EXAMINATION FOR INTESTINAL PARASITES AND ENTERIC BACTERIA IN THE WASTEWATER AND TREATED WASTEWATER FROM THE CITY OF CHIANG MAI, THAILAND

Kanlaya Wongworapat[1], Rassamee Keawvichit[1], Pachern Putsyanant[1], Banyong Khantawa[2], Adung Silprasert[1] and Seni Karnchanawong[3]

[1]Research Institute for Health Sciences, [2]Microbiology Section, Central Laboratory, Maharaj Nakhon Chiang Mai Hospital, Faculty of Medicine; [3]Department of Environmental Engineering, Faculty of Engineering, Chiang Mai University, Chiang Mai, Thailand

Abstract. An attempt to use treated wastewater for agriculture in the Chiang Mai area was made, but the re-use process had to be performed under a condition that limited the risks liable to leave pathogens present in the water. The objective of our study was to examine the intestinal parasites and enteric bacteria in the wastewater and treated wastewater from the Chiang Mai University campus as well as the treated wastewater from the Chiang Mai municipality. The raw wastewater (RW), primary treatment effluent water (PE), treated wastewater using the activated sludge system (AS) from the Chiang Mai University campus and treated wastewater using the aerated lagoon (AL) system from the Chiang Mai municipality were examined for intestinal parasites and enteric bacteria by using the centrifugal sedimentation and conventional methods respectively. The ground water (GW) and the irrigation water (IW) were used for comparison. All kinds of water were collected and examined twice a month for 6 months (February to July 2000). None of human intestinal parasites were found from any wastewater, whereas the RW and PE water contained hookworm larva, *Ascaris* egg and *Taenia* egg on some occasions. A small amounts of pathogenic bacteria that can cause severe diarrhea were detected. *Salmonella enteritidis* gr E was isolated from the AL water in April, while *Vibrio cholerae* type O139 was detected from the PE water in June. Some pathogenic bacteria that might cause gastroenteritis, such as *Aerobacter* spp, *Citrobacter* spp, *Pseudomonas* spp and *Escherichia coli* were also found in all kinds of water. Between the two types of treated wastewater, the bacteria found in AS water was less than that in AL water in terms of both amount and type of bacteria. The treated wastewater from the city of Chiang Mai, compared to natural water such as irrigation water, appears to be safe to use for agriculture.

INTRODUCTION

Water consumption has increased due to the increasing world population. Therefore, a shortage of fresh water and accumulation of wastewater have occurred in some countries. Treated wastewater has been processed in order to solve these problems. The reclaimed water could be used for many purposes such as flushing sanitary sewers, soil compaction, commercial cooling water, fire fighting, cleaning roads or sidewalks and growing plants (State of California 1978; Tchobanoglous, 1979). The reuse of treated wastewater in agriculture has been performed in many countries such as Australia, Israel, Mexico, Saudi Arabia, Egypt and Tunisia (Asano and Levine, 1996, the World Bank Group, 2000). It cannot only release fresh water for high-value use, but also reduce fertilizer consumption. The Thai government has set a policy for the treatment of wastewater collected from every municipality. Methods of treatment depend on the budget and the area available for the system. For example, the activated sludge treatment requires a high technology that makes it the most expensive, but it can be performed in a small area. The aerated lagoon treatment is less expensive, but that requires a larger area to be processed than the space needed for activated sludge treatment. The facultative or stabilization pond is the cheapest treatment, but it requires the largest area of treatment among these three methods.

Chiang Mai, the second biggest city of Thailand, has two stations for the treatment of domestic wastewater. The main station belongs to Chiang Mai municipality, which uses the aerated lagoon system that can treat wastewater of up to 25,000 m^3/day. The other station belongs to Chiang Mai University which uses the activated sludge system and releases 5,000 m^3 of treated wastewater per day. 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 and the Research Institute for Health Sciences, Chiang Mai University. Therefore, the project named "Reuse of Effluent from Domestic Wastewater Treatment Plant in Agriculture" was established. Several aspects concerned with health safety were studied. To examine the intestinal parasites and enteric bacteria in wastewater and treated wastewater is one part of this study.

MATERIALS AND METHODS

Six types of water were investigated for intestinal parasites and enteric bacteria twice a month from February to July 2000. Three types of water from the Chiang Mai University campus were the raw wastewater (RW), primary treatment effluent water (PE) and treated wastewater using the activated sludge system (AS). The others were treated wastewater using the aerated lagoon system (AL) from the Chiang Mai municipality, ground water (GW) and irrigation water (IW). AS and AL water were the types of treated wastewater aimed for agricultural use, while the RW, PE, GW and IW water were used for comparison. The parasite investigation in water was performed by using the centrifugal sedimentation method, which is a modified WHO recommended method for the determination of helminth eggs in wastewater (WHO, 1989). In brief, 400 ml of water were filled in a beaker and left to stand for 2 hours. The supernatant was then discarded by suction pump until the leftover was 50 ml. 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 by a microscope for larva and parasites eggs. The enteric bacteria were examined by the conventional method (Koneman, 1992). Ten microliters of water samples were inoculated onto Salmonella-Shigella (SS) agar, McConkey (MC) agar, phenyl ethyl alcohol agar (PEA) and thiosulfate-citrate bile salts-sucrose agar (TCBS) and incubated for 24 hours at 37˚C. For enrichment, selenite-F broth, peptone water and alkaline peptone water were inoculated and incubated for 24 hours at 37˚C. Following that, suspected colonies were subcultured onto SS agar and MC agar. All isolates were further identified by a standard microbiological method and commercially available antisera.

RESULTS

Parasite examination (Table 1)

PE and RW water samples were the most contaminated. A total of 209 unidentified free living nematode (UFLN), 35 hookworm (HW) larvae, 1 *Taenia* egg and 1 *Ascaris* egg were found in 12 PE samples. All RW water samples (12) were also contaminated with parasites: 214 UFLN, 7 HW, and 1 *Ascaris* egg. HW larvae were found in RW samples colleced in June and July while contamination with this parasite was found in PE water samples collected in February, May, June and July. All GW and AL Water samples were negative for parasites. Only UFLNs were found in 5 of 12 IW samples and 11 out of 12 AS samples.

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

Month		RW	PE	AS	IW	AL	GW
February	I	15UFLN	11UFLN,1HW	1UFLN	Neg	Neg	Neg
	II	6UFLN	1UFLN	Neg	1UFLN	Neg	Neg
March	I	26UFLN	13UFLN	16UFLN	Neg	Neg	Neg
	II	24UFLN	6UFLN	9UFLN	4UFLN	Neg	Neg
April	I	25UFLN	18UFLN	6UFLN	1UFLN	ND	Neg
	II	10UFLN	14UFLN	1UFLN	Neg	Neg	Neg
May	I	13UFLN	18UFLN,9HW	5UFLN	Neg	Neg	Neg
	II	11UFLN	20UFLN	1UFLN	Neg	Neg	Neg
June	I	31UFLN	15UFLN	5UFLN	Neg	Neg	Neg
	II	18UFLN,3HW	39UFLN,21HW 1*Taenia* egg 1*Ascaris* egg	3UFLN	1UFLN	Neg	Neg
July	I	1HW 1*Ascaris* egg	44UFLN 4HW	6UFLN	Neg	Neg	Neg
	II	35UFLN,3HW	10UFLN	3UFLN	1UFLN	Neg	Neg

[a] The number of parasites found in 100 ml of water; UFLN = Unidentified free living nematode
HW = Hookworm egg; ND = Not determined.

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

Month		RW	PE	AS	IW	AL	GW
February	I	*E.coli* >10^4	*E.coli* >10^4 *A.hydrophila*>10^4	Neg	*E.coli* 10^2-10^3 *A.sobria* 10^2-10^3	Neg	Neg
	II	*E.coli* 10^3-10^5 *A.sobria* 10^3-10^4 *C.freundii* 10^3-10^5	*E.coli* >10^4 *A.sobria* >10^4 *C.freundii* >10^4	Neg	*A.sobria* 10^2	Neg	Neg
March	I	*A.hydrophila* 10^3-10^4	*V.cholerae* Type non-O1,non-O139 *A.sobria* 10^3-10^4 *A.hydrophila* 10^3-10^4	Neg	*V.cholerae* Type non-O1,non-O139 *A.hydrophila* 10^2 -10^3	*A.sobria* 10^2-10^3	Neg
	II	*V.cholerae* Type non-O1,non-O139 *E.coli* 10^3-10^4 *A.sobria* 10^3-10^4 *C.freundii* 10^3-10^4	*E.coli* 10^3-10^4 *A.sobria* 10^3-10^4	Neg	*E.coli* 10^2-10^3	*A.sobria* 10^3	Neg
April	I	*V.cholerae* Type non-O1,non-O139 *E.coli* 10^3-10^4 *A.sobria* 10^3-10^4 *C.freundii* 10^3-10^4	*V.cholerae* Type non-O1,non-O139 *E.coli* >10^4 *A.sobria* >10^4 *P.aeruginosa*>10^4	Neg	*E.coli* 10^2-10^3 *A.sobria* 10^2-10^3 *P.aeruginosa* 10^2-10^3	ND	Neg
	II	*E.coli* 10^3-10^4 *C.freundii* 10^3-10^4	*E.coli* 10^3-10^4 *A.sobria* 10^3-10^4 *C.freundii* 10^3-10^4	Neg	Neg	*S.enteritidis* *V.cholerae* Type non-O1,non-O139 *A.sobria* 10^2-10^3	Neg
May	I	*E.coli* >10^4 *A.hydrophila* 10^3-10^4 *C.freundii* 10^3-10^4	*E.coli* 10^3-10^4 *A.hydrophila* 10^3-10^4	Neg	*E.coli* 10^2-10^3 *A.sobria* 10^2-10^3	*V.cholerae* Type non-O1,non-O139	Neg
	II	*V.cholerae* Type non-O1,non-O139 *E.coli* >10^4 *A.sobria* >10^4 *P.aeruginosa*	*V.cholerae* Type non-O1,non-O139 *E.coli* 10^3-10^4 *A.hydrophila* 10^3-10^4 *P.aeruginosa*	*A.sobria* 10^3-10^4 *P.aeruginosa* 10^3-10^4	*V.cholerae* Type non-O1,non-O139 *E.coli* 10^3-10^5 *C.freundii* *P.aeruginosa*	*V.cholerae* Type non-O1,non-O139 *A.sobria* 10^2-10^3	Neg
June	I	*E.coli* 10^3-10^4 *A.hydrophila* 10^3-10^4	*V.cholerae* O139 *E.coli* 10^3-10^4 *A.sobria* 10^3-10^4 *C.freundii* 10^3-10^4	Neg	Neg	*V.cholerae* Type non-O1,non-O139	*E.coli* 10^3-10^4
	II	*V.cholerae* Type non-O1,non-O139 *E.coli* 10^4-10^5 *A.hydrophila* 10^4-10^5	*E.coli* 10^4-10^5 *A.sobria* 10^4-10^5 *P.shigelloides* 10^4-10^5	Neg	*E.coli* 10^2-10^3 *A.sobria* 10^3-10^4 *A.hydrophila* 10^3-10^4	*V.cholerae* Type non-O1,non-O139	Neg
July	I	*E.coli* 10^4-10^5 *A.sobria* 10^4-10^5 *A.hydrophila* 10^4-10^5 *S.aureus* 10^3-10^4	*E.coli* 10^4-10^5 *A.sobria* 10^4-10^5 *C.freundii* 10^4-10^5 *P.aeruginosa*	*A.hydrophila*	*V.cholerae* Type non-O1,non-O139 *A.sobria*10^2-10^3 *A.hydrophila* 10^3-10^4	*A.sobria*	Neg
	II	*E.coli* 10^4-10^5 *A.sobria* 10^4-10^5 *P.aeruginosa*	*E.coli* 10^4-10^5 *A.sobria* 10^4-10^5 *C.freundii* 10^4-10^5	Neg	*A.hydrophila* 10^3-10^4	*V.cholerae* Type non-O1,non-O139	Neg

[a] The number of bacteria indicated as a colony forming unit (CFU) found in 1 ml of water.
ND = Not determined.

Bacteria examination

The results of enteric bacterial examinations are shown in Table 2. All RW and PE water samples were positive for enteric bacteria while only one of GW and 2 of AS samples contained some bacteria. RW and PE samples were more contaminated with bacteria than other types of water. The concentrations of the bacteria in RW and PE samples are in the ranges of 10^3-10^5 CFU/ml compared to 10^2-10^4 CFU/ml in most of AS, IW, AL and GW water samples. *Salmonella enteritidis* gr E and *Vibrio cholerae* type O139, that can cause severe diarrhea, were found in AL and PE water, respectively. Other enteric bacteria found in this study were *Aeromonas sobria*, *Aeromonas hydrophila*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Plesiomonas shigelloides* and *Staphylococcus aureus*.

DISCUSSION

Since the use of reclaimed water has been increasing in many countries, the quality of treated wastewater becomes necessary. The current quality requirements are slightly different, depending on the type of use. According to the World Health Organization (1989) microbiological quality guidelines and criteria for irrigation, the treated wastewater used for growing food crops must not contain intestinal nematodes (*Ascaris*,*Trichuris* and hookworm) in more than 1/liter of water, but there is no standard recommendation for fecal coliforms. Nevertheless, irrigation of crops likely to be eaten uncooked must not have fecal coliforms of more than 10^3 /100 ml of water.

In this study, parasites, hookworm larvae, *Ascaris* egg and *Taenia* egg were found in RW and PE water samples. In addition, a high number of enteric bacteria (10^3-10^5 CFU/ml) was also found in these two types of wastewater. However, after treatment by the activated sludge system (AS), the treated wastewater became cleaner, since there was no intestinal parasites and only a small amount (10^2-10^4 CFU/ml) of enteric bacteria such as *A. sobria, A. hydrophila* and *P.aeruginosa* were presented in AS water. The treated wastewater from the Chiang Mai municipality or AL water seems to be clean, since none of parasites were found in this kind of water. Moreover, the enteric bacteria found in AL water were similar to those in IW water which is natural water. *S. enteritidis* grE was found once in April, but only in a small amount. It was shown that treated wastewater from the wastewater treatment plant of Chiang Mai University and the one from the Chiang Mai municipality were rather safe from the pathogenic organisms. Thus, these two types of treated wastewater could be used for several purposes: cleaning roads, watering flowers and growing crops. However, for using of treated wastewater to grow food crops, the study of pathogenic contamination in certain food crops should be performed in order to assure food safety.

REFERENCES

Asano T, Levine AD. Wastewater reclamation, recycling and reuse: past, present, and future. *Wat Sci Tech* 1996;33:1-14.

Koneman KW, Allen AD, Janda WM, Schreckenberger PC, Winn Jr WC. Diagnostic Microbiology, 4th ed. Philadephia: JB Lippincott, 1992:1154 pp.

State of California. Wastewater reclamation criteria, an excerpt from the California Code of Regulation, Title 22, Division 4, Environmental Health, Department of Health Services, Sacramento, California,1978.

Tchobanoglous G. Wastewater engineering treatment, disposal, reuse. New York: McGraw.Hill, 1979: 920pp.

The World Bank Group. Wastewater re-use. Available at http://wbln0018.worldbank.org. December 1, 2000.

WHO. Health guidelines for the use of wastewater in agriculture and aquaculture. Report of a WHO Scientific Group. *WHO Tech Rep Ser* 1989;778.