PARASITIC AND BACTERIAL CONTAMINATION IN COLLARDS USING EFFLUENT FROM TREATED DOMESTIC WASTEWATER IN CHIANG MAI, THAILAND

Rassamee Keawvichit[1], Kanlaya Wongworapat[1], Pachern Putsyainant[1], Adung Silprasert[1] and Seni Karnchanawong[2]

[1]Research Institute for Health Sciences, [2]Department of Environmental Engineering, Faculty of Engineering, Chiang Mai University, Chiang Mai, Thailand

Abstract. Thailand often has inadequate water supply for agriculture during the dry season. The reuse of treated wastewater treatment plants could solve this problem. Treatment of domestic wastewater of Chiang Mai municipality by the aerated lagoon system (AL) releases more than 25,000 m^3 of treated water everyday. The reuse of wastewater in agriculture is an efficient use of water, especially in tropical countries or in drought zones. The objective of this study is to demonstrate the possibility of using treated wastewater in growing edible vegetables, *ie* collards (kale), without pathogenic parasite and bacterial contamination. Collards (*Bassica oleracea* var *acephala*) were grown using either the treated wastewater from the aerated lagoon system (AL) or ground water (GW). Three cropping times were scheduled in February, May and July, 2000. Samples of water from AL system and GW were taken two times per month (the consecutive weeks) from February to July and examined for bacteria and parasites. Irrigation water (IW) that was normally used in agriculture was also collected, at the same time of the AL and GW collection, for bacteria and parasite investigation. A soil sample was taken before and after each crop for parasite examination. Collards were also collected at the end of the crop for parasite investigation. The results showed that GW seems to be a clean water since no pathogenic bacteria were found although small amount of *Escherichia coli* was noted in May. For AL and IW, similar number and types of bacteria were found. They were *Aeromonas sobria, A. hydrophila, E. coli, Citrobacter fruendii, Pseudomonas aeruginosa*, non-pathogenic type of *Vibrio cholerae*. The small number of *Salmonella enteritidis* gr E was found in AL in April. After investigating 12 samples in 6 months of each kind of water, *ie* GW, Al, and IW, no parasite was found. Only unidentified free living nematodes were found in IW but those parasites are non pathogenic. A small number of unidentified free living nematodes (UFLN), a natural parasite, were found in soil after cropping. After each cropping time, similar number of hookworm was found in the plots which used either GW or AL. Collards grown by using either GW or AL showed no harmful parasite contamination. We conclude that the effluent from wastewater treatment, using aerated lagoon system, of Chiang Mai municipality could be safely used for growing collards.

INTRODUCTION

Wastewater from a community, without any treatment, is normally released into the public water resources, such as river or lagoon, causing problems in the public water consumption and environment. The Thai government set a policy of setting up a Central Wastewater Treatment Plant in every municipality. The treated wastewater will be useful if it can be reused in agriculture. The reuse of wastewater is an efficient use of water, which may be called sustainable development, especially in tropical countries or in drought zones where lack of water is a considerable problem. The advantage of the reuse of wastewater is not only reduce the problem of lacking of water, but also reduce the farmer's fertilization expense (Asano and Levine, 1996) since the treated wastewater retained a lot of nutrients such as nitrogen in the form of urea or nitrate, and also phosphorus.

The suitable treated wastewater can be used in growing edible plants which can be consumed without cooking and shows no effect on the environment (Sheikh *et al*, 1990). The wastewater which is treated to improve its quality can be called reclaimed water. It should be cleared from toxic substances, bacteria and parasites. There have been reports of toxic substances in water causing health hazard in human and living animals. These are organic pollutants such as polycyclic aromatic hydrocarbons (PAHS) and some heavy metals such as lead, cadmium, copper, zinc and chromium. Most of them can cause cancer in human. Therefore, the water must be tested for those toxic substances before use.

The reclaimed water which passed the tertiary treatment containing chlorination process will be cleared from bacteria. Apart from bacteria and toxic substance contamination, parasite contamination in water is also a serious concern. Very high numbers of both pathogenic and nonpathogenic parasites had been found in vegetables available in Songkhla and Chiang Mai markets (Sritherawisarn *et al*, 1986; Keawvichit *et al*, 1989). The possible causes were due

to the use of water contaminated with parasitic eggs in growing vegetables. Therefore, before using treated wastewater in growing of vegetables, one should be sure that there is no contamination with any pathogenic parasite.

Chiang Mai, the second biggest city of Thailand, has a very fast growing population. The aerated lagoon system (AL) is use for the treatment of domestic wastewater of Chiang Mai municipality. More than 25,000 m^3 of wastewater were treated everyday. As a large amount of treated wastewater is released, an attempt to use it for agriculture was made by a group of researchers from the Faculty of Engineering, Faculty of Agriculture, Social Research Institute and the Research Institute for Health Sciences, Chiang Mai University. As a result, the project named "Reuse of Effluent from Domestic Wastewater Treatment Plant in Agriculture", supported by Thailand Research Fund, was established. Several aspects concerned with health safety were studied. To show the possibility of using treated wastewater in growing edible vegetables, *ie* collards (kale), without pathogenic parasite and bacteria contamination was a part of this study.

MATERIALS AND METHODS

Farms in an area next to the central wastewater treatment plant of Chiang Mai consisting municipality of 12 plots for growing collards (Kale) (*Bassica oleracea* var *acephala*), 100 m^2 each with buffer zones. Six plots were the experimental sites which used the treated wastewater from the aerated lagoon system (AL) and another 6 plots were the control sites where the ground water (GW) was used. Three cropping times were designed in this study. The first crop was harvested in February, 2000 while the second and the third crops were harvested in May and July, respectively. It took, on average, 25-28 days for each crop for growing collards.

Samples of water from the AL system and GW were taken two times per month (the consecutive weeks) from February to July and examined for bacteria and parasites. For comparison, irrigation water (IW) that was normally used in agriculture was also collected, at the same time of AL and GW collection and examined for bacteria and parasites.

Soil samples in each plot were taken before and after each crop and examined for parasites. Collards were also collected at the end of growing for parasite investigation. Three methods were used together to explore the parasites in soil. They are centrifugal sedimentation method which can look for both eggs and larva, centrifugal floatation method (Quinn *et al*, 1980) in which eggs can be easily investigated, and Baermann's method (Monica, 1987) which was used for larva collection. For the investigation of parasites in water and collards, centrifugal sedimentation method was used. Centrifugal sedimentation of soil sample was done by adding 40 ml of distilled water to 10 g of soil and mixed thoroughly. The mixture was filtered through 4 pieces of gauze into 50 ml tube and left to stand for 10 minutes. The supernatant was then taken and centrifuged at 1,500 rpm for 2 minutes. After centrifugation, the supernatant was discarded and the pellet was resuspended with 5ml of distilled water and mixed well. Then 100 μl of the mixture was placed on a microscopic slide (5 slides) to look for the ova and larva of parasite under the microscope. Centrifugal floatation for soil sample was performed by mixing 15 g of soil with 30 ml of 1% Tween 80 and filtered through a 1 mm^2 nylon sieve on a Buchner funnel into 50 ml tube. The filtrate was centrifuged at 1,500 rpm for 3 minutes. The soil pellet was kept and washed twice with distilled water. Next, the pellet was resuspended with 30 ml of floatation medium ($NaNO_3$ specific gravity = 1.35) and was placed in two of 15 ml tubes. The tubes were centrifuged at 3,000 rpm for 20 minutes then the floatation medium was topped into the tubes to form a minicus upon which a coverslip was superimposed. The tubes were let to stand for 5 minutes and the coverslips were placed on microscopic slides and examined under the microscope. Baermann's method for soil was carried on by placing 50 g of soil on the 1 mm^2 nylon sieve and 2 pieces of gauze in the Baermann's apparatus. The warm water (40˚C) was filled above the soil and left to stand for 2 hours. After that all of the water was collected in 50 ml tube and centrifuged at 2,200 rpm for 5 minutes. Then supernatant was discarded until 5 ml was left. This supernatant was mixed well and 1 ml of the suspension was mixed with 3 ml of distilled water then 100 μl of the mixture was placed on a slide (10 slides) for microscopic examination.

Centrifugal sedimentation method for water samples (WHO, 1989) was done by filling 400 ml of water into a beaker and left to stand for 2 hours. The supernatant was discarded by a suction pump until 50 ml remained. The mixture of supernatant and pellet was poured into a 50 ml centrifuge tube and spun down at 1,500 rpm for 10 minutes. The pellet was kept and examined microscopically for larva and ova of parasites.

Centrifugal sedimentation method for vegetable samples was performed by mixing vigorously 200*g* of chopped collard in 800 ml of Lipon F (concentrated dishwashing liquid) in saline (specific gravity =1.010) for 15 minutes. The mixture was filtered through 2

pieces of gauze into a beaker and let stand for 2 hours. The top level of supernatant was then discarded until 50 ml was left in the tube. The solution was centrifuged at 2,000 rpm for 10 minutes. The top supernatant was discarded, only 5 ml was kept and mixed well and then 100 μl of the suspension was placed on a slide (10 slides) and examined under the microscope.

Two conventional methods were used for bacteria culture of water samples. The first is Direct Method, 10 μl of water samples were inoculated onto Salmonella-Shigella (SS) agar, McConkey (MC) agar, phenyl ethyl alcohol (PEA) and thiosulfate-citrate bile salts-sucrose agar (TCBS) and incubated for 24 hours at 37˚C. The pathogenic bacteria and other bacteria that may cause diarrhea were identified by a standard method and reported as Colony Forming Unit per one milliliter of water (CFU/ml). The second method was the Indirect Method or Concentration Method that used enrichment media. The selenite -F broth, peptone water and alkaline peptone water were inoculated with 50 ml of water sample and incubated at 37˚C for 24 hours. Following that, the culture broth was subcultured onto SS agar and MC agar. All isolates were identified further by a standard microbiological identification method and with commercially available antisera.

RESULTS

The results of bacterial contamination in samples of GW, AL and IW taken two times per month are shown in Table 1. Table 2 shows the results of parasite investigation in GW, Al, and IW. The results of parasites found in collard growing soil are shown in Table 3. Table 4 shows parasite contamination in collards cropped in February, May and July, respectively.

Table 1
The bacterial contamination in the studied waters[a].

Water type		Feb	Mar	April	May	June	July
GW	I	No S,S,V	No S,S,V	No S,S,V	No S,S,V	*E.coli* 10^3-10^4	No S,S,V
	II	No S,S,V	No S,S,V	No S,S,V	No S,S,V	No S,S,V	No S,S,V
AL	I	No S,S,V	*A.sobria* 10^2-10^3	ND	*V.cholerae*, non-O1, non-O139	*V.cholerae*, non-O1, non-O139	*A.sobria*
	II	No S,S,V	*A.sobria* 10^3	*S.enteritidis* *V.cholerae*, non-O1, non-O139 *A.sobria* 10^2-10^3	*V.cholerae*, non-O1, non-O139 *A.sobria* 10^2-10^3	*V.cholerae*, non-O1, non-O139	*V.cholerae*, non-O1, non-O139
IW	I	*E.coli* 10^2-10^3 *A.sobria* 10^2-10^3	*V.cholerae* Type non-O1, non-O139 *A.hydrophila* 10^2-10^3	*E.coli* 10^2-10^3 *A.sobria* 10^2 -10^3 *P.aeruginosa* 10^2-10^3	*E.coli* 10^2-10^3 *A.sobria* 10^2-10^3	*V.cholerae*, non-O1, non-O139	*A.sobria*
	II	*A.sobria* 10^2	*E.coli* 10^2-10^3	No S,S,C	*V.cholerae*, non-O1, non-O139 *E.coli* 10^3-10^5 *C.freundii* *P.aeruginosa*	*V.cholerae*, non-O1, non-O139	*V.cholerae*, non-O1, non-O139

[a] The number of bacteria are reported as a colony forming unit (CFU) found in 1ml of water; ND = Not determined. No S,S,V = No *Salmonella, Shigella, Vibrio cholerae.*

Table 2
The parasitic contamination in the studied waters[a].

Water type		Feb	Mar	April	May	June	July
GW	I	Neg	Neg	Neg	Neg	Neg	Neg
	II	Neg	Neg	Neg	Neg	Neg	Neg
AL	I	Neg	Neg	ND	Neg	Neg	Neg
	II	Neg	Neg	Neg	Neg	Neg	Neg
IW	I	Neg	Neg	1UFLN	Neg	Neg	Neg
	II	1UFLN	4UFLN	Neg	Neg	1UFLN	1UFLN

[a] The number of parasites found in 100 ml of water; UFLN = Unidentified free living Nematode
ND = Not determined

Table 3
The parasitic contamination in soil by type of water and method of parasite examination.

Water type	Method	1st Croping		2nd Croping		3rd Croping	
		Before	After	Before	After	Before	After
GW	Sediment	Neg	400UFLN	200UFLN	67UFLN	67UFLN	67HW, 133UFLN
	Float	Neg	1HW, 4UFLN	2UFLN	42UFLN	2000UFLN	15UFLN
	Baerman	20UFLN	93UFLN	222UFLN	47HW, 786UFLN	1HW, 60UFLN	147UFLN
AL	Sediment	Neg	800UFLN	Neg	133UFLN	Neg	67HW, 133UFLN
	Float	Neg	1UFLN	6UFLN	30UFLN	600UFLN	39UFLN
	Baerman	20UFLN	26HW, 306UFLN	387UFLN	40HW, 880UFLN	113UFLN	7HW, 260UFLN

UFLN = Unidentified free living Nematode; HW = Hookworm larva

Table 4
The parasitic contamination in the vegetable.

Month	Crop	Parasite/100 g of collard	
		GW	AL
February	I	Neg	Neg
May	II	15UFLN	18UFLN
July	III	Neg	Neg

UFLN = Unidentified free living Nematode

DISCUSSION

Pathogenic bacteria such as *Salmonella* spp, *Shigella* spp, *Vibrio* spp can cause gastroenteritis and diarrhea. Other bacteria such as *Aerobacter* spp, *Citrobacter* spp, *Pseudomonas* spp and *Escherichia coli* in suitable amounts and conditions may also cause other GI disorders.

GW seems to be a clean water since no pathogenic bacteria, *ie Salmonella, Shigella* and *Vibrio,* was found although small amount of *E. coli* was noticed in June (Table 1). For AL and IW, a similar extent and type of bacteria was found. They were *Aeromonas sobria, A. hydrophila, E. coli, Citrobacter fruendii* and *Pseudomonas aeruginosa. Vibrio cholerae* was also found but it is a non-pathogenic type which are non-O1and non-

O139. Small amount of *Salmonella enteritidis* gr E which can cause gastroenteritis, was found in AL in April. Since Indirect method (Con-centration method) was used to detect this bacteria, the quantity could not be determined.

From Table 2, it was found that after investigating 12 samples in 6 months of each kind of water (GW, AL, and IW), no parasite was found. Only unidentified free living nematodes were found in IW.

Table 3 showed parasites found in soil used for growing collards for 3 cropping times. Soil samples were randomly taken before and after collard growing from each field which either GW or AL was used. Three methods, *ie* sedimentation, floatation and Baermann's method were used for parasite studies. Results revealed that small amount of unidentified free living nematodes (UFLN), a natural parasite, were found before and subsequently increased after collard growing. This is probably because the humidity from farming process produced a good environment for the growth of UFLN.

After each growing time, a similar amount of hookworm was found in the plots in which either GW or AL was used. These hookworm did not come from any GW or AL since there were no egg or larva of hookworm found in the water. These hookworm were probably from dog or cat feces or other animal feces used as fertilizer. Before growing time in the third crop (July), 11 larva per 100 g of soil were found only in the plots using GW.

Collards growing by both GW and AL in February, May and July showed no harmful parasite contamination (Table 4).

In conclusion, the effluent from wastewater treatment, using aerated lagoon system, of Chiang Mai municipality could be safely used for growing collards. Using the treated wastewater for growing of wrapped-leaf vegetables such as celery and cabbage will be investigated, since their wrapped leaves may cause an accumulation of parasite or bacteria.

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