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Short Communication

The effect of domestic wastewater from Thailand's Saen Saeb canal on plant growth and rhizosphere microorganisms

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Abstract

The Saen Saeb canal, a transportation route in the central of Bangkok, nowadays is polluted with domestic wastewater. People along the canal still use the Saen Saeb water for watering plants. However, the effect of Saen Saeb water usage on plant growth and soil microorganisms is not well understood. The present study showed the effect of Saen Saeb water on the growth of Chinese kale (*Brassica alboglabra*), tomato (*Lycopersicon esculentum*), and *Dendrobium* orchid (*Dendrobium* Sonia 'Earsakul') at greenhouse and sterile conditions. We found that the Saen Saeb water reduced the growth of Chinese kale and *Dendrobium* orchid in the greenhouse condition as well as the growth of the Chinese kale and tomato in the sterile condition. Moreover, the Saen Saeb water reduced the amount of rhizosphere microorganisms of the Chinese kale to five times less than that of tap water. Thus, this may affect the plant growth.

Keywords: domestic wastewater, rhizosphere microorganisms, Saen Saeb canal, Chinese kale, tomato, *Dendrobium* orchid

1. Introduction

One of the problems resulted from city expansion is the increase of wastewater and lack of good quality water. The main causes of wastewater are often man-made and related to household activities. In general, domestic wastewater contains feces, urine and wastewater from dishwasher, laundry, sinks, and bath. The domestic wastewater may contain harmful disease-causing microorganisms. Moreover, it may also contain toxic inorganic substances. Phosphorous and nitrate are common inorganic pollutants that are plant nutrients. However, the increasing amount of these nutrients may cause algae bloom and eutrophication (Pescod, 1992).

The depleting volume of good quality water results in consideration of the domestic wastewater usage in agriculture. Although it contains nutrients useful for plant growth, wastewater may contain high dissolved salt content. Excessive amount of sodium salts such as sodium chloride, sodium sulfate, and sodium carbonate increases the pH in soil and causes alkali injury in plants. The effects of this injury may range from chlorosis to stunting (Agrios, 2005). Additionally, long-term use of wastewater and phosphate fertilizer would pollute soil with metal toxicity such as nickel, cobalt, arsenic, cadmium, chromium, copper, lead, mercury, and zinc (Yadav, 2009). Despite the fact that toxic materials in present concentration are not harmful to humans, they exhibit phytotoxic level that would be harmful to plant growth (Agency for Toxic Substances and Disease Registry, 2006). Toxic materials are also harmful to soil microorganisms, which in turn may limit wastewater usage in agriculture. For example, the toxicity of copper, manganese, or zinc induces a deficiency of iron in plant (Agrios, 2005). Toxic effects of chromium on plant growth are alterations in the germination process or in the root and stem growth (Shanker *et al*., 2005). High lead concentration induces oxidative stress by increasing the reactive oxygen species (ROS) in plants (Reddy *et al*., 2005).

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The relationship between plants and microorganisms in the rhizosphere, an area encircling the plant root system, is symbiosis. Microorganisms in rhizosphere obtain nutrients excreted from roots, for example amino acids, organic acids, enzymes, and complex carbohydrates. In contrast, the bacteria release nutrients and some mineral required for plant growth. Especially, plant growth-promoting rhizobacteria (PGPR), which is abundant in rhizosphere, promote plant growth directly by either facilitating plant nutrient resource acquisition or producing plant hormone levels, or indirectly in the forms of biocontrol agents inhibiting plant pathogens (Ahemad and Kibret, 2013). Soil conditions play a major role in plant-microorganisms interaction. Soil pollutants can alter root exudates and the plant nutrients released from bacteria. Excessive metal concentrations in contaminated soils decrease soil microbial activity and fertility (McGrath *et al*., 1995).

The Saen Saeb canal is a famous transportation canal in Bangkok. The canal connects the Chao Phraya River to the Bangpakong River with a total length of 72 kilometers. Wastewater is an obvious outcome from commuters along the canal, especially in inner and middle areas of Bangkok (Charuvan *et al*., 2013; Suwandee *et al*., 2013). However, some people still use the Saen Saeb water for watering plants. The effect of Saen Saeb water usage on plant growth and soil pollution has not been well studied. The present study showed the effect of Saen Saeb water on the growth of Chinese kale, tomato, and *Dendrobium* orchid in greenhouse and sterile (tissue culture) conditions. The effect of that on Chinese kale and tomato's rhizosphere microorganisms was also studied. The results of this study would benefit gardeners who live along the Saen Saeb canal. The scope of this study on the water usage in agriculture is limited to the inner and middle areas of Bangkok. According to the studies by Suwandee *et al*. (2013) and Charuvan *et al.* (2013), the water quality around these two areas was quite similar. In particular, water quality index of the Prasanmit pier was relatively close to that of other piers. Therefore, the Prasanmit pier was chosen as a representative area for our study. The above study by Suwandee *et al.* (2013) also concluded that the water around those areas cannot be used for agriculture.

2. Materials and Methods

2.1 Effect of Saen Saeb water on plant growth

To study the effect of Saen Saeb water on plant growth, Chinese kale (*Brassica alboglabra*), tomato (*Lycopersicon esculentum*), and *Dendrobium* orchid (*Dendroium* Sonia 'Earsakul') were selected as representative plants because they were often planted in households. The Chinese kale and tomato were seed in comercial soil in 300 ml-size pots. Two-week old healthy plants were used. The plants were divided into four groups subjected to different watering conditions, tap water, sterile tap water, Saen Saeb water, and sterile Saen Saeb water. The tap water was sterilized by autoclaved at 121°C for 15 minutes and the Saen Saeb water was sterilized by filtration using 0.45 µm pore size cellulose acetate (CA) membrane filter. The similar weight and height tissue cultured *Dendrobium* orchids were selected. Then, they were planted in sterile coconut sheets and were watered as described above. All kinds of plants were tested for two months. Then, root length, root weight, shoot length and shoot weight were measured. All plants were cultured in a greenhouse. The Saen Saeb water was sampled from the Saen Saeb canal at Prasanmit pier during dry period.

To study the effect of Saen Saeb water on plant growth in sterile condition, the plants were cultured in tissue culture condition. Murashige and skoog (MS) medium was used for Chinese kale and tomato, whereas Vacine and Went (VW) medium was used for *Dendrobium* orchids. Both media were sterilized by autoclave application. After autoclaving, both media at approximately 50°C were mixed with the Saen Saeb water, which was sterilized by 0.45 ìm pore size CA membrane filter. The Chinese kale and tomato seeds were surface sterilized with 10% NaOCl for 5 minutes. Then, they were washed with sterile water three times and placed in the MS medium containing 50% or 25% of the Saen Saeb water. The similar weight and height tissue cultured *Dendrobium* orchids were selected. They were cultured in the VW medium containing 50% or 25% of the Saen Saeb water. The plants were tested for two months. Then, root length, root weight, shoot length, and shoot weight were measured. The plants which were cultured in the MS or VW medium without the Saen Saeb water were used as a control in this study.

2.2 Effect of Saen Saeb water on microorganisms in rhizosphere

The rhizosphere soil from the roots of Chinese kale and tomato, which were tested with the Saen Saeb water, sterile Saen Saeb water, tap water, or sterile tap water for two months, were used to test for the effect of Saen Saeb water on the rhizosphere microorganisms. The amount of viable miroorganisms in the rhizoshere soil was counted by the dilution plate count method. Briefly, serial soil dilutions were made and suitable dilutions were poured in nutrient agar (NA).

2.3 Statistic anlysis

The experiments in plants were performed at least ten replicates and the dilution plate count experiments were performed five replicates. Data were analyzed for their homogeneity by Komologov-Smirnov test. The parametric data were analyzed by ANOVA and means of the data were compared by least significant difference test (lsd). The non-parametric data were analyzed by Kruskal-Wallis test and means of the data were compared by Mann-Whithney U test.

3. Results and Discussion

3.1 Effect of Saen Saeb water on plant growth

Hereafter in this section, a prefix "abbreviated watering method name-" is introduced over samples to aid readers. Samples in this study included Chinese kale, tomato, and *Dendrobium* orchid. These samples were tested with watering methods, represented by the prefix "method name-". For example, the Chinese kale tested with the Saen Saeb water (SW) would be shortly referred to as SW-Chinese kale. See Table 1 for how method names are abbreviated.

The effects of Saen Saeb water on the growth of Chinese kale, tomato and *Dendrobium* orchid were investigated at greenhouse conditions. The plants were tested for two months with different watering conditions, tap water, sterile tap water, Saen Saeb water, and sterile Saen Saeb water. As shown in Table 1, we found that the Saen Saeb water affected the Chinese kale and *Dendrobium* orchid growth. SW-Chinese kale had shorter shoots than these of TW-Chinese kale (8.60±1.46 < 11.88±1.16 cm) with a statistical significance ($p<0.05$). The shoot weight was, however, slightly decreased, but the difference was not statistically significant ($p=0.44$). It should be noted that the volume of shoots or roots was not measured in this study. This result was consistent with that of the sterile water; SSW-Chinese kale had shorter shoots than those of STW-Chinese kale (7.46±1.14 < 9.13±0.83 cm), $p<0.05$. In *Dendrobium* orchid, the use of Saen Saeb water significantly reduced root length and root weight compared with the use of tap water (root length: 5.66±0.47 < 6.58±0.42 cm; root weight: 0.19±0.01 < 0.27±0.05 g). These results were also consistent across samples; SSW-*Dendrobium* orchid's roots were lighter than STW-*Dendrobium* orchid's roots (0.13±0.01 < 0.18±0.03 g), $p<0.05$. The results showed that the Saen Saeb water had no effect on tomato growth at greenhouse conditions when SW-tomato was compared with TW-tomato, and SSW-tomato was compared with STW-tomato.

To study the effect of Saen Saeb water on plant growth in sterile conditions, plants were grown in tissue culture system with plant media, with the plant media containing 25% or 50% of the Saen Saeb water. We found that the Saen Saeb water affected the Chinese kale and tomato growth. As shown in Table 2, the Chinese kale that was cultured in the MS medium containing 50% of the Saen Saeb water had root length and root weight less than that in the MS medium with $p<0.05$ (root length: 5.74±1.63 < 13.1±0.89 cm; root weight: 0.05±0.01 < 0.12±0.02 g). A similar result was found in tomato. The shoot weight of tomato that was cultured in the MS medium containing 50% of the Saen Saeb water was lighter than that in the MS medium with $p<0.05$ (0.06±0.02 < 0.11±0.01 g). *Dendrobium* orchid in the VW medium showed the same growth rate with that in the VW medium contained 25% or 50% of the Saen Saeb water. However, the root weight of *Dendrobium* orchid that was cultured in the VW medium containing 50% of the Saen Saeb water was lighter than that

Table 1. Effect of Saen Saeb water on plant growth in greenhouse.

	Chinese kale				Tomato				*Dendrobium* orchid			
	Tap water (TW)	Sterile tap water (STW)	Saen Saeb water (SW)	Sterile Saen Saeb water (SSW)	Tap water (TW)	Sterile tap water (STW)	Saen Saeb water (SW)	Sterile Saen Saeb water (SSW)	Tap water (TW)	Sterile tap water (STW)	Saen Saeb water (SW)	Sterile Saen Saeb water (SSW)
Shoot length (cm)	11.88±1.16 ^{a}	9.13±0.83 ^{b}	8.60±1.46 ^{bc}	7.46±1.14 ^{c}	4.44±0.62 ^{ns}	3.85±1.10 ^{ns}	5.12±1.19 ^{ns}	4.40±0.88 ^{ns}	1.64±0.11 ^{ns}	1.68±0.08 ^{ns}	1.52±0.23 ^{ns}	1.62±0.08 ^{ns}
Root length (cm)	3.08±1.00 ^{a}	2.22±1.32 ^{ab}	2.56±0.72 ^{a}	1.79±0.30 ^{b}	2.44±1.24 ^{a}	1.15±0.30 ^{b}	2.31±0.70 ^{a}	1.47±0.53 ^{b}	6.58±0.42 ^{a}	5.14±0.61 ^{b}	5.66±0.47 ^{b}	6.43±0.27 ^{a}
Shoot weight (g)	0.32±0.14 ^{ns}	0.25±0.07 ^{ns}	0.30±0.13 ^{ns}	0.33±0.06 ^{ns}	0.11±0.05 ^{ns}	0.09±0.02 ^{ns}	0.07±0.04 ^{ns}	0.12±0.12 ^{ns}	0.50±0.47 ^{a}	0.47±0.04 ^{a}	0.44±0.03 ^{ab}	0.32±0.03 ^{b}
Root weight (g)	0.02±0.01 ^{ns}	0.04±0.08 ^{ns}	0.01±0.01 ^{ns}	0.01±0.00 ^{ns}	0.04±0.02 ^{ns}	0.02±0.01 ^{ns}	0.02±0.01 ^{ns}	0.03±0.03 ^{ns}	0.27±0.05 ^{a}	0.18±0.03 ^{b}	0.19±0.01 ^{b}	0.13±0.01 ^{c}

Data were shown as mean ± standard deviation. Different letters in each row for each plant indicate that there were significant differences ($p<0.05$). ns means no significant differences.

of *Dendrobium* orchid that was cultured in the VW medium containing 25% of the Saen Saeb water ($p<0.05$). While the root weight of *Dendrobium* orchid decreased, the shoot weight slightly increased. However, the difference was not statistically significant ($p=0.33$). It should be noted that plant media (both MS and VW) contains macronutrients and micronutrients required for plant growth. It was possible that adding the Saen Saeb water to the plant media can increase some nutrients to the level that produced phytoxicity.

The Saen Saeb water reduced the *Dendrobium* orchid growth in the greenhouse condition (Table 1) but did not reduce the growth in the sterile condition when comparing the *Dendrobium* orchid growth in the VW medium with that in the VW medium with 25% or 50% of the Saen Saeb water (Table 2). At greenhouse conditions, *Dendrobium* orchids were planted on sterile coconut sheets, which contained a few plant nutrients. It could be hypothesized that heavy metals in the Saen Saeb water induced plant nutrient deficiency.

The use of wastewater for agriculture is widespread in many regions, for example the cities Cairo and Tehran, as well as India and the United States (Braatz and Kandiah, 1996). There are many benefits for the use of domestic wastewater, including low-cost treatment, conservation of good quality water and recharge of groundwater. Moreover, domestic wastewater contains plant nutrients such as nitrate and phosphate. However, a controversy was found in this study that the Saen Saeb water reduced the growth of Chinese kale and Tomato (See Table 2), which further suggested that the Saen Saeb water is not suitable for agricultural use. The Saen Saeb water may contain heavy metal ions, which are excessively absorbed by roots and translocated to shoots, leading to impaired metabolism and reduced growth.

The results in Table 1 show that the Saen Saeb water affects the growth of Chinese kale and *Dendrobium* orchid, except for tomato. It was hypothesized that plant species play a role in phytotoxic stress. The Chinese kale belongs to the Brassicaceae family, while the tomato and *Dendrobium* orchid belong to Solanaceae and Orchidaceae families, respectively. Kumar *et al*. (1995) reported that Brassicaceae was able to accumulate high concentrations of heavy metals in shoots. Similarly, Hajiboland (2005) found that under metal stress, bean (*Phaseolus vulgaris* L.) showed a low reduction of their dry weight, whereas the growth of alfalfa (*Medicago sativa* L.) and wheat (*Triticuma estivum* L.) were poor.

3.2 Effect of Saen Saeb water on rhizosphere microorganisms

To assess the amount of microorganisms, the rhizosphere soil from each plant root was used for performing dilution plate count. The effect of Saen Saeb water on rhizosphere microorganisms of the *Dendrobium* orchids was not studied because it was difficult to obtain rhizosphere microorganisms from their aerial roots and they were planted on

Table 2. Effect of Saen Saeb water on plant growth in sterile conditions.

	Chinese kale			Tomato			*Dendrobium* orchid		
	MS medium	MS with 25% Saen Saeb water	MS with 50% Saen Saeb water	MS medium	MS with 25% Saen Saeb water	MS with 50% Saen Saeb water	VW medium	VW with 25% Saen Saeb water	VW with 50% Saen Saeb water
Shoot length (cm)	5.68 ± 0.60^{ns}	5.72 ± 0.46^{ns}	4.42 ± 0.53^{ns}	7.62 ± 2.40^{ns}	6.14 ± 2.12^{ns}	5.38 ± 1.11^{ns}	0.66 ± 0.23^{ns}	0.96 ± 0.31^{ns}	0.88 ± 0.20^{ns}
Root length (cm)	13.1 ± 0.89^{a}	10.4 ± 0.36^{b}	5.74 ± 1.63^{c}	6.29 ± 1.41^{ns}	7.86 ± 2.72^{ns}	7.31 ± 0.75^{ns}	4.54 ± 0.88^{ns}	4.17 ± 0.62^{ns}	3.90 ± 0.67^{ns}
Shoot weight (g)	0.12 ± 0.01^{ns}	0.16 ± 0.06^{ns}	0.09 ± 0.01^{ns}	0.11 ± 0.01^{a}	0.13 ± 0.03^{a}	0.06 ± 0.02^{b}	0.11 ± 0.06^{ns}	0.14 ± 0.06^{ns}	0.18 ± 0.08^{ns}
Root weight (g)	0.12 ± 0.02^{a}	0.13 ± 0.02^{a}	0.05 ± 0.01^{b}	0.02 ± 0.01^{ns}	0.03 ± 0.02^{ns}	0.02 ± 0.01^{ns}	0.08 ± 0.02^{ab}	0.10 ± 0.02^{a}	0.06 ± 0.02^{b}

Data were shown as mean ± standard deviation. Different letters in each row for each plant indicate that there were significant differences ($p<0.05$). ns means no significant differences.

the sterile coconut sheets before experiments were conducted. To inspect the effect of Saen Saeb water on rhizosphere microorganisms of the Chinese kale and tomato, we found that the Saen Saeb water reduced the microorganisms in the rhizosphere for the Chinese kale. As shown in Table 3, the amount of microorganisms in rhizosphere of TW-Chinese kale ([22.47±2.63] x 10^4 cell/g) or STW-Chinese kale ([19.12±1.15] x 10^4 cell/g) was more than that of SW-Chinese kale with $p<0.05$ ([4.47±1.16] x 10^4 cell/g) or SSW-Chinese kale ([2.53±0.90] x 10^4 cell/g), respectively. Using the Saen Saeb water for watering tomatoes did not reduce the microorganisms in the rhizosphere when SW-tomato ([2.83±0.55] x 10^4 cell/g) was compared with TW-tomato ([2.40±0.29] x 10^4 cell/g). However, the SSW-tomato ([1.77±0.32] x 10^4 cell/g) contained rhizosphere microorganisms in lower amounts than the STW-tomato with $p<0.05$ ([4.55±0.66] x 10^4 cell/g). This result agreed with the previous finding that the Saen Saeb water reduced the Chinese kale growth but did not affect the tomato growth in the greenhouse condition (Table 1).

It was possible that the use of Saen Saeb water reduced rhizosphere microorganisms in the Chinese kale that in turn affected the plant growth. Rhizosphere microorganisms are involved in acceleration of metal mobility, or immobilization. Plants and some rhizosphere microorganisms release inorganic and organic compounds possessing acidifying or chelating functions that are involved in plant metal uptake. Some beneficial microorganisms enhance phytoremediation process by controlling the metal accumulation in plant tissues and promoting the shoot and root biomass production (Rajkumar *et al*., 2012). The cadmium uptake in roots and shoots of *Cucurbit apepo* and *Brassica juncea* could be reduced by the inoculation of siderophore producing *Pseudomonas aeruginosa* strain KUCd1 (Sinha and Mukherjee, 2008). Additionally, Sheng *et al*. (2008) and Wu *et al*. (2006) found that the microorganisms associated with the inoculation of plant growth increased the metal extraction from soil as a result of increased biomass production in *B. juncea* and *Brassica napus*.

In this study, the Saen Saeb water affected the Chinese kale rhizosphere microorganisms but did not affect the tomato rhizosphere microorganisms when compared that with tap water (Table 3). It was possibly due to the difference in rhizosphere microbial communities among plant species. It should be noted that the sterile tap water unexpectedly increased microorganisms in tomato's rhizosphere when the result was compared to tap water. This issue was overlooked because this study focused on the usage of the Saen Saeb water. Therefore, a comparison of the direct results between the tap water and sterile tap water is neglected. However, the issue is worth of further investigations in the future. With our limited hypothesis, we speculate that chlorine in the tap water has an active role for reducing certain microorganisms in rhizosphere of tomato only. We hypothesized that the result is due to the difference in rhizosphere microbial communities among plant species. Supporting evidence was found in Kourtev *et al.* (2002) that rhizosphere soils differed in microbial community structure and functions under three plant species, Japanese barberry (*Berberis thunbergii*), Japanese stilt grass (*Microstegium vimineum*), and native species blueberry (*Vaccinium* spp.).

4. Conclusions

We found that the Saen Saeb water affected the growth of Chinese kale and *Dendrobium* orchid at greenhouse conditions and also affected the Chinese kale and tomato in sterile conditions. The Saen Saeb water itself was phytotoxic. Additionally, the usage of Saen Saeb water in plant watering resulted in the reduction of rhizosphere microorganisms, which may in turn affect plant growth.

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Table 3. Effect of Saen Saeb water on rhizosphere microorganisms.

	Amount of microorganisms in one gram of rhizosphere soil (x 10^4 cells)			
	Tap water (TW)	Sterile tap water (STW)	Saen Saeb water (SW)	Sterile Saen Saeb water (SSW)
Chinese kale	22.47±2.63^a	19.12±1.15^b	4.47±1.16^c	2.53±0.90^c
Tomato	2.40±0.29^b	4.55±0.66^a	2.83±0.55^b	1.77±0.32^c

Data were shown as mean ± standard deviation. Different letters in each row for each plant indicate that there were significant differences ($p<0.05$). ns means no significant differences.

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