REMOCIÓN DE COLIFORMES FECALES DE AGUAS RESIDUALES DOMÉSTICAS TRATADAS POR INFILTRACIÓN CON MEDIOS INTERMITENTES PARA LA IRRIGACIÓN

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TESIS

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COMPENDIO

Remoción de coliformes fecales de aguas residuales domésticas tratadas por infiltración con medios intermitentes para irrigación

POR

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DOCTORADO EN CIENCIAS

EN INGENIERÍA DE SISTEMAS DE PRODUCCIÓN

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Palabras clave: bacteria, cultivo, irrigación, zeolita modificada, suelo, reuso de agua residual

El reuso del agua residual en la agricultura es una alternativa para solventar su escasez en las regiones áridas y semi-áridas de México. Se utilizó la prueba de infiltración con medios intermitentes (IMI, por sus siglas en inglés) para evaluar la remoción de coliformes fecales (CF) en aguas residuales domésticas durante cuatro semanas en el laboratorio de química de suelos del Departamento Ciencias del Suelo de la UAAAN. Se prepararon doce columnas (diámetro de 12 cm y altura de 30 cm) con suelo y medios porosos (arena, zeolita, vermicomposta y carbón) en proporciones de 25%, 50% y 75%. La relación 75/25 dió el mejor resultado, siendo 1.25±0.60, 0.75±0.20, 1.21±0.11 y 2.46±0.29 valores de log respectivamente. El análisis de regresión mostró un buen ajuste entre el porcentaje de suelo y la reducción de CF, excepto para suelo/carbón. Los medios filtrantes fueron caracterizados química, física y morfológicamente. Los mecanismos de reducción de CF están directamente afectados por el tamaño de las partículas finas y el incremento de cargas del ion para la retención y adsorción de bacterias. La metodología de predicción por reglas de simulación difusas con redes (FREN, por sus siglas en inglés) dió un buen ajuste para el pronóstico de la remoción de CF en el rango de contenidos de suelo 20-80%. La zeolita natural modificada con Zn^{2+} (Zeo-Zn) demostró que las CF fueron removidos significativamente (2.99±0.92 log) debido a la acción antimicrobial del zinc. Los tratamientos IMI más efectivos (suelo, suelo/carbón y Zeo-Zn), secundario y agua residual clorada se usaron para el riego de acelga (*Beta vulgaris*) cultivados en un suelo franco arcilloso bajo condición de invernaderos en la UAAAN durante cuatro meses y se encontró un efecto residual significativo de CF cuando se analizaron por el método del número más probable (NMP). El riego con IMI de Zeo-Zn dió el menor contenido de CF en suelo (1.66±1.07 NMP g^{-1}) y en acelga durante los dos periodos de cosecha, los cuales fueron 5.45±2.13 y 16.38±8.00 NMP g^{-1} de materia seca

respectivamente. Estos, fueron similares al agua residual clorada. De este modo las aguas residuales tratadas con medio filtrante bactericida por ejemplo Zeo-Zn, fue satisfactoriamente adecuado para su uso agrícola sin restricciones y cumpliendo con la legislación mexicana, con bajo riesgo de contaminación de CF en planta y suelo.

ABSTRACT

Fecal coliform removal of reclaimed domestic wastewater by intermittent media infiltration for irrigation.

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Keywords: Bacteria, crop, irrigation, modified zeolite, soil, wastewater reuse

Wastewater reuse in agriculture is one alternative to solve the water scarcity in arid and semi-arid regions of Mexico. The pilot-scale intermittent media infiltration (IMI) was studied to evaluate the efficiency of fecal coliform (FC) removal in domestic wastewater for four weeks in the chemical laboratory at the Department of Soil Science in the UAAAN. Twelve IMI columns (diameter

12 cm and height 30 cm) were composed of soil and porous media (sand, zeolite, vermicompost and charcoal) in proportions of 25%, 50% and 75%, and the ratio of 75/25 (soil/media) gave a best result of FC removal as 1.25±0.60, 0.75±0.20, 1.21±0.11 and 2.46±0.29 log, respectively. The regression analysis showed a good relationship between the soil percentage and FC reduction except soil/charcoal. The filter media were determined and characterized for chemistry, physics, and morphology. The mechanisms of FC reduction are directly affected by fine particle size and increasing of ion charges for retention and adsorption the bacteria. The prediction methodology by fuzzy rules emulated network (FREN) gave a good performance to forecast FC removal with the range of soil contents (20-80%). The IMI contained natural zeolite modified with Zn^{2+} (Zeo-Zn) demonstrated that FC were removed significantly (2.99±0.92 log) due to the antimicrobial action of zinc. The effective IMI treatments (soil, soil/charcoal and Zeo-Zn), secondary and chlorinated wastewater were used to irrigate swiss chard (*Beta vulgaris*) growing in a clay loam soil under greenhouse conditions at UAAAN during four months and had a significant effect on their residual FC bacteria by a most probable number (MPN). Irrigation with IMI of Zeo-Zn had lower residual FC in soil (1.66±1.07 MPN g^{-1}) and swiss chard in two and four months of harvest as 5.45±2.13 and 16.38±8.00 MPN g^{-1}, respectively, similar results of the disinfection. Thus domestic wastewater treated by IMI with bactericide filter media, for instance Zeo-Zn, was adequately suitable for unrestricted agricultural use and complied with Mexican regulations with low risk of FC contamination in plant and soil.

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I. INTRODUCTION

The components of the environment (air, water and land) nowadays are severely damaged from human activities and natural causes. Although, the legislation to protect the human health and to preserve the ecosystems exist, it is compulsory to find the conservation in an environmentally sustainable development and to clean up the polluted environment for future generations.

The global water crisis is one serious problem that faces the freshwater shortage and wastewater pollution increment, due to the adaptation to climate change and a result of growth population, economic development and urbanization, respecting the limits on the hydrological cycle (UNESCO, 2009).

Mexico has the variety of geographical regions and climate variations according to the altitude. The south is a tropical portion hot and humid all year long. The northern portion is semi-arid or arid condition then the climate is drier than the rest of the country. These conditions provoke water shortage reflected in food crisis, especially in the north region.

Water reuse and reclamation is an efficient tool for water and wastewater management. They are preferred using in irrigation agriculture to reduce the treatment cost, negative impacts on health and environment, and increase the agricultural productivity. However pathogenic bacteria and chemical contaminants in wastewater must be removed within the standard limits.

Fecal coliform (FC) bacteria live in the digestive tract of warm-blooded animals. Thus it can be used as an indicator associated with the potential presence of pathogens (Huertas *et al*., 2008). The suitable treatments (advanced or tertiary) are required to lower FC significantly and can be made by removal or inactivation processes. The FC removal processes can be mentioned as membrane technology, activated sludge, filtration, constructed wetland and membrane bioreactor. Many disinfection (chemical and physical) processes have been applied such as chlorination, ultraviolet light and ozone.

The challenge is to find the alternative disinfection or alterations of chlorination to control common occurrence of disinfection by-products (DBPs). The infiltration-percolation technique and the bactericidal material, for instance the natural zeolites supporting metal ions, could be a good alternative to solve this problem.

Prediction methodologies play an important role for water and wastewater management as the quantitative tool. In the case of bacteria, it was proposed to predict FC concentrations and evaluate the relationships between treatment and fecal pollution in wastewater quality. However the effects on plant and soil by using reclaimed water must be monitored. Thus in this study, the pathway of FC bacteria for wastewater, plants and soil were investigated.

Objectives

The aims of this research were (i) to evaluate the efficiency of FC removal in pilot-scale by intermittent media infiltration (IMI) treatment, to study the mechanism and to predict the FC removal by applying fuzzy rules emulated network (FREN), (ii) to improve the antibacterial capacity by functionalizing the natural zeolite with zinc, and (iii) to evaluate the FC residual effects using reclaimed domestic wastewater in plant and soil tested in fieldwork under greenhouse condition.

The investigation was divided into 3 parts with 4 sections. The first part was investigated by using the IMI treatment to evaluate FC removal efficiency with different proportions and types of natural filtering materials (soil, sand, zeolite, vermicompost and charcoal), later it was applied FREN to predict their FC removal percentages. Due to the interesting and useful properties of zeolite, the second part was to modify for improving the antibacterial capacity by functionalizing the clinoptilolite contained in the natural zeolite by substituting the alkaline and alkaline earth cations on its surface with Zn^{2+}. The last part of this study, the residual FC bacteria using reclaimed domestic wastewater were investigated the effects on plant and soil, and compared with chlorinated and three IMI treated wastewaters in a four months monitoring period.

Publications

The publications were results of the investigation, which are:

- Khamkure, S., Peña-Cervantes, E. Gamero-Melo, P. and Zermeño-González, A. Fecal Bacteria Removal Enhanced by Zinc-Modified Zeolites in An Intermittent Media Infiltration System. 2012. Environmental Engineering and Management Journal. In press. (http://omicron.ch.tuiasi.ro/EEMJ/accepted.htm)

- Khamkure, S., Treesatayapun, C., Peña-Cervantes, E. Gamero-Melo, P. and Zermeño-González, A. 2012. Prediction of fecal coliform removal on intermittent media infiltration by varying soil content based on FREN. International Journal of Environmental Research. In press.

- Khamkure, S., Peña-Cervantes, E. Gamero-Melo, P. and Zermeño-González, A. Clay soil content effect on fecal bacteria removal in an intermittent media infiltration system. Environmental Engineering and Management Journal. (Submitted on Dec 2, 2011: In review).

- Khamkure, S., Peña-Cervantes, E., Zermeño-González, A., López-Cervantes, R., Gamero-Melo, P. and Ramírez, H. Residual effects of fecal coliform using reclaimed domestic wastewater on plant and soil. Water Science and Technology. (Submitted on June 8, 2012: In review).

The other publications were results of this thesis and were presented in the conferences, which are:

- Khamkure, S., Peña Cervantes, E., Gamero Melo, P., López Cervantes, R., Zermeño González, A. and Mireles, M. S. 2010. Fecal Coliform removal by intermittent media infiltration for reclamation of municipal wastewater in agricultural irrigation. XXXV Congreso Nacional de la Ciencia del Suelo y XIII Congreso Internacional en Ciencias Agrícolas, Mexicali, Baja California, México. ISBN: 978-607-00-3557-9. pp. 326-329.

- Khamkure, S., Peña Cervantes, E., Gamero Melo, P. and Zermeño González, A. 2011. Intermittent media infiltration of municipal wastewater treatment and prediction based on FREN for fecal coliform removal. World Environmental and Water Resources Congress 2011. Palm Springs, California. ISBN 978-0-7844-1173-5. pp. 3359-3368. (http://cedb.asce.org/cgi/WWWdisplay.cgi?279399)

- Peña Cervantes, E., Khamkure, S., López Cervantes, R. y Zermeño González, A. 2011. Tratamientos para eliminación de coliformes fecales de aguas residuales: impacto en planta y suelo. XXXVI Congreso Nacional de la Ciencia del Suelo, Campeche, México. ISBN: 978-607-00-5107-4. pp. 548-551.

II. **LITERATURE REVIEW**

2.1 Wastewater reuse for agriculture

Wastewater is defined as a combination of the liquid or water-carried wastes after it has been used in variety of applications and discharged together with surface water and stromwater (Tchobanoglous *et al.*, 2003). It can be classified by source of application such as domestic, industrial or agricultural wastewater. Sometimes wastewater is defined as either black or grey. Blackwater is the dirty wastewater from toilet or polluted water from industries that requires sophisticated treatments. By contrast, greywater is a type of wastewater originated from baths, showers, sinks or laundries that does not contain sewage and has lower levels of impurities (Widiastuti *et al.*, 2008).

Water reuse is the use of reclaimed or repurified wastewater for beneficial use. Wastewater reuse from treated municipal wastewater can be categorized according to the typical applications, which are: agricultural and landscape irrigation, industrial recycling and reuse, groundwater recharge, recreational uses, nonpotable and potable reuse (Asano *et al.*, 2007). Agricultural wastewater reuse is an element of water resources development and management that provides the innovative and alternative options for agriculture (Bahri, 1999).

Agricultural water use is the largest water consumption in the world (70%), however, the use of wastewater in agriculture is only 4% of the total. Mexico is considered as one of the ten largest water users in the world and the main use of water is agriculture (77%) (UNESCO, 2009).

The evolution of water demands in Mexico depends on their large different regions, population and economic growth shifting from rural to urban areas, and the limits on the hydrological cycle. The report of CONAGUA (2010) demonstrated that the annual average precipitation water is 1,489 km^3/year. The major components are for evapotranpiration and return to the atmosphere (73.2%), drained by rivers or streams (22.1%) and infiltration into the ground and recharge aquifers (4.7%). Due to this limitation, some regions of Mexico affect the problem of water availability, for instance Mexico City (Spring, 2011) and especially the northern regions such as Baja California (Medellín-Azuara *et al.*, 2009) and Chihuahua (Espino *et al.*, 2004).

According to municipal and industrial wastewater in Mexico, those are treated at 35% and 18%, which the estimated volumes of wastewater reuse are 5.051 km^3 (equivalent at a rate of 160 m^3 s^{-1}) and most of them are reuse in agricultural irrigation (CONAGUA, 2010). The annual average of FC concentration in surface water in Mexico were greater than 1000 MPN 100 mL^{-1} at 59% of monitoring sites in this country (CONAGUA, 2005), which is the threshold for using in public services or occasional indirect contact established by NOM-003-ECOL-1997 (SEMARNAP, 1997).

The reclaimed wastewater in Mexico is used for crop irrigation and has a significant increase crop yield due to high organic matter (OM) and nutrients in wastewater (Espino *et al.*, 2004; Jimenez and Chávez, 2004). However, it is found a large quantity of water-borne disease such as FC bacteria (Chávez *et al*., 2011; Solís *et al*., 2006) and the risks of high accumulation of heavy metal (Lucho-Constantino *et al.*, 2005; Mireles *et al.*, 2004) in soils and plants.

The Mezquital Valley in Mexico is one of the oldest and largest examples worldwide of an agricultural irrigation system using municipal wastewater (Jimenez and Chávez, 2004). The epidemiological studies in this area were determined diarrhoeal risks contacted with partially treated irrigation water (10^3-10^4 FC MPN 100 mL^{-1}), where people worked in the wastewater-irrigated fields and children less than 15 years are exposed (Mara *et al.*, 2007). The Tula Valley receives untreated wastewater from Mexico City for agricultural irrigation. Although the concentrations of microorganisms in the infiltrated water were generally very low but the incidence of FC (68% of samples) recommended a health risk (Chávez *et al.*, 2011).

The main risks and constraints for considering agricultural wastewater reuse are the presence of pathogenic and chemical contaminants that caused soil and aquifer pollution, salinity and toxicity (Kamizoulis, 2008; Toze, 2006). Therefore, the quality of the wastewater reuse for unrestricted agricultural irrigation is limited by the Mexican regulations (NOM-001-ECOL) at FC less than 1000 MPN 100 mL^{-1} (SEMARNAP, 1996), corresponding with the world health organization guidelines (WHO, 1989) to avoid the effects on human and environment (soils and plants).

2.2 Wastewater treatment and natural filter media

Soil infiltration and soil clay have been investigated for microorganism, organic and heavy metal removal on wastewater. Mosaddeghi *et al.* (2009) demonstrated the influence of soil texture and structure, and organic waste type, which are important for bacteria filtration. Unsaturated intact soil columns (25 cm height) between sandy clay loam (SCL) and loamy sand (LS) are evaluated on bacterial filtration with organic waste loading. LS have high efficiency to filtrate total bacteria more than SCL approximately 1.24 times due to weaker structure and pore discontinuity.

Oladoja and Ademoroti (2006) showed the ratio 3:1 (soil-clay/pebbles) and double column treatment provide high quality of water purification related to mineralogical accumulation and the amount of clay. Adsorption and ion exchange are the principal physicochemical surface reactions on clay. High swelling rate of clay reduces the pore spaces in their structures affecting to the reduction of permeability and the increase of residence time. The stone pebbles assist the water percolation and act as inert media for microbial attachment and growth. Total bacteria in their effluents can be removed approximately 90% because they behave as charged particle then adsorb on the clay surface.

Van Cuyk and Siegrist (2007) discovered the virus removal by applying soil infiltration within the infiltrative surface zone (4 cm) at 3 log removal. Sandy loam has higher virus removal than medium sand that affected by effluent composition, soil type, hydraulic loading rate (HLR) and dosing method. These results agree with the study of Zhang *et al*. (2007) that HLR and temperature are the most important factors for organic removal. Domestic wastewater is treated by shallow soil infiltration with effective depth 30 cm. The applied artificial soil is composed of different proportions of soil, coal slag, dewatering sludge and packing medium.

Sand filtration has been studied by Healy *et al.* (2007) whom recommended the single-pass filtration with intermittent dose. Virus removal can be achieved in the 30 cm of a stratified sand filter. The use of multi-layer intermittent media filter (sand, gravel, soil, textile fabric) has significant to the organic removal and the reduction of clogging problem. Clay particles reduce the hydraulic conductivity and cause the clogging problem due to the deposit of clay in the pore spaces. Similar study was obtained by Koivunen *et al.* (2003) whom evaluated the pilot-scale tertiary treatment by rapid sand contact filter, chemical contact filter and biological-chemical contact filter. They obtained the FC reduction as 99%, 39% and 71%, respectively. Linear regression use to model the microbial numbers in MWTP effluents as a function of effluent residual organic matter, suspended solids and total phosphorus concentrations.

Natural zeolite has widely used for chemical and microorganism removal in water and wastewater treatment. Zeolites are hydrated crystalline aluminosilicates with a three-dimensional framework structure from SiO_4 and AlO_4^- tetrahedra linked together through the sharing of oxygen molecules (Auerbach *et al.*, 2003). Zeolites possess a regular pore structure, regular cavities and a considerably higher specific area; thus, they can serve as an effective filter media for removing bacteria. They are considered as abundant, low cost, high cation-exchange ability, adsorption capacity, and modifiable and regenerated material (Wang and Peng, 2010). Clinoptilolite is the most abundant natural zeolite and commonly used as an ion exchange or sorbent for inorganic or organic compounds (Ostroski *et al.*, 2009) and has a potential to remove bacteria in drinking water (Widiastuti *et al.*, 2008).

Charcoal filters have been applied for objectionable taste and odor removal from both of water and wastewater treatment in the past decade. Many types of wood charcoal have been investigated as low-cost absorbent material in filtration units. Zhu *et al.* (2010) demonstrated that activated bamboo charcoal has the rough surface, high adsorptivity, richness in micronsized pores and a good immobilization substrate provided a suitable dwelling for bacteria to reside. Park *et al.* (2003) reported that activated carbon (AC) acts in a similar manner. The microbes can easily attach onto the surface of AC than zeolite due to high specific area, nanometer-sized micro pores and cavities of AC. Furthermore, Busscher *et al.* (2008) and Hijnen *et al.* (2010) indicated that AC removes waterborne bacteria in water through van der Waals force (long distance) despite electrostatic repulsion (short distance) between negatively charged cells and carbon surfaces.

Vermicompost has been evaluated as the effective and alternative bioadsorbent for heavy metal removal. Matos and Arruda (2003) used vermicompost to remove metal ions (Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}). The dependence of metal adsorption on the particle size relies on the surface contact area and the ionic radius of each metal. The adsorption percentage has decrease significantly with the increase of the particle size of vermicompost (Jordão *et al.*, 2009).

The concentration of pathogenic microorganisms in wastewater can be decreased by using infiltration through porous media affected to the retention and elimination of pathogenic bacteria (Stevik *et al.*, 2004). The major factors in straining are sizing of filter media, bacterial cell size and shape, hydraulic loading and clogging. The important factors in adsorption are characteristic of porous media, content of OM, biofilm, temperature, water flow velocity, ionic strength and species, pH, hydrophobicity, electrostatic charges on the cell surface and concentration of bacteria. Bali *et al.* (2010) confirmed that the microorganism removal using infiltration percolation influenced on filter depth and temperature.

The bacterial removal mechanisms in filter media have been investigated by Foppen and Schijven (2006), Harvey and Garabedian (1991) and Jamieson *et al.* (2002). The main transport and attachment mechanisms for FC removal are straining, physical adsorption by surface forces (van der Waals and electrostatic forces) due to the difference of surface charge between collector and bacteria, sedimentation and grain surface roughness. This result is generally consistent with those reported by Brown and Jaffe (2001) that identified the bacterial transport through porous media. The transport mechanism is due to the expansion of the electric double layer between the bacteria and aquifer sand through displacement of the counterions. The expanded electric double layer increases the electrostatic repulsion. Ionic strength increases and thus bacterial attachment increases and transport decreases. Knappett *et al.* (2008) pointed out the small charges in ionic strength and grain size of silica sand impact to pathogenic virus and bacteria removal.

Clogging is one of the major problems in filtration-perculation unit. Verlicchi *et al.* (2009) identified the clogging occurs due to an accumulation of organic and inorganic materials among the pores causing a decrement of the effective volume available within the substratum, the hydraulic conductivity, the infiltration rate and the hydraulic retention time. Healy *et al.* (2007) and Jung-Woo *et al*. (2010) stated that the occurrence of biological clogging of filter media on the media surface resulted in a similar manner. The accumulation of microorganisms at the upper layer as biolfilms, is the main reason of surface sealing due to pore size and hydraulic conductivity reduction, affected the increasing of water retention and reducing the effective area for infiltration.

2.3 Prediction of FC removal based on FREN

Artificial intelligence (AI) technologies are integrated machine learning capabilities into numerical modeling systems based on advanced computer technology, in order to contain the demands on human experts. The algorithms and methods of AI technologies include knowledge-based systems (KBSs), genetic algorithms (Gas), artificial neural networks (ANNs), and fuzzy inference systems (Chau, 2006).

Microbiology prediction is a widely used method in food processing, especially the risk assessment (Ross and McMeekin, 2003). Thus it is a useful contribution to wastewater treatment in the case of FC removal. ANNs model is considered to be an alternative and powerful technique for modeling microbial survival and growth, due to high accuracy, adequacy and quite promising applications in engineering (Maier and Dandy, 2000).

ANNs models have been successfully implemented to predict coliforms and escherichia coli on tomato fruits and lettuce leaves after sanitizing by hypochlorous and peracetic acids (Keeratipibul *et al.*, 2011), the concentration of BOD and COD of wastewater (Rene and Saidutta, 2008), the performance and a basis for controlling the operation of the wastewater treatment process (Mjalli *et al.*, 2007). ANNs have the ability to adjust its parameters according to the target signal obtained by the experimental results. Unfortunately, this ability cannot include human knowledge due to the system properties especially with IMI treatment.

Fuzzy Rules Emulated Network (FREN) is automatic self-adjustable network which can be included the human knowledge about the system and material properties (Treesatayapun, 2009). These studies are represented a significant advantage of FREN thus the prediction algorithm is developed by using FREN based on the IF-THEN rules to follow the knowledge of IMI system. Furthermore, the convergence analysis can be guaranteed by the main theorem with the parameter selection for learning algorithm (Treesatayapun, 2011a; 2011b). The applications of FREN have been applied for several systems such as a blood pressure control system by minimized sodium nitroprusside dosing (Treesatayapun, 2010) and a positioning control system with hertzian contact estimation based on ultrasonic sensor (Armendariz *et al*., 2012).

2.4 Modification of natural zeolite

As a result of the zeolite structure, zeolites can be functionalized with heavy metals to increase their antibacterial activity for the pathogenic bacteria removal in wastewater treatment (Hrenovic *et al.*, 2012). Zeolites have negative charge within the pores balanced by positively ions (Na^+, K^+, Ca^{2+} and Mg^{2+}) with weaker electrostatic bonds. Those cations can be easily exchanged with cation in wastewater thus zeolites can serve as an effective filter media to remove bacteria (Widiastuti *et al.*, 2008). Zeolites like Montmorillonite (MMT) loaded with metallic cations (Ag^{2+}, Cu^{2+} and Zn^{2+}) have antibacterial and antifungal properties. These effects are described to the interactions of metal ions released from structure of MMT with the bacteria and fungi (Malachová *et al.,* 2011).

Wang and Peng (2010) have reviewed the modification of natural zeolites that can be applied in many methods such as ion exchange, acid treatment and surfactant functionlization to achieve higher adsorption capacity of anionic ions and organics.

Zinc is an essential trace element for living organisms and is associated with a number of processes essential for growth and metabolism; however excess zinc concentrations can be toxic. Zinc resistance mechanisms in bacteria are binding proteins and regulation of expression of zinc resistance genes (Choudhury and Srivastana, 2001). Bong *et al.* (2010) identified that high zinc concentration is toxicity and inhibition to microbial processes affecting on the reduction of the enzyme level, especially aminopeptidase activity. Sugarman *et al.* (1982) indicated that zinc can bind to bacteria pili and augment bacterial adherence. These results are similar to that obtained by Atmaca *et al*. (1998) where zinc accelerates exhibit antibacterial activity in bacterial growth due to binding to membranes of microorganisms, prolonging the lag phase of the growth and increasing the generation time of the organisms.

De La Rosa-Gomez *et al.* (2008a) investigated silver-modified clinoptilolite-heulandite rich tuff as an antibacterial agent against coliform microorganisms from water in a continuous column system at 0.6 mg mL^{-1} of silver concentration. Many mechanisms are proposed for bacterial action of silver zeolite, which are: the action of Ag^{+} released from zeolite, the reactive oxygen species generated from Ag^{+} in the matrix and the adsorption of the bacteria from solution then immobilization on the surface of the modified clay.

De La Rosa-Gomez *et al.* (2008b) investigated municipal wastewater disinfection using Mexican zeolites from Oaxaca (Ag-OZ) and Sonora (Ag-SZ) by the uptake of silver ions into the zeolitic network. Under the same preparation conditions, Ag-OZ is more efficient as a bactericide on E. coli than Ag-SZ because the amount of silver in the Ag-OZ is higher than the amount of silver in the Ag-SZ (4.6 times). Furthermore, it depends on their origin place of zeolites and remains constant during the critical disinfection period.

2.5 Effect of reclaimed domestic wastewater on plant and soil

The microorganisms contamination of vegetables irrigated with treated wastewater have been evaluated by Blumenthal *et al.* (2000). Some vegetable crops (cabbages, carrots, green tomatoes, red tomatoes, onions, chillies, lettuce, radishes, cucumbers and coriander) are reported to be risk from bacterial and viral infections, especially FC bacteria. Al-Sa`ed (2007) indicated that FC residual in plants irrigated with treated wastewater were increased following the harvest time in short period (4-6 months.

However, Cirelli *et al.* (2012) have recently shown that fecal contamination has low value of agricultural products (eggplants and tomatoes) in a long term effect (2 years). The FC contaminations are found from only two eggplant samples irrigated by surface drip irrigation and tomato at 10^2 and 10^3 CFU 100 g^{-1}, respectively. The FC contaminations of tomato are found on samples in contact with soil or plastic mulch, due to a significant increase of microbial biomass activity in these substrates.

The raw-eaten vegetables (tomato and eggplant) irrigated with treated wastewater (TWW) by macrophyte ponds have been evaluated the FC residual in plants and soil. FC and helminth eggs were observed in treated wastewater and irrigated soils at rate over the WHO recommended limits for vegetable to be eaten uncooked. Tomato fruits are observed FC contamination with the direct on-foliage irrigation with treated wastewater. The FC contamination in soil watered with TWW had 4.9±0.3, 7±0.8 and 5±2.4 log (CFU 100 g^{-1}) respectively in 2001, 2002 and 2003 (Akponikpè *et al.*, 2011).

This result is similar to that obtained by Agrafioti and Diamadopoulos (2012). As the feasibility of recycling treated municipal wastewater for agricultural irrigation on the Greek island of Crete has recently been shown. Three types of raw-eaten vegetables crops (olive trees, vineyards and lettuce) were irrigated with treated municipal wastewater to solve the problem of water shortage and stress in islands. However, analysis of effluents of WWTPs did not meet the criteria for unrestricted irrigation of the crops, due to absence of tertiary treatment.

Furthermore, Kalavrouziotis *et al.* (2008) demonstrated the effect of municipal reclaimed wastewater on soil and plants for vegetable irrigation (Broccoli and Brussels sprots). The high FC and E. coli of wastewater contribute high health risk, thus the secondary or advanced treatment is required.

Many studies investigate the impact of wastewater irrigation on the quality of soils. Pedrero *et al.* (2010) reviewed the use of treated municipal wastewater in irrigated agriculture and present the most common water quality problems which are: salinity, specific ion toxicity (sodium, chloride, boron), soil permeability, nutrients, microbiological content and other problems (clogging and excessive residual chlorine).

This result is similar to that obtained by Heidarpour *et al.* (2007) where the salt content (EC) increases in the top soil layer with subsurface irrigation and might inhibit plant growth. The behavior of FC bacteria survival in soil may indicates that they have a directly influence on soil moisture and temperature (Entry *et al*., 2000), affecting other factors such as moisture, soil type, temperature, pH, manure application rate, nutrient availability, and competition (Jamieson *et al.*, 2002).

Kiziloglu *et al.* (2008) studied the effects both of wastewater and treated wastewater irrigation on soil and plants (cauliflower and red cabbage). The wastewater application increase soil salinity, OM, exchangeable Na, K, Ca, Mg, plant available phosphorus and microelements, and decreased soil pH. Wastewater irrigation treatments also increased the yield as well as N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, Pb, Ni and Cd contents.Thus untreated wastewater can be used in agriculture in the short term and treated wastewater can be used in sustainable agriculture in the long term.

This result is generally consistent with those reported by Adrover *et al.* (2012) where the use of secondary-treated municipal wastewater in irrigation for more than 20 years. Soil water-soluble organic carbon, soil microbial biomass and alkaline phosphatase activities increase under treated wastewater irrigation. Biological activity of soils irrigated with treated wastewater is affected mainly by soil OM content.

However, the irrigation with treated wastewater is a strategy with many benefits to agricultural land management. Rusan *et al.* (2007) studied the long term (10, 5, and 2 years) wastewater irrigation that increase salts, OM and plant nutrients in the soil, however the barley biomass increase also.

Travis *et al.* (2010) demonstrated that treated greywater (GW) can be effectively irrigated without detrimental effects on soil or plant growth; however, raw GW may significantly change soil properties that can impact the movement of water in soil and the transport of the contaminants in the vadose zone. FC bacteria were absent or less than 10 CFU g^{-1} irrigated with fresh water or treated GW, but at least 1 order of magnitude higher in raw GW.

Rajeb *et al*. (2009) pointed out that the infiltration-percolation technique can improve the disinfection and treated wastewater quality but the problem of soil microbial growth and biofilm expansion always occur in infiltration percolation process. FC pollution can be used as bacteria indicator and always retain between 10 and 25 cm depths. The microbial biomass growth is low below the 25 cm of soil depth because the limitation of the nutriments or an increase in the interactions between the various micro-organisms of the biofilm.

Candela *et al.* (2007) discovered that FC bacteria acted in a similar manner. The limitation of the nutrients or biodegradable organic matter in soil, increase with depth and affect to a decrease in the FC content. FC can be detected in the top soil 5-10 cm depth and ranged between 100-1000 CFU g^{-1} soil. Palese *et al*. (2009) observed that the bacteria slightly increase in the wastewater-irrigated soil for olive during the irrigation season and especially in the top 10 cm. Jamieson *et al.* (2002) review that bacteria survival in top soil is more favorable than in subsoil.

Furthermore, Aiello *et al.* (2007) determined the persistence of microbial contaminants (E.coli and fecal streptococci) in the irrigated soil with reclaimed wastewater at 0.1 m depth that is found higher than other soil profiles (0.2 and 0.4 m). Irrigated wastewater reduces soil porosity, translation of pore distribution towards narrow pores and a consequent decrease in permeability; however tomato crop production yield is increase.

Clay soil content effect on fecal bacteria removal in an intermittent media infiltration system

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Abstract

Water scarcity, degradation of freshwater resources and wastewater pollution become a serious crisis; especially in the arid regions of the world. The reuse of wastewater treatment system was investigated by using the intermittent media infiltration (IMI) in order to evaluate fecal coliform (FC) removal efficiency. 12 IMI columns were composited with soil and natural filtering materials (sand, zeolite, vermicompost and charcoal) at percentages of 25, 50 and 75. The municipal wastewater was treated for FC analysis during four weeks. The filter media were determined and characterized for chemistry, physics, and morphology related to FC reduction. Fine particle size and adsorption capacity are important factor to retain the FC. Their FC reductions were variable during the period of the study. The compositions of soil and filter media at 75/25 gave the best performance of FC removal and their average FC reductions for sand, zeolite, vermicompost and charcoal were 94.4%, 82.2%, 93.8% and 99.6%, respectively. The IMI of soil/charcoal gave the highest log removal (2.19-2.46 log) than other media and complied with Mexican regulations. These results showed a good performance of FC removal and possibilities for implementation to reclaim municipal wastewater in unrestricted irrigation.

Key Words: Bacteria, column, filter media, soil, wastewater reuse

1. Introduction

Water shortage and water pollution problems still are some of the crucial issues in the world and have a cumulative effect on the environment. It becomes critically and can cause severe consequences, due to the annual growth rate of world population increased dramatically about 1.2% or 77 million people per year in 2002 but their estimation in 2050 will have between 7.9-10.3 billion (Asano et al., 2007). Moreover, freshwater withdrawal on global scale is found that irrigation water use is 70% whereas, industrial and domestic water usages are 20% and 10% respectively.

In Mexico, 77% of freshwater is for irrigation, public supply is 14%, industrial use is 4% and the other 5% is for thermoelectric (CONAGUA, 2010). Their wastewater reuses are applied for agricultural irrigation and industrial 33%

and 26% respectively. Moreover, the cost of treated wastewater is cheaper than producing potable water about 2.67-2.80 times (Escalante et al., 2003). Therefore the high water consumption of agricultural irrigation seems to have high considerable ability for wastewater reclamation and reuse which is important mechanism to sustain water resource management.

However, its application is quite sensitive to human health and environment due to the potential transmission of infection diseases of waterborne microorganisms (Bahri, 1999). Fecal coliform (FC) can be predicted a potentially high pathological level (Huertas et al., 2008) and it is the most used as biological indicator in wastewater reuse (Salgot et al., 2006). Thus, the Mexican regulations (NOM-001-ECOL, 1996) limit the FC less than 1000 MPN/100 ml in the wastewater reuse for unrestricted agricultural irrigation, which is the same as the world health organization guideline (WHO, 1989).

The elimination of residual bacteria in wastewater can be made by inactivation or removal processes, which can lower FC significantly, to repurify wastewater and meet the standards (Mujeriego and Asano, 1999). Many technologies have been widely studied and can be mentioned as membrane filtration (Gómez et al., 2006; Reimann, 2002), activated sludge (Ellouze et al., 2009; George et al., 2002; Kassab et al., 2010), natural treatment (Karathanasis et al., 2003; Kim et al., 2006; Kirsten et al., 2007; Roberto et al., 2008) and filtration with different media (Ausland et al., 2002; Jiménez et al., 2000, 1999; Koivunen et al., 2003; Olson et al., 2005).

In the case of the disinfection different physical or chemical treatments such as heat, light and the very common use of the hypochlorite (Veschetti et al., 2003) and iron (VI) (Cho et al., 2006; Sharma, 2007) have been applied. With regard to chlorine, it is widely use for many applications but their disinfection by-products (DBPs) can produce and affect to human health. Therefore the alternative disinfection technique is necessary for wastewater treatment especially in agricultural reuse (Liberti and Notarnicola, 1999).

The advanced primary treatment followed by filtration was recommended for wastewater reuse in unrestricted irrigation areas in Mexico. The sand and synthetic medium was applied filtration to investigate the removal efficiency of helminth eggs which met the Mexican standard, except FC (Jiménez et al., 2000). At present, technologies of wastewater reuse have developed dramatically for beneficial uses. The selection of these technologies is important to consider cost-effective treatment system, human health, environmental impact and objective of wastewater reuse also (Bahri, 1999; Mujeriego and Asano, 1999).

Therefore soil infiltration is an alternative for applying wastewater reuse into irrigation. This treatment includes of physical processes, chemical reactions and biological transformations (Ausland et al., 2002). The concentration of pathogenic microorganisms in wastewater can be decreased by using infiltration through porous media affected to the retention and elimination of pathogenic bacteria (Bali et al., 2010; Stevik et al., 2004). In this experiment, it was applied intermittent media infiltration (IMI) with selected natural filtering materials to improve quality of FC removal in wastewater reuse.

The natural filtering materials selected in this study were soil, sand, zeolite, vermicompost and charcoal. Soil infiltration and soil clay have been investigated for microorganism (Mosaddeghi et al., 2009; Van Cuyk and Siegrist. 2007), organic (Oladoja and Ademoroti, 2006) and heavy metal (Pode et al, 206) removal on wastewater. Natural zeolite has widely use for water and wastewater treatment (Wang and Peng, 2010; Widiastuti et al., 2008). Vermicompost has been evaluated as the effective and alternative bioadsorbent for heavy metal removal (Matos and Arruda, 2003; Urdaneta et al., 2008) and has the significant BOD reduction (Taylor et al., 2003). Charcoal filters have been applied for objectionable taste and odor removal from both of water and wastewater treatment in the past decade. Many types of wood charcoal have been investigated as low-cost absorbent material in filtration units (Mukherjee et al., 2007; Serpieri et al., 2000; Wang et al., 2010).

In this study, the objectives were (i) to evaluate the efficiency of fecal coliform removal by intermittent media infiltration and achieve wastewater quality established by the Mexican regulations for agricultural irrigation (ii) to study the relation with filter media composition in different ratios and determine the chemical and physical mechanisms for FC removal. The proposed IMI treatment with a low cost treatment technology, simple to operate and minimum energy consumption, had a significant FC reduction.

2. Materials and methods

The study was conducted at the Department of Soil Science in the Universidad Autónoma Agraria Antonio Narro located in an arid region at south of Saltillo city, Coahuila, Mexico. The climate is semiarid with an average annual precipitation of 369 mm and average temperature of 18.2 ^{o}C.

2.1 Filtering materials

Soil, sand and vermicompost were obtained from this university. Soil sampling was selected from soil surface and sand was collected from a stream at agricultural field. Vermicompost was generated from biological degradation and stabilization of organic matter obtained when earthworms decompose feces of dairy cattle by the Californian red worm (*Eisenia foetida*) which is a commercial agricultural product. Natural zeolite was a clinoptilolite from San Luis Potosí, Mexico, commercialized by Zeomex, Monterrey, Mexico. The charcoal was a commercial product made from natural mesquite wood which is the common plant in Mexico.

For media preparation, all filtering media were air-dried for 2 days ground and screened with standard laboratory sieve (2 mm). They were analyzed as soils following the method of Black et al. (1965a, 1965b); whereas description for pH, organic matter (OM), electrical conductivity (EC), total nitrogen (N), carbonate (CO_3^{2-}) and saturated hydraulic conductivity (Ks) are summarized in Table 1.

Table 1. The physical and chemical properties of studied filtering materials.

Sample	% Particle size			pH	EC^a	% OM^b	% N	% CO_3^{2-}	Ks^c
	>2 mm	0.02-0.002 mm	<0.002 mm		($dS\ m^{-1}$)				(cm/h)
Soil	42	34	24	7.6	1.0	1.87	0.09	23.37	0.16
Sand	60	28	12	7.5	0.7	0.63	0.03	20.21	1.38
Zeolite	64	26	10	6.3	0.8	0.95	0.05	8.82	1.30
Vermicompost	-[d]	-	-	7.4	2.1	32.98	1.65	34.52	12.25
Charcoal	84	16	0	8.0	3.0	-	-	-	2.98

[a] EC=Electrical conductivity; [b] OM=Organic matter; [c] Ks=Saturated hydraulic conductivity; [d] not measured

The texture of soil was classified as loam but other media, except vermicompost, were classified as percent of particle size. pH of all filtering materials (6.3-8.0), soil, sand and vermicompost media had neutral pH but zeolite had a acid and charcoal had a weakly alkalinity than others. The EC of charcoal was higher than other media (about 3 times), except vermicompost had high EC. On the other hand, their EC could not cause the salinity problem. Ks is a measure of soil water movement by using of Darcy's law.

These results were obtained by the constant-head permeameter method. Vermicompost had the highest ability of the soil to transmit the water than charcoal, zeolite, sand and soil, respectively. The X-ray diffractometer analysis showed the mineral composition of the soil. It was: quartz (32.94%), calcite (10.80%), albite low (11.16%), illite (12.03%), merlionite (2.17%) and amorphous material (30.90%). Almost 87% of zeolite was clinoptilolite in crystal phase (De la Rosa-Gómez et al., 2008) and the main natural zeolites found in Mexico are mordenite, erionite, and clinoptilolite (Hernandez et al., 2005).

Due to the fact that particle size is an important parameter for FC removal, in this study the particle size distribution of filtering materials was applied and determined by standard sieving method. Furthermore, the chemical composition of filtering materials that indicated their percent of major oxides was obtained by X-Ray fluorescence (XRF) spectroscopy, on press powder pellets using a Bruker AXS spectrometer. Scanning electron microscopy (SEM) can identify the relative particle shape, texture and size of the filtering materials. Energy dispersive X-ray (EDX) indicates the elemental composition of an interesting area of media samples. A SEM (model JSM-8440; JEOL) was applied to obtain SEM images of studied media by coated with graphite and cooperated with EDX.

2.2 Wastewater

A sample of domestic wastewater used in this experiment was obtained from municipal wastewater treatment plant (MWWTP) located in south of Saltillo, Coahuila, Mexico and its capacity is 43.2 m^3/day. Their processes include screening, digester tank by activated sludge process, 4 aeration tank series, clarification tank and disinfection tank by using Sodium hypochlorite.

Raw water, unchlorinated and chlorinated treated wastewater were collected from MWWTP 2 times per week following the sampling standard method (NMX-AA-003, 1980). They were analyzed according to standard method (APHA, 1998) for evaluating the physicochemical performance of the MWWTP. It was found that the mean effluent concentration for other parameters (TSS, BOD, Cu, Zn, Pb) fulfilled the Mexican regulations. For FC bacteria, the average data for raw, unchlorinated treated and chlorinated treated wastewater were 199.2×10^3, 30.0×10^3 and 15.8×10^3 MPN/100 ml respectively, in which the effluent was higher than its limitation (1.0×10^3 MPN/100 ml).

Wastewater influent and the effluent from IMI columns were analyzed pH and temperature by using pH meter (model 420A; Orion). Conductivity and TDS were measured by using conductance meter (model 32; YSI). Cations (Ca, K, Mg, Na) and heavy metal (Cu, Zn, Fe, Pb) were analyzed by Atomic Absorption Spectrophotometer (model SpectrAA-5; Varian).

2.3 IMI columns

The study was conducted in the laboratory using 12 clear plastic columns of 12 cm diameter and 30 cm height. All columns were supported with small size of gravel having an average diameter of approximately 2 mm at 3 cm depth and placed with cotton wool at the bottom of each column. Then, the homogeneous mixture was filled into the columns (22 cm length) at 80% of the total volume of IMI column. Due to the high biogeochemical activity within the infiltrative zone of soil, core analyses were from 0 to 15 cm depth (Van Cuyk et al., 2001).

The composite of filter media were prepared by mixed with different combination ratios (v/v) of other filtering materials as soil/sand (Ss), soil/zeolite (Sz), soil/vermicompost (Sv) and soil/charcoal (Sc). All of them were used without any additional pretreatment. Then, soil was added according with the proportion established in percentage of 25, 50 and 75 as IMI column number 1 to 3, respectively. In this experiment, 12 IMI columns were studied in different proportion of mixed filter media.

After IMI columns were saturated with deionized water for 24 h, the unchlorinated treated municipal wastewater was introduced into each IMI column 500 ml per time per day and pass through the IMI column by gravity flow. The effluent samples of each column were collected manually in 100 ml sterile bag (Whirl-pak) and analyzed in the laboratory for 4 monitoring weeks.

2.4 Fecal coliform removal

FC bacteria were analyzed as microbiological parameter by the most probable number (MPN) method to estimate their concentrations. Five 1ml aliquots of each three dilutions were examined by A-1 medium (Bection, Dickinson and company USA) accepted by USEPA, which can be used in a single-step procedure (Standridge and Delfino, 1981). The tube contained an inverted inner vial (Durham tube) for gas collection was incubated at 35 ± 0.5 °C for 3 h and transferred to 44.5 ± 0.2 °C for 21 h.

For waterborne pathogenic microorganism analysis, percentage of FC reduction and log removal were calculated on each IMI column between influent and their effluents (Asano et al., 2007). The FC concentrations of the effluent of each IMI columns were evaluated following Mexican regulation. Regression analysis was applied in this study to measure the reliability of the linear relationship between the percent of soil filling and the percent of FC reduction.

3. Results and discussion

3.1 The wastewater quality on the IMI performance

3.1.1 Performance of IMI columns

The results of pH, conductivity, TS and TSS of each IMI columns are shown in Table 2. pH of all the effluents were more alkaline than the influent and in the range of 7.6-8.2. Conductivity, TS and TSS were high in the first week but decreased the rest of the time. Due to the IMI operation of this experiment, it was run without flush cleaning in the first time compared with the studied of Oladoja and Ademoroti (2006). All solids and heavy metal (Cu, Zn, Fe, Pb) in the effluents of IMI columns were lower than the Mexican regulations. Plant nutrients (Ca, K, Mg, and Na) were analyzed; calcium contents in the effluent IMI were higher than the influent.

It could be observed that when percent of soil increased (from 25-75%), calcium content in the wastewater was also increased, due to high calcium contents of soil following the analysis of chemical composition. The result of chlorinated wastewater and effluent from IMI columns were considered.

Furthermore, their physical and chemical characteristics were similar but the effluents of IMI columns were utilizable for watering the plant due to free of chlorine.

Table 2. Physical and chemical properties of the IMI influent (n=10) and effluents (n=4) of wastewater, mean (± S.E.M). All concentrations in mg L^{-1}.

Parameter	Influent	Effluents											
		Ss1[a]	Ss2	Ss3	Sz1[b]	Sz2	Sz3	Sv1[c]	Sv2	Sv3	Sc1[d]	Sc2	Sc3
pH	7.2 (0.1)	7.9 (0.2)	7.9 (0.2)	8.0 (0.0)	8.0 (0.1)	7.8 (0.2)	8.1 (0.3)	7.7 (0.2)	7.9 (0.1)	7.8 (0.1)	7.7 (0.1)	7.6 (0.1)	7.6 (0.1)
EC[e] (μS cm^{-1})	837 (21)	908 (33)	928 (24)	943 (48)	899 (25)	884 (9)	848 (42)	1082 (36)	944 (13)	920 (17)	1488 (240)	1301 (122)	1060 (53)
TS[f]	828 (59)	793 (43)	761 (48)	711 (65)	686 (93)	705 (118)	904 (73)	1031 (58)	795 (85)	775 (49)	1082 (176)	984 (147)	776 (74)
TSS[g]	11 (3)	9 (1)	11 (5)	7 (5)	2 (1)	9 (3)	10 (5)	10 (0)	12 (2.5)	9 (1)	6 (1)	10 (2)	7 (3)
Ca	56.5 (10.1)	66.3 (9.7)	96.3 (21.9)	197.5 (84.7)	80.0 (10.8)	96.3 (8.0)	122.5 (33.3)	32.5 (2.5)	25.0 (2.9)	32.5 (6.3)	50 (4.1)	42.5 (4.8)	37.5 (7.5)
K	21.5 (6.3)	13.3 (1.3)	20.8 (1.3)	17.3 (1.1)	22.0 (1.4)	18.5 (1.5)	17.8 (1.3)	88.8 (15.5)	66.5 (9.3)	152.3 (100)	174.8 (49.5)	111.75 (26.3)	71.5 (13.1)
Mg	18.5 (1.1)	13.7 (1.3)	21.3 (4.7)	26.3 (4.7)	17.5 (1.4)	15.0 (0.0)	20.0 (3.5)	27.5 (2.5)	17.5 (2.5)	17.5 (2.5)	20 (0.0)	20 (0.0)	17.5 (2.5)
Na	308.0 (76.7)	818.8 (201.9)	562.5 (80.7)	625.0 (97.4)	731.3 (219.0)	593.8 (204.2)	662.5 (96.6)	110.0 (13.5)	115.0 (15.5)	92.5 (13.1)	112.5 (32.0)	137.5 (21.4)	125.0 (15.5)
Zn	0.03 (0.02)	N.D.[h]	N.D.	N.D.	N.D.	N.D.	N.D.	0.03 (0.03)	0.05 (0.03)	0.03 (0.03)	0.1 (0.00)	0.1 (0.00)	0.1 (0.00)
Pb	0.03 (0.02)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.08 (0.05)	0.10 (0.04)	0.1 (0.04)	0.1 (0.04)	0.13 (0.03)	0.13 (0.03)

[a] Ss = soil/sand; [b] Sz = soil/zeolite; [c] Sv =soil/vermicompost; [d] Sc = soil/charcoal; [e] EC= Electric conductivity; [f] TS = Total solid; [g] TSS = Total suspended solid; [h] N.D = Not detectable

3.1.2 FC removal efficiency

FC concentrations in the influent were highly variable during the sampling periods. The average FC concentration in the influent was 30.0 X 10^3 MPN/100 ml (ranging from 17.0×10^3 - 54.0×10^3 MPN/100 ml). The average effluent concentrations were also variable (ranging from 1.0×10^3 - 9.0×10^3 MPN/100 ml). The IMI-Sc columns and only IMI-Ss3 with soil/sand at 75/25 had average effluent FC concentration lower than the limitation and met these criteria during the monitoring periods. The FC log removal of IMI columns in each different proportion were calculated and presented in Fig.1. It can be observed that log removal of IMI-Sc (2.19-2.46 log) showed the highest among other compositions of filter media and the increase of percent of soil filling gave the higher efficiency of FC reduction.

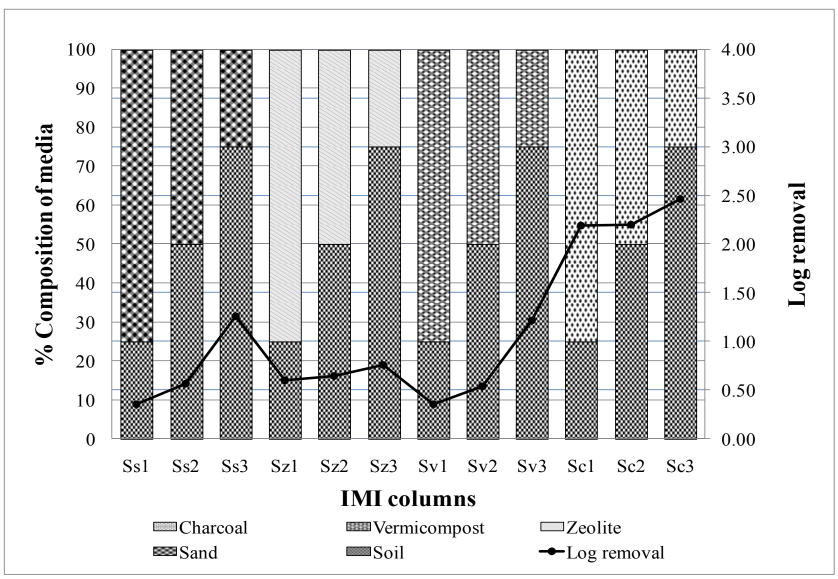

Fig. 1. FC log removal and percent composition of

Ss (soil/sand), Sz (soil/zeolite), Sv (soil/vermicompost) and Sc (soil/charcoal).

The results of FC removal during 4 monitoring weeks are presented in Fig. 2. The results showed that the percents of FC reduction were variable during the period of the study due to the fact that wastewater influent was variable as discussed above. The compositions of soil and filter media at 75/25 gave the best performance of FC removal and their average FC reductions for sand, zeolite, vermicompost and charcoal were 94.4%, 82.2%, 93.8% and 99.6%, respectively. FC reduction of IMI-Sc columns was moderately stable during the studied period (98.5-99.8%).

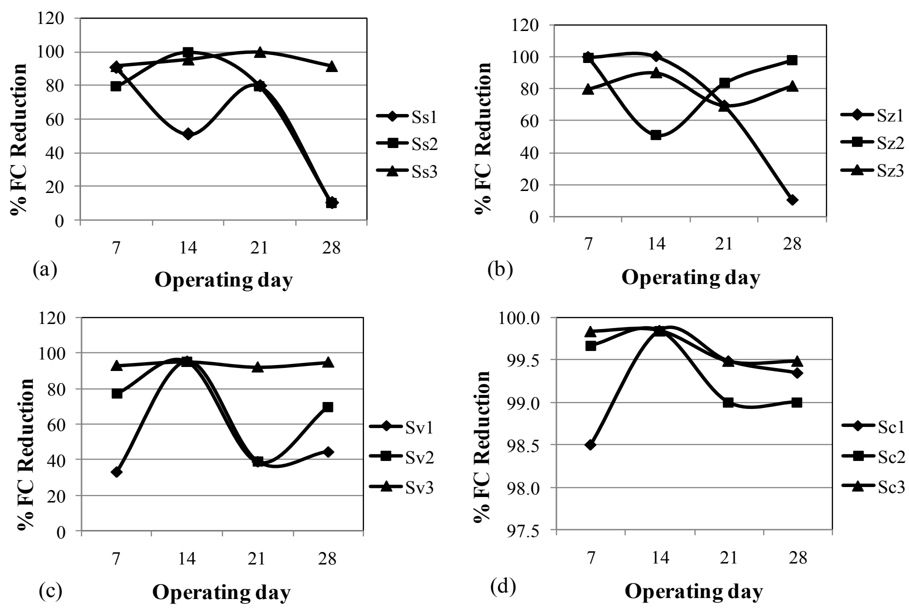

Fig. 2. Operating period of IMI columns vs % FC reduction;
Ss (soil/sand), Sz (soil/zeolite), Sv (soil/vermicompost) and Sc (soil/charcoal).

3.2 Filter media composition on FC bacteria removal

In order to identify the significant factors which may be associated with the FC removal and IMI efficiency, the physical and chemical characterization of media are presented in Table 1. The smallest particle was clay (<0.002 mm) which was most frequently found in soil, sand and zeolite, respectively.

Vermicompost had highest chemical properties of OM, N and CO_3^{2-} because it was directly obtained from earthworms decompose wastes by the process of biological degradation and stabilization of OM (Matos and Arruda, 2003). The characteristic of vermicompost (pH, N) was similar values but content of OM was lower than the minimum limit (40%) (Jordão et al., 2009). Ks of vermicompost had the highest ability to transmit the water than other filtering materials.

Because of porosity and permeability, vermicompost contained large pore space and particle size, which affect to have the lowest residence time in the IMI column than the other media. It was observed that Ks decreased when clay content in media increased ($Ks_{soil} < Ks_{sand} < Ks_{zeolite}$). When the IMI column had high Ks and consequently, the residence time of each column was low due to it directly affected the relationship with their soil structure and porosity and small microorganism removal (Bali et al., 2010; Gonçalves et al., 2007).

The particle size distribution was done by sieve analysis and prepared curves of 5 filtering materials, which are illustrated in Fig. 3. According to this figure, the mean grain size at 50% finer particle (d_{50}) of soil, sand, zeolite, vermicompost and charcoal were 0.20, 0.12, 0.20, 0.32 and 0.85 mm, respectively. The effective size, uniformity coefficient (C_u), and coefficient of gradation (C_c) of filtering materials can be calculated also.

For the C_u of the particle size distribution (d_{60}/d_{10}) of soil, sand, zeolite, vermicompost and charcoal were 5.887, 2.376, 8.647, 7.319 and 14.296, respectively. Sand had the smallest C_u than other media that meant the majority of grains were mostly the same size, which is classified as a uniformly graded media. In the other hand, charcoal had the highest C_u which was the least uniform than the others. For the C_c of soil, sand, zeolite, vermicompost and charcoal were 0.730, 0.828, 1.064, 0.763 and 1.573, respectively. Therefore, zeolite and charcoal were considered as well grade sand due to their C_u (higher than 6) and C_c (between 1 to 3).

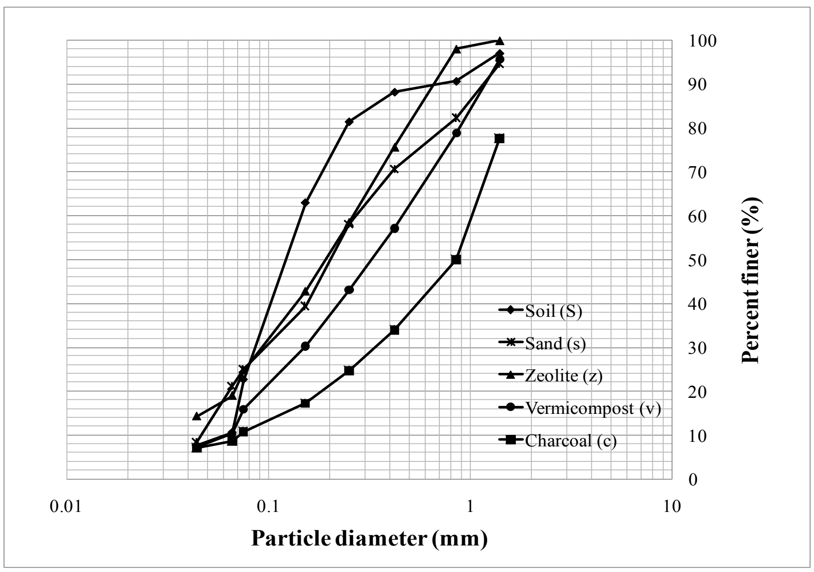

Fig. 3. Particle size distribution curve of filtering materials used to form the composite filter media

It was also observed that sand contained the finest particles in the diameter larger than 0.075 mm while charcoal contained the coarsest particles. According to the previous study of texture analysis showed in Table 1, the finest particles size smaller than 0.02 mm was most presented in soil and sand about 58% and 40%, respectively.

Due to different process for finding the particle size, sieve analysis which is mechanical technique, can only determine the particle sizes larger than 0.05 mm in diameter (Black et al., 1965a). On the other hand, the hydrometer method which is a sedimentation procedure, can determine the particle size smaller than 0.05 mm in diameter. As a direct result of sieve analysis, the large particles cannot liberate fine particles like the other method. Therefore, it could be concluded that texture analysis of filtering materials defined the finest particle size as soil and particle size distribution defined the coarsest particle as charcoal.

The morphology of the filtering materials is presented in Fig. 4 identified by SEM. It illustrates that SEM images of soil, sand, zeolite and vermicompost were mainly composed of amorphous materials and vermicompost had the largest particle size. While charcoal was irregular shaped particles and the longest and largest particle size confirmed by the analysis of particle size distribution.

The composition of soil had an influence on other filtering media such as sand, zeolite, vermicompost and charcoal because the high percentage of soil filling was directly affected to high FC reduction as presented in Fig. 2. It can be seen that the composition of soil and filtering materials at 75/25 gave the best performance of FC removal for all of IMI columns (Oladoja and Ademoroti, 2006) because soil texture contained a highest percentage of clay than other media and fine particle of clay content affected by their filtration. Physicochemical characteristic of clays had the high surface area and the platy morphology. Not only the finest particle sizes give the highest surface area but also a great potential for adsorption (Mackie et al., 2010).

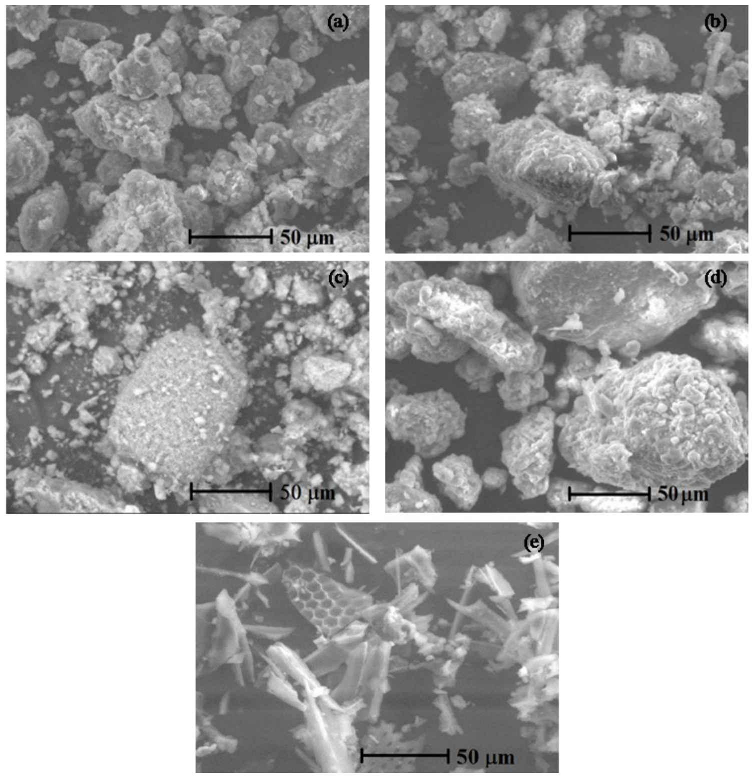

Fig. 4. SEM images of filtering materials

(a) soil; (b) sand; (c) zeolite; (d) vermicompost (e) charcoal.

Comparison of FC log removal for all IMI columns (Fig.1), it showed that the composition of Sc performed the highest FC removal efficiency in more than 3 compositions of Ss, Sz and Sv. Although the fine particle was the important mechanism of FC removal but charcoal was largely pure carbon and composed of porous structure which was a highly efficient media for adsorption also.

Moreover, their surface morphology could be identified that their sizing of micro pores was appropriated and more easily attached the bacteria onto their surface. These results are in agreement with expertise reported by Park et al. (2003) indicating that their surfaces are available for microbes and their adsorption capacities of organic materials are higher than zeolite. Although natural zeolite is considered that has high cation-exchange ability, adsorption capacity and modifiable and regenerated material (Wang and Peng, 2010). Vermicompost has a very high porosity aeration, drainage and water holding capacity as well as a good adsorption capacity (Jordão et al., 2009).

However, the capacity of the filter media (sand, zeolite and vermicompost) for FC removal was lower than our expectation because they did not contain the fine particle for producing the micro pores. Thus they could not work as fine filter to help the mechanical retention and increase surface contact area (Matos and Arruda, 2003). The adsorption capacity decreased when particle size of filter media (vermicompost) increased (Jordão et al., 2009).

Although the OM content in the filter media is associated with the increase of cation exchange capacity, surface area and adsorption (Bali et al., 2010). Vermicompost had the highest OM content than other media; however its FC removal efficiency was quite low. Thus, it can be concluded that the significant factor for straining mechanism could be grain size of porous media than the OM content.

The study of chemical composition of filtering materials (soil, sand and zeolite) indicated the percent of chemical composition of major oxides analyzed by XRF as presented in Table 3. Thirteen major oxides were determined and found that the principal chemical compositions of filtering materials (soil, sand and zeolite) were silica (SiO_2), calcium oxide (CaO) and alumina (Al_2O_3), but vermicompost and charcoal did not determine due to highly contain of OM and purely compose of carbon, respectively. It shows that the content of SiO_2, CaO and Al_2O_3 were high for all samples, similar to the results obtained by Oladoja and Ademoroti (2006).

Table 3. Chemical composition of major oxides of the studied filtering materials, wt%

Material	Percentage of chemical composition													LOI[a]
	SiO_2	CaO	Al_2O_3	Fe_2O_3	K_2O	MgO	Na_2O	TiO_2	P_2O_5	SO_3	MnO	ZrO_2	SrO	
Soil	42.35	21.19	10.24	4.44	2.43	1.66	0.86	0.66	0.24	0.18	0.07	0.05	0.05	15.49
Sand	48.70	18.36	11.10	4.58	2.38	1.57	1.12	0.76	0.18	0.10	0.05	0.06	0.05	10.92
Zeolite	62.53	3.05	13.02	1.89	3.67	0.75	0.41	0.10	N.D.[b]	N.D.	0.04	0.04	0.08	14.37

[a] LOI = Loss on ignition at 950 ^{o}C, 1h; [b] N.D. = Not detectable

Moreover, the mainly small particles on the morphology surface of filtering materials were detected by EDX and determined the percentage of element composition (Fig. 5). It was found that the principal elements of filtering materials were C, O, Al, Si, K, Ca and Fe. The highest element percentage of soil and vermicompost was Ca but for sand, zeolite and charcoal samples were Si. These results corresponded with the analysis of chemical compositions by XRF.

Clay particles play a critical role for reducing bacteria concentrations in wastewater due to their smaller size (2 μm), and its direct influences to adhesion of bacteria to clay by electrostatic force of the counter ions. Moreover, the bacteria attraction of filter media surface depends on the thickness of double layer containing concentration of ion and valency (Stevik et al., 2004). This behavior can be explained in term of the chemical composition of filtering media, which contained the quantity of oxide of CaO, Al_2O_3, Fe_2O_3, K_2O and MgO.

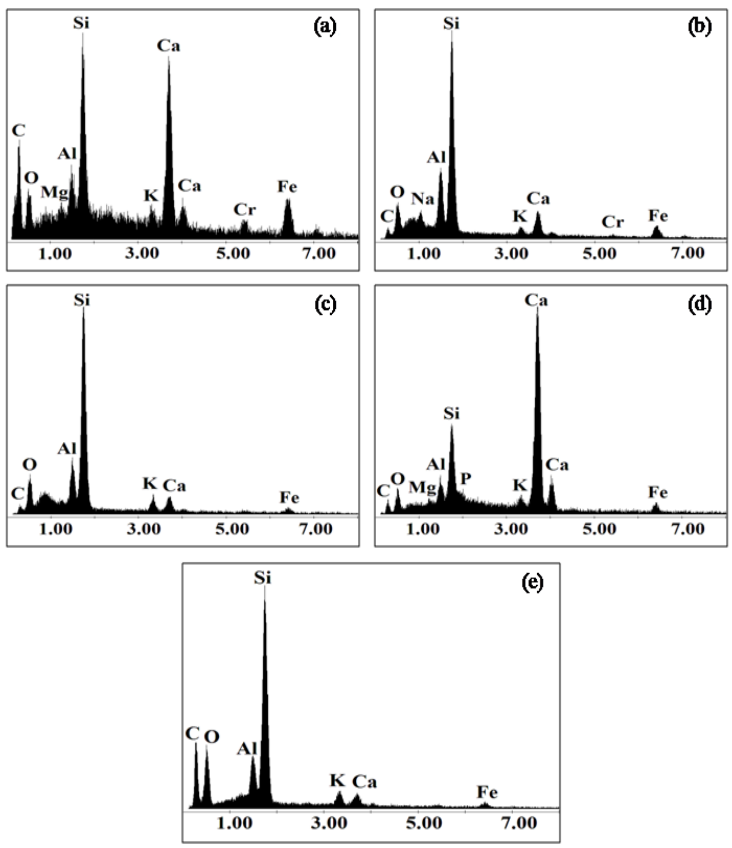

Fig. 5. EDX of filtering materials

(a) soil; (b) sand; (c) zeolite; (d) vermicompost (e) charcoal.

Their cations (Ca^{2+}, Al^{3+}, Fe^{3+}, K^{+} and Mg^{2+}) increased more positive charges in the electrolyte solution; at the same time, it was significantly reduced their thickness of double layers at the media surface. Cation exchange capacity (CEC) of soil can retain and release elements such as K, Ca, Mg and Na. Soil highly contained clay or OM, may tend to have high CEC. Then, bacteria cells come closer to the media surface by the van der Waals force and their adsorption capacities were increased to attach more FC. Furthermore, bacteria have more adsorption capacity with divalent and trivalent cations than monovalent cations, respectively (Stevik et al., 2004). According to the highest content of CaO in soil, it can be summarized that FC can be efficiently removed when percent of soil filling increased.

Moreover, it can be explained that bacteria had been removed by porous filter media of IMI columns during the wastewater passed through the media by physical (straining, adsorption) and biological (microbial degradation) processes (Oladoja and Ademoroti, 2006; Stevik et al., 2004). Straining is the important factor to retain bacteria in the IMI columns by filter media which is the physical process to obstruct the transit of wastewater through pores smaller than the bacteria.

FC bacteria are considered as colloids in the wastewater. They are attached to the surface of filter media by driving forces which are electrostatic for long distance and van der Waals attraction for short distance (Hijnen et al., 2010). Filter media like clay and silt have the pore sizes within the range of bacteria cell size and bacteria act as charged particles. Regard to negative charge of bacteria cell and negative charge of media surface (clay particles), it can be attached each other by electrostatic forces of positive charge around the edge of media surface and this could be consequence of bacteria removal in the IMI columns also (Van Loosdrecht et al., 1989).

Therefore the physicochemical process by adsorption can occurred at the surface reaction between bacteria and clays. The biological process can be explained in terms of the microbial attachment and growth at the filter media to retain the bacteria in the IMI columns. Moreover, these microorganisms also help to digest the solids in wastewater (Koivunen et al., 2003).

Regression analysis was applied to measure the reliability of the linear relationship between percent of soil filling in each IMI columns and percent of FC reduction as presented in Fig. 6.

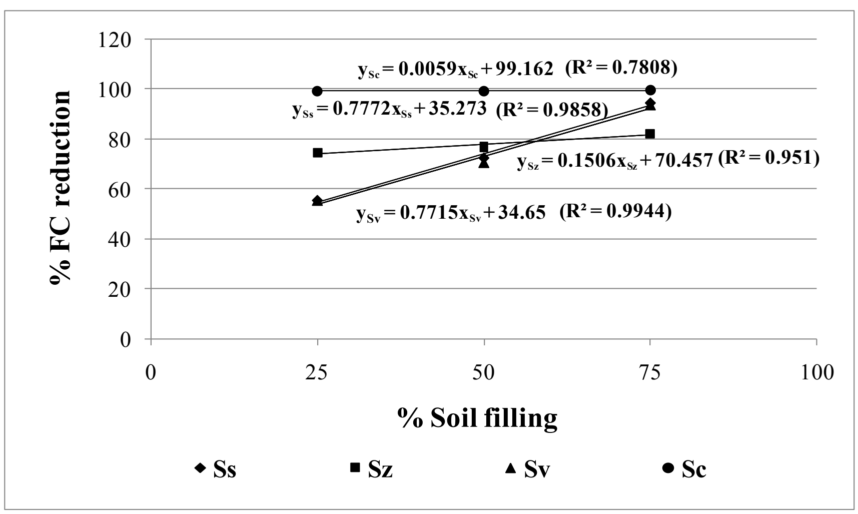

Fig. 6. Linear regression between the percent of FC reduction and soil filling; Ss (soil/sand), Sz (soil/zeolite), Sv (soil/vermicompost) and Sc (soil/charcoal).

Their correlation coefficients of Ss, Sv and Sz indicated a clearly significant relationship (R^2 = 0.99, 0.98 and 0.95, respectively), but no relationship was shown for Sc (R^2 = 0.78). As it can be seen, when soil was increased into the IMI-Sc and IMI-Sv columns at percentage of 25, 50 and 75, the percent of FC reduction increased too due to clay particle associate to reduce large pore of filtering materials such as vermicompost expanding the retention time in the IMI column. In contrast, charcoal seemed to work well as filter media without composed of soil because their relations when soil mixing was less significant.

4. Conclusions

The composition of soil and filtering materials (sand, zeolite and vermicompost) at 75/25 gave the best performance of FC removal for all of IMI columns. The increase percent of clay soil was influenced on filtering media and directly gave the higher efficiency of FC reduction. Fine particle size is an important factor to retain FC by their physical and physicochemical mechanisms for retention and adsorption. The IMI-Sc gave the highest FC log removal due to high adsorption capacity. Further research of wastewater reuse should investigate the effects on plants and soils due to survival of FC bacteria and soil salinisation.

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Prediction of fecal coliform removal on intermittent media infiltration by varying soil content based on FREN

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ABSTRACT

Current global water shortage and water pollution problem are some of the crucial issues in the world, especially in the arid zones. The wastewater reuse was investigated the efficiency of fecal coliform (FC) removal using the intermittent media infiltration (IMI) with varying soil content and natural porous media (sand, zeolite, vermicompost and charcoal), and its prediction was introduced by applying fuzzy rules emulated network (FREN). The physicochemical properties of the porous media were determined and the mechanisms of FC removal were discussed as the effect of fine particle size and increasing of ion charges. The compositions of soil and porous media at a ratio of 75/25, respectively, gave the best performance of FC reduction. The network architecture was constructed by the knowledge regarding to the relation between soil content (25, 50 and 75) and FC removal, and was introduced IF-THEN rules for FREN construction as their predicted curves at 20 iterations. The learning rate η was selected as 5 following the main theorem and the convergence of FREN prediction could be guaranteed. The results showed that the prediction methodology gave a good performance to forecast FC removal with the range of soil content (20-80%) and several compositions of porous media in IMI system.

Key words: Bacterial removal, fuzzy logic, infiltration, porous media, prediction

INTRODUCTION

According to the UNESCO (2009), water consumption in agriculture is by far the main user and expands rapidly due to population growth, rapid economic development and impact of climate change. Mexico is considered to be one of the ten largest water users in the world (CONAGUA, 2010). Therefore water reclamation and reuse is crucial to sustain water resource management, especially for agricultural irrigation.

However, one of the concerns to its application is related to hygienically safe for human health and the environment, due to the potential transmission of infection diseases of waterborne microorganisms (Salgot *et al.*, 2006). The observation of fecal coliform bacteria (FC) in wastewater is evidence of fecal contamination and possibility of having the pathogenic risks. FC is always present in the intestinal tract of warm-blooded animals and is the most used as biological indicator in wastewater reuse (Huertas *et al.*, 2008). Thus, the Mexican regulations NOM-001-ECOL (1996) limit the FC less than 1000 MPN/100 ml in the wastewater reuse for unrestricted irrigation, which is the same as the world health organization (WHO) guideline.

At present, technologies of wastewater reuse have developed dramatically for beneficial uses. It is important to consider cost-effective treatment system, standards, human health, environmental impact and reuse applications (Salgot, 2008). The infiltration-percolation process is the most effective filtration process that has highest removal percentages for physical and chemical parameters and improves microbiological quality due to physical processes, chemical reactions and biological transformations (Ausland *et al.*, 2002; Mosaddeghi *et al*., 2009; Stevik *et al.*, 2004).

Therefore, the intermittent media infiltration (IMI) was proposed in this research as an alternative for applying wastewater reuse into irrigation. Natural porous media (sand, zeolite, vermicompost and charcoal) have been widely use for water and wastewater treatment. The media mixtures of soil were prepared to investigate the quality of FC removal in reuse of domestic wastewater. FC was considered as a colloid contaminated in wastewater but it differs from colloids due to the presence of cell structure (Scheuerman *et al.*, 1998). The mechanical removal of FC bacteria achieved by IMI with porous media during the wastewater passed through them is accomplished in two steps: transport and attachment.

Transport mechanisms move a particle into and through a pore of filter media so it comes very close to its surface or existing deposits. Then attachment associate to remove the particle by contact with media surface or previously deposited solids by one or more phenomenon which are: straining, sedimentation, impaction, interception, adhesion, flocculation, chemical and physical adsorption and biological growth (Asano *et al.*, 2007). Physical and chemical forces between suspended particles promote the removal of particles from suspension (Zamani and Maini, 2009).

The other mechanisms (interception, impaction and adhesion) participate with straining where smaller particle can be removed through the filtration mechanism by interception. The main transport and attachment mechanisms for FC removal are straining and physical adsorption by surface forces (van der Waals and electrostatic forces), respectively (Harvey and Garabedian, 1991).

Prediction methodologies are important for wastewater treatment in this case of FC removal. Artificial intelligent neural networks (ANN) have been successfully implemented to predict coliforms and escherichia coli on fruits and leaves (Keeratipibul *et al.*, 2011) and the concentration of BOD and COD of wastewater (Rene and Saidutta, 2008). ANN has ability to adjust its parameters according to the target signal obtained by the experimental results. Unfortunately, this ability cannot include human knowledge due to the system properties especially with IMI treatment. There is a relation between content of soil and porous media in the column for FC reduction (Khamkure *et al.*, 2011), that can be introduced by IF-THEN rules like fuzzy logic systems.

The study of Treesatayapun and Uatrongjit (2005) represents a significant advantage of Fuzzy rules emulated networks (FREN) thus the prediction algorithm is developed by using FREN based on the IF-THEN rules to follow the knowledge of IMI system. Furthermore, the convergence analysis can be guaranteed by the main theorem with the parameter selection for learning algorithm.

Therefore, the objectives of this study were to evaluate the efficiency of FC removal by IMI, and to predict the FC removal according to IMI system with varying composited natural porous media and soil content by applying FREN.

MATERIALS AND METHODS

IMI columns

In this research, 12 IMI columns were studied in different proportion of mixed porous media of 12 cm diameter by 30 cm height. The columns were prepared following the method described by Khamkure *et al*. (2011) and were set up in the laboratory at the Department of Soil Science in the Universidad Autónoma Agraria Antonio Narro located in an arid region to the south of Saltillo city, Coahuila, Mexico.

Soil, sand and vermicompost were obtained from this location and others were local materials. Their compositions were prepared by mixing soils with different combination ratios (v/v) of other porous media as soil/sand (Ss), soil/zeolite (Sz), soil/vermicompost (Sv) and soil/charcoal (Sc). All of studied porous media were used without any additional pretreatment. Soil was added according to the proportion established in percentages of 25, 50 and 75 as IMI column number 1 to 3, respectively (Oladoja and Ademoroti, 2006).

The porous media were analyzed as soils according to the method of Black *et al*. (1965a, 1965b) as summarized in Table 1. The soil texture was classified as loam but other media, except vermicompost, were classified as percentage of particle size. The saturated hydraulic conductivity (Ks) was measured for each porous media to evaluate the speed of water movement throughout the columns.

Table 1. Physical and chemical properties of the different porous media.

	Particle size (%)			pH	EC[a]	OM[b]	N	CO_3^{2-}	Ks[c]
Porous media	>2 mm	0.02-0.002 mm	<0.002 mm		(dS m^{-1})	(%)	(%)	(%)	(cm/h)
Soil	42	34	24	7.6	1.0	1.87	0.09	23.37	0.16
Sand	60	28	12	7.5	0.7	0.63	0.03	20.21	1.38
Zeolite	64	26	10	6.3	0.8	0.95	0.05	8.82	1.30
Vermicompost	-[d]	-	-	7.4	2.1	32.98	1.65	34.52	12.25
Charcoal	84	16	0	8.0	3.0	-	-	-	2.98

[a] EC=Electrical conductivity; [b] OM=Organic matter; [c] Ks=Saturated hydraulic conductivity; [d] not measured

The particle size distribution (PSD) was applied because it is an important parameter for FC removal that give a the better understanding of filter performance (Ausland et al., 2002). Scanning electron microscopy (SEM, model JSM-8440; JEOL) was also carried out and cooperated by energy dispersive X-ray (EDX) to indicate the elemental analysis or chemical characterization of an interesting area of media samples. Furthermore, the porous media were characterized by X-ray diffraction (XRD, model D550; Bruker) a powder diffractometer with a graphite monochromator to identify their mineralogical and morphological characterizations.

Wastewater used in this study was from a municipal wastewater treatment plant (MWWTP) located to the South of Saltillo, Coahuila, Mexico. Raw wastewater and unchlorinated treated wastewater were collected from MWWTP twice a week and analyzed according to APHA (1998) and their FC averages were 199.2×10^3 and 30.0×10^3 MPN/100 ml, respectively. The mean effluent concentration for other parameters (TSS, BOD, Cu, Fe, Pb and Zn) fulfilled the Mexican regulations.

The unchlorinated treated municipal wastewater was introduced into each IMI column, in a volume of 500 ml daily and passes through by gravity flow. Effluent samples of each column were collected manually in a 100 ml sterile bag (Whirl-Pak) and analyzed for chemical and biological parameters in the laboratory every week for four weeks. FC bacteria were analyzed by the most probable number (MPN) technique. Five 1ml aliquots of each three dilutions were examined by the A-1 medium (Becton, Dickinson and company, USA) accepted by the USEPA; this medium can be used in a single-step procedure (Standridge and Delfino, 1981).

The results of FC reduction percentage were statistically compared between treatments for each IMI column by one-way analysis of variance

(ANOVA) and Tukey's HSD (Honestly Significant Difference) test at significance levels of $p < 0.05$. Statistical analyses were carried out using R statistic version 2.14.1 (R development Core Team, 2011). Regression analysis was applied to measure the reliability of a linear relation between the percentage of soil filling and FC reduction according to the functional model described by Koivunen *et al.* (2003).

The mechanisms of FC removal

The bacterial transportation in the porous media can be described by filtration theory for understanding the mechanisms of FC removal (Harvey and Garabedian, 1991). The colloid filtration theory was described Brown and Jaffe (2001) as follows:

Assuming that bacteria are removed at a constant rate along a flow path (x) in a porous media by

$$\frac{\partial C}{\partial x} = -\lambda C$$

where λ is the filter coefficient and C is colloid or bacterial concentration existing the filter column.

Integration along a column of length L, C can be obtained as

$$C = C_0 \exp(-\lambda L)$$

where C_0 is the initial colloid concentration (inlet). The parameter λ is dependent on the porous media and can be determined from the experiment.

During the transportation of bacteria on the column, the collector efficiency (η_c) can describes the approach of the bacteria to the surface of porous media (Brown and Jaffe, 2001) and is written as

$$\eta_c = 4A_S^{1/3}N_{Pe}^{-2/3} + A_S N_{vdW}^{1/8} N_R^{15/8} + 0.00338 A_S N_G^{1.2} N_R^{-0.4}$$

where A_S represents the influence of neighboring collector on the flow, and N_{pe}, N_{vdW}, N_R and N_G are dimensionless numbers accounting for diffusion, van der Waals, interception, and sedimentation. This equation can describe the phenomenon for FC removal as discussed above.

Electrostatic interactions play an important role in bacterial attachment to their porous surfaces and can be explained by the classical Derjaguin, Landau, Verwey and Overbeek (DLVO) theory, which consider the balance between the attractive van der Waals and the repulsive electrostatic interactions with bacterial and solid surfaces (Adamczyk and Weronski, 1999; Jucker *et al.*, 1996).

Moreover, the DLVO theory is based on concept of diffusion double layer (DDL), which is a layer of counterions that forms around a charge surface, is given by the Debeye length (K^{-1}). The DDL thickness as a function of ionic strength (I) at 20 °C of aqueous system, it can be written as:

$$\kappa^{-1} = \frac{0.301}{\sqrt{I}} (nm)$$

The Debeye length has the inversely relation with the ionic strength. In the case of bacterial transportation, ionic strength increases and thus bacterial attachment increases and transport decreases (Jucker *et al.*, 1996; Brown and Jaffe, 2001).

Fuzzy Rules Emulated Network Prediction

Prediction based on FREN

FREN is an automatic self-adjustable network which can include the human knowledge about the system and material properties (Treesatayapun and Uatrongjit, 2005; Treesatayapun, 2009). In this application, the percentages of each composition are assigned to be the inputs of FREN and log removal is denoted as the network's output. Let consider the relation between log removal with the percentage of soil, we desire to use this percentage as the first input of FREN and the second input is type of other materials (sand, zeolite, vermicompost and charcoal).

According to this implementation, these IF-THEN rules are firstly defined as follows:

"For compositions Ss or Sz or Sv or Sc"

1. IF %Soil is small AND another material is Sand or Zeolite or Vermicompost or Charcoal THEN log removal is Low,
2. IF %Soil is middle AND another material is Sand or Zeolite or Vermicompost or Charcoal THEN log removal is Medium,

3. IF %Soil is massive AND another material is Sand or Zeolite or Vermicompost or Charcoal THEN log removal is High.

Thus, the network configuration can be illustrated as in Fig. 1. In Fig. 1, according to these defined rules, “S”, “m” and “M” denote as “small”, “middle” and “massive”, respectively and LCs are linear consequence nodes which can be tuned theirs parameters inside by the learning algorithm (Treesatayapun, 2010). Those membership functions can be designed in Fig. 2, according to the knowledge based on the level of soil percentage which can take the effect with FC removal together with another composition.

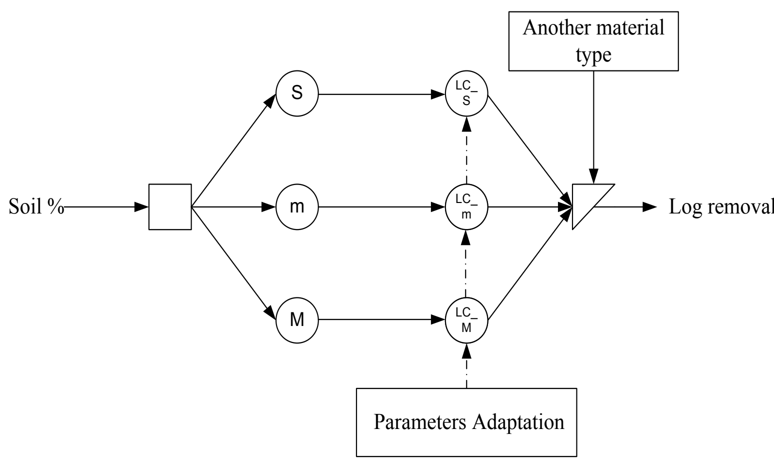

Fig. 1. FREN configuration for FC removal

The back propagation technique is performed for off-line tuning parameters. Let the log removal value “*y*(*k*)” at “*k*” iteration be calculated by

$$y(k) = \sum LC_a(k)\mu_a(k) = \beta^T F,$$

when $\beta = [\beta_S, \beta_m, \beta_M]^T$ and $F = [f_S, f_n, f_M]^T$ where β_S denotes as the linear parameter that needed to be tuned at the membership grade f_S and so on.

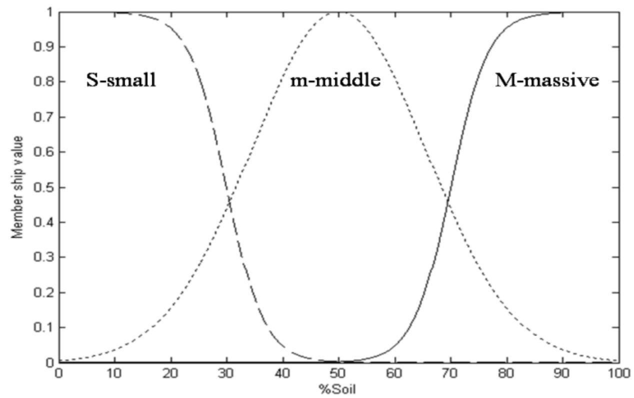

Fig. 2. Membership functions of percentage of soil

Parameters adaptation

Those adjustable parameters β are considered as the time varying as $\beta(k)$ which can be tuned by the following algorithm. Let consider the target point or setting log removal as $y_p(k)$ thus the error function can be obtained as

$$e(k) = y_p(k) - y(k)$$

where $y_p(k)$ is the desired response and $y(k)$ is the prediction output of FREN at time index *k*, respectively. The system configuration can be briefly represented in Fig. 3.

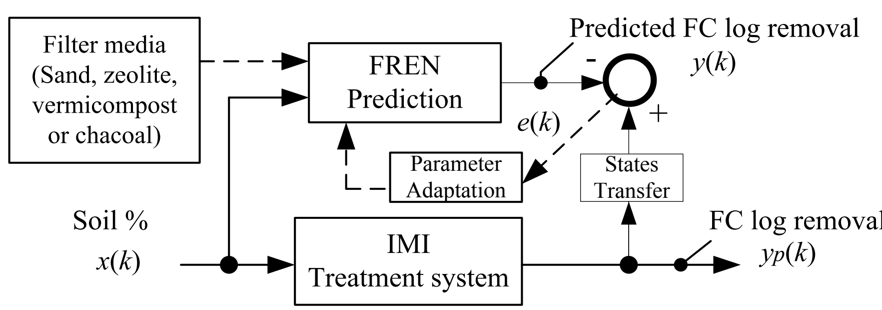

Fig. 3. Block diagram of FREN prediction for IMI treatment system

The proposed algorithm needs only “Soil%” as the input signal during the computation process. This system can be designed by using only one FREN model to predict “FC log removal”. The learning mechanism can tune parameters inside FREN with difference natural filter materials. The common membership functions given in Fig. 2 have the ability to predict the FC removal effect on different filter media types in each IMI column.

It is desired to adjust $\beta(k)$ such that *e*(*k*) becomes zero. The value of parameter $\beta(k)$ is updated at each time *k* by

$$\beta(k) = \beta(k-1) + \alpha(k)\gamma(k),$$

when

$$\gamma(k) = \frac{F(k)}{||F(k)||^2}\left(1 - \eta\frac{|e(k-1)|}{|\alpha(k)|+\epsilon}\right),$$

and

$$\alpha(k) = y_p(k) - \beta(k-1)^T F(k),$$

where η and $\epsilon > 0$ are predefined constants.

The convergence property of FREN prediction can be demonstrated by the following theorem with that parameter adaptation for $\beta(k)$.

Theorem : If the error *e*(*k*) is finite for all *k* and all parameters are adjusted according to the proposed learning algorithm with $\eta < 1$, then $\lim_{k\to\infty} |e(k)| = 0$.

Proof :

The output of FREN *y*(*k*) is calculated by,

$$y(k) = \beta(k)^T F(k).$$

Substitute in to the error equation, we obtain

$$
\begin{aligned}
&e(k) = y_p(k) - y(k),\\
&= y_p(k) - \beta(k)^T F(k) = y_p(k) - \left[\beta(k-1)^T + \alpha(k)\gamma^T(k)\right] F(k),\\
&= y_p(k) - \beta(k-1)^T F(k) - \alpha(k)\gamma(k)^T F(k).
\end{aligned}
$$

Recall $\alpha(k) = y_p(k) - \beta(k-1)^T F(k)$ again, we have

$$
\begin{aligned}
&e(k) = \alpha(k) - \alpha(k)\gamma^T(k)F(k) = \alpha(k) - \alpha(k)\tfrac{F^T(k)F(k)}{\|F(k)\|^2}\left(1 - \eta\tfrac{|e(k-1)|}{|\alpha(k)|+\epsilon}\right),\\
&= \eta\tfrac{|e(k-1)|}{|\alpha(k)|+\epsilon}\alpha(k).
\end{aligned}
$$

By using the absolute, it can be rewritten as

$|e(k)| = |\eta|\frac{|\alpha(k)|}{|\alpha(k)|+\epsilon}|e(k-1)|$.

Since $|\eta| < 1,\ |\alpha(k)| < |\alpha(k)| + \epsilon$ and *e*(*k*) is finite for all *k*, thus, we obtain $\lim_{k\to\infty} |e(k)| = 0$.

Moreover, the learning rate parameter η can be determined to guarantee the convergence with the following.

Let us define Lyapunov function $V(k)$ as

$V(k) = e^2(k)$,

Thus the change of Lyapunov function can be obtained by

$$
\begin{aligned}
&\Delta V(k) = V(k) - V(k-1),\\
&= e^2(k) - e^2(k-1),\\
&= \tfrac{\eta^2\alpha^2(k)}{(|\alpha(k)|+\epsilon)^2}e^2(k-1) - e^2(k-1),\\
&= \left[\tfrac{\eta^2\alpha^2(k)}{(|\alpha(k)|+\epsilon)^2} - 1\right] e^2(k-1).
\end{aligned}
$$

According to the decreasing of Lyapunov function for $\Delta V(k) < 0$, this following argument must be obtained as

$\frac{\eta^2\alpha^2(k)}{(|\alpha(k)|+\epsilon)^2} < 1,$

or

$$|\eta| < \frac{|\alpha(k)| + \epsilon}{|\alpha(k)|}.$$

This statement can be acquired until $|\eta| < 1$ or in such a case $0 < \eta < 1$.

$\square$

This performance proof guarantees the convergence of the FREN prediction and does not have the relation with filter media materials. Furthermore, the experiment results will confirm this theorem and the prediction property with difference filter media by using only one FREN prediction model, which will be discussed in the next section.

RESULTS AND DISCUSSION

The performance of IMI columns on FC bacteria removal

The physical and chemical parameters (pH, conductivity, TSS, TS and heavy metals) of the effluents were lower than the values established in Mexican regulations. The FC reductions of effluents had a great variation because the FC concentrations in the influent were highly variable during the sampling periods. The FC log removals of effluents were in a range of 0.35-2.46 as presented in Table 2. The results show that the composition of soil and porous media at a ratio of 75/25 (IMI no.3), respectively, gave the best performance of the FC removal for all of the IMI columns in accordance with the results of a previous study (Oladoja and Ademoroti, 2006).

The Sc composition performed the highest FC removal efficiency (2.19-2.46 log) among other 3 compositions of Ss, Sz and Sv. Regarding to IMI-Sc (1-3) columns, their FC were reduced from 4.48 to 2.12 log unit and the effluent FC concentrations were lower than the limitation (3 log unit) that met these criteria during the monitoring periods.

Table 2. FC (log MPN/100ml; mean ± standard deviation) of IMI influent and effluent and FC log removal.

Parameter		Influent	Effluent											
			Ss1[a]	Ss2	Ss3	Sz1[b]	Sz2	Sz3	Sv1[c]	Sv2	Sv3	Sc1[d]	Sc2	Sc3
FC	Mean	4.48	3.80	3.39	2.88	2.41	3.03	3.58	4.02	3.87	3.26	2.15	2.31	2.12
	SD [e]	4.05	0.51	0.84	0.65	1.94	1.02	0.09	0.54	0.46	0.09	0.28	0.38	0.26
FC log removal	Mean	- [f]	0.35	0.56	1.25	0.60	0.64	0.75	0.35	0.53	1.21	2.19	2.20	2.46
	SD	-	0.43	0.97	0.60	2.06	0.91	0.20	0.55	0.47	0.11	0.40	0.39	0.29

[a] Ss = soil/sand; [b] Sz = soil/zeolite; [c] Sv =soil/vermicompost; [d] Sc = soil/charcoal; [e] Standard Deviation; [f] not calculated

Each composition of IMI (Ss, Sz, Sv and Sc) at different soil filling percentage (25, 50 and 75%), were no different in the treatment of effects on FC reduction, only IMI-Sc was statistically significant from IMI-Ss1 and IMI-Sv1 ($p < 0.05$), as illustrated in Fig. 4. However it was observed that when percent of soil increased (from 25-75%), the FC reduction percentage also increased, especially in the composition of Ss and Sv, their FC reductions were increased about 40% (Fig. 4). As a result of the increase of the soil filling percentage, it had an influence on porous media (sand and vermicompost) due to the association of clay particle to reduce large pore of these porous media and to expand the retention time in the IMI column. These results agreed with those reported by Jamieson *et al.* (2002) for FC transport in a finer grained soil.

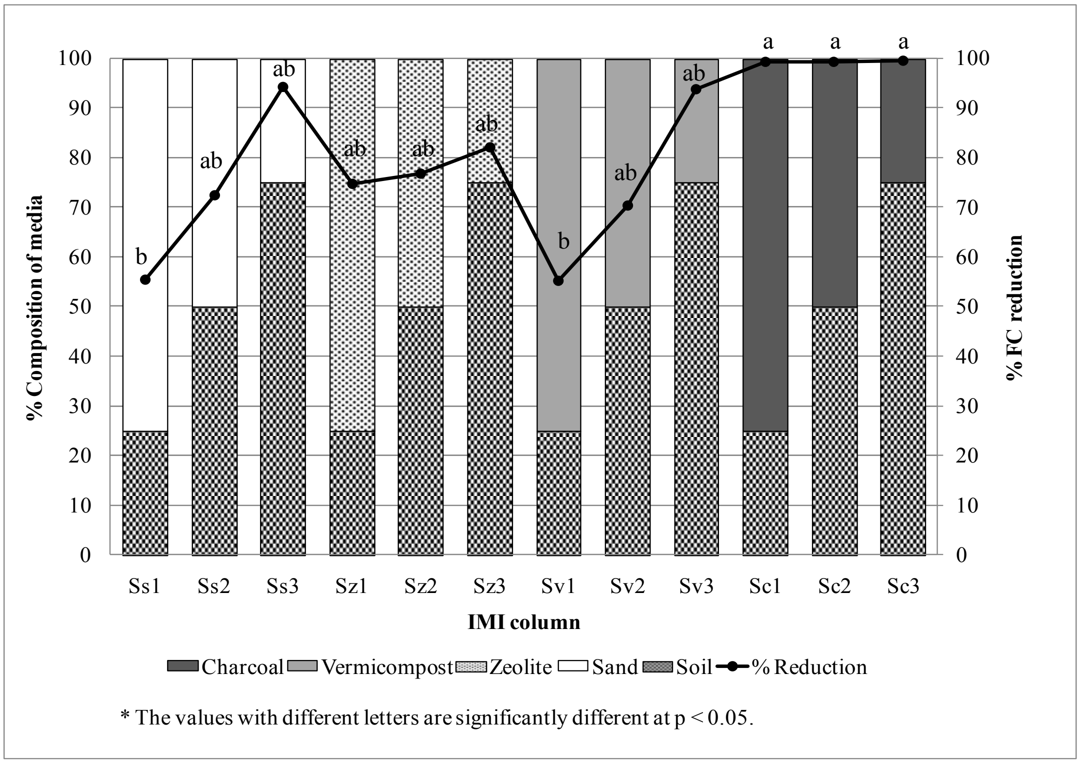

Fig. 4. The percentage of FC reduction and composition of Ss (soil/sand), Sz (soil/zeolite), Sv (soil/vermicompost) and Sc (soil/charcoal) in IMI columns.

The regression analysis was applied to measure the reliability of the linear relationship between the percentage of soil filling in each IMI columns and FC reduction as shown in Table 3. Their correlation coefficients of Ss, Sv and Sz indicated a clearly significant relationship (R^2=0.99, 0.98 and 0.95, respectively), but relationship lower coefficient was shown for Sc (R^2 = 0.78).

Table 3. Linear regression between the percentage of FC reduction (y) and soil filling (x); Ss (soil/sand), Sz (soil/zeolite), Sv (soil/vermicompost) and Sc (soil/charcoal).

IMI	Regression equation	R^2
Ss	y = 0.7772x + 35.273	0.99
Sz	y = 0.1506x + 70.457	0.95
Sv	y = 0.7715x + 34.65	0.99
Sc	y = 0.0059x + 99.162	0.78

Table 1 shows that Ks of vermicompost had the highest ability to transmit the water than other porous media. Because of porosity and permeability, vermicompost contained large pore space and particle size, and had the lowest residence time in the IMI column than the other media. It was observed that Ks decreased when clay content in filter media increased ($Ks_{soil} < Ks_{sand} < Ks_{zeolite}$). When the IMI column had high Ks and consequently, the residence time of each column was low because it directly affected the relationship with their soil structure and porosity and small microorganism removal (Gonçalves *et al.*, 2007).

In contrast, charcoal seemed to work well as filter media without composed of soil because their relations when soil mixing was less significant. Although the fine particle was the most important mechanism of FC removal, charcoal was largely pure carbon and composed of roughly porous structure which was a highly efficient media for adsorption due to more surface area available for attachment (Scheuerman *et al*., 1998). Moreover, their surface morphology could be identified that their sizing of micro pores was appropriated and more easily attached the bacteria onto their surface (Park *et al*., 2003).

The average grain size at 50% finer particle (d_{50}) of soil, sand, zeolite, vermicompost and charcoal were 0.20, 0.12, 0.20, 0.32 and 0.85 mm, respectively. On this study, texture analysis of porous media defined the finest particle size as soil and PSD defined the coarsest particle as charcoal. The effect of soil filling into IMI columns can be explained that soil contained high percentage of clay (Table 1), which considered as colloidal particles. Their surface area and surface charge of soil are the most reactivity and surface area is a direct result of particle size and shape (Bohn *et al*., 2001). Not only the finest particle sizes give the highest surface area but also a great potential for adsorption.

To evaluate clay content influence on filtration, XRD was applied for identifying mineral compounds in porous media (Oladoja and Ademoroti, 2006) and their powder diffraction patterns are illustrated in Fig. 5. It can be observed that the most abundant primary minerals in soil and sand were quartz (SiO_2) (Fig. 5a, b) and similar contained crystalline mineral components at 70%. Composition of soil was considered as mineral and obtained as quartz (32.94%), calcite (10.80%), albite low (11.16%), illite (12.03%), merlionite (2.17%) and amorphous material (30.90%). Calcite ($CaCO_3$) was the most carbonate mineral of soil and sand at 10-13% which is common in semiarid and arid region soil.

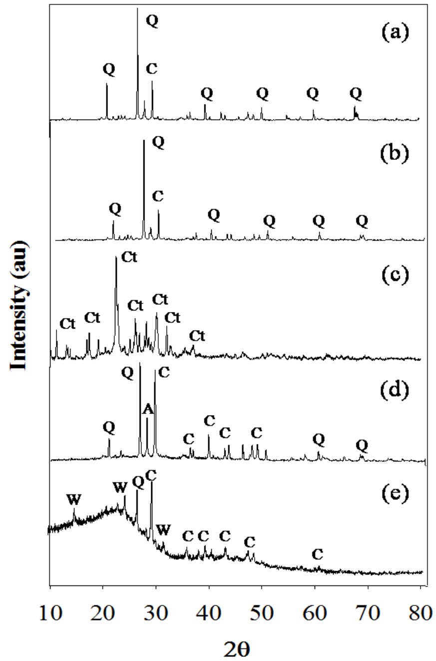

Fig. 5. XRD analysis of porous media (a) soil; (b) sand; (c) zeolite; (d) vermicompost (e) charcoal. Calcite (C), quartz (Q), clinoptilolite (Ct), albite (A), and whewellite (W).

Moreover, this soil has high concentrations of $CaCO_3$ and has high ability of Ca^{2+} for exchangeable capacity, which affected to soil filling in other composition of IMI columns and their FC reductions also. It has been found that natural zeolite (Fig. 5c) composed most of clinoptilolite (87%) in crystal phase, is one of most minerals found in Mexico. Natural zeolite normally includes another crystalline phases and a significant amount of an amorphous phase. 73% of charcoal was in amorphous phase thus charcoal has the highest surface area and low degrees of crystallinity than other media.

Regarding the negative charge of bacteria cell and the negative charge of media surface (clay particles), it can be described that FC removal is account for the bacteria interactions with porous media. They can be attached each other by electrostatic forces of positive charge around the edge of media surface and this could be consequence of bacterial removal in the IMI columns also (Van Loosdrecht *et al*., 1989).

This behavior can be explained in term of the element composition which detected by EDX (Fig. 6) following the function of K^{-1} as mentioned above. It was found that the principal elements of porous media were C, O, Al, Si, K, Ca, Mg and Fe. The chemical composition percentage of soil (%wt) was Ca (25.96%), O (21.36%), Si (16.43%), Fe (11.30%), P (9.41%), Al (6.08%), Mg (3.11%) and K (2.30%). Not only the presence of cations (Ca^{2+}, K^{+} and Mg^{2+}) can associate the adsorption of FC to the solid surface but the metallic cation in solution (Fe^{3+}) can enhance bacterial adsorption also (Stevik *et al*., 2004).

Their cations increased more positive charges in the electrolyte solution; at the same time, it was significantly reduced their thickness of double layers at the media surface. It meant that ionic strength increased, Debeye length decreased and affected to decrease the capacity of bacterial transportation then bacterial attachment increased therefore it can obtain high quality of the effluent with lower FC contamination. According to the highest content of Ca in soil, FC can be efficiently removed when soil filling percentage increased.

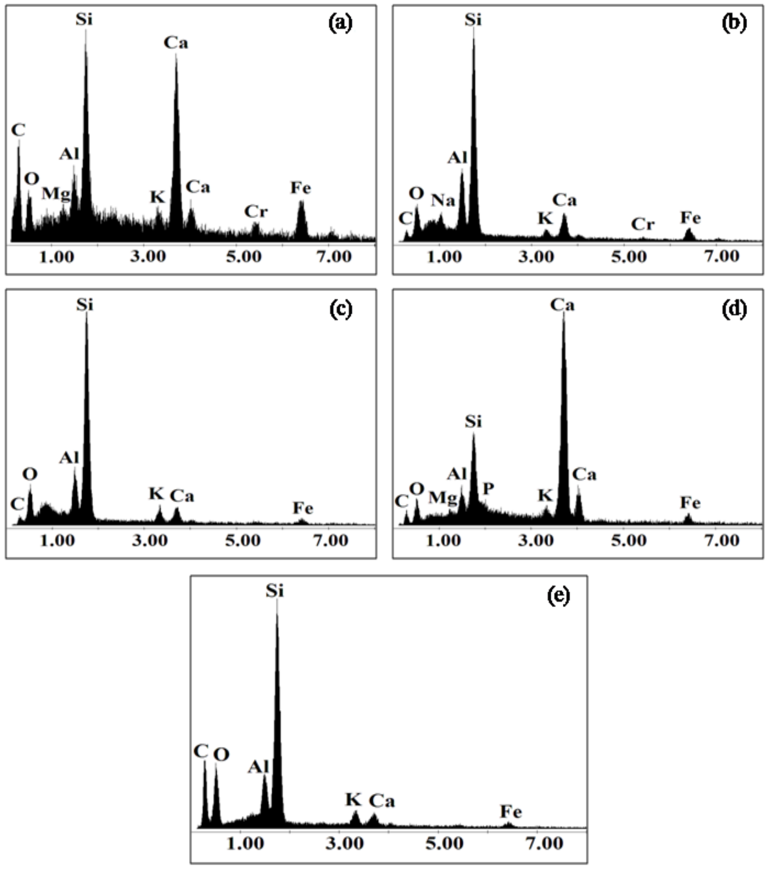

Fig. 6. EDX images of porous media (a) soil; (b) sand; (c) zeolite; (d) vermicompost (e) charcoal.

Therefore the physicochemical process by adsorption can occurred at the surface reaction between bacteria and clays. The biological process can be explained in terms of the microbial attachment and growth at the porous media to retain the bacteria in the IMI columns. In the other hand, the solids removal efficiency is affected by the changes in the pores due to deposit, which are both time and depth dependant. Then biological clogging in the porous media was found from operated IMI column since 4 weeks ago due to the excessive growth of bacteria (Jung-Woo *et al*., 2010), which should be recommended for further research.

Prediction based on Fren and their results

In this application, levels of soil percentage (25, 50 and 75%) were selected as the learning points to FREN. The conventional back propagation was realized to tune those linear parameters inside LC nodes. According to those testing points, the prediction curves for Ss-composition could be illustrated in Fig. 7 with 10 and 20-iteration, respectively.

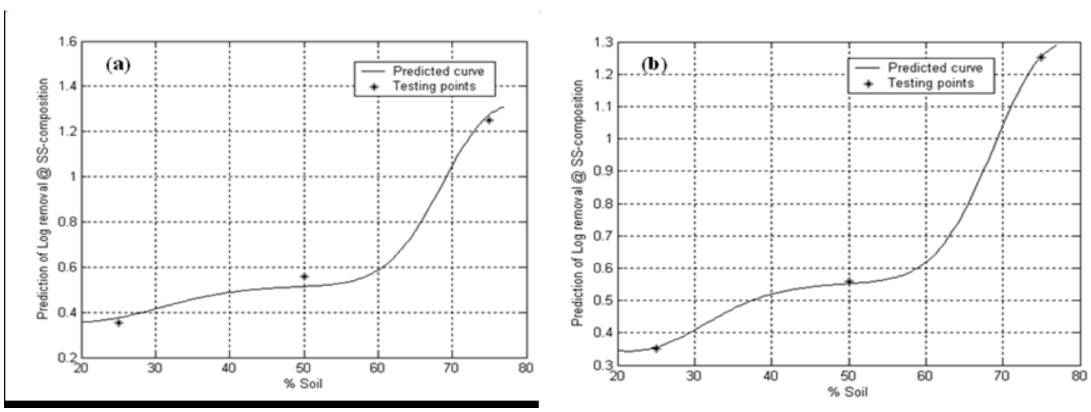

Fig. 7. Predicted curve for Ss with (a) 10 iterations and (b) 20 iterations

According to the above results, we selected to test the FREN prediction at 20 iterations for the rest of material compositions. The prediction curves can be illustrated in Fig. 8 for Sz, Sv and Sc, respectively.

In those experimental results, the learning rate η has been selected to follow the main theorem as $\eta = 0.5$. With this setting, the convergence of FREN prediction can be guaranteed. Refer to the main theorem again, another parameter ϵ doesn't play important role for the convergence. The propose of this parameter is to prevent the division by zero when $\alpha(k) = 0$. In this study, $\epsilon = 0.02$ was selected when $|\alpha(k)| < 0.05$. Furthermore, the better performance can be obtained with the increasing of iteration number.

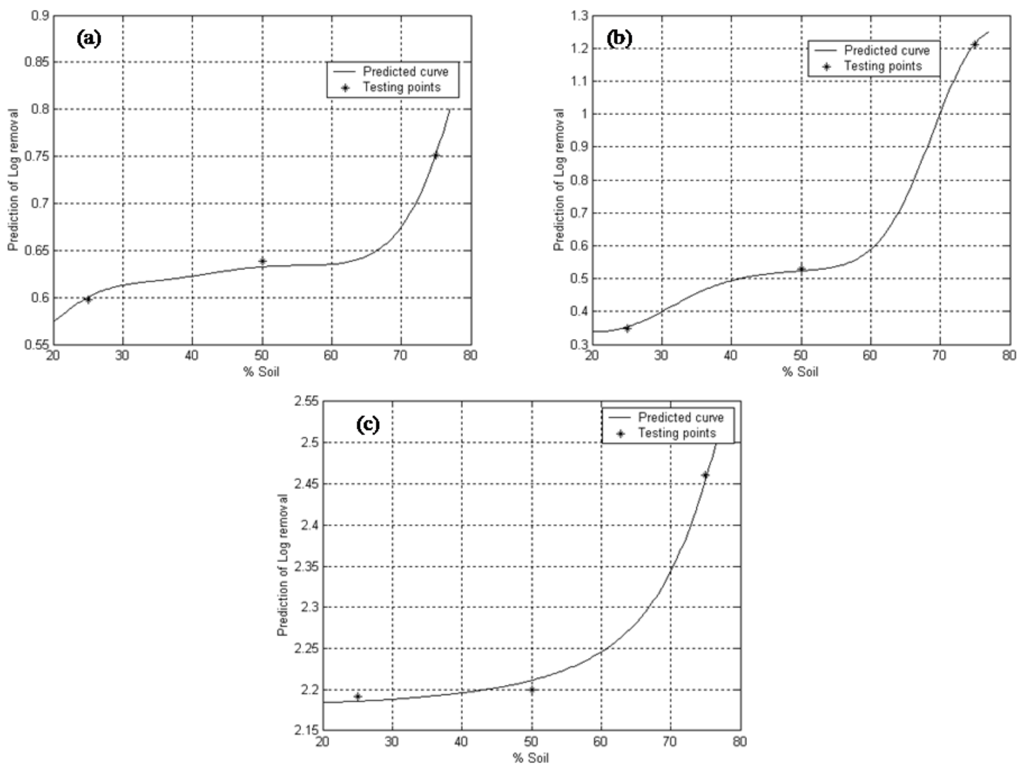

Fig. 8. Predicted curve with 20 iterations for (a) Sz, (b) Sv and (c) Sc

CONCLUSIONS

The IMI demonstrated the greatest FC removal of the compositions of soil and porous media at a ratio of 75/25, respectively. The effect of soil filling on FC bacteria removal depends on the fine particle size and the increasing of ion charges. FC attraction on filter surface increase and ionic strength in the electrolyte solution increase also. The proposed IMI system with simplicity of treatment technology and operation, low capital and operating cost, had significant FC bacteria reduction. It was possible to achieve wastewater reuse with the FC established in the Mexican regulations required for agricultural irrigation.

This technique gave a forecast for the performance of FC removal between the range of soil content as 20-80% with difference type of the compositions Ss, Sz, Sv and Sc. Unlike the linear interpolation, the predicted curves obtained by the proposed algorithm are mostly nonlinear relation regarding to the nature of IMI. The robustness could also be counted because of the learning algorithm for its parameters. The human knowledge related on the treatment materials and FC removal can be included directly as the IF-THEN rules and membership functions. Furthermore, the convergence of prediction could be guaranteed by the proposed theorem and this algorithm can be implemented to predict the FC removal as the application of agriculture irrigation.

ACKNOWLEDGEMENTS

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Fecal bacteria removal enhanced by zinc-modified zeolite in an intermittent media infiltration system

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Abstract

The reuse of a wastewater treatment system was investigated using intermittent media infiltration (IMI) to evaluate the fecal coliform (FC) removal efficiency with natural zeolite containing clinoptilolite from the state of San Luis Potosí, Mexico and its zinc-modified form (Zeo-Zn). The municipal wastewater, which was from Saltillo, Coahuila, Mexico, was treated for FC analysis for four months. The physicochemical properties of zeolite were analyzed, and their behavior as filter media for FC removal with an IMI system was studied using X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and X-Ray fluorescence techniques (XRF). At the conditions under which natural zeolite was treated to functionalize its surface with a bactericide agent (Zn^{2+}), there were no significant structural and chemical changes in the clinoptilolite contained in the natural zeolite. Thus, Zeo-Zn could be regenerated by treatment with a zinc salt after a long-term IMI process. The use of Zeo-Zn resulted in an average FC removal efficiency (2.99 ± 0.92 log) and FC reduction (99.44 ± 1.04%) that were significant better than those obtained with natural zeolite (approximately 47.44%). Zeo-Zn enhanced the removal of FC in the IMI system more than natural zeolite and thus complied with Mexican regulations for unrestricted reuse during the study period. The study showed that Mexican natural zeolite functionalized with zinc can be used as a disinfectant to achieve the antibacterial effect in an IMI system for wastewater treatment.

Keywords: Bacteria, column, modification, wastewater reuse, zeolite

1. Introduction

Many treatment technologies for wastewater reuse have been widely studied and implemented to address the limited amount of fresh water in many countries. The principal criteria for considering wastewater reuse are the objective of its reuse, health concerns, a cost-effective treatment system and the environmental impact (Mujeriego and Asano, 1999). Fecal pollution can lead to the transmission of infection diseases of waterborne microorganisms; thus, fecal coliform (FC) bacteria have been used as a monitoring tool to predict these diseases (Savichtcheva and Okabe, 2006). The Mexican regulations (NOM-001-ECOL/96) limit the FC by a most probable number (MPN) of less than 1000 MPN/100 mL in the wastewater reuse for unrestricted agricultural irrigation (SEMARNAP, 1996), corresponding with the world health organization guideline (WHO, 1989).

In a previous study, intermittent media infiltration (IMI) was performed by mixing the composition of clay soil and natural filter media (sand, zeolite, vermicompost and charcoal) to evaluate the efficiency of the FC reduction in wastewater reuse (Khamkure et al., 2011). The authors found that the compositions of clay soil and filter media at a ratio of 75/25, respectively; in the IMI columns removed the maximum amount of FC in accordance with the results of chemical oxygen demand (COD) removal in a previous study (Oladoja and Ademoroti, 2006).

To manage the removal of FC in an environmentally sustainable manner, the FC removal capacity of the IMI process associated with the lifetime, regeneration and disposal of filter media must be considered. In addition to zeolite filters being successfully used to treat municipal wastewater (Castilla et al., 2009), the high availability, low cost, regenerability, adsorption and ion exchange properties of natural zeolites (Widiastuti et al., 2008) make them a potential material for improving the IMI process.

Zeolites are hydrated crystalline aluminosilicates with a three-dimensional framework structure from SiO_4 and AlO_4 tetrahedra linked together through the sharing of oxygen molecules (Auerbach et al., 2003). Zeolites possess a regular pore structure, cavities and a considerably higher specific area; thus, they can serve as an effective filter media for removing bacteria.

Synthetic zeolites prepared by a straightforward method using soft reaction conditions and industrial byproducts with no commercial value could be an efficient alternative for treating wastewater (Medina et al., 2009). The nature, strength and concentration of specific sites in the zeolite surface can be adjusted; thus, exhausted zeolites can be regenerated and their lifetime can be extended (Medina et al., 2010).

Chemical modifications to the structure and surface of zeolites can be used to adjust their properties such that the zeolites' separation efficiency is improved and their absorbent capacity is maximized. Wang and Peng (2010) reported that acid/base treatment and surfactant impregnation by ion exchange (Apreutesei et al., 2009) are methods that are typically used to modify natural zeolites.

The applications of natural zeolites, clay (montmorillonite) and tobermorite modified with metallic elements (Ag^{+}, Ni^{2+}, Zn^{2+}, Cu^{2+} and Fe^{3+}) have been studied to evaluate their antibacterial activities; these applications been shown to exhibit increased bactericidal properties during water and wastewater treatment (Coleman et al., 2009; De la Rosa-Gómez et al., 2008a, b; Malachová et al., 2011; Milán et al., 2001; Orha et al., 2007; Rivera-Garz et al., 2000; Top and Ülkü, 2004). Although the antibacterial action of the Zn^{2+} ion has been less studied than that of Ag^{+}, its wound-healing and bactericidal properties are similarly used in a range of formulations, composites and coatings (Bright et al., 2002; Bonferoni et al., 2007). In the case of zinc's application in wastewater reuse for agriculture, the cost effectiveness of the treatment and its effect on crop growth must be considered.

Zinc affects microbial growth, the resistance mechanism in bacteria and the bacteria's adherence ability (Atmaca et al., 1998; Choudhury and Srivastava, 2001; Sugarman et al., 1982). Zeolites have the capacity to exchange alkaline or alkaline earth ionic metal from their surface through cations such as Zn^{2+} (Nibou et al., 2009). However, while zinc is an essential trace element for living organisms and is associated with a number of processes essential for growth and metabolism, excess zinc concentrations can be toxic (Choudhury and Srivastana, 2001). Zinc, copper and iron are essential micronutrients required for optimum crop growth; however, only a small amount is required.

Although silver has a high antimicrobial action and is the most use for modifying zeolites as a powerful metal (Coleman et al., 2009; Malachová et al., 2011), it is a precious metal. Furthermore, zinc is the fourth most valuable and widely used metal after iron, aluminum and copper (EPA, 1995). In addition to its price being lower than those of other metals, its influence on plant growth must be considered. Previous studies found that the optimum quantity of zinc use increased the yield and resulted in high-quality products (Ghaffar et al., 2011; Tani and Barrington, 2005).

In this study, the interesting and useful properties of zeolite are used to design a functional material for antibacterial filter media. The exchanged cation, such as Zn^{2+}, is homogeneously dispersed in the entire zeolite surface; thus, only a small amount of metal is required to kill the bacteria. The Zn^{2+} is chemically anchored to the zeolite structure, and the lixiviation under the treatment conditions is negligible in the middle time. Thus, the aim of this study was to evaluate the efficiency of natural zeolite for FC removal in an IMI system and improve its antibacterial capacity by functionalizing the clinoptilolite contained in the natural zeolite by substituting the alkaline and alkaline earth cations on its surface with Zn^{2+}.

2. Experimental

2.1. IMI treatment units

The natural zeolite used in this study was obtained from San Luis Potosí, Mexico and commercialized by Zeomex S.A, a Mexican company located in Monterrey, Nuevo Leon. The natural zeolite was sampled and treated according to the method described by Khamkure et al. (2011) and filled into an IMI column of 12 cm diameter and 30 cm height identified as IMI-Z. The experiments in the IMI treatment unit were carried out in the laboratory at the Department of Soil Science in the Universidad Autónoma Agraria Antonio Narro, which is located in an arid region to the south of Saltillo city, Coahuila, Mexico.

Domestic wastewater samples, which were composed of unchlorinated treated effluent from a municipal wastewater treatment plant (MWWTP) located to the south of Saltillo, Coahuila, Mexico, were collected once a week following the standard sampling method (SEMARNAP, 1980) and analyzed according to APHA (1998). After the IMI columns were saturated with deionized water for 24h, 500 mL of the unchlorinated treated wastewater was introduced into each IMI column daily by gravity flow.

Effluent samples of each column were collected manually in a 100 mL sterile bag (Whirl-Pak) weekly in the morning and transported to the laboratory for FC analysis within one hour. After the samples were analyzed, they were stored at 4 °C so that other parameters could be analyzed. The sampling period was 4 weeks of monitoring. Wastewater influent and effluent from the IMI columns were analyzed pH and temperature by using a pH meter (model 420A; Orion Research, Inc. USA). The conductivity and TDS of the samples were measured using a conductance meter (model 32; YSI Inc. USA). The main elements (Ca, K, Mg, and Na) and heavy metals (Cu, Zn, Fe, and Pb) were analyzed by atomic absorption spectrophotometry.

The FC bacteria were analyzed as a microbiological parameter by the MPN method. Five 1 mL aliquots of each three dilution were examined by the A-1 medium (Becton, Dickinson and Company, USA) accepted by the USEPA; this medium can be used in a single-step procedure (Standridge and Delfino, 1981). The tube contained an inverted inner vial (Durham tube) to collect gas; this tube was incubated at 35 ± 0.5 °C for 3h and transferred to 44.5 ± 0.2 °C for 21h. The characteristics of the wastewater influence are shown in Table 1. The FC log removal is defined as follows (Asano et al., 2007):

Log removal = log (Σ Influent FC/Σ Effluent FC)

Table 1. Physical and chemical properties of the IMI influent (n=10) of the wastewater. All of the concentrations are in mg/L (± standard deviation)

Parameter	FC (Log unit)	pH	EC[a] (μS/cm)	TS[b]	TSS[c]	Ca	K	Mg	Na	Zn	Pb
Influent	4.45	7.20	837.00	827.00	11.00	56.50	21.50	18.50	308.00	0.03	0.03
	(0.16)	(0.4)	(65)	(186)	(10)	(31.8)	(19.9)	(3.4)	(242.7)	(0.05)	(0.05)

[a] EC= Electric conductivity; [b] TS = Total solid; [c] TSS = Total suspended solid

2.2. Modification of natural zeolite

The modification of natural zeolite was prepared in the laboratory at CINVESTAV IPN-Unidad Saltillo. In this study, the natural zeolite was modified with zinc through an ion exchange process with (Zn^{2+}) ions. In the first step, sodium-modified natural zeolite is obtained, and in the second step, zinc-modified natural zeolite was prepared following the method described by De la Rosa-Gómez et al. (2008a).

The chemical composition indicate the percentages of major oxides obtained by X-Ray fluorescence (XRF) spectroscopy on press powder pellets using a Bruker AXS spectrometer, as shown in Table 2. The chemical composition of natural zeolite (wt%) was SiO_2 (62.53), CaO (3.05), Al_2O_3

(13.02), Fe_2O_3 (1.89), K_2O (3.67), MgO (0.75), Na_2O (0.41), TiO_2 (0.10), SrO (0.08) MnO (0.04), ZrO_2 (0.04) and LOI (14.37). The chemical elements in groups I and II of the periodic table were used to calculate the equivalence of each chemical element and metal, and then computed the summation of the Zn^{2+} equivalence.

The sodium-modified zeolite (Zeo-Na) was prepared by stirring 5,750 g of natural zeolite and 4,728 mL of a NaCl solution (0.3 M) at 90 °C for 12h. The obtained product was filtered and retreated with a fresh NaCl solution. The mixture was filtered with a filtration unit and washed with deionized water (DI) until Cl^- ions could not be detected using silver nitrate. The wet sample was dried at 85 °C for 5h. Zeo-Na was prepared to replace the cations typically present in natural zeolite that strongly bonded with the zeolite surface, such as Ca^{2+} and Mg^{2+}, with Na^+, which weakly bonds with the zeolite surface. This pretreatment facilitate the exchange of these cations with Zn^{2+} when creating the Zn-modified zeolite. Zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$), referred to as ZnAC, was used as the source of Zn^{2+} ions to enhance the zeolite's antibacterial effect and reduce the bacterial growth (Atmaca et al., 1998).

Zinc-modified zeolite, identified as Zeo-Zn, was obtained through an exchange process of the corresponding sodic form. Five thousand grams of Zeo-Na was added to 8,400 mL of a ZnAC solution (0.15 M) and kept under reflux conditions in darkness for 12h. The final obtained product was filtered and retreated with a fresh ZnAC solution. The resulting material was filtrated, washed with DI water and dried at 100 °C for 4h. The ion exchange reaction was finally obtained as follows:

Zeo-Na + ZnAC ↔ Zeo-Zn + NaAC

Because Zeo-Na does not have an antibacterial effect on water and wastewater (De la Rosa-Gómez et al., 2008a), it was not applied as the filter media in the IMI column. Zn^{2+} was used to promote the antibacterial capacity of the clinoptilolite contained in the natural zeolite. Zeo-Zn obtained according to the aforementioned methods was used in the IMI columns (IMI-MZ), and its FC removal efficiency was monitored monthly for 4 months following the method in the first experiment (see Section 2.1).

2.3. Characterization of the filter media

The particle size distribution (PSD) was determined by the standard sieving method. The average grain size (d_{50}) of zeolite was 0.20 mm. The uniformity coefficient (Cu) and coefficient of gradation (Cc) of zeolite were 8.647 and 1.064, respectively. X-ray diffraction (XRD, model D550; Bruker) analysis was used to determine the type of zeolite, and scanning electron microscopy (SEM, model JSM-8440; JEOL) was applied to identify the shape, texture and particle size. The punctual chemical composition was determined by an energy dispersive X-ray (EDX). Furthermore, the filtering materials' chemical composition, which indicated the percentage of major oxides, was obtained by

X-Ray fluorescence (XRF) spectroscopy, on press powder pellets using a Bruker AXS spectrometer.

3. Results and discussion

3.1. IMI treatment performance

The average (± standard deviation, referred to as SD) pH, conductivity, TS and TSS of the effluent of the IMI-Z were 7.62 ± 0.20, 878.50 ± 38.58 μS/cm, 843.75 ± 181.63 mg/L and 8.75 ± 4.79 mg/L, respectively. The conductivity and TSS were high in the first sample but decreased over time. Due to the IMI operation, the columns were run without flush cleaning in the first time compared with the study of Oladoja and Ademoroti (2006). All of the solids and heavy metals were at values lower than those established in the Mexican regulations.

To compare the FC removals of the columns packed with natural zeolite (IMI-Z) and zinc-modified zeolite (IMI-MZ), both of the columns were evaluated for 4 weeks. Fig. 1 shows that natural zeolite could only be used as filter media for FC removal during the first operating week, when it resulted in a high FC reduction percentage (99.65%) and FC log removal (2.46).

After the first week, the natural zeolite’s capacity to remove bacteria decreased significantly, and its ability was only approximately 10% by the fourth week of operations. Widiastuti et al. (2008) found that natural zeolite acted in a similar manner. These authors identified that this material needs to be activated by regeneration to be reusable. In this study, the average concentrations of FC influent and effluent of the IMI-Z were 4.32 ± 0.15 and 3.55 ± 1.19 log MPN/100 mL, respectively, because their FC effluents were above the detection limit (3 log MPN/100 mL) (data not shown). Compared to the IMI-MZ results, their FC reduction and log removal were higher and more constant than those of the IMI-Z during the study period. Their average FC reduction percentage and log removal (97.88%, 1.67) were approximately twice those of the IMI-Z (52%, 0.77, data not shown).

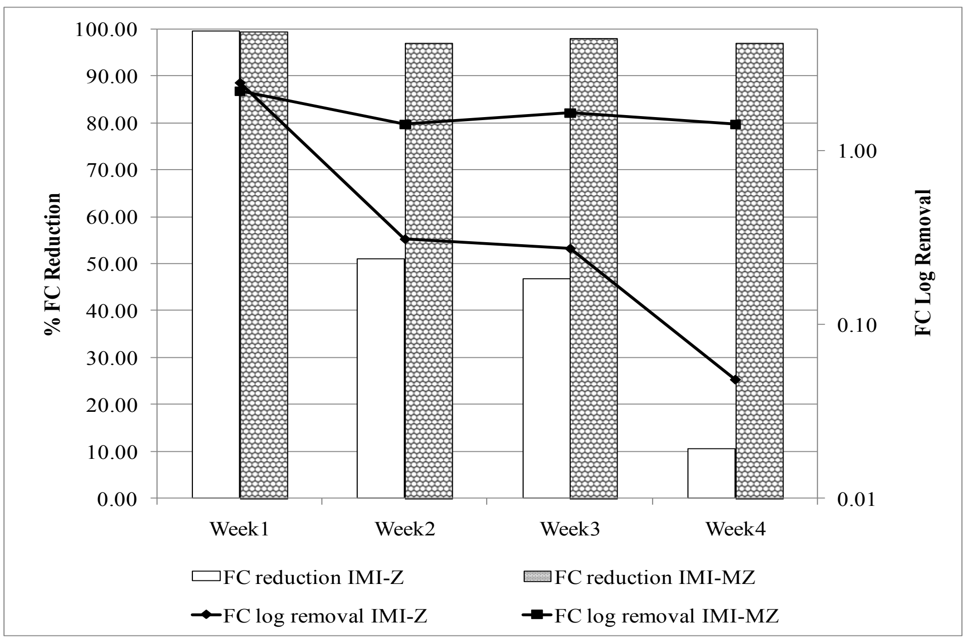

Fig. 1. FC reduction and FC log removal of the IMI-Z (natural zeolite) and IMI-MZ (zinc-modified zeolite) columns over 4 operating weeks.

3.2. Modification of natural zeolite

Due to the promising results obtained from the preliminary experiments, Zeo-Zn was used in the IMI system over a longer period of time (4 months). The average (± SD) pH, conductivity, TSS and TS of the IMI-Z effluent were 7.62 ± 0.20, 878.50 ± 38.58 μS/cm, 843.75 ± 181.63 mg/L and 8.75 ± 4.79 mg/L, respectively. The average FC contents of the influent and effluent decreased from 4.82 ± 0.49 to 1.83 ± 0.46 log MPN/100 mL (data not shown), and the effluent FC concentrations were lower than that specified by the regulations (3 log unit) over 4 operating months, as shown in Fig. 2.

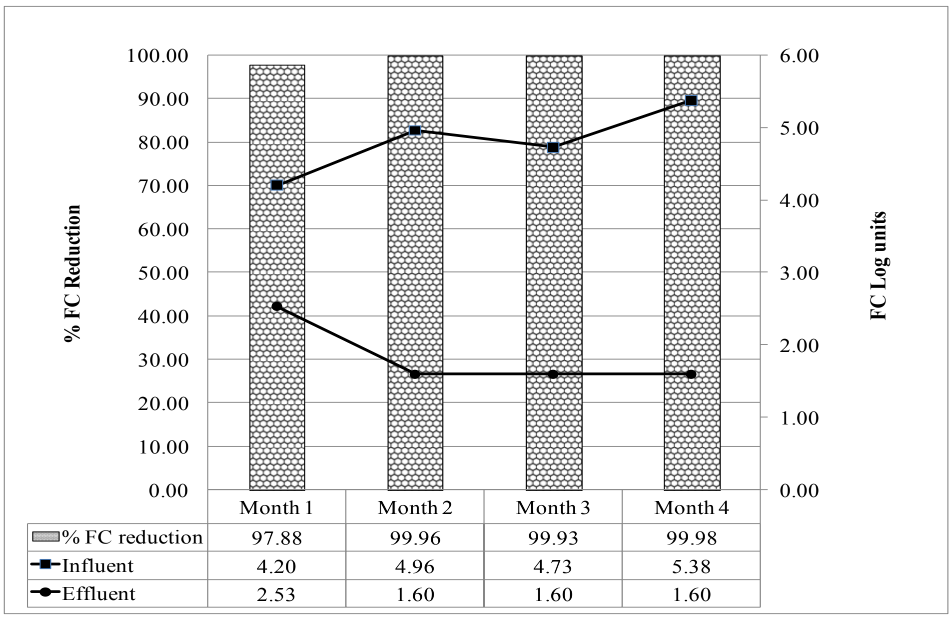

Fig. 2. FC reduction and FC log units (influent and effluent) of the IMI-MZ columns (zinc-modified zeolite)

The IMI-MZ's average FC log removal efficiency (2.99 ± 0.92 log) and FC reduction percentage (99.44 ± 1.04%) indicated that the efficiency of the system was significantly greater than that in the previous IMI-Z results (approximately 47.44%). Therefore, the IMI-MZ enhanced the FC removal more efficiency than the IMI-Z because the chemical conditioning into homoionic form increased the natural zeolites' ion exchange capacity (Trgo et al., 2006). In this case, the uptake of Zn^{2+} by zeolite increased the FC disinfection efficiency, which will be discussed later.

3.3. Characterization of the studied zeolites

The mineralogical characteristics of both the natural zeolite and the zeolites obtained from the modifying process (Zeo-Na and Zeo-Zn) were determined by XRD, as shown in Fig. 3. The typical diffraction spectrums of the studied zeolites were plotted from the reflected intensities *vs.* the detector angle 2θ. XRD analysis software (Traces v3.0) was used to process and calculate the percentage of the mineral composition's corresponding area in the crystalline phase, and then the percentage of the amorphous phase was obtained by subtracting the percentage in the crystalline phase from the total area.

The XRD patterns clearly show that the natural and modified zeolites are crystalline composed mainly of clinoptilolite; the natural and modified zeolites also have similar peak patterns. The incorporation of Na^{+} (Zeo-Na) or Zn^{2+} (Zeo-Zn) into the clinoptilolite crystalline network containing K^{+}, Ca^{2+} and Mg^{2+} modified the X-ray reflections intensity, as shown in the Figs. 3 (a, b, c). The zeolite content calculated according to the aforementioned method indicated that the clinoptilolite contents in the natural zeolite, Zeo-Na and Zeo-Zn decreased by 87.25%, 73.08%, and 61.91%, respectively. These results agreed with those reported of Malla and Komarneni (1995) and Saleh et al. (2011)

indicated that the chemical treatment of zeolites by zinc salt decreased the crystallinity of the zeolites and changed the Si/Al ratio in the structural framework. However, from our point of view, the soft conditions of the cationic exchange process followed in this work appear insufficient to destroy the zeolite structure, reduce the clinoptilolite content, or modify the natural zeolite's Si/Al molar ratio.

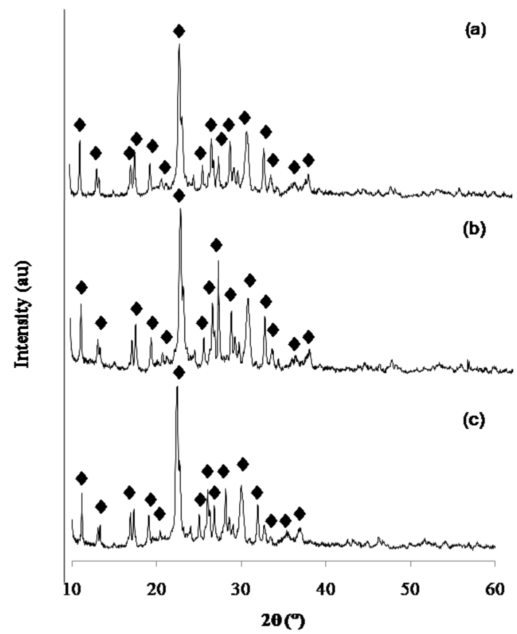

Fig. 3. XRD patterns of (a) natural zeolite, (b) Zeo-Na and (c) Zeo-Zn. Clinoptilolite (♦).

The observed changes in the X-ray reflection intensities and thus zeolite content of Zeo-Na and Zeo-Zn versus the natural zeolite could be explained by the presence of metals with different nature, such as K^+, Ca^{2+} and Mg^{2+}, in the surface of the natural zeolite *vs.* the monovalent cation (Na^+) or divalent cation

(Zn^{2+}) in Zeo-Na and Zeo-Zn, respectively. As demonstrated by the XRF analysis, the total SiO_2 and Al_2O_3 content in the natural, sodic and zinc-modified zeolites remained fairly constant, as presented in Table 2, indicating that Zeo-Zn was chemically stable and could be regenerated by treatment with a zinc salt after a long-term intermittent media infiltration process.

The intensity, broad and area peaks of the modified and natural zeolites differ (Fig. 3), indicating that the applied method was not sufficiently reliable to determine the minerals in zeolite containing with different chemical of nature in its surface, which is similar to the results of Yang et al. (2001) findings. However, quantifying the content of the uniformity zeolite in a defined matrix would be a good alternative.

The SEM identified an irregular morphology in the natural zeolite particles, as illustrated in Fig. 4 (a, c, e). The morphological processing of clinoptilolite and other aluminosilicates contained in the natural zeolite were analyzed by EDX with different particles; however, the analysis showed that the natural and modified zeolites had very similar chemical compositions (Figs. 4b, d, f).

The EDX showed an irregular topography, with the presence of micropores over the entire surface of the zeolites; these micropores are an important factor for improving the capacities of a filter media to adsorb microbes and organic materials (Park et al., 2003). Thus, in the case of FC removal by zeolites in an IMI system, the results show that in the short-term operation, the zeolite surfaces were available and appropriate for the physical adsorption of microbes.

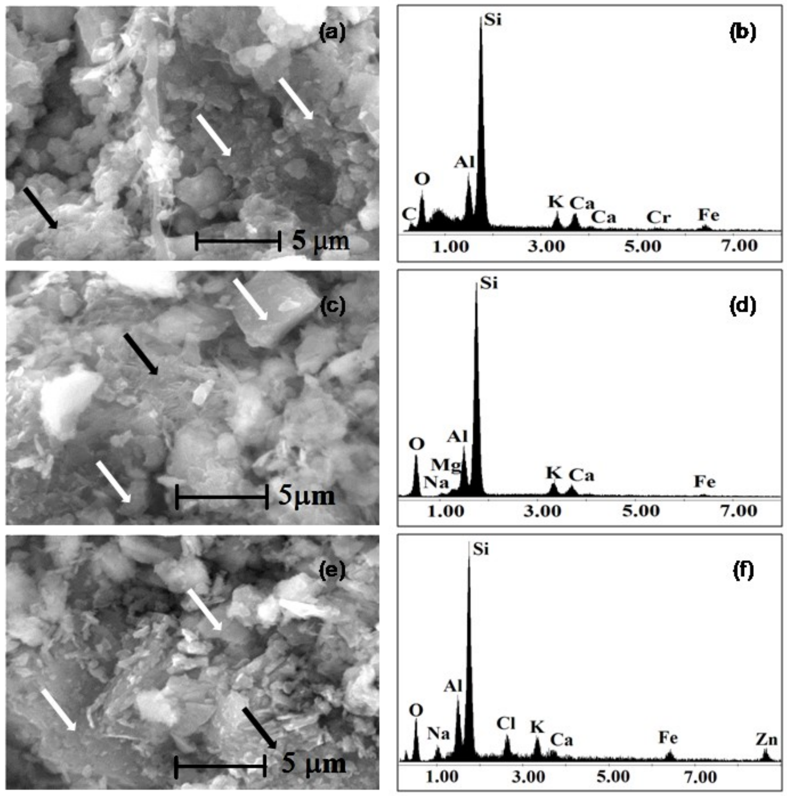

Fig. 4. SEM and EDX of (a, b) Natural zeolite, (c, d) Zeo-Na and (e, f) Zeo-Zn.

The analysis of XRF in Table 2 shows the major oxides of the natural zeolite, Zeo-Na and Zeo-Zn with percentage by weight. The concentration of Na_2O in Zeo-Na (1.49 wt%) was higher than those in the other zeolites due to the preconditioning of the clinoptilolite contained in the natural zeolite to obtain its sodic form; this result is similar to that obtained by Trgo et al. (2006). A similar trend was found for the concentration of ZnO in Zeo-Zn (3.08 wt%). The SiO_2/Al_2O_3 ratio (calculated from the data in Table 2) did not change significantly between the untreated and chemically treated natural zeolites, Zeo-Na and Zeo-Zn, with ratios ranging from 4.63-4.68 (data not shown). The Zn^{2+} appears to remain outside of the chemical framework of zeolites, corresponding to the behavior reported in the literature (Saleh et al., 2011).

Furthermore, the XRD profiles of the natural and modified zeolites had the similar characterization peak patterns (Fig. 3), indicating that the zeolite framework is preserved by the chemical conditioning by Zn^{2+}. In line with the conclusion, Ciobanu et al. (2008) demonstrated that Zn^{2+} cations are only incorporated in the zeolite surface, including the sites in the external surface of the crystals and intra-porous zeolitic centers. Thus, the chemical and structural properties of the zeolite did not change significantly following the chemical treatment.

Table 2. Major oxides of zeolite and its modified forms, wt%

Materials	Percentage of chemical composition												LOI[a]
	SiO_2	Al_2O_3	K_2O	CaO	Fe_2O_3	MgO	Na_2O	TiO_2	SrO	ZrO_2	MnO	ZnO	
Zeolite	62.53	13.02	3.67	3.05	1.89	0.75	0.41	0.10	0.08	0.04	0.04	N.D[b]	14.37
Zeo-Na	62.71	13.54	3.55	2.16	1.92	0.59	1.49	0.10	0.07	0.04	0.02	N.D	13.73
Zeo-Zn	61.97	13.35	3.45	1.81	1.94	0.48	N.D	0.11	0.07	0.04	0.02	3.08	13.62

[a] LOI = Loss on ignition at 950 °C; [b] N.D = Not detectable

3.4. FC removal of Zn^{2+}-exchanged zeolite-clinoptilolite in the IMI system

The results of this experiment (Figs. 1 and 2) showed that Zn^{2+}-exchanged zeolite-clinoptilolite could increase the amount of FC removed in the IMI system, as mentioned above. The mechanical removal of FC bacteria achieved by IMI with filter media is accomplished in two steps: transport and attachment (Asano et al., 2007). Although FC was considered to be a colloid that contaminates wastewater, it differs from colloids due to the presence of a cell structure (Scheuerman et al., 1998). The wastewater contaminated with FC was introduced and passed through the IMI column, at which point it came in contact with the Zeo-Zn contained into the column. The negative charges of the bacteria indicate the electrical potential across their outer layer and thus their potential to interact with microorganisms and antibiotics (Sugarman et al., 1982; Van Loosdrecht et al., 1989). The bacteria or some parts of them interact with the empty orbitals of the zinc atoms chemically anchored to the surface of the clinoptilolite contained in Zeo-Zn. The interaction between the zinc atoms and the bacteria can damage the bacteria's cell walls, inhibit the functions of some enzymes and lead to bacterial death.

Furthermore, zinc ions present several possible disinfection mechanisms; they bind to the membranes of microorganisms (Atmaca et al., 1998), bind to bacterial pili, increase the bacterial adherence (Sugarman et al., 1982), damage cell walls and inhibit certain enzymes (Malachova et al., 2011). As mentioned previously, these results indicate that Zn^{2+} ions could act as chemical agents or have the characteristics of an ideal disinfectant, due to their availability and toxicity to FC bacteria.

However, the presence of alkaline and alkaline earth cations in wastewater could contribute to the loss of Zn^{2+} in the long-term (Malachova et al., 2011), potentially reducing the ability of Zeo-Zn to remove FC. Thus, additional studies should be conducted to investigate the deactivation mechanism of Zeo-Zn, the medium lifetime of the IMI column and the process required to regenerate the active form of zeolite.

4. Conclusions

This study demonstrated that modified natural zeolites, such as Zeo-Zn, could be used as low-cost filter media to treat domestic wastewater in IMI systems. Due to the availability of natural zeolite containing clinoptilolite in Mexico and worldwide, the low cost of zinc salts, the relative simplicity of the procedure used to functionalize zeolites with zinc cations, and the bactericidal capacity of zinc ions, Zeo-Zn could be used as a low-cost disinfectant for FC removal. Further research should be conducted to investigate the regenerability and lifetime of this filter media for environmentally sustainable management.

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Residual effects of fecal coliform using reclaimed domestic wastewater on plant and soil

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Abstract

Reclamation of domestic wastewater for irrigation is one alternative approach to solve the water scarcity crisis but it is essential to control its microbiological quality. Fecal coliform (FC) removal from domestic wastewater was monitored during a four months period by an intermittent media infiltration (IMI) treatment. IMI columns were composed of natural filter media (soil, soil/charcoal and zinc-modified zeolite, Zeo-Zn) and were compared with wastewater and disinfected wastewater for irrigation in greenhouse, in order to examine the residual FC on swiss chard (*Beta vulgaris*) and agricultural soil. The effluents of IMI contained Zeo-Zn had higher FC removal efficient (2.98 log) than other filter media and disinfection (1.87-2.57 log) due to the bactericidal properties of Zn^{2+}. Treated wastewater by Zeo-Zn and disinfection decreased the FC accumulation in plants and soils approximately 1-20 MPN/g dry matter. Domestic wastewater reclamation using the IMI of Zeo-Zn was adequately suitable for unrestricted agricultural use, complied with Mexican regulations the same as the disinfection results and had low risk of FC contamination in plant and soil.

Keywords: Irrigation, reclaimed wastewater, residual bacteria, soil, crop

1. Introduction

Although, domestic wastewater is harmful due to its pathogenic load, wastewater reuse is one important mechanism to sustain water resources management, especially for agricultural irrigation. Wastewater reuse can reduce treatment cost and increase agricultural productivity by improving its quality (pathogen and chemical) to minimize negative impacts on health and the environment (Pedrero et al., 2010).

Mexico is considered as one of the ten largest water users in the world. Its resources of freshwater use are from surface water (63%) and underground water (37%). The distribution of the freshwater use in Mexico for irrigation, public supply, industrial use and thermoelectric are 77%, 14%, 4% and 5%, respectively (CONAGUA, 2010). In some locations of Mexico, the reclaimed wastewater is used for crop irrigation and aquifer recharge to maintain surface resources and to reduce the overexploitation of groundwater aquifers (Espino et al., 2004; Jimenez and Chávez, 2004). The Mezquital valley, an irrigation district of the state of Hidalgo, Mexico, has been using the municipal wastewater of Mexico City for forage crops and vegetable crops irrigation. The results of this implementation have shown a significant increase in crop yields because of the high organic matter (OM) and nutrients in the wastewater but the quantity of water-borne diseases has also increased (Lucho-Constantino, 2005).

Fecal coliform (FC) bacteria are always found in the intestinal tracts of warm-blooded animals and their contaminated feces are a risk factor for different types of diseases; thus, FC is the biological indicator most used in wastewater reuse (Salgot et al., 2006). In accordance with the Mexican regulations (NOM-001-ECOL/96), the limit of the FC by a most probable number (MPN) is less than 1000 MPN/100mL in the wastewater reuse for unrestricted agricultural irrigation, corresponding with the WHO guideline.

Not only the compliance regulations are important to control the wastewater characteristics for reducing the health and environmental problem, but also the planning and implementation of the suitable wastewater treatments are necessary for the cost-effective development (Salgot, 2008). The challenge is to find alternative disinfection processes or improvements of chlorination practice to control commonly occurrence of disinfection by-products (DBPs) (Hrudey, 2009). The residual chlorine causes severe damage to plants in excess of 5 mg/L Pedrero et al. (2010).

The infiltration-percolation technique can improve the disinfection and treated wastewater quality (Rajeb et al., 2009). The fate and survival of bacteria in porous medium is affected by the adsorption, transport to the depth and die-off (Smith and Badawy, 2010). Soil characteristic are affected by microbes and waterborne pathogen, which may survive in soil and potentially compromise to public health (Travis et al., 2010).

Many studies have been published on the effects in soil and plant of irrigation with treated municipal wastewater on nutrients, heavy metal and crop biomass. However, only a few of them have been studied the pathway of bacteria (Akponikpè et al., 2011) for wastewater, soil (Candela et al., 2007; Travis et al., 2010) and especially the residual of microorganism on plant (Al-Sa`ed, 2007; Cirelli et al., 2012).

A previous study of treated wastewater by intermittent media infiltration (IMI) showed a good performance for removing FC effectively over a four weeks period (Khamkure et al., 2012a; 2012b). It is considered to be a low cost treatment and easy operation for possible reuse in unrestricted irrigation. Thus, the aim of this research was to evaluate the residual effects of FC using reclaimed domestic wastewater in plant and soil, compared with chlorinated water and three IMI treated wastewaters in a four months monitoring period.

2. Materials and methods

The experiments were carried out in the greenhouse of the Department of Soil Science at the Universidad Autónoma Agraria Antonio Narro (UAAAN) located in a semiarid region to the south of Saltillo, Coahuila, Mexico, where the average annual precipitation is 369 mm and the average temperature is 18 ^{o}C. The greenhouse ambient temperature was averagely maintained in the daytime and nighttime at 18 and 25 ^{o}C, respectively. IMI columns were prepared and composed of soil 100% (IMI-S), soil 75% and charcoal (Mesquite wood) 25% (IMI-C) and zinc-modified natural zeolite (clinoptilolite) by using zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) 100% (IMI-MZ) following Khamkure et al. (2012a; 2012b).

Swiss chard (*Beta vulgaris*) was planted to study the effects of wastewater reuse as a control plant in pots with five kilograms of agricultural soil (UAAAN source), which were irrigated for four months with the effluent from IMI columns. The experimental design was used a randomized complete block design with five treatments and five replications. The applied treatments were five different types of wastewater watering with one plant represented as the experimental unit. This study was conducted in the greenhouse.

The agricultural soil was classified as clay loam (34% sand, 36% clay and 30% silt), pH, OM, N and carbonate were 7.5, 4.2%, 0.210% and 67.5%, respectively. The soil was screened with a 5-mm sieve to remove stones and was dried at sunlight for 7 days, before it was filled into the plastic pots.

Freshwater was added into each pot for saturation 2 days before seeding (3seeds/pot). Two weeks after, seedling with best development in each plot was selected and kept, whereas the others were discarded.

Treated domestic wastewater (unchlorinated wastewater) was obtained from a wastewater treatment plant located to the south of Saltillo. The studied wastewater were unchlorinated wastewater (WWI), chlorinated wastewater by using sodium hypochlorite (NaOCl) (WWC), effluents from IMI-S (WES), IMI-C (WEC) and IMI-MZ (WEZ). NaOCl (household bleach 5.25%, w/w) was applied at 25 ppm NaOCl with the contact time of 15 min. A water depth of 270 mm during the crop season (60 days) was applied. No fertilizer was applied in this study.

The effluents of each IMI column were collected manually in a 100 mL sterile bag (Whirl-Pak) and analyzed monthly according to the standard method during four months. The FC bacteria were analyzed by the MPN technique and examined by A-1 medium (Becton, Dickinson and Company, USA) accepted by the USEPA, which can be used in a single-step procedure.

Plant samples were cut at two and four months after planting and their leaf samples were taken for analysis. Soil samples were analyzed at the end of the experiment. Undisturbed plants and soil samples were sterilely collected for bacterial analysis. Soil samples were obtained from the surface to a depth of 5 cm from each pot, which corresponds to the soil profile were most of the FC bacteria is found (Entry et al., 2000, Jamieson et al., 2002).

Ten gram (wet weight) samples of swiss chard leaves and experimental soil were placed in a sterile flask and filled with 90 mL of sterile peptone water (5g/L of peptone, MCD LAB). Leaves were gently shaken 2-3 min and soil samples were shaken with low speed shaker for 5 min to rinse off most of the present bacteria (Estrada et al., 2004). Three 1 mL aliquots of each three dilutions of 0.1, 1.0, and 10.0 mL of sample volumes were used to estimate FC by using the MPN test as described above. Plant and soil samples were dried in the oven at 70 °C for 48 hours and until a stable weight was observed, and then the FC bacteria were reported per gram of dry weight.

Statistical analyses were carried out using R statistic version 2.14.1. One-way analysis of variance (ANOVA) of the results were performed to detect the effects of crop irrigation with different types of wastewater in the FC removal on plants and soils at significance levels of p=0.05. Then Tukey's HSD (Honestly Significant Difference) test was used for multiple comparisons.

3. Results and Discussion

FC removal performance

The analytical results of the physical and chemical properties of the irrigation water are shown in Table 1. The values were lower than those established by the Mexican regulations. FC bacteria were most effectively removed in WEZ, followed by WWC, WES and WEC (Table 2). WEZ gave the highest virtually 100% (99.9%) of the FC reduction and 2.98 log removal than those of other filter media, which were in the range of FC reduction percentage and log removal as 98.6-99.7% and 1.87-2.57, respectively. The FC quantity in irrigation water is illustrated in Figure 1. Its presence in the treated wastewaters was relatively lower (1-3 log MPN 100/mL) than WWI approximately 2-4 orders of magnitude.

Table 1 Characteristic of wastewater and treated wastewater used for irrigation and the Mexican water quality guidelines for various water uses.

Characteristic	Wastewater irrigation					Guidelines for discharge[a]	
	WWI	WWC	WES	WEC	WEZ	Water (indirect)	Soil (direct)
Temperature (°C)	23.8±1.0	23.6±3.6	24.4±4.1	24.5±3.8	24.6±3.8	N.A.[b]	N.A.
pH	7.6±0.4	7.7±0.2	8.2±0.8	8.1±0.8	7.5±0.3	5-10	5-10
Concuctivity (μS/cm)	893±113	1037±120	912±77	979±126	817±150	N.A.	N.A.
TS (mg/L)	709±164	841±186	767±147	751±139	622±38	N.A.	N.A.
TSS (mg/L)	12.5±2.9	8.8±6.3	10.0±7.0	9.2±3.0	7.5±2.9	150	N.A.

Note: [a] SEMARNAP (1996); [b] not applicable; The data are the mean and stand deviation for four samples taken at differnet times during the irrigation period.

The main bacterial transport mechanisms in filter media are straining, adsorption, the difference of surface charge between collector and bacteria, and grain surface roughness (Foppen and Schijven, 2006). A similar trend of FC removal was observed between WWC and WEZ, and WES and WEC. It was found that WWC and WEZ had lower FC values than the Mexican limitation during this study. IMI-MZ column composed of the uptake of Zn^{2+} by substituting of alkaline and alkaline earth cations on the surface of clinoptilolite and enhanced the FC antibacterial capacity as good as the chemical disinfection

(Nibou et al., 2009). The bacteria or some parts of them interact with the empty orbitals of the zinc atoms chemically anchored to the clinoptilolite surface. The interaction between the zinc atoms and the bacteria can damage the bacteria's cell walls, inhibit the functions of some enzymes and lead to the bacteria's death (Malachová et al., 2011).

Thus, IMI-MZ may have the disinfecting behavior against FC bacteria due to the availability of Zn^{2+} (Bonferoni et al., 2007). The effluent of IMI column containing zinc-modified natural zeolite (Zeo-Zn) is recommended over the chlorination process for using in wastewater treatment to avoid chlorine toxicity resulted in chlorosis and significant depression of plant growth.

Table 2 Mean, standard error of the mean (S.E.M.), the reduction percentage and log removal of FC in each type of irrigated water (log10 number)

Type of irrigated water	Mean	S.E.M.	% Reduction	Log removal
WWI	4.82	0.24	ND	ND
WWC	1.74	0.64	99.7	2.57
WES	2.74	0.37	98.9	1.94
WEC	2.77	0.37	98.6	1.87
WEZ	1.43	0.52	99.9	2.98

Note: The data are four samples taken at differnet times during the irrigation period. ND = Not detectable.

WES and WEC had lower FC values than the limitation (3 FC log MPN/100mL) within 2 operating months, soon after FC contents seemed to be increased (Figure 1). However, the FC contents of WES and WEC were in the lower range, according to the study of Akponikpè et al. (2011). Due to a long operation period in greenhouse, both IMI-S and IMI-C columns showed green slime at their filter media and IMI-S columns also found low permeability of their effluents.

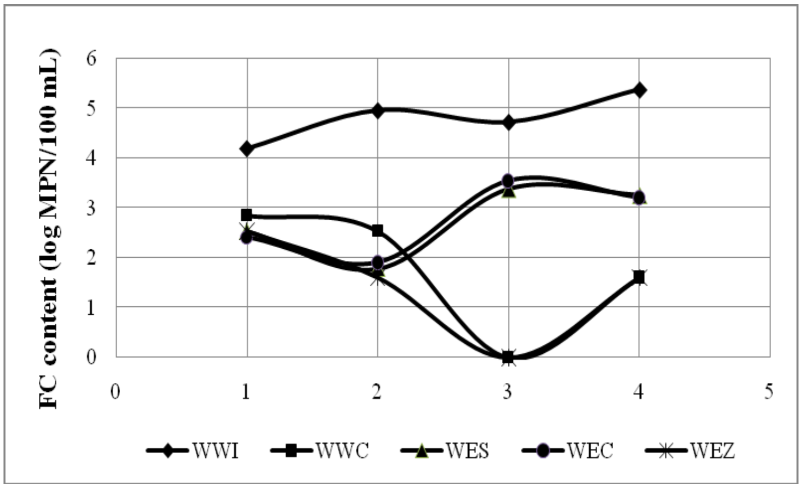


Figure 1 Time variations of the log values of fecal coliform bacteria in the irrigation water during four operating months; WWI (unchlorinated wastewater), WWC (chlorinated wastewater), WES, WEC and WEZ which are effluents from IMI-S, IMI-C and IMI-MZ, respectively.

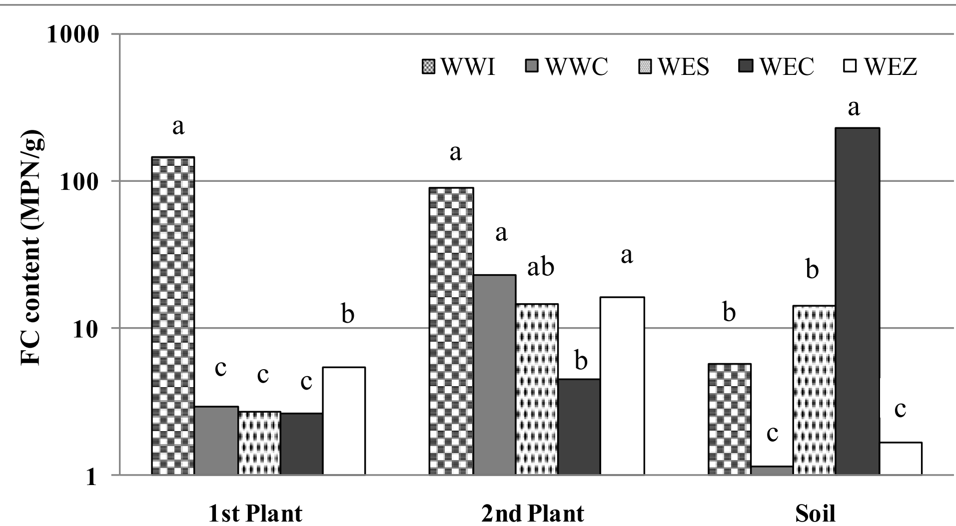
Although the adsorption capacity of charcoal is high, the biofilm concentration is higher also. The occurrence of biological clogging of filter media on the surface was caused for the accumulation of microorganisms at the upper layer as biolfilms, which was the main reason of surface sealing due to pore size reduction and effect on the increasing of water retention and reducing the effective area for infiltration (Jung-Woo et al., 2010). Thus, FC in their effluents increased following the operation time (Table 2) due to the reduction of hydraulic conductivity in the IMI columns.

Effects of watering with wastewater and treated wastewater on plant and soil

The comparison of five different types of irrigated water (WWI, WWC, WES, WEC and WEZ) affecting the FC bacteria contents in the first and second sampling of plant and soil are shown in Figure 2. At the first sampling, plants irrigated with WWI had significantly higher FC bacteria contents than those of plants irrigated with treated wastewater. When compared with soil, in both WWI sampling plants, the FC bacteria contents was higher with a value about 1 order of magnitude. All types of water were directly applied on the plant leaves to achieve the worse result of microbial contamination of the vegetables that are eaten raw.

Moreover, it may be possible for the soil limitation of the nutrients or biodegradable organic matter (Candela et al., 2007), or the increase in the interactions between various micro-organisms in soil matrix (Rajeb et al., 2009). This behavior of FC bacteria survival in soil may indicates that they have a direct influence on soil moisture and temperature (Entry et al., 2000), affecting other factors such as moisture, soil type, temperature, pH, manure application rate, nutrient availability, and competition (Jamieson et al., 2002).

At the first plant sampling, no difference was observed on FC contents on the plants irrigated with WWC, WES, and WEC. However, the FC contents were higher on the plants watered with WWI than on the plants watered with WEZ ($p < 0.05$) (Figure 2). At the second plant sampling, plants watered with WEC had lower FC contents than the plants watered with WWI. It was also observed that at the two plant samplings, the plants watered with WWI had the higher FC bacteria contents, and the plants watered with the treated wastewater (WWC, WEC, WES and WEZ) had lower FC contents. The FC residual trend in plants irrigated with WWC, WEC, WES and WEZ were increased following the harvest time. This result is similar to that obtained by Al-Sa`ed (2007) in short period (4-6 months). However, Cirelli et al. (2012) have recently shown that fecal contamination had low value of agricultural products in a long term effect (2 years).



* The letters a, b and c indicate statistical significance ($p < 0.05$) between treatments.

Figure 2. Fecal coliform bacteria in first and second sampling of plant and soil, irrigated with WWI (unchlorinated wastewater), WWC (chlorinated wastewater), WES, WEC and WEZ which are effluents from IMI-S, IMI-C and IMI-MZ, respectively.

Furthermore, plant and soil watered with WWI had almost the residual FC contents more than the other treated wastewaters, except in soil watered with WES and WEC, which will be explained later. The concentration of FC bacteria in WWI (Table 2) was significantly higher than those of the treated wastewaters (WWC, WEC, WES and WEZ) about 2-3 orders, which it was a reasonable value.

Soil microbiology results show that FC contents significantly decreased from soil irrigated with WWC and WEZ, respectively (Figure 2). It can be referred to Figure 1 that FC contents of the effluents of WWC and WEZ decreased the number of FC bacteria to an acceptable level according to WHO guidelines during the study period. The results of FC in soil irrigated by WWI and treated wastewater (WWC and WEZ) were in good agreement with the report of Travis et al. (2010). A similar trend was found for FC survival from WWC and WEZ irrigation in both plant sampling and soil. These results demonstrated that WWC and WEZ had decrease of FC contents from wastewater, plant and soil, and were effective for reuse in unrestricted agricultural utilization.

Figure 2 also shows that soil containers irrigated with WEC had a FC value significantly higher than those of soils irrigated with other types of wastewater approximately 1-2 orders of magnitude. Although the FC residual of WEC irrigation result was significantly high but they were in the range of coliform contamination (10^2-10^3 CFU/g soil) from treated wastewater in soil at surface (5-10 cm), corresponding to the values reported by Candela et al. (2007). Regarding soil container irrigated with WEC, the numbers of FC could be reincreased due to the detachment of biomass as similar with WES (Rajeb et al., 2009) because the average effluent of WEC and WES (Table 2) were higher than those of other effluents and because the occurrence of microbial biomass growth as previously discussed.

4. Conclusions

Domestic wastewater reclamation by IMI-MZ reduces FC contents up to 99.9%. This FC removal efficiency represents higher (2.98 log) than other filter media (soil and soil/charcoal) and disinfection. FC contents in different types of irrigated wastewater had a significant effect on the plants and soils. Irrigation with WEZ were lower residual FC in soil 1.66±1.07 MPN/g and swiss chard in two and four months of harvest as 5.45±2.13 and 16.38±8.00 MPN/g dry matter, respectively, similar results of the chemical disinfection. The effluent of IMI-MZ is recommended over the chlorination process for using in agricultural irrigation due to possibility of regenerate the filtering media, free of residual chlorine and no toxic effect of the vegetable crops.

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IV. CONCLUSIONS

The composition of soil/sand, soil/vermicompost and soil/zeolite had a good relationships between the soil filling percentage and significantly removed FC contents (0.75±0.20-1.25±0.60 log) at a ratio of 75/25 (soil/media). The filter media analysis of chemistry, physics, and morphology demonstrated that the effect of soil filling on FC bacteria removal depends on the fine particle size and the increasing of ion charges. The main mechanisms to remove FC are the straining and the adsorption between clay particle and bacteria. However, the IMI of soil/charcoal provokes the highest removal (2.46±0.29 log) than other media due to its high adsorption capacity following the standard limits in four monitoring weeks.

IMI of Zeo-Zn significantly and constantly improved FC removal efficiency (2.99±0.92 log) and FC reduction (99.44±1.04%) in a four months operation over the natural zeolite (approximately 47.44%); thus, complied with Mexican regulations for unrestricted agricultural irrigation.

Domestic wastewater reclamation by IMI of Zeo-Zn demonstrated that the accumulation of residual FC bacteria decreased in agricultural soil, swiss chard in two and four months of harvest due to the effectiveness of disinfectants.

Further research is recommended to verify the IMI treatment promising sustainable in agricultural wastewater reuse as follow:

a) Long term operation of IMI treatment associated with lifetime and regenerability of filter media.
b) The study of residual FC bacteria in the experimental filter media and the application of FREN for FC prediction by varying depth of IMI column.

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