa. Wastewater from the river is also used for fish culture as has been common in Vietnam since the 1960's.

The prevalence of the protozoan parasites *Cyclospora*, *Cryptosporidium* and *Giardia* in Vietnam is relatively unknown but due to the status of these infections as emerging infectious diseases mainly in developing countries but also in the industrialised part of the world, knowledge on risk factors for transmission in the 'farm to fork' chain and on the species/genotypes found in food, environmental samples and in human infections is needed both in the Vietnamese setting, in similar settings in the developing world and in more developed countries, where there is now an increasing importance and awareness of these waterborne zoonotic parasites.

Phnom Penh, located on the left bank of the Mekong River, has a population of nearly one million people. Similar to many cities in Southeast Asia, peri-urban areas of Phnom Penh are very important for food production and supply. Aquatic areas around peri-urban wetlands are one of the most important locations for cultivating aquatic plants and fish for the city and other areas around Phnom Penh. These wetlands are mostly fertilized by sewage/wastewater discharged from the city (Chouk B. 2004). The sewerage and wastewater treatment facilities to serve this population are in general inadequate. The sewage networks, mostly built in the 1960s, are a combined sewage overflow from many sources including households, storm water, and industrial effluents. There are about 160 km of sewer networks in the city core including 2.6 km of open channels (JICA 1999). There is no wastewater treatment plant, so 10% of the effluents flow directly into the Mekong River without any treatment. The remaining 90 percent is loaded into retention basins that are the natural wetlands. Every day, about 55,600 m^3 of household wastewater (equivalent to 2,414 tons per year of biological oxygen demand (BOD_5) discharge) and nearly 1 million m^3 of storm water are discharged into three wetlands; Boeng Trabek, Boeng Tumpun and Boeng Cheung Ek, when rainfall intensity is at 30 mm per 24 hours (Muong 2003).

The wetland of Boeng Cheung Ek (BCE) is the largest. Its surface area is as large as the centre of Phnom Penh (about 2,000 ha) in the wet season and shrinks to 1,300 ha in the dry season (Muong 2000). Effluents from the wetlands of Boeng Trabek and Boeng Tumpun are discharged into this wetland, together with industrial effluents from the surrounding areas.

The objectives of the studies were to:

1) determine the numbers of thermotolerant coliforms, helminth eggs and protozoan parasites in water spinach grown in Boeng Cheung Ek (BCE) lake, Phnom Penh and at two peri-urban communes in Hanoi, including samples harvested with and without wastewater (ww) exposure

2) To assess the association between microbiological water quality and the microbiological quality of water spinach.

1.2.1 Study site descriptions Hanoi

1.2.1.1 Hoang Liet

In Hoang Liet commune, Bang B village, waste water from the Kim Nguu river is used in the production of aquatic vegetables. The waste water consists of 60-80% domestic waste water and 20-40% industrial waste water. A pumping station situated near the road, between the river and the fields on the other side of the road pumps water into the field area by demand.
During the dry season which lasts until the beginning of April, the pumping station is generally activated twice a week and daily during harvest. Fields are then flooded with water. During the wet season the pumping station is activated less frequently, typically 1 to 2 times per month. The pumping station pumps the water into a concrete-lined open canal which runs alongside on end of the fields. Outlets from this canal are present for every field and the water is distributed from the canal to the other end of the field area (max. distance approx. 150-200 meter). No waste water is spread onto the fields manually (i.e. with buckets or watering cans). Pesticides are used regularly during vegetable production, whereas commercial fertilizer is not used very often.

Morning glory (water spinach) is the main crop in this area and is grown all year round. Other crops include watercress (grown from September to April), water dropwort (grown from September to April) and water mimosa (grown from April/March until August).
Morning glory is harvested using hands to collect the plants out of the soil. All crops are dipped or washed in a nearby fishpond to keep the vegetables wet and fresh during transport and to remove soil from the roots. The water in the fishpond originates from the Tu Lich river as well as the irrigation water.

Vegetables are transported to local markets or distribution markets on bicycles and motorcycles in chicken wire baskets or sacks. From the distribution market the vegetables are distributed to different markets in Hanoi. Workers wear gumboots during work in the fields. Workers do not wear gloves although their hands are often in the water and soil when harvesting, planting or washing the vegetables in an adjacent fish pond. No toilet is present in the field area; when workers have to urinate and defecate they have to sit somewhere along the fields.
No animals were observed in the field areas or near the fish pond.

1.2.1.2 Long Bien

Long Bien District, Long Bien Commune, Station Village (Vietnamese name: Cum Tram) is situated in the Eastern part of peri-urban Hanoi, across the Red River. In Long Bien, rainwater collected in large pond/lake is used for irrigation. No waste water is used, but as a mean of fertilizing the crops pig manure (faeces + urine) mixed with water is spread onto the crops, usually after harvest. This takes place approximately every 10-12 days when the pigsties are cleaned out. Water for irrigation is spread manually, using a bucket and a small shovel/spoon. The main crop grown all year round is morning glory. Pesticides are applied twice a month using a spray method.

At harvest, morning glory plants are pulled out by hand, leaving part of the root in the soil. Directly after pulling out the plants by hand, they are put into a bucket with water (from the pond). Vegetables are then transported to the house of the farmer and the morning glory is soaked overnight in water before being transported to the Long Bien distribution market early the next morning (2 – 3 am).Motorbikes are used to transport the vegetables to the Long Bien distribution market. Vegetables are transported in chicken wire baskets or plastic buckets; two on each motorbike. Dogs were observed near the field area. No other animals were seen near or in the fields. The pigs providing the manure are kept in stables in the village.

1.2.2 Study site description Phnom Penh

Boeng Cheung Ek (BCE) lake is located in the West of Phnom Penh city, Cambodia. Wastewater from Phnom Penh urban population and from industrial factories (garment and other various factories) and rain-water run-off was discharged into the lake. Currently, there are two main wastewater inlets of Boeng Cheung Ek lake. One is Boeng Trabek pumping station was constructed in 1986 and the other is constructed in 2004 by JICA called Tumpun Station. The one built by JICA is located just near the old one.

There are two main outlets of this lake. Wastewater from Boeng Cheung Ek lake, after being naturally treated, is currently discharged into Prek Thnaot river through a stream named STEUNG CHROV.

Morning glory (white stem morning glory)/water spinach mostly is grown in Boeng Cheung Ek lake by the farmers and is mainly collected for human and animal consumption. Morning glory is harvested by farmers and then stored within the day. At the end of the day, harvested morning glory is collected by wholesalers and is carried to the local market by truck for sales.

In the dry season, the water level in Boeng Cheung Ek lake goes down and some parts of the lake are dry. Therefore, morning glory cannot be grown well in these areas during the dry season. In the rainy season, almost all the area around the lake is flooded. Thus, it is not easy to identify the location of the outlets except STOENG CHROV stream.

Phnom Penh urban wastewater discharged into the Boeng Cheung Ek lake is not undergoing any formal wastewater treatment. Thus, the wastewater passage through Boeng Cheung Ek lake and the associated treatment will be important in reducing any possible negative impacts of the wastewater on recipient rivers.

In summary, there are always two inlets of Boeng Cheung Ek lake in both seasons. In the rainy season, wastewater from the lake flows into recipient rivers via both outlets but in the dry season, wastewater only flows into recipient rivers via STEUNG CHROV stream.

Boeng Samrong (Prek Phnov) lake has been selected as one of the control sites by the PAPUSSA team in Phnom Penh. It is a big lake located 15 km away from the centre of Phnom Penh city. Non-wastewater samples will be collected from this lake during both seasons. Water spinach is not seen to be grown in this lake.

1.3 Methodology

1.3.1 Study design and analysis - Hanoi

The research on microbiological quality of plants was conducted along the food chain from production sites to markets and included two main studies. The first study investigated the microbiological quality of plants irrigated with wastewater (ww) and non-ww at different field sites in peri-urban Hanoi; Hoang Liet (wastewater site) and Long Bien (non-wastewater site) communes. Microbiological quality of crops was assessed in six fields on each of the two sites during wet and dry seasons (duplicate samples).

The microbiological quality of plant samples, mainly morning glory which is the main plant produced with wastewater, but also other types of aquatic vegetables (water dropwort, water cress and water mimosa) were studied. Samples were collected at harvest, after post-harvest washing, before transport and upon arrival to the market.

a) b)

Figure 0-1 Post harvest washing of vegetables at Hoang Liet commune (a), Hanoi and Concrete canal leading the untreated wastewater into the field areas (b). (Photos: Lise Tønner Klank)

Figure 0-2 Getting ready for transport of vegetables to the market – Hoang Liet, Hanoi (Photo: Lise Tønner Klank).

The second study aimed at assessing the microbiological quality of plants originating mainly from wastewater-irrigated fields and subsequently sold at markets. Markets studied include the Hoang Liet market (a local market) and Hang Be (a Hanoi city market). At the markets, duplicate plant samples, including both morning glory and herbs commonly consumed raw by the Vietnamese, were collected weekly from different market traders during a 3-month period from March to June 2005 and again from Nov-Dec 2005.

In total, 216 plant samples were collected in the field study and 96 in the market study.

The microbiological parameters analysed for in both studies were:

- Thermotolerant coliform bacteria
- Helminth eggs (*Ascaris lumbricoides, Trichuris trichiura,* hookworm)
- Protozoan parasites: (*Giardia intestinales*, *Cryptosporidium parvum* and *Cyclospora spp.*)

Numbers of thermotolerant coliforms were measured in all samples by means of a Most Probable Number (MPN) method described by the WHO. To test for helminth eggs, the Bailinger method described by the WHO, was applied, whereas protozoan parasites were detected by use of an immunofluorescent antibody test. Some isolates of protozoan (oo-)cysts were further characterized by molecular characterization, using a PCR-based genotyping method.

Fields designated F1 to F6 in the Bang B village of Hoang Liet Commune in the Thanh Tri district were chosen to be tested both for water quality and food quality. Here untreated wastewater from the Tu Lich river is pumped into the field area via a system of concrete canals.

An immuno-magnetic separation technique (IMS) to detect *Cryptosporidium* oocysts and *Giardia* cysts was applied to water samples taken at both field sites and at markets. These samples were filtered in the laboratory at NIHE and subsequently shipped to Denmark where IMS took place. In this manner, *Giardia* cysts and *Cryptosporidium* oocysts were detectable even in relatively small volumes of water.

1.3.2 Study design and analysis – Phnom Penh

Water spinach samples (total of 68 samples) were collected at the 2 inlets, 1 outlet of Boeng Cheung Ek lake and the control site (a small pond); all sites were sampled four times; with and without wastewater exposure of plants. Plant samples were collected with and without wastewater exposure during harvest to allow for comparison analyses. Samples were collected by NIHE staff during visits to the Cambodian PAPUSSA partner. Water spinach samples were processed and enumerated for thermotolerant coliforms by the Pasteur Institute in Phnom Penh. NIHE researchers brought duplicate samples to Hanoi for detection and enumeration of parasites, including *Giardia intestinales*, *Cryptosporidium parvum* and *Cyclospora spp*, helminth eggs (*Ascaris lumbricoides, Trichuris trichiura* and hookworm).

The microbiological parameters analysed for in both studies were:

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Figure 0-3 Sample collection in the water spinach field – Phnom Penh

1.4 Results and discussion - Hanoi

1.4.1 Bacteriology

Results are summarized in Figure 1-4 where each bar represents the mean of duplicate samples from six fields; i.e. a total of 6 samples per bar in the graph. The levels of thermotolerant coliform bacteria thermotolerant coliform bacteria showed an increasing trend when moving from the field to the market with the increase being most evident at the non-wastewater-site during the wet season. At the non-wastewater site levels of thermotolerant coliform bacteria were lower than at the Wastewater site when plants had been harvested and washed post harvest during the wet season, indicating that there was a lower contamination level of plants when using non-wastewater for irrigation of crops. During the dry season this difference was not present. The lack of difference during the dry season could be due to relatively more water needed for irrigation during this season and as the non-wastewater site was using pig manure to irrigate and fertilise their soils, the contamination with thermotolerant coliform bacteria may have arisen from this use.
At markets, the levels of thermotolerant coliform bacteria were comparable and even slightly higher than those found in the field. This would suggest that some further contamination takes place at the market. This is supported by the occasional finding of very high levels of thermotolerant coliform bacteria in water from bottles using for sprinkling water on to the vegetables. These levels were up to 10^6 or 10^7 MPN/100ml. At markets there were neither any obvious differences between seasons nor between markets.

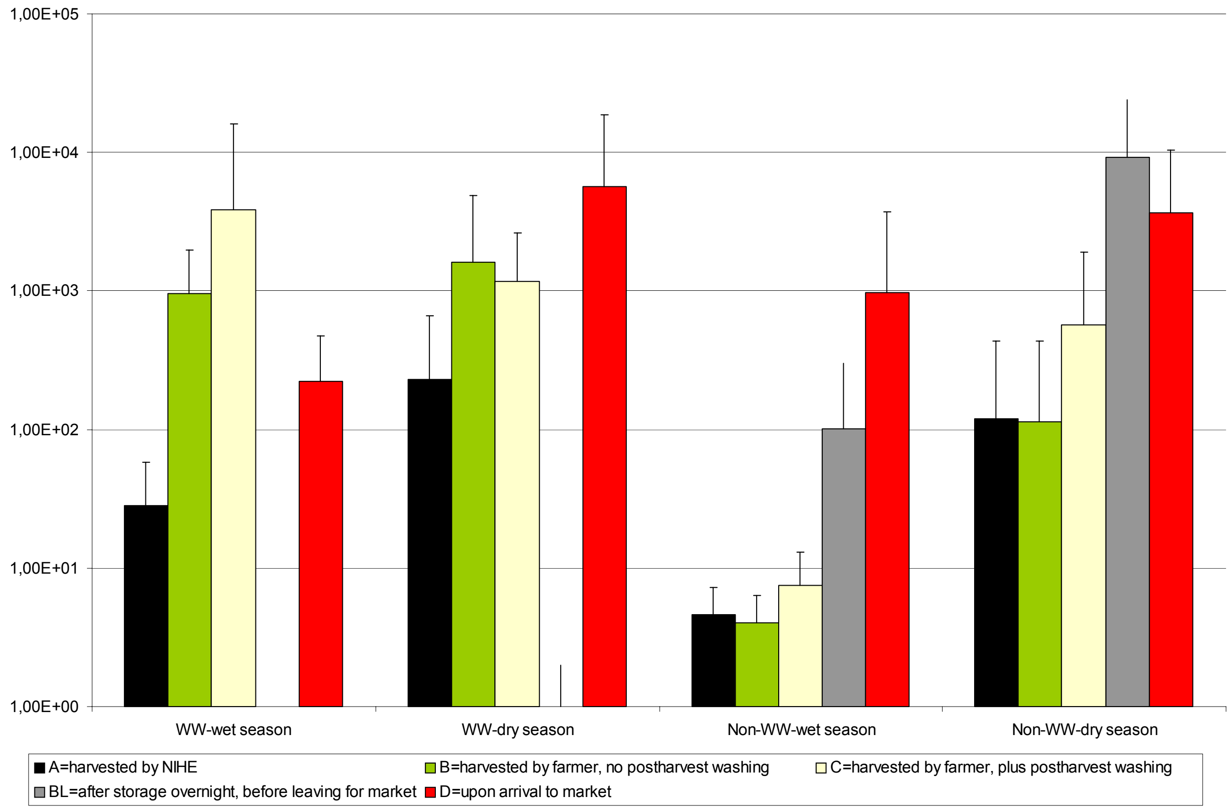

Figure 0-4 MPN numbers (MPN/g vegetable) for thermotolerant coliform bacteria in samples from the field sites

Table 0-1 Levels of thermotolerant coliform bacteria in vegetable and herb samples collected at two markets in Hanoi.

Location	Wet season	Dry season
Small market –Hoang Liet	6×10^4 MPN/g	6×10^3 MPN/g
Large market – Hang Be	7×10^4 MPN/g	5×10^4 MPN/g

1.4.2 Parasitology

1.4.2.1 Protozoan parasites

The overall prevalence of protozoan parasites for the two sites and markets are presented in Table 0-2 below.

At the wastewater-site, the highest prevalences of *Giardia* and *Cryptosporidium* were found during the dry season when fields are irrigated more often. At the non-wastewater-site differences between seasons were not obvious. The prevalence of *Cyclospora* did not vary to any great extent between sites and seasons. At the market the highest prevalence of *Giardia* was found during the wet season and for *Cryptosporidium*, during the dry season, indicating different modes of transmission.

Table 0-2 Overall prevalence of protozoan parasites in field and market samples (% positive samples).

	Giardia	*Cryptosporidium*	*Cyclospora*	**Total number of samples**
Wastewater site, wet season	18.8 %	6.3 %	6.3 %	48
Wastewater site, dry season	45.8 %	33.3 %	2.1 %	48
Non-wastewater site, wet season	10 %	6.7 %	6.7 %	60
Non-wastewater site, dry season	11.9 %	11.9 %	3.3 %	60
Large urban market, wet season	37.5 %	8.3 %	0 %	24
Large urban market, dry season	8.3 %	20.8 %	12.5 %	24
Small peri-urban market, wet season	37.5 %	12.5 %	8.3 %	24
Small peri-urban market, dry season	12.5 %	33.3%	8.3 %	24

Figure 0-5 Market trader at Hoang Liet market, Hanoi (Photo: Lise Tønner Klank)

1.4.2.2 Helminth parasites

Very few samples (less than 2 % of samples) contained helminth eggs (Table 0-3). Of those positive, only eggs of *Ascaris* spp. were found. Surprisingly, no *Ascaris* eggs were found on the non-wastewater site, where pig manure is used to fertilise the vegetable fields. This could indicate that eggs have not survived on the vegetables to be detected at harvest time.

At the wastewater site in Hoang Liet, few *Ascaris* eggs were found. It is not possible to distinguish the human variant of *Ascaris*, *A. lumbricoides* from the swine variant, *A. suum*, but it is most likely that the types of eggs found at Hoang Liet were *A. lumbricoides* as pigs are not kept near this field site.

It is likely that helminth eggs sediment out during the relatively long transport during the system of concrete canals leading to the individual fields and that this phenomenon could explain why we find so few helminth eggs in the field samples. Samples taken from the Tu Lich river after the completion of the fields study, showed presence of helminth eggs, however helminth eggs do not appear to reach the fields to any great extent and hence do not contaminate the vegetables in the field.

Table 0-3 Prevalence of helminth eggs on vegetables at the field sites. Number of samples positive for helminth eggs

Sampling location	Hookworm	*Ascaris* spp	*Trichuris* spp.
WW-irrigated site – Wet season	0/48	1/48	0/48
WW-irrigated site – Dry season	0/48	2/48	0/48
Non-WW-irrigated site – Wet season	0/60	0/60	0/60
Non-WW-irrigated site – Dry season	0/60	0/60	0/60

1.5 Results and discussion - Phnom Penh

1.5.1 Bacteriology

Relatively high levels of *E. coli* were detected, particularly at the Trabek inlet. This suggests a high level of faecal contamination in the wastewater discharged into the lake at this location, both at inlets and at outlets.

The initial analyses showed that water spinach collected close to the wastewater inlets contained higher numbers of thermotolerant coliforms compared with plants collected close to the outlet. Little difference in level of faecal pollution was seen between water spinach harvested with and without direct wastewater exposure (Figure 0-6 and Figure 0-7).

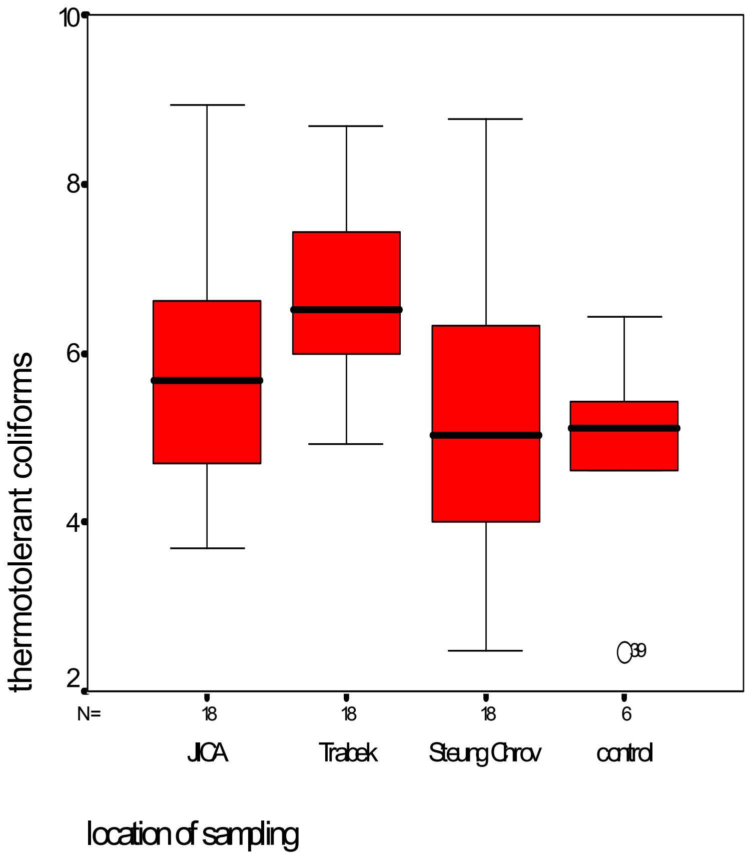

Figure 0-6 Phnom Penh results: Numbers of thermotolerant coliforms in water spinach (JICA and Trabek = inlet; Steung Chrov = outlet)

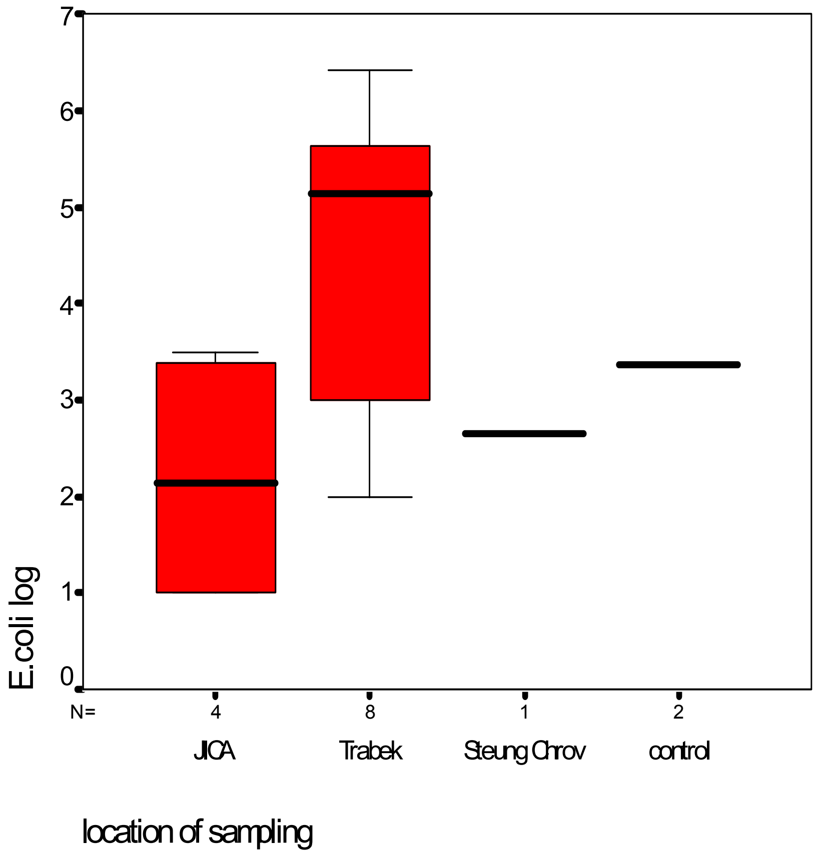

Figure 0-7 Phnom Penh results: Numbers of *E. coli* in water spinach (JICA and Trabek = inlet; Steung Chrov = outlet)

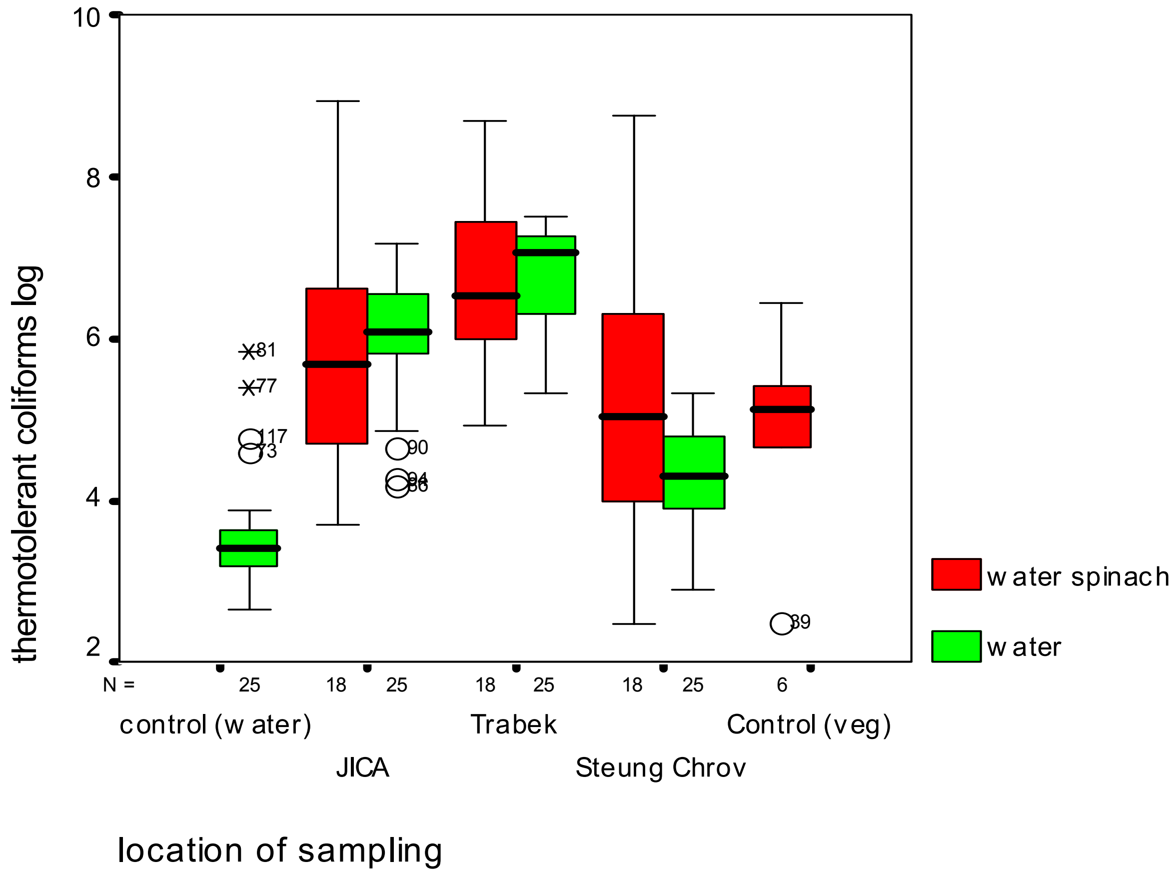

Figure 0-8 Correlation of microbiological quality of water and water spinach (JICA and Trabek = inlet; Steung Chrov = outlet)

1.5.2 Parasitology

Protozoan parasites were found in some samples with the highest occurrence of *Giardia* in plant samples from the inlets. *Cryptosporidium* was more prevalent in plants from the Trabek inlet, whereas *Cryptosporidium* levels were similar in plants from the JICA inlet and the Steun Chrov outlet. Interestingly, *Cyclospora* oocysts were detected in plants harvested from both inlet and outlet locations.

Few samples contained helminth eggs, but nevertheless eggs of *Ascaris* were found in all categories of samples. It was not possible to distinguish the human and the pig variant of *Ascaris*, but as both can infect humans it is safe to say that *Ascaris* is present in this setting and that the eggs could pose an occupational health and food safety risk for farmers and consumers.

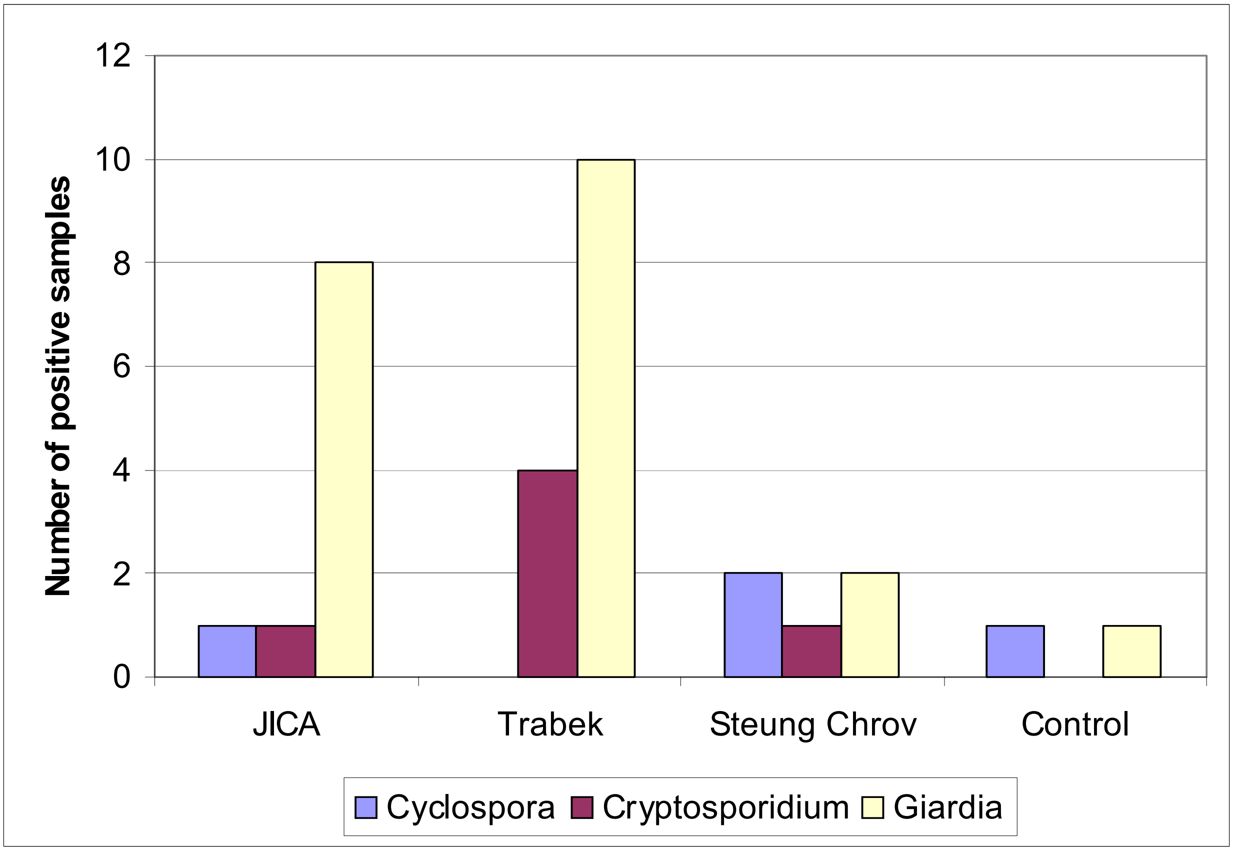

Figure 0-9 Protozoan parasites on water spinach (total 68 samples). Number of positive samples (n = 68). (JCA and Trabek = inlet; Steung Chrov = outlet)

Table 0-4 Phnom Penh: helminth eggs qualitative analysis

Sampling location	Hookworm	*Ascaris* spp	*Trichuris* spp.
JICA (inlet)	0/12	1/12	0/12
Trabek (inlet)	0/12	1/12	0/12
Steung Chrov (outlet)	1/12	2/12	0/12
Control	0/8	1/8	0/8

1.6 Conclusions

1.6.1 Hanoi study

Thermotolerant coliforms

- Relatively low levels were found on water spinach (<10^4/g)
- Slightly higher levels were found at markets
- No apparent differences in levels of faecal pollution levels between seasons

Protozoan parasites

- Higher prevalences during dry season at wastewater-site in Hoang Liet commune
- *Giardia* was most prevalent, particularly at the wastewater-site
- *Cryptosporidium*: higher levels during dry season at wastewater site
- *Cyclospora* is less prevalent than *Giardia* and *Cryptosporidium*, but was present at both sites and at markets and during both seasons
- Protozoan parasites are present in Vietnam in the farm to fork chain and may pose a threat to occupational health as well as to consumers' health.

Helminth eggs

- Very low levels detected at both sites and at markets, no apparent difference between seasons
- Appear not to pose a big food safety problem in the field as they are likely to sediment out in the irrigation canal system

1.6.2 Phnom Penh study

E. coli

- Relatively high levels detected on water spinach, indicating a high level of faecal contamination, particularly at Trabek inlet.

Thermotolerant coliforms

- Relatively higher levels (10^5-10^6 /g) on water spinach
- Positive correlation between microbiological water quality and microbiological vegetable quality

Protozoan parasites

- *Giardia* was more prevalent that *Cryptosporidium* and *Cyclospora*, particularly in plant samples collected at inlets
- Positive correlation between levels of faecal contamination (*E. coli*) and prevalences of *Giardia*, particularly at the Trabek inlet.

Helminth eggs

- Helminth were found in low levels both at inlets and outlets, indicating that there could be some further contamination taking place after the sedimentation treatment in the lake where eggs would otherwise tend to be removed.

2 ELEMENTAL CONTENT AND FOOD SAFETY OF WATER SPINACH (*Ipomoea aquatica* Forssk.) CULTIVATED WITH WASTEWATER IN HANOI, VIETNAM

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2.1 Abstract

Extensive aquatic or semi-aquatic production of water spinach (*Ipomoea aquatica* Forssk.) for human consumption takes place in Southeast Asia. The aim of this study was to assess the concentration level of 39 elements in soil and water spinach cultivated under different degrees of wastewater exposure in Hanoi, Vietnam. The results indicate that there was no clear effect of wastewater use on the elemental concentrations in soil and water spinach. Mean soil concentrations for selected potentially toxic elements at the studied field sites were 9.11-18.7 As, 0.333-0.667 Cd, 10.8-14.5 Co, 68-122 Cr, Cu 34.0-62.1 Cu, 29.9-52.8 Ni, 32.5-67.4 Pb, 0.578-0.765 Tl and 99-189 Zn mg kg^{-1} dry weight (d.w.). In all samples, Cd and Zn soil concentrations were below the Vietnamese Guideline Values (TCVN 7209-2002) for agricultural soils whereas As and Cu exceeded the guideline values. Maximum site concentration of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in water spinach were 0.139, 0.0320, 0.135, 2.01, 39.1, 57.3, 0.16, 0.189 and 6.01 mg kg^{-1} fresh weight (f.w.), respectively. A significant correlation between soil and plant content were only seen for the elements Al, Cu, Tl, and Zn. The estimated average daily intake of As, Cd, Cu, Fe, Pb and Zn for adult Vietnamese consumers amounts to < 11% of the maximum tolerable intake proposed by WHO for each element. It is assessed that the occurrence of the investigated elements in water spinach will pose low health risk for the consumers.

2.2 Introduction

Contamination of agricultural land in Southeast Asia due to irrigation with industrial effluents is of increasing concern due to fast growing populations, increasing urbanization, industrialization and often non-existent or ineffective wastewater treatment facilities. Coupled with this there is an increasing demand for fresh vegetables by urban populations. To comply with this demand vegetables are often produced in peri-urban areas where wastewater may be used as a source of water and nutrients. Due to mixing of industrial and domestic wastewater streams, wastewater often contains Potentially Toxic Elements (PTEs). Consequently, when utilized as a source of irrigation, there is a risk that these PTEs may accumulate to critical levels in soils and agricultural produce. It should also be noted that some PTEs are essential trace elements to plants, e.g. copper (Cu), zinc (Zn), nickel (Ni) and molybdenum (Mo), whereas other elements such as lead (Pb) and cadmium (Cd) are toxic at low concentrations and should not be present in any plants used for consumption. Plants may accumulate both essential and non-essential elements at concentrations which are not phytotoxic but constitute a health risk for consumers (Kabata-Pendias and Pendias, 2001). Elevated concentrations of PTEs have been found in plants grown in wastewater polluted environments (Kisku at al., 2000; Mehar et al., 2000; Nan and Cheng, 2001). However, the ability of plants to accumulate different PTEs varies between plant species and is element specific (Mehar et al., 2000; Kabata-Pendias and Pendias, 2001).

Hanoi, the capital of Vietnam, covers an area of 1,000 km^2 and had a population of about 2.3 million persons in 2002 (Peoples Committee of Hanoi, 2003). Hanoi has nearly 400 medium and large scale industries and more than 14,000 small scale industries located in 14 industrial zones (Peoples Committee of Hanoi, 2003). The To Lich river system drains wastewater from Hanoi and consists of four connected rivers; To Lich, Kim Nguu, Set and Lu rivers and has a total length of 40 km. A total of 458,000 m^3 wastewater is generated daily in Hanoi of which 57% is of industrial origin, 41% of domestic origin and 2% is discharged from hospitals. About 4% of the industrial wastewater and 21% of hospital wastewater are subjected to some kind of treatment (Peoples Committee of Hanoi, 2003). The Hanoi drainage water and wastewater is pumped from the Kim Nguu and To Lich rivers to a system of connected canals and distributed to about 800 ha of intensively cultivated agricultural land located south of the capital in Thanh Tri district.

Water spinach (*Ipomoea aquatica* Forssk.) is a perennial herbaceous aquatic or semi-aquatic green vegetable belonging to the morning glory family (*Convolvulaceae*). It is extensively cultivated in peri-urban areas of Southeast Asia and achieves optimal growth in temperatures between 24 and 30ºC (Rubatzky and Yamaguchi, 1997). Water spinach is cultivated free floating in lakes and ponds, or rooted in wet or moist soils at conditions similar to paddy rice. Under optimal conditions it grows several centimetres per day. Water spinach makes up an important staple food in Vietnam and is the second most commonly eaten food after rice (Duc et al., 1999). The upper part of the stem with foliage is harvested, bundled and sold for human consumption. Re-growth occurs and plants can be harvested again after three to six weeks. Annual water spinach yields range from 40 to 90 t ha^{-1} f.w. (Rubatzky and Yamaguchi, 1997). Other plant parts or older plants are fed to livestock. Water spinach has a high nutritional value and is rich in vitamin A (Rubatzky and Yamaguchi, 1997). According to a survey carried out among Vietnamese consumers, Vietnamese are aware and concerned about the potential risk due to residues of chemical contaminants in food (Figuié, 2003). Furthermore, 88.5% of the interviewed Vietnamese perceived vegetables as the most unsafe food category and 46.9% of the interviewees specified that water spinach is the most unsafe commodity.

The specific aim of this study was to assess the concentration of PTEs in water spinach and soil from agricultural production sites which have been using wastewater for over 13 years in peri-urban Hanoi and to investigate if wastewater usage affected the overall elemental content of water spinach and soil.

2.3 Materials and methods

2.3.1 SAMPLING SITES

Samples were collected at five water spinach production field sites in peri-urban Hanoi (Figure 1).

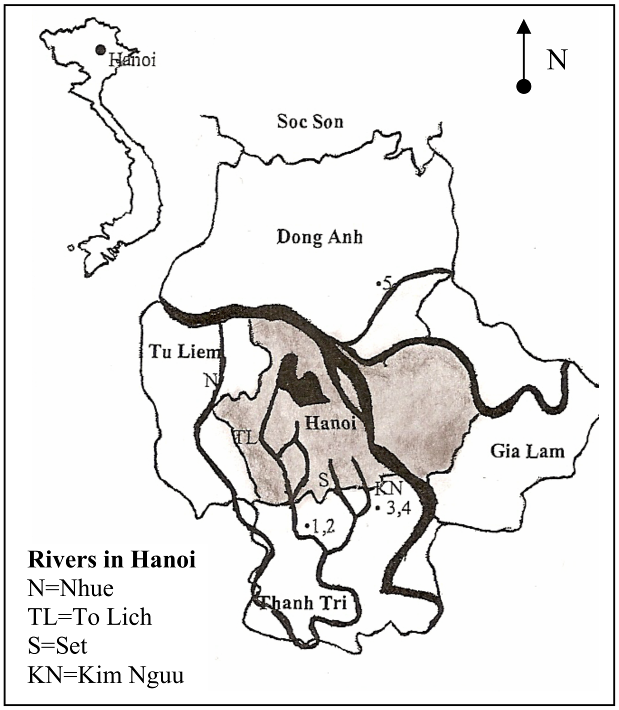

Figure 2-1 Map of Vietnam and Hanoi. City districts (grey) are surrounded by four peri-unban districts. Sampling sites are marked with numbers.

Table 2-1 Characteristics of study sites located in peri-urban Hanoi. Sampled in September and October 2004.

Site no.	District / commune / village	Wastewater exposure
1	Thanh Tri / Hoang Liet / Bang B	High
2	Thanh Tri / Hoang Liet / Bang B	High
3	Thanh Tri / Tran Phu / Khuyen Luong	Low
4	Thanh Tri / Tran Phu / Khuyen Luong	Low
	Dong Anh / Duc Tu / Duc Tu	None (Red River water)

Wastewater is discharged to the To Lich and Kim Nguu rivers in peri-urban Hanoi and is actively pumped from the rivers into production systems. Most of the wastewater used for production of water spinach does not undergo any formal treatment. However, dilution and natural attenuation occur during the flow of the wastewater in the rivers, canals or e.g. when the wastewater is used for fish production before being used for cultivation of water spinach. Sites no. 1 and 2 are defined as high wastewater exposure sites based on frequency of irrigation. They are located in Bang B village, Hoang Liet commune in Thanh Tri district south of Hanoi (Figure 1; Table I). In Bang B village wastewater is pumped to the production system from the To Lich River twice a week and water spinach production plots receive approximately 25 cm of wastewater at each irrigation. Sites no. 3 and 4 which are defined as low wastewater exposure sites are located in Tran Phu village in Tran Phu commune, Thanh Tri district (Figure 1; Table I). In this area water spinach production sites are conjunctively irrigated with rain water and wastewater which has passed through fish ponds and only to a low degree directly with raw wastewater. Site no. 5 which is non-wastewater exposed is located in Duc Tu commune in Dong Anh district north of Hanoi (Figure 1; Table I). In Duc Tu water from the Red River is used to irrigate water spinach.

2.3.2 SAMPLE COLLECTION

Water spinach and soil samples were collected in September and October 2004 at the five field sites in peri-urban Hanoi. Water spinach samples ready for harvest were collected as composite samples with four replicates. All replicates were collected within one producer's field to ensure exposure to the same agricultural practice. All sampling was carried out according to the sampling principles by Gy (1999). A field plot of about 16 m^2 was selected for collection of each sample; it was divided into four smaller plots of about 4 m^2 for collection of replicates. Each replicate sample consisted of the upper 30 cm of a total of 25 plants complete with stem and foliage. Five plants were collected in the centre and five in each of the four corners of the 4 m^2 plots. Fresh weight was determined. To remove surface contamination adhering from dust or attached soil the water spinach samples were washed by dipping them 20 times in three sets of distilled water. Samples were oven dried for seven days at 45 ºC and dry weights (d.w.) were determined. Samples were subjected to being ground in a kitchen blender with titanium knife blades until a fine powder was obtained. Approximately 1 g sub-sample was selected by representative mass reduction following the sampling principles stated by Petersen et al. (2005) and retained in a poly ethylene (PE) bag for subsequent analysis.

Soil samples were collected as composite samples with four replicates in the same field plots as water spinach. Each replicate was made up of five sub samples collected as the upper 10 cm soil with a KC Kajak core sediment sampler (KC Denmark) with a diameter of 60/52mm; as for water spinach, sub samples were collected in the centre and each of the four corners of the 4 m^2 plot. Sub samples were mixed and one fifth of the sample mass was extracted by representative mass reduction. Sediment was spooned into five new samples by adding one spoon in turn to each new sample until no more sediment was left in the original sample; one of the five samples was randomly selected for further work. The soil samples were dried at 45 ºC for seven days and ground in an agate mortar. Approximately 1 g of each of the soil samples was selected for further work by representative mass reduction and stored in a PE bag until further characterisation.

2.3.3 DIGESTION

Digestion of water spinach and soil samples was carried out using a modified version of the EPA (Environmental Protection Agency, USA) Method 3052 (USEPA, 1996). Accurate sample weights of about 0.25 g d.w. water spinach was digested in a mixture of 5 ml conc. nitric acid, 3 ml ultra pure water (Millipore element), 3 ml conc. hydrogen peroxide and 1 ml conc. hydrofluoric acid in a closed Teflon vessel microwave assisted system (Multiwave 3000, Anton Paar GmbH) at 1400W with a ramp time of 10 min and a hold time of 15 min. The addition of hydrofluoric acid was necessary to obtain total digestion of water spinach plant material due to high siliceous content. Digestion of about 0.25 g d.w. soil sample was carried out in 9 ml conc. nitric acid, 1 ml conc. hydrogen peroxide, 2 ml conc. hydrochloric acid and 3 ml conc. hydrofluoric acid at 180 ºC for 25 min. using temperature control and the same microwave system as applied for water spinach digestion. Following digestion 6 ml of saturated boric acid were added to all samples per 1 ml of hydrofluoric acid applied. Quality assurance was carried out by including analytical blanks and standard reference materials (SRM) in every digestion; these were prepared in the same manner as the samples. The applied SRMs were NIST-SRM-1515 (Apple Leaves; National Institute of Standards and Technology (NIST)) for water spinach digestion and NIST-SRM-2709 (San Joaquin Soil) and NIST-SRM-2711 (Montana soil) for soil digestion.

2.3.4 ANALYSES

Determination of As in plant material was carried out by graphite furnace atomic absorption spectrometry (GFAAS) using Zeeman-effect background correction (Perkin Elmer 5100 AAS, HGA-600 graphite furnace) and a Mg-Pd modifier (Marcussen and Holm, unpublished). Concentrations of 39 elements including Al, Ag, As, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Eu, Er, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Nd, Ni, Pb, Pr, Sb, Sc, Se, Sm, Sr, Tb, Th, Tl, Tm, U, V, Y, Yb and Zn were determined by inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent 7500C, Agilent Technologies, Manchester, UK) equipped with an octopole reaction system (ORS) which was operated in non-pressurized standard mode or pressurized with He or H_2 to remove polyatomic interferences. Analysis sensitivity drift was corrected for by carrying out recalibration for every eight samples analysed; one mid-range calibration standard was chosen for this purpose. Limit of detection (LOD) was calculated as three times the standard deviation (SD) of a minimum for eight replicate analyses of the calibration blank. LOD for all elements are reported in Table II.

Table 2-2 Limits of detection (LOD) for all determined elements by inductively coupled plasma mass spectroscopy.

Ele-Ment	LOD Instrumental µg/L	Water spinach µg/g d.w.	Water spinach µg/g f.w.	Soil µg/g d.w.	Ele-ment	LOD Instrumental µg/L	Water spinach µg/g d.w.	Water spinach µg/g f.w.	Soil µg/g d.w.
Al	0.0040	11	1.1	27	Lu	0.00068	0.0025	0.00025	0.0020
Ag	0.0040	0.015	0.0015	0.039	Mg	0.59	2.2	0.22	8.0
As	0.10/0.13[a]	0.24[a]	0.024[a]	0.23	Mn	0.066	0.25	0.025	0.34
Ba	0.0041	0.02	0.002	0.041	Nd	0.0011	0.0041	0.00041	0.0062
Be	0.0020	0.0075	0.00075	0.012	Ni	0.010	0.038	0.0038	0.12
Cd	0.017	0.064	0.0064	0.032	Pb	0.0045	0.017	0.0017	0.11
Ce	0.00038	0.0014	0.00014	0.0037	Pr	0.00040	0.0015	0.00015	0.0030
Co	0.0026	0.0097	0.00097	0.028	Sb	0.0022	0.0081	0.00081	0.010
Cr	0.019	0.071	0.0071	0.11	Sc	0.032	0.087	0.0087	0.070
Cu	0.050	0.19	0.019	0.16	Sm	0.0013	0.0049	0.00049	0.017
Dy	0.00093	0.0035	0.00035	0.0054	Sr	0.0027	0.010	0.0010	0.010
Er	0.00053	0.0020	0.00020	0.0029	Tb	0.00031	0.0012	0.00012	0.0025
Eu	0.0025	0.0092	0.00092	0.013	Th	0.0020	0.0074	0.00074	0.010
Fe	1.4	1.4	0.14	19	Tl	0.0019	0.0072	0.00072	0.016
Gd	0.00095	0.0036	0.00036	0.0070	Tm	0.00021	0.00080	0.000080	0.0018
Ho	0.0017	0.0064	0.00064	0.012	U	0.0022	0.0082	0.00082	0.010
K	56	211	21.1	193	Y	0.00048	0.0018	0.00018	0.0036
La	0.0018	0.0068	0.00068	0.011	Yb	0.0023	0.0088	0.00088	0.010
Li	0.028	0.11	0.011	0.27	Zn	0.022	0.082	0.0082	0.19

[a] Determined by GFAAS.

2.3.5 STATISTICS AND MULTIVARIATE DATA ANALYSES

Before statistical tests and multivariate data analyses was carried out concentrations of all soil elements were standardized with respect to the geogenic element Sc according to eq. (1) to prevent different soil parent material and their natural concentrations of the different elements from influencing the statistical test too much and thereby disguise any effect not linked to parent material as e.g. wastewater use.

$$c_{\mathrm{El}}^{\mathrm{standardized}} = \frac{c_{\mathrm{El}}^{\mathrm{measured}}}{c_{\mathrm{Sc}}^{\mathrm{measured}}} \times 1\mathrm{mg\ kg}^{-1} \tag{1}$$

Where $c_{\mathrm{El}}^{\mathrm{standardized}}$ is the standardized element concentration, and $c_{\mathrm{El}}^{\mathrm{measured}}$ and $c_{\mathrm{Sc}}^{\mathrm{measured}}$ are the measured concentrations all in mg kg^{-1} d.w. Scandium is known to be a conservative element in soil formed on various rock types which is minimally affected by weathering (Gouveia et al. 1992; Nesbitt and Markovics, 1997). Nesbitt and Marckovics (1997) recommended standardization with respect to Sc for soils with different parent material. However, Sc has only been used for standardization in a few studies possibly because this element is rarely determined. Shotyk et al. (2000), Aulinger et al., (2002) and Brown et al. (2003) used Sc for element standardization.

Statistical testes were carried out to elucidate whether there was significant difference between soil and plant elemental concentrations from sites of different wastewater exposure. In addition, relationship between soil and plant concentration of each element was investigated. Data analyses were carried out as linear regression analyses using the procedure General Linear Models (GLM) in the statistical computer program SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA).

Correlations between element concentrations, pH and organic content in soil were investigated through Principal Component Analysis (PCA). For plants relations between different element concentrations were investigated through PCA and relations between soil and plant elemental content were elucidated through Partial Least Square (PLS) regression analysis with introduction of soil data as the X-matrix and plant data as the Y-matrix. PCA and PLS analyses were performed using The Unscrambler version 9.2 (Camo Technologies Inc., New Jersey, USA). The distribution of plant and Sc-standardized soil elements were found to be approximately normally distributed by evaluation of skewness and kurtosis. Data were mean centred and scaled to unit variance before analysis. Plant and soil elements determined with high analytical noise compared to other elements may gain unintended high importance when data are scaled to unit variance. Therefore elements were removed in a step wise procedure leaving out an increasing number of elements starting with those with lowest diagnostic power (DP). DP is defined as the relative standard deviation for samples of different origin (RSD_v) divided by the standard deviation for replicate analyses of one sample (RSD_s) eq. (2).

$$\mathrm{DP} = \frac{\mathrm{RSD_V}}{\mathrm{RSD_S}} \tag{2}$$

Al, Be and Li had a diagnostic power below 1 for soil samples; these elements were therefore removed from the soil PCA model. PCA score and loading plots were not affected by removal of other elements with a low diagnostic power for both soil and plant data and no further elements were excluded. The number of Principal Components (PC) was determined by cross validation and inspection of score and loading plots.

2.4 Results and discussion

2.4.1 ACCURACY AND PRECISION OF ELEMENTAL ANALYSIS

For most elements the analytical blanks had concentrations below the detection limits or negligible concentrations compared to the sample concentrations. For the SRMs NIST-SRM-2709, San Joaquin Soil and NIST-SRM-2711, Montana Soil recoveries of Al, Ba, Ce, Co, Cr, Cu, Fe, K, La, Mg, Mn, Ni, Sr, Sb, Sm, Th, Tl, Y, Yb and Zn were in good agreement with the certified values; recoveries were within ± 15 % of the certified value (Table III). Concentrations of Sc, Ce, Nd, Eu, Dy, Ho and U in NIST-SRM-2711 and NIST-SRM-2709 deviated up to 25 % from the value given by NIST. However, these values are not certified and can only be used as guiding values. The recovery of Pb for NIST-SRM-2711 was 112 – 131 % and for NIST-SRM-2709 it was 103 – 108 %. The diluted digestion of NIST-SRM-2711 had Pb concentrations above the calibration range, which explains the up to 131 % recovery for NIST-SRM-2711. Recoveries of Cd for NIST-SRM-2711 were excellent; however poor Cd recoveries were observed for NIST-SRM-2709. The certified value for NIST-SRM-2709 was below the quantification limit; which could be the reason for the poor accuracy.

The recoveries for the apple leaves NIST-SRM-1515 were not as good as for the soil NIST-SRMs (Table III). Considering that the certified values for many elements are close to the detection limits and under the quantification limit then good to acceptable recoveries are seen for Al, Ba, Ce, Cu, Fe, K, La, Mg, Mn, Nd, Ni, Pb, Sm, Sr, Th, and Zn; all recoveries were within ± 30% of the certified value (Table III). Values given for Eu, Nd, Sb, Th and Yb are not certified; considering the uncertainties connected to these values the measured values are also acceptable. The 95 % confidence interval for some of the elements are high compared to the mean value; however this is expected when operating near the quantification limit. Determination of As by ICP-MS in the NIST-SRM-1515 resulted in recoveries from 995-1435 %. High recoveries at about 400 % for ICP-MS determination of As in the NIST-SRM-1515 have been found by others (Ivanova et al., 2001; Husted et al., 2004). The NIST-SRM-1515 has a high content of Eu, Nd and Sm compared to the content of As. $^{150}Eu^{2+}$, $^{150}Eu^{2+}$ and $^{150}Nd^{2+}$ may cause interference with $^{75}As^{+}$ since they have the

Table 2-3 Measured and certified values ± 95 % prediction interval and % recovery for 8 digestions of standard reference materials NIST-SRM-2709 San Joaquin Soil , NIST-SRM-2711 Montana Soil and NIST-SRM-1515 Apple Leaves from National Institute of Standards and Technology.

Ele-	NIST-SRM-2709			NIST-SRM-2711			NIST-SRM-1515		
ment	Measured[a]	Certified[a]	Recovery	Measured[a]	Certified[a]	Recovery	Measured[a]	Certified[a]	Recovery
	µg/g	µg/g	%	µg/g	µg/g	%	µg/g	µg/g	%
Al	64203 ±2145[b]	75000 ±600	82-89	59226 ±4112[b]	65300 ±900	87-103	325 ±7	286 ±9	111-117
Ag	0.474 ±0.044	0.41 ±0.03	106-131	4.75 ±0.14	4.63 ±0.39	99-107	0.019 ±0.027	-	-
As	20.1 ±1.2	17.7 ±0.8	106-121	102.4 ±2.1	105 ±8	95-101	<0.24	0.038 ±0.007	-
Ba	1052 ±20[b]	968 ±40	107-111	780 ±11[b]	726 ±38	107-111	57.4 ±3.1	49 ±2	110-124
Cd	0.46 ±0.11	0.38 ±0.01	87-138	41.6 ±1.1	41.7 ±0.25	94-105	<0.064	0.013 ±0.002	-
Ce	49.54 ±0.90	42[c]	109-115	77.2 ±1.2	69[c]	116-121	3.52 ±0.13	3[c]	112-121
Co	13.31 ±0.39	13.4 ±0.7	97-102	9.68 ±0.24	10[c]	94-102	0.182 ±0.028	0.09[c]	158-226
Cr	127.6 ±3.5	130 ±4	96-101	44.4 ±1.3	47[c]	89-98	<0.071	0.3[c]	-
Cu	36.3 ±2.2	34.6 ±0.7	99-114	115 ±4	114 ±2	98-109	6.34 ±0.19	5.64 ±0.24	109-116
Dy	3.02	3.5[c]	83-93	4.855	5.6[c]	85-89	1.99	-	-

	±0.11			±0.048			±0.20		
Eu	1.067 ±0.025	0.9 [c]	115-121	1.245 ±0.033	1.1 [c]	109-120	0.285 ±0.019	0.2 [c]	132-154
Fe	40504 ±3318[d]	35000 ±11000	107-124	28900 ±600	29740 ±1115	97-110	101 ±10	83 ±5	108-135
Ho	0.637 ±0.018	0.54 [c]	114-122	0.992 ±0.018	1 [c]	95.3-103	0.338 ±0.057	-	-
K	20684 ±468	20300 ±600	100-104	23900 ±997	24500 ±800	93-106	18898 ±786	16100 ±200	112-122
La	22.88 ±0.58	23 [c]	96-103	37.71 ±0.57	40 [c]	91-97	23.2 ±1.2	20 [c]	109-122
Mg	15673 ±474	15100 ±500	100-107	10104 ±546	10500 ±300	90-108	3055 ±26	2710 ±80	112-113
Mn	592 ±40	538 ±17	104-116	660 ±26	638 ±28	97-109	62.0 ±2.4	54 ±3	111-119
Nd	21.57 ±0.48	19 [c]	111-117	33.60 ±0.52	31 [c]	105-111	20.1 ±2.2	17 [c]	106-129
Ni	86.0 ±5.2	88 ±5	93-104	19.48 ±0.90	20.6 ±1.1	89-104	0.911 ±0.202	0.91 ±0.12	80-123
Pb	19.92 ±0.56	18.9 ±0.5	112-131	1376 ±64d	1162 ±31	103-108	0.423 ±0.087	0.470 ±0.024	70-105
Sb	7.44 ±0.19	7.9 ±0.6	91-97	19.65 ±0.40	19.4 ±1.8	99-106	0.0142 ±0.0059	0.013 [c]	65-171
Sc	13.59 ±0.58	12 [c]	107-118	10.07 ±0.57	9 [c]	102-125	<0.087	0.03 [c]	-
Sm	3.816 ±0.070	3.8 [c]	99-102	5.974 ±0.052	5.9 [c]	99-103	3.12 ±0.10	3 [c]	101-108
Sr	235.5 ±7.9	231 ±2	99-106	237.0 ±4.7	245.3 ±0.7	94-102	28.0 ±0.7	25 ±2	108-115
Th	11.27 ±0.28	11 [c]	100-105	14.18 ±0.23	14 [c]	98-105	0.0201 ±0.012	0.03 [c]	77-93
Tl	0.716 ±0.027	0.74 ±0.05	92-101	2.655 ±0.052	2.47 ±0.15	102-111	0.0107 ±0.0024	-	-
U	3.152 ±0.029	3 [c]	104-106	3.008 ±0.069	2.6 [c]	111-121	0.012 ±0.005	0.006 [c]	159-296
Y	16.40 ±0.64	18 [c]	89-94	26.52 ±0.59	25 [c]	102-112	12.3 ±0.2	-	-
Yb	1.693 ±0.078	1.6 [c]	100-112	2.871 ±0.056	2.7 [c]	102-110	0.215 ±0.028	0.3 [c]	65-85
Zn	106.2 ±6.6	106 ±3	95-107	342 ±10	350.4 ±4.8	94-103	13.4 ±0.6	12.5 ±0.3	102-113

[a] the 95 % confidence interval for each mean
[b] concentration of element was higher than the highest standard
[c] Value not certified, since a bias is suspected or two independent methods are not available

same mass to charge ratio. Arsenic in plant material was therefore determined by GFAAS. Recoveries for Co, Cr and U were not acceptable for NIST-SRM-1515 and concentrations of these elements in plant samples are therefore excluded from this work.

2.4.2 ELEMENTAL CONTENT IN SOIL

The content of the determined elements in soil samples and the related statistics are summarised in Table IV. Vietnamese limit values for agricultural soils have been developed for As, Cd, Cu, Pb and Zn (VMSTE, 2002). No such Vietnamese limit values exist for Cr and Ni; however for these elements Dutch target values have been established based on natural soil levels and negligible risk concentrations (Lamé and Leenaers, 1998). Limit values for Cd, Cu, Ni, Pb and Zn have also been set by in the European Union (CEC, 1986). These are developed for soils with pH 6 and 7. Only the values for soils with pH 6 will be considered since the investigated soils had pH ranging from 5.3 - 6.7. Soil from all five sites had Cd concentrations below the Vietnamese and European limit values of 2 and 1 mg kg^{-1} d.w, respectively. The concentrations of Zn were below the Vietnamese limit value of 200 mg kg^{-1} d.w.; however site no. 3 showed concentrations above the European limit value of 150 mg kg^{-1} d.w. Lead concentrations were below the Vietnamese limit value of 70 mg kg^{-}

1 d.w. although sites no. 3 and especially no. 5 had elemental soil concentrations close to the limit value and these two sites had concentrations above the European limit value of 50 mg kg^{-1} d.w. Sites no. 3 and 5 contained Cu concentrations above the Vietnamese and European limit values of 50 mg kg^{-1} d.w. and site no. 3 also had Cr concentration above the Dutch target value of 100 mg kg^{-1} d.w. Nickel concentrations at sites no. 1, 3, 4 and 5 were above the Dutch target value and the European limit value of 35 and 30 mg kg^{-1} d.w., respectively. Only site no. 4 did not contain As concentrations above the Vietnamese limit value for agricultural soils of 12 mg kg^{-1} d.w. Berg et al. (2001) demonstrated that aquifer sediments in Hanoi city and the surrounding districts contained As concentrations ranging from 0.6 to 33 mg kg^{-1} and that 48 % of 196 investigated wells had As groundwater concentrations above the Vietnamese limit value of 50 µg/L in drinking water. This indicates that the high As concentration observed in this study are likely to be of geogenic origin. In accordance with the Vietnamese limit, Dutch target and European limit values sites no. 3 and 5 are not suited for agricultural production. No target value exists for Tl however since this element has higher toxicity to mammals than mercury, cadmium and lead it is important to monitor its occurrence in the environment (Nriagu, 1998). Mean Tl concentrations in this study ranged from 0.578 to 0.765 mg kg^{-1} d.w. which is within the background range for soils considered to be 0.1 to about 1.0 mg kg^{-1} (Kazantzis, 2000).

Table 2-4 Mean and standard deviation (n=4) for elemental concentration (µg/g d.w.) in soil. The level of significance differences between the elemental concentrations at the five Hanoi sites are given.

	Site no.				
Element	1	2	3	4	5
Al^{ns}	7057 ± 1811	41960 ± 3073	53633 ± 7607	39008 ± 3037	48988 ± 15307
Ag ***	0.287 ± 0.029^{c}	0.266 ± 0.008^{c}	2.73 ± 1.14^{a}	1.08 ± 0.27^{b}	0.281 ± 0.028^{c}
As**	12.6 ± 0.8^{c}	15.4 ± 3.3^{ab}	16.3 ± 0.8^{bc}	9.11 ± 0.75^{c}	18.7 ± 4.6^{a}
Ba*	390 ± 7^{ab}	382 ± 15^{a}	394 ± 28^{b}	330 ± 13^{a}	395 ± 82^{ab}
Be^{ns}	1.97 ± 0.03	1.76 ± 0.08	2.14 ± 0.14	1.66 ± 0.27	2.36 ± 0.24
Cd^{ns}	0.433 ± 0.206	0.333 ± 0.009	0.667 ± 0.129	0.485 ± 0.083	0.418 ± 0.128
Ce**	62.6 ± 2.5^{ab}	61.1 ± 6.0^{a}	63.0 ± 11.4^{c}	48.4 ± 1.6^{bc}	63.4 ± 21.6^{bc}
Co^{ns}	12.4 ± 0.5	11.8 ± 0.85	14.5 ± 1.1	10.8 ± 0.7	14.3 ± 1.9
Cr^{ns}	80.7 ± 2.6	67.5 ± 1.9	122 ± 16	83.4 ± 6.0	85.1 ± 11.1
Cu*	34.3 ± 0.9^{b}	34.0 ± 1.1^{b}	62.1 ± 6.3^{a}	35.5 ± 4.8^{ab}	51.9 ± 7.6^{a}
Dy***	3.20 ± 0.08^{a}	3.10 ± 0.19^{a}	3.31 ± 0.48^{b}	2.45 ± 0.15^{b}	3.57 ± 1.27^{a}
Er***	1.75 ± 0.05^{b}	1.71 ± 0.11^{a}	1.82 ± 0.29^{c}	1.35 ± 0.07^{c}	1.91 ± 0.67^{ab}
Eu***	1.00 ± 0.03^{b}	0.983 ± 0.098^{a}	1.04 ± 0.18^{c}	0.801 ± 0.062^{cb}	1.06 ± 0.37^{b}
Fe^{ns}	31058 ± 1460	31198 ± 2956	36019 ± 3001	26591 ± 1776	37473 ± 5452
Gd ***	4.35 ± 0.10^{ab}	4.21 ± 0.38^{a}	4.46 ± 0.79^{c}	3.41 ± 0.13^{bc}	4.62 ± 1.58^{ab}
Ho***	0.630 ± 0.014^{a}	0.597 ± 0.035^{a}	0.637 ± 0.097^{b}	0.471 ± 0.028^{b}	0.675 ± 0.225^{a}
K^{ns}	16013 ± 732	14289 ± 617	18154 ± 1452	14606 ± 633	16317 ± 1647
La**	28.1 ± 1.3^{ab}	22.4 ± 2.9^{a}	28.5 ± 5.6^{c}	22.2 ± 1.0^{bc}	28.1 ± 9.0^{bc}
Li^{ns}	34.0 ± 1.5	29.1 ± 1.6	41.0 ± 2.9	30.8 ± 1.8	43.9 ± 4.8
Lu***	0.257 ± 0.013^{b}	0.264 ± 0.009^{a}	0.270 ± 0.048^{c}	0.213 ± 0.017^{b}	0.276 ± 0.097^{b}
Mg***	4866 ± 288^{b}	4497 ± 460^{b}	7115 ± 1071^{a}	5030 ± 474^{a}	4862 ± 1977^{b}
Mn**	282 ± 121^{c}	536 ± 34^{a}	473 ± 299^{bc}	223 ± 23^{c}	535 ± 233^{ab}
Nd**	27.1 ± 0.7^{ab}	26.2 ± 2.5^{a}	27.4 ± 4.8^{c}	21.3 ± 0.6^{bc}	27.5 ± 9.3^{b}
Ni^{ns}	37.6 ± 1.4	29.9 ± 1.6	52.8 ± 2.1	36.9 ± 3.8	37.7 ± 6.3
Pb**	37.0 ± 1.8^{c}	34.4 ± 2.0^{bc}	59.9 ± 6.65^{ab}	32.5 ± 3.3^{bc}	67.4 ± 11.5^{a}
Pr **	6.80 ± 0.19^{ab}	6.55 ± 0.63^{a}	6.84 ± 1.23^{c}	5.35 ± 0.16^{bc}	6.87 ± 2.33^{bc}
Sb^{ns}	2.07 ± 0.17	1.82 ± 0.11	2.86 ± 0.56	2.08 ± 0.20	2.46 ± 0.25
Sc	10.5 ± 0.6	9.45 ± 0.71	12.1 ± 1.9	8.76 ± 0.72	11.0 ± 3.4
Sm**	4.89 ± 0.10^{ab}	4.78 ± 0.48^{a}	5.02 ± 0.88^{c}	3.92 ± 0.14^{bc}	5.11 ± 1.78^{b}
Sr***	71.0 ± 4.1^{b}	75.2 ± 2.1^{a}	72.7 ± 6.4^{c}	65.6 ± 2.6^{a}	62.1 ± 16.6^{c}
Tb***	0.586 ± 0.016^{bc}	0.579 ± 0.035^{a}	0.601 ± 0.098^{d}	0.454 ± 0.016^{cd}	0.637 ± 0.225^{ab}
Th^{ns}	9.18 ± 0.37	9.06 ± 0.78	10.4 ± 2.0	7.87 ± 0.435	9.75 ± 3.30
Tm***	0.266 ± 0.010^{b}	0.257 ± 0.015^{a}	0.275 ± 0.043^{c}	0.202 ± 0.014^{c}	0.283 ± 0.101^{b}
Tl^{ns}	0.671 ± 0.021	0.578 ± 0.036	0.759 ± 0.034	0.587 ± 0.031	0.765 ± 0.060
Y**	13.2 ± 1.1^{b}	13.4 ± 1.2^{a}	14.6 ± 2.7^{b}	10.4 ± 0.8^{b}	14.3 ± 4.5^{ab}
Yb**	1.74 ± 0.08^{ab}	1.69 ± 0.12^{a}	1.84 ± 0.23^{b}	1.35 ± 0.07^{b}	1.83 ± 0.65^{b}
U^{ns}	3.02 ± 0.19	2.66 ± 0.11	2.86 ± 0.14	2.30 ± 0.06	3.37 ± 0.33
Zn*	94.6 ± 5.9^{b}	90.8 ± 4.4^{b}	189 ± 17^{a}	106 ± 14^{b}	127 ± 26^{b}

^{ns} non-significant; *,**, *** significant at 5, 1 and 0.1 % significance level, respectively. There is no significant difference between mean concentrations followed the same letter. ^{a} indicate the highest Sc correct values, ^{b} the next highest and so on.

No background level has been established for PTEs in Vietnam and it is therefore not possible to evaluate whether the relatively high soil elemental concentrations compared to the Vietnamese agricultural limit values are due to pollution or locally natural high soil concentration. However, Zarcinas et al. (2004a, b) carried out a study of 241 and 318 agricultural, forested and uncultivated soils in Malaysia and Thailand, respectively and established investigation levels as the 95th percentile of concentrations of As, Cd, Co, Cr, Cu, Ni, Pb and Zn in the investigated soils. The investigation levels do not indicate that soils with concentrations above this level are toxic, only that soils from Malaysia and Thailand with higher concentrations are likely polluted and further investigation is needed. Investigation levels for Malaysian and Thai soils are reported in Table V together with the range of soil elemental concentrations found in the present study of Hanoi soils.

Arsenic soil concentrations determined in Hanoi were below the investigation levels for Malaysia and Thailand. All Hanoi soil samples had Cd concentrations above the investigation levels of Malaysia and Thailand, for Co this was only the case for the Malaysian investigation level. For Cr, Cu, Ni, Pb and Zn several samples had concentrations above the Malaysian and Thai investigation levels. This indicates a relatively high PTE load in the Hanoi soils compared to other soils in Southeast Asia. It is not known whether the high PTE concentrations are due to natural higher concentrations of geogenic origin in the Red River delta. However, the Hanoi soils are intensively cultivated; regular fertilizer applications may lead to accumulation of some PTEs (McLaughlin et al., 1996).

Table 2-5 Thai and Malaysian 95 percentile investigation level for soils (Zarcinas et al., 2004a, b) and range of elemental soil concentrations determined in Hanoi, all in mg kg^{-1} d.w.

Element	Investigating levels		Range for this study
	Thai	Malaysia	
As	30	60	8.20-15.4
Cd	0.15	0.30	0.281-0.799
Co	20	10	10.6-17.1
Cr	80	60	65.0-131
Cu	45	50	30.3-67.1
Ni	45	45	28.1-54.2
Pb	55	65	29.2-83.2
Zn	70	95	84.3-207

It was tested whether there was any significant difference between the elemental soil concentrations at the five field sites, and whether such difference could be linked to different degrees of wastewater exposure. There was no significant influence of site on the Sc-standardized soil values of Al, Cd, Co, Cr, Fe, K, Li, Mo, Ni, Sr, Tl, Th and U at the 5 % significances level. The site had a significant influence on the Sc-standardized soil values of Ag, Dy, Er, Eu, Gd, Ho, Lu and Mg at the 0.1 % significance level, As, Ce, La, Mn and Nd at the 1 % significance level and Ba and Cu at the 5 % significance level (Table IV). Sc-standardized Ce and Sr soil values had significantly higher concentrations at sites no. 1 and 2 which are exposed to higher wastewater application as compared to site no. 5 which is non-exposed. However, site no. 5 had higher Cu and Pb Sc-standardized values than sites no. 1 and 2. Compared to sites no. 3 and 4 with low wastewater exposure the non-exposed site no. 5 had significantly higher As, Er, Ho, Tb and Tm Sc-standardized values and significantly lower Ag and Mn Sc-standardized values. Significant difference between samples collected in the same commune at a distance of not more than 300 m was observed for some elements. For As, Er, Eu, Lu, Mn, Sr, Tb, Tm and Y Sc-standardized concentration values were significantly different at site no. 1 as compared to site no. 2 and for the elements Ag, Ba, Lu, Sr and Zn there were significant differences between sites no. 3 and 4.

The statistical tests carried out by linear regression and comparison of sites with significantly different concentrations of single elements showed that there is no indication of increased elemental concentrations as a result for wastewater use. However, exploring several elements simultaneously can possibly show patterns that are not visible when looking at single elements and correlations between elements can be found. PCA was therefore carried out for the soil data. The score plot for the resulting PCA model is presented in Figure 2a. The first two PCs of the resulting model explain 53 and 18 percent of the data. There is a tendency for samples from the same site to group into clusters but these are not very well defined. It is clear that samples from the sites no. 1 and 2 with high wastewater exposure cannot be distinguished from the non-wastewater exposed site no. 5.

However samples from sites no. 1, 2 and 5 seems to differ from sites no. 3 and 4. The loading plot for the soil PCA is presented in Figure 2b. The main characteristic is that elements whose concentrations are suspected to be influenced by anthropogenic addition have low loadings for PC1 whereas elements which are known to be present in high geogenic concentrations in soil compared to anthropogenic inputs e.g. Fe and Mn have high loadings for PC1. It is therefore likely that the first component states the origin of the elements. The group of elements attributed to anthropogenic inputs are Ag, Cd, Cr, Cu, Mg, Ni, Sb and Zn. These elements show a higher degree of correlation to soil pH and Soil Organic Matter (SOM) determined as percent carbon (%C) than the geogenic elements. The Rare Earth Elements (REE) including Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Y and Yb have the highest loadings for PC1 and make up a very well defined group in the loading plot.

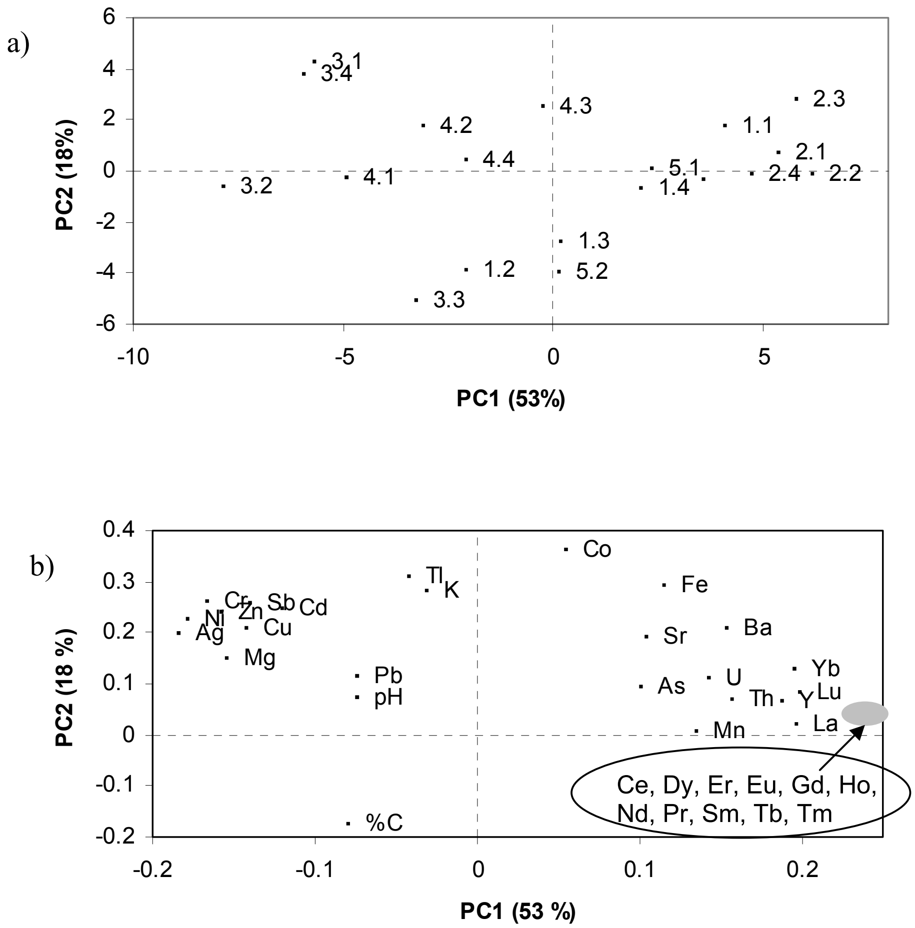

Figure 2-2 (a) Score plot and (b) loading plot for the principal component analysis of soil samples from peri-urban Hanoi, Vietnam. Principal component 1 and 2 explains 53 and 18 % of the data, respectively.

The REEs have very similar chemical behaviour and will usually be present as trivalent cations in soil which is probably the reason for this group being so highly correlated (Figure 2b). The REEs have the lowest correlation to the content of organic matter and pH. SOM may affect the mobility of REEs in a number of ways. Nikonov et al. (1999) found that the presence of stable and immobile organic matter will prevent transport of REEs whereas the concentration of dissolved organic carbon has been found important for the concentrations of REEs in the soil solution (Tyler and Olsson, 2002). Tl, K and Co are located in between the anthropogenic and geogenic elements and their concentrations in the soils are probably controlled both by anthropogenic and geogenic sources. From the score and loading plots it seen that samples from the low wastewater exposed sites no. 3 and 4 have a higher correlation with the anthropogenic elements than samples from sites no. 1 and 2 with high wastewater exposure and site no. 5 which is non-wastewater exposed. One replicate from site 5 had much higher Sc-standardized concentrations of most elements compared to the other samples and it clearly deviated from all the other samples in the score plot (plot not shown). This sample was removed from the model to describe the major variation between most samples instead of focusing on the variation between this sample and the other samples.

Generally, the elemental concentrations at the study sites are not correlated to the degree of wastewater application. The differences in Sc-standardized values between the field sites is likely to be caused by natural variation, differences in the parent which the Sc-standardization can not fully account for or differences due to agricultural practices e.g. fertilizer application which may result in increased Cd concentrations (McLaughlin et al., 1996). This may also be the reason for significant differences in elemental concentrations between neighbouring samples as seen for site no. 1 and 2 and site no. 3 and 4.

2.4.3 ELEMENTAL CONTENT IN WATER SPINACH

The content of the determined elements in water spinach samples and the results of the statistical data analyses are summarised in Table VI. In one out of the 20 samples analysed the concentration of the elements Be, Cd, La, Ce, Pr, Nd and Th were below the detection limit. In such case it was assumed that the concentration was half the detection limit and this value was used in all further data treatment.

A normal range for concentration of PTEs in water spinach cannot be established since only a few studies have focused on this topic. Göthberg et al. (2002) and Zarcinas et al. (2004a) investigated the accumulation of PTEs in water spinach in Malaysia and Bangkok, respectively. Their results are reported in Table VII together with the current results from Hanoi. For Cd, Cr and Cu concentrations in water spinach were at the same level in Hanoi and Malaysia; however, Cd concentrations were much lower in Bangkok. Concentrations of As and Zn in samples from Hanoi are much lower than observed for Malaysia. Nickel and Pb concentrations are higher in Hanoi compared to Malaysia which may be explained by high soil concentrations of these elements in Hanoi compared to Malaysia (Zarcinas et al., 2004).

Table 2-6 Mean and standard deviation (n=4) for elemental concentration (µg/g f.w.) in water spinach. The level of significance differences between the elemental concentrations at the five Hanoi sites are given.

	Site no.				
Element	1	2	3	4	5
Al*	12.2 ± 3.98^{ab}	7.86 ± 1.73^{b}	21.2 ± 18.6^{ab}	6.94 ± 1.57^{b}	6.71 ± 1.79^{b}
As***	0.139 ± 0.041^{a}	0.033 ± 0.018^{c}	0.101 ± 0.007^{b}	0.051 ± 0.011^{c}	0.089 ± 0.027^{b}
Ba***	3.66 ± 0.57^{b}	4.52 ± 1.01^{ab}	1.62 ± 0.38^{c}	2.04 ± 0.19^{c}	4.81 ± 0.65^{a}
Be*	0.00209 ± 0.00039^{a}	0.00174 ± 0.00024^{ab}	0.00222 ± 0.00072^{a}	0.00125 ± 0.00017^{b}	0.00119 ± 0.00015^{b}
Cd*	0.0236 ± 0.0193^{ab}	0.0320 ± 0.0116^{a}	0.0083 ± 0.0018^{b}	0.0240 ± 0.0052^{ab}	0.0101 ± 0.0037^{b}
Ce^{ns}	0.0118 ± 0.00133	0.0112 ± 0.0026	0.02421 ± 0.02159	0.00802 ± 0.00532	0.00951 ± 0.00258
Cr*	0.0621 ± 0.0088^{b}	0.0687 ± 0.0175^{b}	0.1350 ± 0.0708^{a}	0.0635 ± 0.0175^{b}	0.0706 ± 0.0178^{b}
Cu**	0.98 ± 0.53^{cd}	1.71 ± 0.45^{ab}	1.26 ± 0.43^{bc}	2.01 ± 0.42^{a}	0.507 ± 0.241^{d}
Fe*	39.1 ± 7.8^{a}	20.8 ± 4.6^{b}	31.4 ± 18.5^{ab}	25.5 ± 2.12^{ab}	20.8 ± 4.6^{a}
K**	3556 ± 571^{a}	2405 ± 538^{b}	3645 ± 388^{a}	3839 ± 74^{a}	3635 ± 532^{a}
La^{ns}	0.00601 ± 0.00082	0.00607 ± 0.00184	0.0133 ± 0.0111	0.00451 ± 0.00278	0.00526 ± 0.00164
Li^{ns}	0.030 ± 0.004	0.037 ± 0.003	0.044 ± 0.021	0.039 ± 0.008	0.028 ± 0.011
Mg*	386 ± 40^{abc}	416 ± 58^{a}	414 ± 94^{ab}	323 ± 33^{bc}	310 ± 33^{c}
Mn***	17.0 ± 1.4^{c}	34.0 ± 11.0^{b}	16.9 ± 5.6^{c}	14.1 ± 1.2^{c}	55.5 ± 17.4^{a}
Nd^{ns}	0.00496 ± 0.00092	0.00511 ± 0.00149	0.0106 ± 0.0087	0.00314 ± 0.00204	0.00348 ± 0.00103
Ni**	0.113 ± 0.051^{ab}	0.0854 ± 0.0239^{bc}	0.0698 ± 0.0397^{bc}	0.160 ± 0.023^{a}	0.0472 ± 0.0183^{c}
Pb**	0.128 ± 0.007^{bc}	0.164 ± 0.032^{ab}	0.104 ± 0.044^{c}	0.185 ± 0.014^{a}	0.189 ± 0.033^{a}
Pr^{ns}	0.00135 ± 0.00017	0.00130 ± 0.00030	0.00280 ± 0.00237	0.0009 ± 0.0005	0.00106 ± 0.00030
Sb***	0.0078 ± 0.0016^{b}	0.00593 ± 0.00046^{b}	0.00615 ± 0.00191^{b}	0.0120 ± 0.0011^{a}	0.00593 ± 0.00046^{c}
Sr*	2.76 ± 0.24^{ab}	4.22 ± 0.90^{a}	3.05 ± 0.65^{b}	2.76 ± 0.24^{b}	3.46 ± 0.35^{ab}
Th^{ns}	0.00156 ± 0.00040	0.00463 ± 0.00718	0.00418 ± 0.00369	0.00108 ± 0.00057	0.00084 ± 0.00032
Tl**	0.0143 ± 0.0113^{ab}	0.0227 ± 0.0063^{a}	0.0271 ± 0.0071^{a}	0.0206 ± 0.0047^{ab}	0.0075 ± 0.0052^{b}
Y*	0.00585 ± 0.00076^{ab}	0.00605 ± 0.00079^{ab}	0.01056 ± 0.00598^{a}	0.00319 ± 0.00219^{b}	0.00462 ± 0.00095^{b}
Zn*	5.06 ± 1.06^{ab}	5.55 ± 0.90^{ab}	4.11 ± 1.28^{b}	6.01 ± 0.35^{a}	5.12 ± 0.52^{ab}

^{ns} non-significant; *,**, *** significant at 5, 1 and 0.1 % significance level, respectively. There is no significant difference between mean concentrations followed the same letter. ^{a} indicate the highest Sc correct values, ^{b} the next highest and so on.

Table 2-7 Metal concentrations found in water spinach in Hanoi (this study) compared with reported values from Malaysia and Bangkok.

Element	Malaysia[a] mg kg^{-1} f.w.	Hanoi mg kg^{-1} f.w.	Bangkok[b] mg kg^{-1} d.w.	Hanoi mg kg^{-1} d.w.
As	0.28	0.083	-	0.82
Cd	0.026	0.019	0.060	0.19
Cr	0.13	0.079	-	0.79
Cu	0.8	1.29	-	12.8
Ni	0.18	27.5	-	272
Pb	0.034	27.5	280	272
Zn	3.9	0.095	-	0.94

[a] Zarcinas et al., 2004a, mean concentrations, n=6
[b] Göthberg et al., 2002, highest concentration observed in plant top

Through linear regression it was tested if the site had any significant influence on the plant elemental concentrations. The site was found to have significant influence on the plant concentration of As, Ba, Mn and Sb at the 0.1 % significance level, Cu, K, Ni, Pb and Tl at the 1 % significance level and Al, Be, Cd, Cr, Fe, Mg, Sr, Y and Zn at the 5 % significance level (Table VI). However, site had no significant effect on plant elemental concentrations for La, Li, Nd and Pr. The differences in plant elemental concentration between sites seem to be caused by natural variation and not wastewater application. Only Sb had significantly higher concentrations at site no. 1 and 2 which are exposed to high wastewater application as compared to the non-exposed site no. 5. Conversely, Mn was observed at significantly higher concentrations at site no. 5 compared to site no. 1 and 2. Compared to site no. 3 and 4 with low wastewater exposure site no. 5 had significantly higher Ba, Mn concentration and significantly lower Cu and Sb concentrations. Significant differences in water spinach elemental concentrations for sites located at short distances in the same commune were observed for As, Cu, Fe, K and Mn for sites no. 1 and 2 and for As, Be, Cr, Cu, Ni, Pb, Sb, Y and Zn at sites no. 3 and 4. It is clear that concern about wastewater use for production of aquatic vegetables is unfounded in this case and that a high variation exists in the elemental concentration between plants grown in close proximity. This variation can maybe be caused by few days' difference in age of the plant at harvest time and small differences in the application of fertilizers (Gothberg et al., 2004). Gothberg et al., (2004) investigated distribution of Cd, Hg and Pb in water spinach and observed that all elements accumulated at higher proportions in the root as compared to shoots. This distribution become more pronounced at higher Cd, Hg and Pb concentrations in the growth media. In this study elemental concentrations were only determined in shoots as this poses the direct ingestion pathway of PTEs via human consumption.

PCA was carried out to investigate whether the concentrations of the different elements are correlated in water spinach. The resulting loading plot is presented in Figure 3. Cross validation showed that the elements Ba, K, Mn, Pb and Sr were disturbing the model and they were therefore removed. According to the score plot there was no observable difference of overall metal content between samples of high and none wastewater exposure (results not shown). The REEs Ce, La, Nd, Pr and Y have high loadings for PC1 and correlates with the elements Al, Be, Th and Mg (Figure 3). It is not clear what the reason for this correlation is however REEs are known to replace other elements in life supporting functions; REEs have been found to increase the activity of photosystem II in a fern and there is indications that Ce can replace Mg in Mg-chlorophyll (Hong et al., 1999a; 2001). The other elements in the model are less influenced by PC1. Copper, Cd, Ni, Zn and Sb have high loadings for PC2 whereas As and Fe has low loadings. This distribution of elements is quite similar to the one seen for PC1 for the soil samples and is possible that the PC2 for the plant data describes the origin of the elements; whether the plant uptake of a specific element is dominated by geogenic or anthropogenic sources.

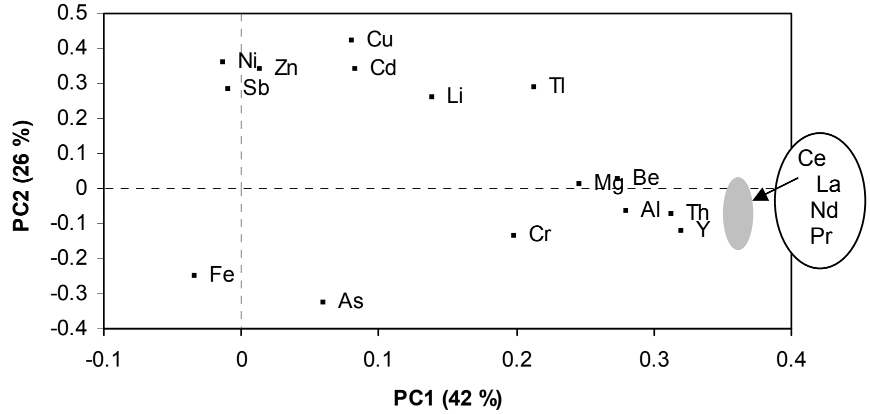

Figure 2-3 Loading plot for the principal component analysis of water spinach samples cultivated in peri-urban Hanoi, Vietnam. Principal component 1 and 2 explains 39 and 25 % of the data, respectively.

Standard deviations for replicate water spinach samples are generally high (Table VI). Samples were collected as composite samples with 25 plants in each replicate and sub samples for analysis were collected by representative mass reduction it is therefore likely that the high standard deviation is due to high natural variation between replicates grown just a few meters apart. High standard deviations compared to mean total concentrations of Pb, Cd and Hg in water spinach have also been observed by Göthberg et al. (2002).

Twelve of the determined elements had concentrations below the detection limit in the majority of the analysed samples. All water spinach samples had Lu concentrations below the detection limit of 0.00025 mg kg^{-1} f.w. The concentration of Ag, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Sc and Sm were below the detection limit for 10 to 19 of the 20 analysed water spinach samples. Due to the large proportion of samples with concentrations below the detection limit, estimates of actual sample concentrations would have a high level of uncertainty and the estimated values would have high influence on the calculated mean value and results of statistical analyses. Hence, the concentrations of Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Sc and Sm are not shown in Table VI. The following concentration intervals were determined: <0.0015-0.0057 Ag, <0.00092-0.00118 Eu, <0.00036-0.00409 Gd, <0.00012-0.00074 Tb, <0.00035-0.00348 Dy, <0.00064-0.00751 Ho, <0.00020-0.00864 Er, <0.00080-0.00181 Tm, 0.00088-0.00170 Yb, <0.0087-0.0200 Sc, 0.00049-0.00437 Sm mg kg^{-1} f.w.

2.4.4 ELEMENTAL RELATIONS BETWEEN SOIL AND WATER SPINACH

The influence of soil on plant elemental concentration was tested for the different elements by linear regression. The test was carried out both for Sc non-standardized and standardized soil concentrations. The statistical tests showed that soil concentrations had no significant influence on the plant concentrations of As, Ba, Be, Cd, Ce, Cr, Fe, K, La, Li, Mg, Mn, Nd, Ni, Pb Pr, Sb, Sr, Th and Y. This is not surprising since poor correlations are known to exist between wetland plants and soil metal concentrations (Greger, 1999; Deng et al., 2004). Intuitively, this is to be expected, as the oven-dry soil sample does not reflect the complex redox/pH interactions associated with the flooded soils which may result in sorption, desorption and precipitation processes for the different elements. Plant elemental uptake depends on many factors such as pH, competition between ions, redox potential, etc. Göthberg et al., (2004) found that uptake of Cd, Hg and Pb in water spinach decreased with increasing levels of fertilizers. Different levels of fertilizers applied at the study sites could obscure any effects that soil element concentration would have on plant element concentrations. Finally the total pool of elements in the soil will not be available because part of it is incorporated into the three dimensional ion lattice of soil components.

The Sc-standardized Al soil concentrations had significant influence on the water spinach Al concentrations but this was not the case for the non-Sc-standardized soil concentrations. This indicates that Al inherent to the parent material had no effect on the Al uptake in the plants whereas non-geogenic Al is correlated to plant uptake. The site of production had significant influence on Al transfer from soil to plant and the Biological Absorption Coefficients (BAC) eq. (3) were different from site to site; this effect was significant at the 5 % significance level.

$$BAC = \frac{C_{plant} \text{ mg/kg f.w.}}{C_{soil} \text{ mg/kg d.w.}} \tag{3}$$

Total soil concentrations of Cu, Zn and Tl not standardized with respect to Sc had a significant influence on the plant concentrations of these elements at the 5 % significance level. For these elements the site had no influence on the soil plant transfer; this means that there is no significant difference between the BAC for the different sites. The observed BAC for thallium in water spinach ranged from 0.05-0.49. The BAC value is low compared to values published for other vegetables. LaCoste et al. (2001) found BAC's in 11 plants ranging from 0.76-87.0 with the lowest value for tomato and high values for cabbage family (*Brassicaceae*). Uptake of Tl depends on the soil conditions; it will increase with increasing soil acidity and Tl originating from pollution sources has been found more available than Tl with a geogenic origin (Kazantzis, 2000). Fertilizers are known to be a source of Tl pollution in agriculture with concentrations up to 0.62 mg kg^{-1} having been determined in NPK fertilizers (Mermut et al., 1996).

For the major part of the element there was no correlation between the elemental concentration in soil and water spinach. It is however likely that such correlation can be seen for groups of elements instead of single elements. A PLS analysis was carried to test whether groups of elements in soil and plant are correlated. However cross validation showed that no PLS model is able to describe the data even if elements are removed from the model. It can therefore be concluded that no correlation exists between plant and soil elemental concentrations for the major part of the elements.

2.4.5 ASSEMENT OF WATER SPINACH FOOD SAFETY

According to WHO/FAO, the daily average consumption of vegetables other than tomatoes and onions in Vietnam is of 220.5 g/person/day (FAO, 2003). This figure has been derived from Food Balance Sheets (FBS). It is known that data derived from FBSs gives a rough estimation of the food consumption as they are based on statistics of annual food production, imports, and exports, post-harvest losses, etc (WHO, 1997). Furthermore, data derived from FBS gives no information on the food consumption levels for people living in different geographical areas of the country. Such information can only be obtained from food consumption surveys. A household survey (24-hour recall, 7686 households), carried out in Vietnam in 2000 by the Vietnamese National Institute of Nutrition has shown that the daily consumption of vegetable leaves amounts to 147 g $person^{-1}$ day^{-1} (Khoi, 2000). The same survey showed that the vegetable consumption pattern among urban and rural inhabitants was very similar, as the mean vegetable leaves consumption amounted to 153.1 and 145.1 g person^{-1} day^{-1} for the two sub-groups of the population, respectively. Finally, a household consumption survey carried out in the urban and peri-urban Hanoi (Anh et al., 2004) has shown that water spinach is the major vegetable consumed in Hanoi and that the per capita daily consumption was 75.0, 77.3, and 78.7 g person^{-1} day^{-1} in the urban, peri-urban, and all Hanoi, respectively.

Even if the WHO consumption data are internationally used, in order to risk assess the potential health risks posed to consumers, it has been decided to make use of the consumption data reported by Anh et al. (2004) for the following reasons: *i)* The consumption data refers specifically to consumers living in Hanoi; *ii)* The mean daily consumption has been derived from a survey and not from FBS. As water spinach is often grown and traded locally, e.g. for subsistence farming, the statistical data used to compile the FBS can hardly take into account this feature; *iii)* The consumption figures reported in FBSs refer to a group of food commodities (e.g. vegetables other than onions and tomatoes) and not specifically to water spinach.

As the uncertainties on the consumption data available are not known and because of the low measured elemental concentrations in water spinach, an accurate quantitative risk assessment on potential health risks posed to consumers is not feasible. As a result, the risk assessment reported below has to be meant as a screening tool only.

The maximum mean concentration of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn observed for the production sites were 0.139, 0.032, 0.135, 2.01, 39.1, 57.3, 0.16, 0.189 and 6.01 mg kg^{-1} f.w., respectively. FAO/WHO (2003) standards for the provisional tolerable intake (PTI) of As, Cd, Cu, Fe, Pb and Zn are set to 0.0021, 0.001, 0.5, 0.8, 0.0036 and 1.0 mg kg^{-1} body weight day^{-1}, respectively. A study of 673 adults in Southern Vietnam showed an average bodyweight of 48 kg with a standard deviation of 8 kg (Keane et al., 1997). Assuming that a person with a mean body weight of 48 kg consumes 77.3 g f.w. water spinach a day, and conservatively assuming that the average concentration levels of the investigated elements correspond to the highest reported mean concentrations, the intake of As, Cd, Cu, Fe, Pb and Zn will correspond to 10.7, 5.2, 0.6, 7.9, 8.5 and 1.0 % of the maximum tolerable intake, respectively. As the estimated exposures do not exceed its safety limits, it is considered that no more refined estimations of the intake are necessary. Furthermore, it is assessed that the health risk related to the dietary exposure to As, Cd, Cu, Fe, Pb and Zn from water spinach consumption is low. However, a health risk cannot be excluded if consumers are exposed to other foods which contain these elements in elevated concentrations, or if consumers are long-term exposed to these elements in other ways. In particular, As may constitute a problem since drinking water in Hanoi has elevated As concentrations. Seventy two percent of 68 private drinking water wells in rural districts around Hanoi may have As concentrations above the WHO threshold limit of 10 µg/L (Berg et al., 2001).

Considerable research has established that levels of dietary Zn as well as Fe and to a lesser extent Ca are known to influence the absorption of Cd and its distribution in organs and tissues (Flanagan et al., 1978; Koo et al., 1978; Fox, et al., 1979; Brzóska and Moniuszko-Jakoniuk*,* 1988*;* Berglund et al., 1994*;* Reeves and Chaney, 2001). Iron and Zn deficiency predisposes individuals to a higher Cd absorption and therefore women are a more vulnerable group since they are more prone to Fe deficiency (Berglund et al., 1994). In 1995, 40% of Vietnamese women between 15 and 40 yrs were considered anaemic (Fe deficient). In 2004, the national average had through a national Fe supplementation program declined to 28% (National Institute of Nutrition in Vietnam (National

Institute of Nutrition, 2004). Rice is the staple food of Vietnam. However, rice grain Fe, Zn and Ca contents are insufficient for human needs (Hallberg et al., 1977; Pedersen and Eggum 1983). In addition, milling rice grain results in further Fe and Zn losses (Pedersen and Eggum 1983; Zhang et al., 1997). The high levels of Zn and Fe in water spinach may in part give protection against Cd uptake and be a vital source of dietary Fe and Zn. Further Chaney et al. (1996) indicate that a Cd:Zn ratio of <0.015 effectively provides protection from Cd-induced health impacts. The Cd:Zn ratio found for water spinach in this study ranged from 0.002 to 0.006 indicating that consumption of water spinach produced in peri-urban Hanoi will not contribute negatively to the uptake of Cd.

Tl concentrations ranging from 0.0036 to 0.0353 mg kg^{-1} f.w. were found in water spinach samples. A limit value ranging from 0.25-0.50 µg/g f.w. for Tl in foods have been proposed (Grössmann, 1984) and it is unlikely that the consumption of water spinach produced in Hanoi is associated with any health risk with respect to Tl.

2.5 Conclusion

Mean concentrations of selected PTEs in soils at the five investigated sites had ranges 9.11-18.7 As, 0.333-0.667 Cd, Co 10.8-14.5 Co, 68-122 Cr, Cu 34.0-62.1 Cu, 26591-34473 Fe, 29.9-52.8 Ni, 32.5-67.4 Pb and 99-189 Zn mg kg^{-1}. Cadmium, Pb and Zn soil concentrations were below the Vietnamese limit values for agricultural soils at all sites but the Zn and Pb soil concentration were above the European limit value at one and two sites, respectively. Arsenic and Cu soil concentrations were above the Vietnamese and European limit values at more sites. Nickel and Cr soil concentration above the Dutch target value were observed. Thallium concentrations ranged from 0.578 to 0.765 mg kg^{-1} d.w. which is a typical range for soils. There was no significant effect of wastewater use on elemental soil concentrations. Maximum mean concentration of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in water spinach were 0.139, 0.0320, 0.135, 2.01, 39.1, 57.3, 0.16, 0.189 and 6.01 mg kg^{-1} f.w., respectively. An adult Vietnamese person consuming 77.3 g water spinach per day will have an intake of As, Cd, Cu, Fe, Pb and Zn corresponding to 10.7, 5.2, 0.6, 7.9, 8.5 and 1.0 % of the maximum tolerable intake, respectively. Tl concentrations ranged from 0.0036 to 0.0353 mg kg^{-1} f.w. in water spinach, which is well below the suggested limit value. It is therefore assessed that the health risk due to water spinach consumption is low. There was no correlation between wastewater use and water spinach elemental concentrations. For Al, Cu, Tl and Zn there was a significant positive correlations between soil and water spinach concentrations.

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3 ARSENIC, CADMIUM AND LEAD IN FISH FROM POND PRODUCTION SYSTEMS IN HANOI, VIETNAM

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3.1 Introduction

Extensive production of fresh water fish with reuse of wastewater takes place in the peri-urban areas of major cities in Southeast Asia. Fish produced in wastewater constitute an important part of the daily stable food in many of these cities. It is well known that toxic elements may accumulate in fish grown in presence to these (Al-Yousuf et al., 2000; Licata et al., 2005) and there is concern that consumption of fish produced with wastewater constitutes a food safety problem.

Hanoi, the capital of Vietnam, covers an area of 1,000 km^2 and had a population of about 2.3 million persons in 2002 (Peoples Committee of Hanoi, 2003). Hanoi has nearly 400 medium and large scale industries and more than 14,000 small scale industries located in 14 industrial zones (Peoples Committee of Hanoi, 2003). The To Lich river system drains wastewater from Hanoi and consists of four connected rivers; To Lich, Kim Nguu, Set and Lu rivers and has a total length of 40 km. A total of 458,000 m^3 wastewater is generated daily in Hanoi of which 57% is of industrial origin, 41% of domestic origin and 2% is discharged from hospitals. About 4% of the industrial wastewater and 21% of hospital wastewater are subjected to some kind of treatment (Peoples Committee of Hanoi, 2003). The Hanoi drainage water and wastewater is pumped from the Kim Nguu and To Lich rivers to a system of connected canals and distributed to fish ponds and agricultural land located south of the capital in Thanh Tri district.

The specific aim of this study was to assess the concentration of As, Cd and Pb in fish from pond cultures, which have been using wastewater for over 13 years in peri-urban Hanoi.

3.2 Materials and methods

3.2.1 SAMPLING

Fish samples were collected in two polyculture pond production systems located in Thanh Tri district in Yen So and Hoang Liet communes, respectively. Wastewater is discharged to the To Lich and Kim Nguu rivers in peri-urban Hanoi and is actively pumped from the rivers into pond production systems. The Yen So pond production system receives wastewater from Kim Nguu River. It is pumped into to the lake four days a week for 24 hours. The Hoang Liet production system receives wastewater from the To Lich River; water is pumped into the lake three to four days a week. The ponds are both used for polyculture production.
The ponds were harvested with nets and 10 fish of marketable size of the three species common carp, silver carp and tilapia were randomly selected. Weight and length of the fish were recorded and they were desiccated into muscles, liver and skin. Muscles were subjected to ground in a

kitchen blender with titanium knife blades until a smooth mass was obtained and skin was cut into small pieces. Samples were stored in a freezer until further treatment.

3.2.2 DIGESTION AND ANALYSES

Digestion of fish tissue was carried out in 6 ml conc. HNO_3 and 2 ml H_2O_2 using a closed Teflon vessel microwave assisted system (Speedwave MW3+, Berghof). Quality assurance was carried out by including analytical blanks and standard reference materials (SRM) in every digestion; these were prepared in the same manner as the samples.

Determination of arsenic, cadmium and lead in fish digestions was carried out by graphite furnace atomic absorption spectrometry (GFAAS) using Zeeman-effect background correction (Perkin Elmer 5100 AAS, HGA-600 graphite furnace)

3.3 Results and discussion

Concentrations of arsenic, cadmium and lead in fish tissue from fish grown in Yen So and Hoang Liet are presented in Table 3-1 and Table 3-2, respectively. Concentrations of arsenic in fish were generally low. In Yen So arsenic concentrations all in mg kg^{-1} fresh weight (f.w.) ranged from <0.011-0.120 in muscle, <0.011-0.105 in liver and <0.011-0.020 in skin for common carp. For silver carp the following concentrations were observed: <0.011-0.064, <0.011-0.106 and <0.011-0.020 mg kg^{-1} f.w. in muscle, liver and skin, respectively. In tilapia similar concentrations were observed with <0.011-0.042 and <0.011-0.104 mg kg^{-1} f.w. in muscle and liver, respectively. However, skin of tilapia had higher arsenic concentrations compared to common carp and silver carp with a range of <0.011-0.216 mg kg^{-1} f.w. The same pattern with relatively high arsenic accumulation in tilapia skin was observed for fish from Hoang Liet. The fish from Hoang Liet all had liver arsenic concentration below the detection limit.

The EU has established threshold values for Cd, Pb and Hg in fish for human consumption in the Commission Regulation (EC) No. 211/2002. The threshold values are 0.05 and 0.2 mg kg^{-1} f.w. for cadmium and lead, respectively. Concentrations of cadmium and lead were below the threshold values for all fish tissues from Yen So except in liver from tilapia. The same was seen for the fish from Hoang Liet, but one sample of liver from common carp also had cadmium concentrations above the threshold limit value. The cadmium concentrations in tilapia liver ranged from 0.077-0.233 mg kg^{-1} f.w. in Yen So and 0.117-0.841 mg kg^{-1} f.w. in Hoang Liet. All tilapia liver samples are above the EU threshold value and this tissue should not be eaten. Concentration of lead in liver from tilapia ranged from 0.037-0.231 in Yen So with a mean value of 0.162 mg kg^{-1} f.w. For tilapia liver from Hoang Liet the lead concentration ranged from 0.154-0.681 mg kg^{-1} f.w. and the mean concentration was 0.447 mg kg^{-1} f.w. The mean lead concentration in tilapia liver from Yen So are below the threshold value, but consumption of tilapia liver from Yen So cannot be recommended since four of the 10 samples had concentrations above the threshold value. The mean lead concentration in tilapia liver from Hoang Liet is 2 times higher than the threshold value.

Table 3-1 Concentration of arsenic, cadmium and lead (mg kg-1 fresh weight) in tissue from fish grown in Yen So, Hanoi.

		Common carp			Silver carp			Tilapia		
Tissue	**Sample**	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**
Muscle	**1**	0.021	0.007	<0.033	0.041	<0.005	<0.033	<0.011	<0.005	<0.033
	2	0.025	0.009	<0.033	<0.011	<0.005	<0.033	0.032	0.012	0.067
	3	0.029	0.011	0.043	0.024	<0.005	<0.033	<0.011	<0.005	<0.033
	4	0.120	<0.005	<0.033	<0.011	<0.005	<0.033	0.042	0.009	<0.033
	5	0.104	0.051	0.035	0.043	<0.005	0.025	<0.011	<0.005	<0.033
	6	<0.011	0.013	<0.033	<0.011	0.019	<0.033	<0.011	<0.005	<0.033
	7	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.016	0.010	<0.033
	8	0.036	0.007	<0.033	0.064	0.009	<0.033	<0.011	<0.005	<0.033
	9	0.044	0.009	<0.033	0.047	0.011	<0.033	0.013	<0.005	<0.033
	10	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.036	0.010	<0.033
	Range	<0.011-0.120	<0.005-0.051	<0.033-0.043	<0.011-0.064	<0.005-0.019	<0.033-0.025	<0.011-0.042	<0.005-0.012	<0.033-0.067
Liver	**1**	0.062	0.022	<0.033	<0.011	0.012	<0.033	<0.011	0.199	0.231
	2	<0.011	0.006	0.065	<0.011	0.006	0.065	0.056	0.215	0.215
	3	0.044	0.028	<0.033	0.034	0.008	<0.033	<0.011	0.176	0.189
	4	0.052	<0.005	<0.033	0.050	<0.005	<0.033	0.078	0.170	0.199
	5	0.074	0.055	0.073	0.077	0.015	0.073	<0.011	0.104	0.104
	6	0.061	<0.005	<0.033	0.059	<0.005	<0.033	0.104	0.233	0.213
	7	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	<0.011	0.077	0.037
	8	0.024	<0.005	0.052	0.024	<0.005	0.052	<0.011	0.070	0.113
	9	<0.011	0.008	<0.033	<0.011	0.008	<0.033	0.076	0.210	0.223
	10	0.105	0.009	<0.033	0.106	0.009	<0.033	0.044	0.092	0.098
	Range	<0.011-0.105	<0.005-0.055	<0.033-0.073	<0.011-0.106	<0.005-0.015	<0.033-0.073	<0.011-0.104	0.077-0.233	0.037-0.231
Skin	**1**	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.023	0.012	<0.033
	2	<0.011	0.008	<0.033	<0.011	0.008	<0.033	0.103	0.019	<0.033
	3	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.019	0.009	<0.033
	4	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.204	0.022	0.057
	5	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.030	0.010	<0.033
	6	0.019	<0.005	<0.033	0.019	<0.005	<0.033	0.174	0.012	<0.033
	7	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.216	0.017	<0.033
	8	<0.011	<0.005	0.048	<0.011	<0.005	0.054	<0.011	<0.005	<0.033
	9	0.020	<0.005	<0.033	0.020	<0.005	<0.033	0.145	0.015	<0.033
	10	0.014	<0.005	<0.033	0.014	<0.005	<0.033	0.130	0.013	<0.033
	Range	<0.011-0.020	<0.005-0.008	<0.033-0.048	<0.011-0.020	<0.005-0.008	<0.033-0.054	<0.011-0.216	<0.005-0.022	<0.033-0.057

Table 3-2. Concentration of arsenic, cadmium and lead (mg kg-1 fresh weight) in tissue from fish grown in Hoang Liet, Hanoi.

		Common carp			Silver carp			Tilapia		
Tissue	**Sample**	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**
Muscle	**1**	<0.011	0.079	<0.033	<0.011	0.016	<0.033	<0.011	0.024	0.045
	2	<0.011	0.021	<0.033	<0.011	0.019	0.042	<0.011	0.019	0.067
	3	0.022	0.046	<0.033	0.024	0.021	<0.033	<0.011	0.035	0.039
	4	<0.011	0.033	<0.033	<0.011	0.013	<0.033	<0.011	0.019	0.047
	5	<0.011	0.019	<0.033	<0.011	0.11	<0.033	<0.011	<0.005	<0.033
	6	<0.011	0.013	<0.033	<0.011	0.013	<0.033	<0.011	0.027	0.056
	7	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033
	8	<0.011	0.007	<0.033	<0.011	0.015	0.045	<0.011	0.016	0.082
	9	0.029	0.009	<0.033	<0.011	0.011	<0.033	<0.011	0.015	<0.033
	10	<0.011	<0.005	<0.033	<0.011	0.009	<0.033	<0.011	<0.005	<0.033
	Range	<0.011-0.029	<0.005-0.079	<0.033	<0.011-0.024	<0.005-0.021	<0.033-0.045	<0.011	<0.005-0.035	<0.033-0.082
Liver	**1**	<0.011	0.019	0.067	<0.011	0.014	<0.033	<0.011	0.734	0.487
	2	<0.011	0.023	0.231	<0.011	0.016	0.038	<0.011	0.341	0.201
	3	<0.011	0.016	0.213	<0.011	0.013	<0.033	<0.011	0.685	0.478
	4	<0.011	0.013	0.054	<0.011	0.011	<0.033	<0.011	0.721	0.681
	5	<0.011	0.016	0.057	<0.011	0.017	<0.033	<0.011	0.543	0.571
	6	<0.011	0.025	0.097	<0.011	0.015	<0.033	<0.011	0.697	0.531
	7	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	<0.011	0.717	0.571
	8	<0.011	0.019	0.251	<0.011	0.041	<0.033	<0.011	0.841	0.613
	9	<0.011	0.024	0.198	<0.011	0.026	<0.033	<0.011	0.368	0.179
	10	<0.011	0.011	<0.033	<0.011	<0.005	<0.033	<0.011	0.117	0.154
	Range	<0.011	<0.005-0.024	<0.033-0.231	<0.011	<0.005-0.041	<0.033-0.038	<0.011	0.117-0.841	0.154-0.681
Skin	**1**	<0.011	0.011	<0.033	<0.011	<0.005	<0.033	<0.011	0.009	<0.033
	2	0.019	0.009	<0.033	0.023	<0.005	<0.033	0.342	0.011	<0.033
	3	<0.011	0.012	<0.033	<0.011	<0.005	<0.033	0.241	0.007	<0.033
	4	<0.011	0.017	0.121	<0.011	0.011	0.045	0.124	0.013	0.049
	5	<0.011	0.016	0.118	<0.011	0.015	0.061	<0.011	0.014	<0.033
	6	<0.011	0.013	<0.033	<0.011	0.014		0.235	0.012	<0.033
	7	0.023	<0.005	<0.033	<0.011	<0.005	<0.033	0.156	<0.005	<0.033
	8	<0.011	0.006	<0.033	<0.011	0.009	<0.033	0.278	0.014	<0.033
	9	0.026	0.011	0.057	0.027	0.007	0.039	0.076	0.013	<0.033
	10	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	0.053	0.013	<0.033
	Range	<0.011-0.026	<0.005-0.017	<0.033-0.121	<0.011-0.027	<0.005-0.015	<0.033-0.061	<0.011-0.342	<0.005-0.014	<0.033-0.049

3.4 Conclusion

Consumption of common carp, silver carp and tilapia produced in Thanh Tri district south of Hanoi with use of water from the wastewater exposed rivers Kim Nguu and To Lich rivers does not constitute a food safety problem with respect to cadmium and lead concentration. However, liver from tilapia should not be consumed since all samples from this tissue had cadmium concentration above the EU threshold value of 0.05 mg kg^{-1} f.w. and most samples had lead concentrations above the threshold value of 0.02 mg kg^{-1} f.w.

The highest arsenic concentrations were observed in skin from tilapia and in this tissue the concentration ranged from <0.011-0.342 mg kg^{-1} f.w.

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4 TOXIC ELEMENTAL CONTENT IN WATER SPINACH, FISH AND SEDIMENT FROM A WETLAND PRODUCTION SYSTEM RECEIVING WASTEWATER IN PHNOM PENH, CAMBODIA

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4.1 Introduction

Extensive production of water spinach (*Ipomoea aquatica* Forssk.) and fishing for human consumption takes place in the highly wastewater exposed Boeng Cheung Ek lake situated west of Phnom Penh city.

Phnom Penh is the capital of Cambodia it covers an area of 375 km^2 and had a population of 1,250,000 people in 2003 and a demographic growth of 4.14 % per year (Phnom Penh Municipality, 2004). About 750 factories and 4,000 domestic workshops are located in Phnom Penh (Takahashi et al., 2002). 31 % of Phnom Penh's gross domestic product (GDP) comes from the textile industry (Phnom Penh Municipality, 2004). Domestic and industrial wastewater from Phnom Penh together with storm water is drained out of the city through a sewage system consisting of old sewer pipes and open canals. About 10 % of the city's wastewater is discharged directly to the Bassac River the rest is discharged to a wetland area. Boeng Cheung Ek lake covers about 80 % of this wetland area the rest consist of agricultural low land (Muong, 2004). There is no sewage treatment plant in Phnom Penh and all domestic wastewater is discharged non-treated (Takahashi et al., 2002). It was estimated that a total of 55,600 m^3 domestic wastewater and about 1 million m^3 storm water is discharged to these wetlands every day (Muong, 2003). The majority of the industries located in Phnom Penh were constructed in the 1950s and 1960s without wastewater treatment facilities but also most new industries have been constructed without such facilities (Takahashi et al., 2002). Non-treated industrial wastewater is discharged directly to wastewater canals, rivers and lakes (JICA, 1999). More than 3,000 industrial firms are located in the Boeng Cheung Ek lake catchment area including battery fabric and repair, paint manufacture, zinc and metal products, pulp and paper, textiles, plastics, etc. (Muong, 2004). The estimated discharge of industrial wastewater in 2002 was 4.6 million m^3 based on water consumption (MOE, 2003).

The Boeng Cheung Ek lake covers an area of about 2,000 ha in the rainy season and decrease to 1,300 in the dry season (Muong, 2000). There are two main wastewater inlets to Boeng Cheung Ek lake with operating pumping stations both located at the north bank of the lake; Boeng Trabek pumping station and Tompun pumping station both constructed in 1960 (JICA, 1999). During the years of operations the pumping stations have received new pumps to keep up with the demand for pumping and in 2004 a new pumping station with improved wastewater canals replaced the old Tompun pumping station (JICA, 1999). The lake has one main outlet to the southeast running into the Prek Thnaot river through the Steung Chrow stream.

Water spinach for human consumption is extensively cultivated in the in the north end of the Boeng Cheung Ek lake at the wastewater inlets. Muong (2004) found that 205 farmers are involved in the production that and the estimated production area was of 100 ha. In Boeng Cheung Ek lake water spinach is cultivated free floating or rooted in the sediment at low water level. In the dry season water spinach is also grown on dry sediment. The upper part of the stem with foliage is harvested, bundled and sold for human consumption. Re-growth occurs and plants can be harvested again after three to four weeks. Other plant parts or older plants are fed to life stock.

Concerns have been raised that potentially toxic elements (PTE) may accumulate in the Boeng Cheung Ek lake and that toxic metal concentrations in water spinach may exceed safe levels for food due to the discharge of household and industrial wastewater (Muong, 2004). Plants may accumulate both essential and non-essential elements at concentrations which are not phytotoxic but constitute a health risk for consumers (Kabata-Pendias and Pendias, 2001). Elevated concentrations of PTEs have been found in plants grown in wastewater polluted environments (Kisku at al., 2000; Mehar et al., 2000; Nan and Cheng, 2001). However, the ability of plants to accumulate different PTEs varies between plant species and is element specific (Mehar et al., 2000; Kabata-Pendias and Pendias, 2001).

Wild living fish are caught in the Boeng Cheung Ek lake and they make up a part of the daily stable food for people living around the lake or are sold at the marked. A wide variety of fish are living in the lake including walking catfish, spineless eel and snakehead. It is well known that toxic metals may accumulate in fish cultivated in polluted environments (Al-Yousuf et al., 2000; Licata et al., 2005).

The specific aim of this study was to assess the concentration of PTEs in water spinach, fish and sediment from Boeng Cheung Ek which have received wastewater from Phnom Penh since the 1960's. Concentrations were determined in plants and sediment in a gradient from the point of wastewater discharges to the lake outlet.

4.2 Materials and methods

4.2.1 SAMPLING SITES

Sampling sites for water spinach and sediment are described in Table 4-1. Fish were caught with net or cages in the north part of the lake.

Table 4-1 Water spinach and sediment sampling sites in Phnom Penh.

Site	Description
1.1	Tompun pumping station in Boeng Cheung lake
1.2	50 m from the Tompun pumping station in Boeng Cheung lake
1.3	100 m from the Tompun pumping station in Boeng Cheung lake
1.4	200 m from the Tompun pumping station in Boeng Cheung lake
1.5	400 m from the Tompun pumping station in Boeng Cheung lake
1.6	600 m from the Tompun pumping station in Boeng Cheung lake
2.1	Trabek pumping station in Boeng Cheung lake
2.2	50 m fr0m Trabek pumping station in Boeng Cheung lake
2.3	100 m from Trabek pumping station in Boeng Cheung lake
2.4	200 m from Trabek pumping station in Boeng Cheung lake
2.5	400 m from Trabek pumping in Boeng Cheung lake
2.6	600 m from Trabek pumping in Boeng Cheung lake
3.1	East in Boeng Cheung lake 1, point with direct discharge from industries
3.2	East in Boeng Cheung lake 2, point with direct discharge from industries
4	Outlet of Boeng Cheung lake

5	Control point not receiving wastewater located Cheung Ek village
6.1	Canal leading wastewater to Tompun pumping station, 0.1 km from the lake inlet
6.2	Canal leading wastewater to Tompun pumping station, 0.3 km from the lake inlet
6.3	Canal leading wastewater to Tompun pumping station, 0.6 km from the lake inlet
6.4	Canal leading wastewater to Tompun pumping station, 1.6 km from the lake inlet
6.5	Canal leading wastewater to Tompun pumping station, 1.9 km from the lake inlet
6.6	Canal leading wastewater to Tompun pumping station, 2.4 km from the lake inlet

4.2.2 SAMPLE COLLECTION

Water spinach samples ready for harvest were collected as composite samples with four replicates. All sampling was carried out according to the sampling principles by Gy (1999). An area of about 16 m^2 was selected for collection of each sample; it was divided into four smaller plots of about 4 m^2 for collection of replicates. Each replicate sample consisted of the upper 30 cm of a total of 25 plants complete with stem and foliage. Five plants were collected in the centre and five in each of the four corners of the 4 m^2 plots. Fresh weight (f.w.) was determined. To remove surface contamination adhering from dust or attached soil the water spinach samples were washed by dipping them 20 times in three sets of distilled water. Samples were oven dried for seven days at 45 ºC and dry weights (d.w.) were determined. Samples were subjected to being ground in a kitchen blender with titanium knife blades until a fine powder was obtained.

Soil samples were collected as composite samples with four replicates in the same area as water spinach. Each replicate was made up of five sub samples collected as the upper 10 cm soil with a KC Kajak core sediment sampler (KC Denmark) with a diameter of 60/52mm; as for water spinach, sub samples were collected in the centre and each of the four corners of the 4 m^2 plot. Sub samples were mixed and one fifth of the sample mass was extracted by representative mass reduction. The soil samples were dried at 45 ºC for seven days and ground in an agate mortar.

4.2.3 DIGESTION

Digestion of water spinach and sediment samples was carried out using a modified version of the EPA (Environmental Protection Agency, USA) Method 3052 (USEPA, 1996). 0.25 g fish samples were digested in 6 ml conc. HNO_3 and 2 ml H_2O_2. A closed Teflon vessel microwave assisted system was applied in the digestion procedure.

4.2.4 ANALYSES

Determination of selected toxic elements was carried out by graphite furnace atomic absorption spectrometry (GFAAS) using Zeeman-effect background correction (Perkin Elmer 5100 AAS, HGA-600 graphite furnace) and inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent 7500C, Agilent Technologies, Manchester, UK) equipped with an octopole reaction system (ORS).

4.2.5 STATISTICS

Data analyses were carried out as linear regression analyses using the procedure General Linear Models (GLM) in the statistical computer program SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA).

4.3 Results and discussion

4.3.1 TOXIC ELEMENTAL CONTENT IN SEDIMENT

The content of the determined elements in soil samples and the related statistics are summarised in Table 4-2. There was no significant difference in the arsenic concentration between the different sites in the Boeng Cheung Ek lake and the wastewater canal. This indicates that the observed arsenic concentrations are due to inherent high concentrations in the parent material. Cadmium concentrations decreased with increasing distance from the wastewater discharge. The highest cadmium concentrations were observed in the wastewater canal transporting wastewater to the Tompun pumping station (sites 6.4, 6.5, 6.6) and at the Trabek pumping station (site 2.1). Cadmium concentrations are elevated at the Tompun pumping station and to a distance of 100 m from the pumping station compared to further away where cadmium could not be detected in the sediment. This indicated that the sediments of Boeng Cheung Ek lake are slightly polluted with cadmium by the discharge of wastewater. The European limit value for cadmium in agricultural soil is 1 mg kg^{-1} d.w. (CEC, 1986) since all cadmium sediment concentrations found were below this level the lake is not considered unfit for agricultural production. At the sites with direct industrial discharge of wastewater into the lake (sites 3.1 and 3.2) cadmium concentrations were below the detection limit and there is no indication of cadmium pollution from industries located on the east bank of the lake.

Table 4-2 Mean and standard deviation of As, Cd and Pb sediment concentrations in mg kg^{-1} d.w.

Site	As ^{ns}	Cd ***	Pb ***
1.1	10.1±3.2 ^{a}	0.24±0.08 ^{c}	45±3.4 ^{c}
1.2	10.2±2.3 ^{a}	0.21±0.05 ^{c}	45±3.7 ^{c}
1.3	8.9±3.4 ^{a}	0.23±0.04 ^{c}	38±4.1 ^{c}
1.4	11.2±3.2 ^{a}	<0.14 ^{d}	37±2.9 ^{c}
1.5	10.7±2.5 ^{a}	<0.14 ^{d}	38±4.1 ^{c}
1.6	10.8±3.6 ^{a}	<0.14 ^{d}	39±7.3 ^{c}
2.1	13.1±2.9 ^{a}	0.83±0.09 ^{a}	99±3.6 ^{a}
2.2	10.5±3.4 ^{a}	0.31±0.010 ^{c}	56±7.1 ^{b}
2.3	13.3±1.7 ^{a}	0.35±0.09 ^{c}	50±6.2 ^{b,c}
2.4	8.9±2.3 ^{a}	0.25±0.05 ^{c}	44±4.5 ^{c}
2.5	11.2±2.5 ^{a}	<0.14 ^{d}	37±3.2 ^{d}
2.6	11.7±1.2 ^{a}	<0.14 ^{d}	38±4.5 ^{c,d}
3.1	13.3±3.6 ^{a}	<0.14 ^{d}	46±7.2 ^{c}
3.2	12.6±5.1 ^{a}	<0.14 ^{d}	45±5.1 ^{c}
4	14.1±2.6 ^{a}	<0.14 ^{d}	30±2.8 ^{e}
5	<0.81 ^{b}	<0.14 ^{d}	18±2.9 ^{e}
6.1	9.4±2.4 ^{a}	0.30±0.10 ^{c}	46±4.1 ^{c}
6.2	10.1±4.2 ^{a}	0.29±0.07 ^{c}	50±5.6 ^{c}
6.3	14.2±4.3 ^{a}	0.32±0.06 ^{c}	60±3.2 ^{b}
6.4	12.3±2.3 ^{a}	0.64±0.10 ^{a,b}	111±15 ^{a}
6.5	9.3±3.4 ^{a}	0.66±0.07 ^{a,b}	118±13 ^{a}
6.6	13.1±3.4 ^{a}	0.51±0.09 ^{b}	120±20 ^{a}

^{ns} non-significant; *** significant at 0.1 % significance level. There is no significant difference between mean concentrations followed the same letter. ^{a} indicate the highest Sc correct values, ^{b} the next highest and so on.

The highest lead concentrations were observed in the wastewater canal transporting wastewater to the Tompun pumping station (sites 6.4, 6.5, 6.6) and at the Trabek pumping station (site 2.1) as also seen for cadmium concentrations. The lead concentrations in sediment from the pumping stations to a distance of 600 m are elevated compared to the concentration at the lake outlet and the non wastewater exposed control point (sites 4 and 5). This shows that the lake is being polluted with lead from the wastewater discharge. According to European limit value lead in agricultural soils should not exceed 50 mg kg^{-1} d.w. Concentrations above this value are found at the Trabek pumping station and 100 m away from the pumping station (sites 2.1 to 2.3). It can be concluded that the lake is slightly polluted with lead however natural attenuation processes result in fixation of lead close to the source of discharge and lead is therefore not found in highly elevated concentrations throughout the wetland system. However, production of water spinach in the area around the Trabek pumping station could be of concern.

4.3.2 TOXIC ELEMENTAL CONTENT IN WATER SPINACH

The content of the determined elements in water spinach samples and the results of the statistical data analyses are summarised in Table 4-3.

Table 4-3. Mean and standard deviation (n=4) for elemental concentration (mg kg^{-1} f.w.) in water spinach.

Site	As ^{ns}	Cd***	Cr ***	Cu*	Mn ^{ns}
1.1	0.184±0.034	0.0181±0.0014 ^{b}	0.439±0.202 ^{a,b}	1.28±0.10 ^{b}	27.1±10.9
1.2	0.193±0.010	0.0103±0.0006 ^{c}	0.273±0.090 ^{b}	0.937±0.643 ^{b}	27.8±6.1
1.3	0.188±0.014	0.0114±0.0009 ^{c}	0.156±0.010 ^{b,c}	1.21±0.20 ^{b}	29.8±6.5
1.4	0.184±0.003	0.0124±0.0001 ^{c}	0.132±0.002 ^{c}	1.29±0.32 ^{b}	30.4±3.1
1.5	0.178±0.044	0.0124±0.0024 ^{c}	0.134±0.024 ^{c}	1.28±0.41 ^{b}	30.8±3.5
1.6	0.147±0.044	0.0122±0.0033 ^{c}	0.128±0.034 ^{c}	1.46±0.43 ^{b}	35.3±4.8
2.1	0.147±0.045	0.0224±0.0024 ^{a}	0.460±0.035 ^{a}	2.95±0.54 ^{a}	42.4±14.8
2.2	0.140±0.064	0.0197±0.0009 ^{a}	0.251±0.035 ^{b}	1.48±0.08 ^{b}	43.0±10.7
2.3	0.148±0.044	0.0199±0.0033 ^{a,b}	0.187±0.031 ^{b,c}	1.30±0.31 ^{b}	31.9±6.6
2.4	0.101±0.017	0.0033±0.003 ^{c}	0.157±0.033 ^{c}	1.19±0.19 ^{b}	26.4±6.5
2.5	0.108±0.047	0.0049±0.0023 ^{d}	0.151±0.053 ^{c}	1.46±0.33 ^{b}	29.9±5.9
2.6	0.158±0.054	0.0053±0.0011 ^{d}	0.131±0.022 ^{c}	1.23±0.42 ^{b}	36.7±3.7
3.1	0.118±0.053	0.0071±0.0013 ^{c}	0.156±0.023 ^{c}	1.49±0.42 ^{b}	39.9±7.0
3.2	0.152±0.044	0.0063±0.0021 ^{c}	0.139±0.022 ^{c}	1.50±0.39 ^{b}	39.7±5.9
4	0.168±0.034	0.0068±0.0038 ^{c,d}	0.134±0.066 ^{c}	1.25±0.40 ^{b}	36.9±6.7
5	0.148±0.047	0.0074±0.0018 ^{c}	0.129±0.039 ^{c}	1.35±0.22 ^{b}	30.7±4.0

Site	Ni*	Pb ***	Tl ^{ns}	Zn **
1.1	0.274±0.155 ^{a,b}	0.177±0.022 ^{a}	0.0128±0.0011	4.91±1.74 ^{c,d}
1.2	0.181±0.069 ^{b}	0.133±0.013 ^{b}	0.0139±0.0018	4.43±1.01 ^{d}
1.3	0.159±0.066 ^{b}	0.067±0.015 ^{c}	0.0114±0.0050	4.33±1.02 ^{d}
1.4	0.109±0.026 ^{c}	0.059±0.018 ^{c}	0.0119±0.0004	4.71±0.28 ^{d}
1.5	0.131±0.035 ^{c}	0.056±0.015 ^{c}	0.0134±0.0025	4.81±0.38 ^{d}
1.6	0.127±0.045 ^{c}	0.056±0.023 ^{c}	0.0132±0.0074	5.08±1.14 ^{d}
2.1	0.375±0.051 ^{a}	0.206±0.023 ^{a}	0.0132±0.0074	9.08±1.14 ^{a}
2.2	0.412±0.077 ^{a}	0.198±0.025 ^{a}	0.0083±0.0040	8.33±1.93 ^{a,b}
2.3	0.209±0.015 ^{b}	0.166±0.015 ^{a}	0.0114±0.0055	6.35±0.91 ^{b,c}
2.4	0.112±0.011 ^{c}	0.072±0.005 ^{c}	0.0120±0.0006	3.48±0.46 ^{d}
2.5	0.109±0.023 ^{b}	0.076±0.015 ^{c}	0.0134±0.0046	3.61±0.66 ^{d}
2.6	0.145±0.071 ^{b,c}	0.069±0.025 ^{c}	0.0122±0.0035	4.03±1.01 ^{d}
3.1	0.203±0.047 ^{b}	0.079±0.023 ^{c}	0.0134±0.0024	4.61±0.56 ^{d}
3.2	0.189±0.074 ^{b,c}	0.080±0.013 ^{c}	0.0122±0.0035	4.55±1.09 ^{c,d}
4	0.155±0.089 ^{b,c}	0.057±0.028 ^{c}	0.0124±0.0045	4.34±1.69 ^{c,d}
5	0.132±0.059 ^{c}	0.064±0.018 ^{c}	0.0135±0.0043	3.39±1.39 ^{d}

[ns] non-significant; *** significant at 0.1 % significance level. There is no significant difference between mean concentrations followed the same letter. [a] indicate the highest Sc correct values, [b] the next highest and so on.

There is no significant difference between the arsenic, manganese and thallium concentrations in water spinach at the different sites. For cadmium, chromium, copper, nickel, lead and zinc difference in plant concentrations were observed between sites. For copper, water spinach grown at Trabek pumping station had significantly higher concentrations than for plants grown at all other sites.
Zinc concentrations were also elevated in plants grown in the Trabek pumping station area compared to the other sites. Cadmium, chromium, nickel and lead concentration were elevated in plants grown in front of both pumping stations compared to the other production sites.

The maximum mean concentration of As, Cd, Cr, Cu, Mn, Ni, Pb and Zn observed for the production sites were 0.193, 0.022, 0.460, 2.95, 43.0, 0.41, 0.206 and 9.08 mg kg^{-1} f.w., respectively. FAO/WHO (2003) standards for the provisional tolerable intake (PTI) of As, Cd, Cu, Pb and Zn are set to 0.0021, 0.001, 0.5, 0.0036 and 1.0 mg kg^{-1} body weight day^{-1}, respectively. Assuming that a person with a mean body weight of 50 kg consumes 125 g f.w. water spinach a day, and conservatively assuming that the average concentration levels of the investigated elements correspond to the highest reported mean concentrations, the intake of As, Cd, Cu, Pb and Zn will correspond to 24.1, 5.5, 1.5, 14.3 and 2.3 % of the maximum tolerable intake, respectively. As the estimated exposures do not exceed its safety limits, it is considered that no more refined estimations of the intake are necessary. Furthermore, it is assessed that the health risk related to the dietary exposure to As, Cd, Cu, Fe, Pb and Zn from water spinach consumption is low.

The maximum mean thallium concentration in water spinach was 0.0139. A limit value ranging from 0.25-0.50 µg/g f.w. for Tl in foods have been proposed (Grössmann, 1984) and it is unlikely that the consumption of water spinach produced in the lake is associated with any health risk with respect to Tl.

4.3.3 TOXIC ELEMENTAL CONTENT IN FISH

The content of the determined elements in different fish tissues are shown in Table 4-4. Generally concentrations are low for most tissues below or near the detection limit. Arsenic, cadmium and lead does not seem to accumulate in the fish analysed except for walking catfish, with this species showing a high accumulation of all three metals in skin.

Threshold values for Cd and Pb in fish for consumption are set to 0.05 and 0.2 in the Commission Regulation (EC) No. 211/2002, respectively. Only the skin of walking catfish has concentrations above these threshold values. Cadmium concentrations up to nine times the threshold value were observed and the highest lead concentration in skin from walking catfish was two times the threshold value. Skin of walking catfish should not be used for cooking.

Table 4-4. As, Cd and Pb concentrations (mg kg-1 f.w.) in muscle, liver and skin of three fish species from Boeng Cheung Ek lake.

		Spineless eel			Walking Catfish			Snakehead		
Tissue	**Sample**	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**
Muscle	1	<0.011	0.012	0.047	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033
	2	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033

	3	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033
	4	<0.011	0.017	<0.033	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033
	5	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033
	6	<0.011	0.014	<0.033	-	-	-	<0.011	<0.005	<0.033
	Range	<0.011	<0.005-0.017	<0.033-0.047	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033
Liver	**1**	<0.011	0.016	0.056	<0.011	<0.005	<0.033	<0.011	0.012	<0.033
	2	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033	<0.011	<0.005	<0.033
	3	<0.011	<0.005	<0.033	<0.011	0.009	<0.033	<0.011	0.014	<0.033
	4	<0.011	<0.005	0.039	<0.011	<0.005	<0.033	<0.011	0.016	<0.033
	5	<0.011	<0.005	<0.033	<0.011	0.013	0.052	<0.011	0.008	<0.033
	6	<0.011	0.013	<0.033	-	-	-	<0.011	0.011	<0.033
	Range	<0.011	<0.005-0.017	<0.033-0.056	<0.011	<0.005-0.013	<0.033-0.052	<0.011	<0.005-0.016	<0.033
Skin	**1**	<0.011	0.028	<0.033	2.89	0.247	0.051	<0.011	0.011	<0.033
	2	<0.011	<0.005	<0.033	10.4	0.456	0.371	<0.011	<0.005	0.043
	3	0.017	<0.005	<0.033	9.61	0.217	0.412	0.087	0.014	<0.033
	4	<0.011	<0.005	<0.033	17.3	0.371	0.362	0.057	0.025	<0.033
	5	<0.011	<0.005	<0.033	13.2	0.113	0.374	<0.011	<0.005	0.068
	6	<0.011	<0.005	<0.033	-	-	-	<0.011	<0.005	<0.033
	Range	<0.011-0.017	<0.005-0.028	<0.033	2.89-17.3	0.113-0.456	0.051-0.412	<0.011-0.087	<0.005-0.025	<0.033-0.068

4.4 Conclusion

A slight pollution of sediment with cadmium due to wastewater discharge was observed in the Tompun wastewater canal and in the area in front of the Trabek pumping station. The highest observed concentration was 0.83 mg kg^{-1} d.w. which is below the EU threshold value of 1.0 mg kg^{-1} d.w. for agricultural soils. As for cadmium the highest lead concentrations in sediment were observed in the Tompun wastewater canal and at the Trabek pumping station. Lead concentrations were elevated at the two pumping stations to a distance of 600 m compared to the concentration at the lake outlet and the control point. The highest lead concentration in the lake was 99 mg kg^{-1} d.w. well above the EU threshold value of 50 mg kg^{-1} d.w. Sediment concentrations of arsenic in the Boeng Cheung Ek lake were not affected by the discharge of wastewater.

Cadmium, chromium, copper, nickel, lead and zinc concentrations in water spinach were elevated at the Trabek pumping station and cadmium, chromium, nickel and lead concentrations were also elevated at the Tompun pumping station compared to the other production sites. A person with a body weight of 50 kg who consumes 125 g f.w. water spinach a day with the highest metal concentrations observed will have an As, Cd, Cu, Pb and Zn intake which corresponds to 24.1, 5.5, 1.5, 14.3 and 2.3 % of the maximum tolerable intake, respectively. Consumption of water spinach from Boeng Cheung Ek lake does not seem to constitute a food safety problem with regards to toxic elements.

Skin of walking catfish had cadmium and lead concentrations up to nine and two times the EU threshold value for fish and it should not be used for cooking.

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5 Microbiological quality of fish grown in wastewater-fed and non-wastewater-fed fishponds in Hanoi, Vietnam: influence of hygiene practices in local retail markets

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5.1 ABSTRACT

Mean water quality in two wastewater-fed ponds and one non-wastewater-fed pond in Hanoi, Vietnam was ~10^6 and ~10^4 presumptive thermotolerant coliforms (pThC) per 100 ml, respectively. Fish (common carp, silver carp and Nile tilapia) grown in these ponds were sampled at harvest and in local retail markets. Bacteriological examination of the fish sampled at harvest from both types of pond showed that they were of very good quality (2−3 pThC g^{-1} of flesh), despite the skin and gut contents being very contaminated (10^2−10^3 pThC g^{-1} and 10^4−10^6 pThC g^{-1}, respectively). These results show that there is a factor of ~3 orders of magnitude in the WHO guideline quality of ≤1000 faecal coliforms per 100 ml of fishpond water. However, when the fish from both types of pond were sampled at the point of retail sale, quality deteriorated to 10^2−10^5 pThC g^{-1} of chopped fish (mainly flesh and skin contaminated with gut contents); this was due to the practice of the local fishmongers in descaling and chopping up the fish from both types of pond with the same knife and on the same chopping block. Fishmonger education is required to improve their hygienic practices; this should be followed by regular hygiene inspections.
Key words │ Aquaculture, coliforms, enterococci, fish, hygiene, retail markets, Vietnam, wastewater

5.2 INTRODUCTION

Fish production in excreta-fertilized fishponds is a very ancient practice, especially in the Far East and notably China where the practice is believed to have been initiated over 3,000 years ago (Zhiwen 1999). In Vietnam wastewaters are used for aquaculture as a source of both water and nutrients (Vo 2001). The nutrients supports the growth of plankton and other micro-organisms which are consumed by the fish with little additional feeding taking place. In peri-urban Hanoi there are ~2,500 ha of aquaculture ponds, over 99 percent of which are used for fish culture, mainly carp and tilapia, with a small area (<1 percent) for shrimp production (Mai *et al*. 2004). Most of the wastewater-fed fishponds are located in Thanh Tri district in the south of the city, where there are ~330 ha of wastewater-fed fishponds (Vo & Edwards 2005); there is also widespread wastewater use for rice culture which is often alternated with fish production (Tran 2001).

In order to assure the microbiological safety of fish raised in wastewater-fed fishponds the World Health Organization's guideline is that the fishpond water should have a faecal coliform count of ≤1000 per 100 ml (WHO 1989); this guideline value is expected to be retained in the new guidelines which are currently being prepared (WHO, 2005). Various bodies have made recommendations for the microbiological quality of fish rather than the fishpond water. For example, the International Commission on Microbiological Specifications for Foods (1986) recommended an '*m*' value of 11 *E. coli* g^{-1} and an '*M*' value of 500 *E. coli* g^{-1} of uncooked fresh and frozen fish flesh, where *m* and *M* are defined as follows: if the *E. coli* count is <*m* the quality is 'satisfactory'; if it is >*M* it is 'unsatisfactory'; and, if no more than three out of five fish samples have values between *m* and *M*, it is 'acceptable'. In Vietnam the national standards are ≤100 *E. coli* g^{-1} of uncooked fresh and frozen fish flesh and ≤3 *E. coli* g^{-1} of cooked fish flesh (Ministry of Health 1998). A comprehensive review of, and the corresponding rationales for, microbiological criteria for safe fish are given in Institute of Medicine (2003).

Studies on the microbiological quality of fish raised in wastewater-fed fishponds are few with some studies indicating that faecal bacteria may penetrate the fish flesh when fish is grown in highly polluted water (Buras *et al*. 1985, 1987; Buras 1990), whereas other studies found no or little penetration of micro-organisms in aquaculture environments in which the fish were not stressed (Edwards 1992). Furthermore, the level of microbiological cross-contamination and quality of wastewater-fed fish sold to consumers at retail markets are unknown. In this paper we report the results of an investigation into the microbiological quality of fish from wastewater-fed and non-wastewater-fed fishponds in Thanh Tri district of Hanoi, both at harvest and at the point of sale in local retail markets.

5.3 Methods

5.3.1 Study locations and sampling

Fishponds. The study was carried out in two wastewater-fed ponds and one nominally non-wastewater-fed (control) pond in Yen So commune, Thanh Tri district (Figures 1 and 2). The areas of the wastewater-fed ponds were ~3 and ~15 ha and their liquid depths were ~1.5−2 m. Both ponds were fed with raw wastewater directly from the Kim Nguu river through a pumping station located in the commune; ponds also received direct discharges of domestic wastewater from households around the ponds. The Kim Nguu river is essentially a wastewater canal: CEETIA (1997) found it to be heavily polluted, with BOD and COD concentrations some 3−7 times higher than the Vietnamese permitted standard levels (≤50 mg BOD l^{-1} and ≤100 mg COD l^{-1} for wastewaters discharged into water bodies used for aquaculture and crop irrigation). Toan (2004) found thermotolerant coliform (ThC) numbers of 3×10^7 per 100 ml in the inlet of a fishpond in Yen So commune fed with water from the Kim Nguu river.

The control pond, with an area of ~14 ha and a depth of ~1.5−2 m, was located on the alluvial plain adjacent to the west bank of the Red River beyond a flood-control dyke. Red River water was used to feed the control pond as wastewater could not be economically pumped across the dyke. Before the control pond was selected for the study, samples of its contents were analysed for ThC numbers (details in Results section below).

In Yen So commune the most commonly cultured fish are common carp (*Cyprinus carpio*), silver carp (*Hypophthalamichthys molitrix*), and Nile tilapia (*Oreochromis niloticus)*. The growing season is ~10 months and at harvest common carp weigh ~500−600 g, silver carp ~200−300 g, and tilapia ~150−200 g. In this study, five individual fish of each of these three species were collected at ~7 a.m. immediately after they had been harvested from the wastewater-fed and non-wastewater-fed ponds. Each fish was placed in a sterile plastic bag. At the same time the fish samples were collected, grab samples of the fishpond water were collected from 15−20 cm below the surface in sterile 500-ml glass sampling bottles. The fish and fishpond water samples were then protected against heat and sunlight and transported to the laboratory within 30 minutes. Samples were kept at 4−5ºC upon arrival at the laboratory and analyzed within six hours of collection.

Figure 5-1 A local retail fish market in Yen So commune

Local retail fish markets. There are several retail markets within 4 km of Yen So commune to which fish are transported in bamboo baskets on bicycles or motorbikes early in the morning. At the market the fish are kept alive in small aerated basins filled with tap water (Figure 1); the same basin is used for fish from both wastewater-fed and non-wastewater-fed ponds. The fishmongers, who are usually women, generally sit on small wooden chairs close to the ground. They gut and clean the fish on small wooden chopping boards placed on the ground (Figure 2). Normally the scales are removed and the gut removed through a cut in the side of the fish. Carp are then chopped into pieces, placed in a polythene bag and sold. Tilapia are de-gutted and sold as whole fish after the scales have been removed. The same knife is used for all stages of fish processing. The fishmongers clean the chopping board only twice a day, generally at the end of the morning and afternoon trading sessions.

Figure 5-2 Processing of a carp at a local retail market in Yen So commune

The fish sampled at the markets were 'tracked' from the fishpond at harvesting and accompanied to the market, so that it was known which fish came from the wastewater-fed ponds or the non-wastewater pond. At the market whole fish were purchased and the fishmonger asked to process each fish in the normal way (i.e., to remove the scales and gut the fish, then chop it into pieces). Each fish processed in this way was then placed in a sterile plastic bag and taken immediately to the laboratory for analysis.

5.3.2 Microbiological examination

Fish sampled at harvest. Samples of the skin, muscle and intestinal tract of the whole fish samples were collected separately under aseptic conditions, as follows:

(a) skin samples were taken from a 10-cm^2 (2 × 5 cm) central area of the fish by marking out, using a sterile template and scalpel, the outline of the desired area and then removing, with sterile scalpel and forceps, as thin a layer of the skin as possible (1−2 mm); the skin sample was then placed in a sterile petri dish.

(b) flesh (muscle) samples were taken by first sterilizing the surface with a red-hot knife blade and then removing, with sterile scalpel and forceps, the flesh immediately below the singed surface so that a sample could be taken of the raw flesh below; each sample collected in this way weighed ~5 g.

(c) the whole intestinal tract of each fish was removed aseptically with sterile scalpel and forceps.

Similar sample types (skin, flesh or intestinal tract) from each of five fish of a single species (common carp, silver carp or tilapia) were removed, pooled, placed in a polyethylene bag to give a five-fish composite sample, which was then weighed. Nine times this weight of a solution of 0.1% peptone and 0.85% sodium chloride at ph 7.5 was added and this 1-in-10 dilution was then homogenized in a bagmixer model vw400 stomacher (interscience, st nom, france) for 30 seconds. This dilution was then used for microbiological analyses directly or diluted further, as described below.

Fish sampled at markets. A ~10-g sample of fish flesh was taken from one of the pieces of fish in each of five plastic bags containing the same fish species (common carp, silver carp or tilapia). These samples were then pooled in a polyethylene bag to give a five-fish composite sample which was then weighed. They were then diluted and homogenized, as described above.

Bacteriological analyses. Serial 1-in-10 dilutions to 10^{-7} were made of each fish or wastewater sample using the peptone-NaCl diluent. Bacteriological analyses for presumptive ThC, enterococci and aerobic standard plate counts were then carried out within 30 minutes using the procedures recommended by the Nordic Committee on Water and Food Analysis (Danish Standards Association 1999, 2001, 2002). Spread plates of membrane lauryl sulphate agar (MM0615 broth with 15 g L0011agar l^{-1}; Oxoid Ltd, Basingstoke, Hampshire, England) and Slanetz & Bartley agar (Oxoid CM0377) were used for presumptive ThC and enterococci, respectively, with incubation at $44^{\circ}C$ for 24 h (ThC) and 48 h (enterococci). Pour-plates of tryptone yeast extract agar (Oxoid CM1012 water plate count agar) were used for standard plate counts (SPC) following incubation at $37^{\circ}C$ for 48 h. After incubation colonies growing on the agar plates were enumerated and the counts of CFU per g of fish and CFU per100 ml of fishpond water determined.

Statistical analyses. The student *t* test was used to compare the geometric mean results from the wastewater-fed and the non- wastewater-fed ponds, and ANOVA for those from the three fish species. The data were analyzed in Excel 2003 (Microsoft Corp., Seattle, WA).

5.4 RESULTS

5.4.1 Fishpond water

The two wastewater-fed fishponds had significantly higher mean counts of presumptive ThC ($p<0.0001$) and enterococci ($p<0.001$) than the nominally non-wastewater-fed pond: two orders of magnitude higher for presumptive ThC and one order of magnitude higher for enterococci; there was no difference in the standard plate counts (Table 1). The ThC counts in the wastewater-fed ponds were nearly three orders of magnitude higher than the WHO (1989) guideline value of ≤1000 per 100 ml, whereas those in the nominally non-wastewater-fed pond were less than one order of magnitude above this guideline value.

Table 5-1 Numbers of faecal indicator bacteria and standard plate counts in wastewater-fed and non-wastewater-fed fishponds

Bacterial group	Wastewater-fed ponds[a]			Non-wastewater-fed pond			*p* (*t* test)[b]
	n[c]	Mean[d]	σ	n	Mean[c]	σ	
Presumptive thermotolerant coliforms	9	5.92	0.91	10	3.79	0.57	**0.0001**
Enterococci	7	4.41	0.68	10	3.43	0.47	**0.001**
Standard plate count	8	7.79	1.02	10	7.57	0.79	0.183

[a] There was no significant difference in the bacterial counts in the two wastewater-fed ponds (*t* test: $p>0.05$).
[b] Values in bold indicate significant differences.
[c] Number of water samples analysed.
[d] Log geometric mean bacterial numbers per 100 ml.

5.4.2 Fish sampled at harvest

There was no major significant differences (i.e., those important from a public health perspective) in bacteriological qualities between the skin, gut contents or flesh for the three fish species when comparing their origin from either wastewater-fed or non-wastewater-fed ponds (Tables 2−4). Comparison of bacterial numbers in skin samples from the three fish species revealed no significant differences, except in one case where skin samples from wastewater-fed silver carp had a higher SPC than non-wastewater-fed silver carp.

Fish from both wastewater-fed and non-wastewater-fed ponds contained similar bacterial numbers in their gut contents: 10^5–10^6 presumptive ThC g^{-1} and 10^3–10^5 enterococci g^{-1} (Table 3). Amongst the fish from the wastewater-fed ponds common carp contained significantly higher numbers of presumptive ThC and enterococci than silver carp and tilapia. Common carp are primarily bottom feeders and thus will be exposed to high bacterial numbers in pond sediment, whereas silver carp and tilapia primarily feed in the water column where bacterial concentrations are lower.

Fish flesh samples collected by the stringently aseptic technique contained no or very few faecal indicator bacteria, whereas the SPC were ~10^3 CFU g^{-1} (Table 4). No significant differences were found in bacterial numbers between fish from the wastewater-fed and the non-wastewater fed ponds. Thus the very limited penetration of faecal bacteria into the fish flesh came primarily from the fish gut.

Table 5-2 Numbers of faecal indicator bacteria and standard plate counts on the skin of fish collected immediately after harvest from wastewater-fed and non-wastewater-fed ponds

Bacterial group	Fish	Wastewater-fed ponds[a] (n[b] = 20)			Non-wastewater-fed pond (n = 18)			*p* (*t* test)[c]
		n	Mean[d]	σ	n	Mean	σ	
Presumptive thermotolerant coliforms	Common carp	6	2.53	0.83	6	2.46	0.90	0.44
	Silver carp	6	2.30	0.43	6	2.19	1.71	0.44
	Tilapia	8	2.95	1.10	6	3.27	1.18	0.69
P (ANOVA)			*0.38*			*0.35*		
Enterococci	Common carp	6	1.93	1.35	6	2.51	0.84	0.80
	Silver carp	6	2.07	1.48	6	1.88	1.29	0.40
	Tilapia	8	3.10	1.10	6	3.08	0.99	0.48
p (ANOVA)			*0.20*			*0.18*		
Standard plate counts	Common carp	6	5.35	0.82	6	5.01	0.44	0.20
	Silver carp	6	5.47	0.69	5	4.29	1.14	**0.03**
	Tilapia	8	5.48	1.15	4	5.50	0.61	0.51
p (ANOVA)			0.96			*0.10*		

[a] There was no significant difference in the bacterial counts on the skin of the fish harvested from the two wastewater-fed ponds (*t* test: $p > 0.05$).
[b] Number of skin samples analysed.
[c] Values in bold indicate significant differences.
[d] Log geometric mean bacterial numbers g^{-1}.

Table 5-3 Numbers of faecal indicator bacteria and standard plate count in the gut of fish collected immediately after harvest from wastewater-fed and non-wastewater-fed ponds.

Bacterial group	Fish	Wastewater-fed ponds[a] (n[b] = 20)			Non-wastewater-fed pond (n = 18)			p (t-test)[c]
		n	Mean[d]	σ	n	Mean	σ	
Presumptive thermotolerant coliforms	Common carp	6	5.33	1.12	6	6.19	0.85	0.91
	Silver carp	6	4.67	0.91	6	4.65	0.95	0.48
	Tilapia	8	5.17	1.07	6	4.62	1.47	0.21
p (ANOVA)[c]			**0.53**			**0.04**		
Enterococci	Common carp	6	3.75	1.01	6	5.11	1.35	0.96
	Silver carp	6	3.36	0.64	6	3.25	0.62	0.39
	Tilapia	8	3.42	0.60	6	2.57	0.90	**0.02**
p (ANOVA)			**0.63**			**0.001**		
Standard plate counts	Common carp	6	7.97	0.73	6	8.32	0.21	0.85
	Silver carp	6	7.42	0.48	6	7.85	0.75	0.86
	Tilapia	8	7.58	0.63	6	7.60	0.85	0.52
p (ANOVA)			0.30			*0.20*		

[a] There was no significant difference in the bacterial counts in the gut of the fish harvested from the two wastewater-fed ponds (*t* test: $p > 0.05$).
[b] Numbers of individual fish analysed.
[c] Values in bold indicate significant differences.
[d] Log geometric mean bacterial numbers g^{-1}.

Table 5-4 Numbers of faecal indicator bacteria and standard plate count in the flesh of fish collected immediately after harvest from wastewater-fed and non-wastewater-fed ponds

Bacterial group	Fish	Wastewater-fed ponds[a,b] (n[c] = 20)			Non-wastewater-fed pond (n = 18)			*p* (*t*-test)[d]
		n	Mean[e]	σ	n	Mean[c]	σ	
Presumptive thermotolerant coliforms	Common carp	6	0.41	0.28	6	0.30	0	0.17
	Silver carp	6	0.30	0	6	0.30	0	–
	Tilapia	8	0.30	0	6	0.41	0.28	0.86
p (ANOVA)[d]			***0.32***			***0.39***		
Enterococci	Common carp	6	0.41	0.28	6	0.30	0	0.17
	Silver carp	6	0.51	0.53	6	0.30	0	0.17
	Tilapia	8	0.30	0	6	0.41	0.28	0.86
p (ANOVA)			*0.48*			*0.39*		
Standard plate count	Common carp	6	3.03	0.94	6	3.13	0.97	0.57
	Silver carp	6	3.40	1.20	6	2.65	0.55	0.09
	Tilapia	8	2.72	0.46	6	3.88	0.78	0.99
p (ANOVA)			**0.38**			**0.03**		

[a] There was no significant difference in the bacterial counts in the flesh of the fish harvested from the two wastewater-fed ponds (*t* test: $p > 0.05$).

[b] Only three of the 20 fish examined had measurable numbers of presumptive ThC and enterococci per g of flesh (zero colony formation was recorded as <2 g^{-1}).
[c] Numbers of individual fish analysed.
[d] Values in bold indicate significant differences.
[e] Log geometric mean bacterial numbers g^{-1}.

5.4.3 Fish sampled at point of retail sale

The bacteriological qualities of all three fish species from both types of fishpond were substantially worse after handling, cleaning and purchase in the local retail markets than that at harvest: the geometric mean presumptive ThC and enterococci counts in the fish samples from both the wastewater-fed and non-wastewater-fed ponds were 10^2−10^5 CFU g^{-1} and the SPC ranged from 10^6−10^7 CFU g^{-1} (Table 5). Numbers of presumptive ThC and enterococci were significantly higher in silver carp than in common carp and tilapia. In general, there was no significant difference between the bacteriological qualities of the fish from the wastewater-fed ponds and those from the non-wastewater-fed ponds.

Table 5-5 Numbers of faecal indicator bacteria and standard plate counts in fish samples (flesh, skin, bone) from wastewater-fed and non-wastewater-fed ponds purchased at local retail markets

Bacterial group	Fish	Wastewater-fed ponds[a] (n^b = 52)			Non-wastewater-fed pond (n = 64)			p (t test)[c]
		n	Mean[d]	σ	n	Mean	σ	
Presumptive thermotolerant coliforms	Common carp	10	2.89	0.69	20	3.45	1.80	0.82
	Silver carp	20	4.23	1.35	20	4.28	1.28	0.55
	Tilapia	22	3.49	0.78	24	3.73	0.95	0.81
p (ANOVA)[c]			**0.004**			0.15		
Enterococci	Common carp	10	2.68	0.92	20	3.23	1.29	0.87
	Silver carp	20	4.33	1.26	20	3.70	0.69	**0.02**
	Tilapia	22	3.68	0.63	24	3.54	0.57	0.22
p (ANOVA)			**0.0003**			0.24		
Standard plate count	Common carp	10	6.49	0.54	20	6.65	1.31	0.64
	Silver carp	19	6.93	0.52	19	6.91	0.93	0.46
	Tilapia	22	6.93	0.65	24	6.99	0.95	0.58
p (ANOVA)			0.11			0.57		

[a] There was no significant difference in the bacterial counts in the fish harvested from the two wastewater-fed ponds (t test: p >0.05).
[b] Numbers of individual fish analysed.
[c] Values in bold indicate significant differences.
[d] Log geometric mean bacterial numbers g^{-1}.

5.5 DISCUSSION

The water in the non-wastewater-fed pond receiving water from the Red River was faecally contaminated at a level of just under 10^4 presumptive ThC per 100 ml, but the quality of the flesh of fish from this pond at harvest showed little if any faecal contamination (maximum 2−3 presumptive ThC g^{-1}). The flesh from fish harvested from the much more contaminated wastewater-fed ponds (just under 10^6 presumptive ThC per 100 ml) contained similar levels of presumptive ThC and was thus of an equally satisfactory microbial quality. Thus very few faecal indicator bacteria penetrated into the fish flesh even in the highly faecal polluted wastewater-fed fish pond. However the SPC of the fish flesh was 10^2–10^4 CFU g^{-1}, indicating that bacterial penetration did occur, but at similar levels in the wastewater-fed and non-wastewater-fed ponds.

A limited number of other studies have investigated the association between microbiological qualities of the fishpond water and the fish in both laboratory environments and functioning waste-fed aquaculture ponds. A few studies, mainly conducted in Israel, have suggested a threshold bacterial concentration in the fishpond water above which bacteria enter the edible muscle tissues of fish and thus increase the risk of exposure for consumers of the fish. Buras and co-workers (Buras *et al.* 1985, 1987; Buras 1990) reported such a threshold concentration of total bacteria in fishpond water of 1−5 × 10^6 per 100 ml, but this seems to have been due to a major malfunction in the wastewater treatment plant which introduced such high organic loadings into the receiving fishpond that the fish were extremely stressed and only just able to survive (P. Edwards, personal communication, 2005). A study in Thailand reported an SPC range in septage-fed fishponds of 1.8 × 10^5 − 2.0 × 10^6 per 100 ml of pond water which produced fish with minimal bacterial penetration into their flesh (Edwards *et al*. 1984). Exposure of 132 healthy tilapia to fishpond *E. coli* concentrations of up to 10^6 cfu per 100 ml from wastewater sources led to little or no detectable bacterial or bacteriophage penetration into their flesh (Fattal *et al*. 1988, 1992). In the United States Hejkal *et al*. (1983) found a maximum of 25 faecal streptococci per 100 g of fish muscle even though the gut contained >10^5 per 100 g. The fish in the Buras studies were grown under conditions of high stress which is atypical of normal aquaculture ponds; thus the penetration of micro-organisms into the fish flesh in this study may have been an exceptional case. The results of the current study, together with other studies on well-managed ‘normal’ wastewater-fed fishponds, suggest that the maximum number of faecal indicator bacteria permitted in wastewater-fed fishponds should be less, by one order of magnitude as a safety factor, than that which would lead to significant contamination of the fish flesh.

Our results indicate that these fish flesh qualities were satisfactory in terms of their faecal bacterial indicator counts and complied with the recommendations of the International Commission on Microbiological Specifications for Foods (1986) and the Vietnamese Ministry of Health (1998). They are in partial agreement with the fish quality classification scheme developed by Buras *et al*. (1987); this proposed “that in the case of fish grown in wastewater , the quality of the fish should be determined by the presence of any bacteria in the muscles” and “that the indicators should be bacteria that grow on nutrient and mFC agar , and the bacteriological quality should be expressed as: 0−10 bacteria ml^{-1}, very good; 10−30 bacteria ml^{-1}, medium quality; more than 50 bacteria ml^{-1}, not acceptable” [*sic*; it is not clear what quality was to be assigned for 31−49 bacteria ml^{-1}]. Thus, based on this scheme, the fish flesh qualities at harvest were ‘very good’ on the basis of their *E. coli* counts but ‘not acceptable’ on the basis of their SPC (Table 4). It is difficult to imagine a wastewater-fed (or river-water-fed) aquaculture situation in which the “nutrient and mFC agar” counts are the same as this would imply that all (or essentially all) the bacteria present were faecal coliforms/*E. coli*. We thus accept the classification of Buras *et al*. (1987) but only in terms of the *E. coli* counts g^{-1} of fish flesh and not in terms of the SPC g^{-1}.

Surprisingly, the fish from both the wastewater-fed and the non-wastewater fed ponds contained similar bacterial concentrations in their gut contents (~10^5–10^6 presumptive ThC g^{-1} and ~10^3–10^5 enterococci g^{-1}). This may be partly explained by the relatively high numbers of presumptive ThC) in the non-wastewater pond (~10^4 per 100 ml). Our findings of high numbers of faecal bacteria in the gut content are in agreement with several studies reviewed by Edwards (1992). Fish samples (muscle, skin, bone) purchased at retail markets contained from ~1,000 to ~20,000 presumptive ThC g^{-1}, irrespective of whether the fish originated from the wastewater-fed or the non-wastewater fed ponds. This indicates that significant faecal cross-contamination occurred at the markets during handling and processing of the fish for human consumption. As noted by Buras *et al*. (1987), "exposure to pathogens can occur when fish are handled and cleaned. During the digestive tract removal, the content is usually spilled and contaminates the intestinal cavity of the fish and the hands of the handler. Casual rinsing does not prevent contamination". Clearly, local environmental health officers/assistants need to educate local fishmongers so that (a) they are aware of the risks for faecal contamination of the fish products and possible occupational health risks of their unhygienic practices and (b) they are then able to implement and sustain improved hygiene practices; they also need to be regularly inspected by the local environmental health officers/assistants to ensure that their fish-handling and cleaning practices are always hygienic.

Finally, it should be noted that only the bacteriological quality of fish from wastewater-fed and non-wastewater-fed fishponds was investigated. Thus, the possible occurrence and food safety aspects of fishborne zoonotic parasites, in particular trematode parasites, and any bio-accumulation of toxic chemicals in the wastewater, were not assessed.

5.6 CONCLUSIONS

● Fish grown in both wastewater-fed and nominally non-wastewater-fed fishponds with presumptive ThC counts of ~10^6 and ~10^4 per 100 ml, respectively, were of very good quality at harvest (2−3 presumptive ThC g^{-1} of flesh). This indicates a safety factor of three orders of magnitude in the WHO guideline value for wastewater-fed aquaculture (≤1000 *E. coli* per 100 ml of fishpond water).
● Grossly unhygienic fish handling and cleaning practices at the local retail markets caused significant recontamination (10^2−10^5 presumptive ThC g^{-1}) of the fish grown in both wastewater-fed and nominally non-wastewater-fed ponds.
● The fishmongers in the local retail markets should be informed about their unhygienic fish handling and cleaning practices, and how these can be improved to reduce the faecal cross-contamination of the fish they sell.

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