

Utilization of wastewater on seed germination and physiological parameters of rice (*Oryza sativa* L.)

V Huy^{1,2} and C B Iwai^{1,2}

¹ Department of Land Resources and Environment, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002 Thailand

² Integrated Water Resource Management Research and Development Center in Northeast Thailand, Khon Kaen University, Khon Kaen, 40002 Thailand

E-mail: chuleemas1@gmail.com

Abstract. Due to increasing world population and demand, fresh water availability is becoming a limited resource. Reusing wastewater for agriculture has received attention since it contains nutrients, which are beneficial for growing crops. Even though wastewater can be used as the nutrient source for the plant, the toxicity of wastewater can still be a cause for concern and investigation. The main objective of this paper was to assess the effect of different sources of wastewater on the germination of Jasmine rice (KDML105), White rice (Phatum Thani 1), and Sticky rice (RD6) under laboratory conditions. Petri dish cultures were used with various concentrations (0, 50, and 100%) of wastewater collected from swine farm, aquaculture activity, and domestic. Seed germination, root length, shoot length, seed vigor index, fresh weight and dry weight were measured after each experiment. The results have shown that domestic wastewater and aquaculture activity wastewater did not decrease performance of Jasmine rice (KDML105), White rice (Phatum thani 1), and Sticky rice (RD6) while the germination of Jasmine rice (KDML105), White rice (Phatum thani 1), and Sticky rice (RD6) decreased when irrigated with swine farm wastewater. Therefore, using domestic and aquaculture activity wastewater for irrigation are suitable for growth of these crop.

Introduction

Due to increasing population numbers in the developing countries, demand for natural resources have been put under pressure and stress from excess waste. Access to water has become an essential and limited resource since industrial, agricultural and human activities keep rising to fulfill economic demands. Many countries in Africa, Latin American, Southern Asia, Middle East and more isolated island countries in Oceania may have problems with the supply and disposal of water because of the diverse requirement including irrigation of crops [1]. Increasing human demand for rice has focused attention on increasing agricultural activities by fertilizer use, irrigation and value adding food processes and this requires further access to water resources. In the same way, wastewater continues to increase due to these activities and is a major main problem for the environment. Wastewater is considered one

³ To whom any correspondence should be addressed.



of the worst kinds of pollution because of chemical and waste material from agricultural and food production. The environment can be affected by the additional heat, high or low pH level, and chemical contamination.

However, wastewater may contain various amount of organic matter (OM) and plant nutrients [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), copper (Cu), manganese (Mn), and zinc (Zn)] which benefit agriculture by increasing crop production [2]. A major crop in Asia is rice which consumes a lot of water during its life cycle. Additionally, wastewater from livestock production, aquaculture, and domestic have high nutrient levels and have a biochemical oxygen demand (BOD). After proper dilution, wastewater can be used as a potential nutrient source for plant growth in agricultural production, especially with rice. Therefore, reusing wastewater in agriculture has received much attention for the exploitation of its nutrients. For instance, using wastewater for irrigation can cause an increase in OM, N, P, and K to the soil and can provide significant benefit to farming communities [3]. For example, an experiment on seed germination using jute mill wastewater indicated that jute mill wastewater plays a profound role on germination characteristics and carbohydrate content of *Cicer arietinum* and *Pisum sativum* [4]. An experiment on the effects of treated and untreated wastewater on the seed *Avena sativa* L reported that treated wastewater can improve the seedling growth, and its amylase and lipase activities [3]. An experiment on seed performance of pea, lentil and gram from textile wastewater showed that wastewater can be utilized for irrigation propose [5]. It was also reported that municipal wastewater had an effect on germination, seedling performance, nutrient uptake, and increased chlorophyll content of rice [2]. A study on wastewater treatment on the seed *Abelmoschus Esculentus* L showed that the wastewater is an important nutrient source for seed germination and plant growth [6]. An experiment on rice germination from domestic wastewater reported that with the proper dilution domestic wastewater could be used as a potential source for seed germination [7]. There are many researchers studying the effect of wastewater on seed germination, but most of the studies focus on a single source of wastewater to test seed germination. There is limited information about comparing the different sources of wastewater on seed germination. Therefore, the aim of this study is to investigate the applicability of different sources and different concentrations of wastewater on selected rice varieties in order to improve germination and yield.

Experimental procedure and methodology

1.1. Wastewater sampling

Domestic wastewater (DW) was collected from Khon Kaen University wastewater, Khon Kaen province, Thailand. Swine farm (SW) and aquaculture activity wastewater (AW) were collected from animal farms, also located in Khon Kaen University. Wastewater was stored in plastic containers and put in the refrigerator below 5 °C until use. Wastewater was diluted to 0, 50 and 100 % (pure wastewater without dilution).

1.2. Physical-chemical characteristic of wastewater

Physical-chemical parameters of wastewater were analyzed: pH, Temperature, Electrical Conductivity (EC), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Total Solids (TS), Suspended Solids(SS), Hardness, Fats, Oil, and Grease (FOG), Nitrogen (N), Nitrate (NO₃⁻), Phosphorus (P), Potassium(K), Copper (Cu), Cadmium (Cd), Lead (Pb), Chromium (Cr) and Zinc (Zn) following the Standard Method for Examination of Water and Wastewater (APHA, 2013) [8].

1.3. Seed germination experiment

A seed germination experiment was conducted for 5 days at Ecotoxicology Laboratory, Department of Land Resource and Environment, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. The experiment followed a Completely Randomized Design (CRD) with three replications. Rice seeds (*Oryza sativa* L.) from three different varieties comprising Jasmine rice (KDML105),

White rice (Phatum Thani 1), and Sticky rice (RD6) were sterilized with 1% solution of hydrochloride for 15 minutes to remove microbes. Seeds were then washed with distilled water. Whatman Grade 181 No. 1 filter papers were put into petri dishes to which 10 seeds from each representative rice variety were added. Each petri dish was irrigated with 5 mL of different concentration of wastewater, and then incubated at 25 °C in an Aqualytic Tc 455s incubator. After completion of seed germination, seeding from each treatment was measured for germination percentage, root length, shoot length, seeding vigor index, fresh weight, and dry weight.

1.4. Germination percentage estimation

Seed germination percentage was calculated from each treatment based on the primary root emergence from the seeds and the results were expressed in percentage following the calculation as in Equation 1 [4].

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated in particular treatment}}{\text{Total number of seed treated in particular treatment}} \times 100 \quad (1)$$

1.5. Seeding Length estimation

The shoot length was determined based on the primary seed plumule and root length was determined by the primary seed radical. Seeding length results were expressed as centimeter.

1.6. Fresh weight and dry weight

Fresh weight was measured using an Ohaus PA 2102 electronic balance. Dry weight was measured after drying the seedling in hot air oven at 80 °C for 24 hours and was then recorded using electronic balance [4].

1.7. Seed vigor index

Seed germination, root length, and shoot length were used to calculate seed vigor index, which followed the formula as in Equation 2 [9].

$$\text{Vigor index} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Percentage germination} \quad (2)$$

1.8. Statistical analysis

The Statistic 10 software (version 10, USA) was used to analyze the data including the analysis of variance (One Way ANOVA). Treatment means were compared using least significance difference (LSD) at $P < 0.05$.

Results and Discussions

1.9. Wastewater characteristics

The chemical and physical characteristics of swine farm, domestic, and aquaculture activity wastewater are presented in Table 1. The different sources of wastewater express difference characteristics. Swine farm, domestic, and aquaculture activity wastewater are slightly alkaline with pHs of 7.78, 7.37 and 7.8, respectively. Swine farm wastewater shows the highest electrical conductivity (EC), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Fats, Oil, and Grease (FOG) when compared with the other wastewaters. The concentration of Total Solids (TS), Suspended Solids (SS), Biological Oxygen Demand (BOD), Hardness and Dissolved Oxygen (DO) in swine farm, aquaculture, and domestic wastewater show a similar order. The concentration of Nitrogen (N), Nitrate (NO_3^-), Phosphorus (P), and Potassium (K) in swine farm, domestic and aquaculture activity wastewater are also similar. Copper (Cu) and Zinc (Zn) are higher in swine farm wastewater when compared with other wastewater. It is reported that Copper (Cu) and Zinc (Zn) are found in higher quantity in swine farm wastewater because of supplement added to the pig's feed with higher amounts of Copper (Cu) and Zinc (Zn) to improve feed efficiency and the health of swine [10].

Lead (Pb) concentration is higher in domestic wastewater compared with other wastewater (Table 1). It is reported that the higher concentration of Lead (Pb) in domestic wastewater is due to the use of leaded petrol when undertaking domestic activities [11]. Zinc (Zn) and Lead (Pb) are not detected in aquaculture activity wastewater (Table1).

Table 1. Physical and chemical characteristic of different sources of wastewater

Characteristics	Swine farm	Domestic	Aquaculture
pH	7.78	7.37	7.81
Electrical conductivity ($\mu\text{S}/\text{cm}$)	3081.7	477.3	494.7
Total dissolve solids (mg/L)	1521.7	238.7	247.3
Total Solids (mg/L)	4600	5000	5800
Suspended solids (mg/L)	1500	1500	2000
Biochemical oxygen demand (mg/L)	205	167	140
Chemical oxygen demand (mg/L)	7200	91.33	24
Hardness (mg/L)	72	30	26
Fats, Oil, and Grease (mg/L)	6802.5	1500	2005
Temperature ($^{\circ}\text{C}$)	28.33	29.30	26.29
Salt (mg/L)	1.54	0.22	0.23
Dissolve Oxygen (mg/L)	1.63	1.83	1.36
Nitrate as NO_3^- (mg/L)	0.05	0.09	0.072
Nitrogen as N (mg/L)	2.24	1.49	1.12
Phosphorus as P (mg/L)	1.34	1.52	0.1
Potassium as K (mg/L)	148.3	80.27	70.95
Copper as Cu (mg/L)	0.098	0.003	0.001
Zinc as Zn (mg/L)	0.308	0.031	ND
Lead as Pb (mg/L)	0.002	0.164	ND

Note: ND is not detected

3.1. Germination results

The results of the seed experiment of Jasmine rice (KDML105), Sticky rice (RD6), and White rice (Phatum Thani 1) are showed in Table 2, 3 and 4.

Table 2. Effect of wastewater on Jasmine rice (KDML105) germination (GM), roots length (RL), shoots length (SL), seeding vigor index (SVI), fresh weight (FW) and dry weight (DW)

Sources of WW	Concentration (%)	Jasmine rice (KDML105)					
		GM (%)	RL (cm)	SL (cm)	SVI	FW (g)	DW (g)
DI	0	100 ^a	3.42 ^a	2.07 ^b	550.28 ^a	0.605 ^b	0.20 ^a
SW	50	0 ^b	0 ^b	0 ^c	0 ^b	0 ^c	0 ^c
SW	100	0 ^b	0 ^b	0 ^c	0 ^b	0 ^c	0 ^c
DW	50	90 ^a	3.77 ^a	2.32 ^{ab}	548.92 ^a	0.75 ^{ab}	0.20 ^a
DW	100	95 ^a	4.03 ^a	2.57 ^a	628.30 ^a	0.87 ^a	0.21 ^a
AW	50	90 ^a	3.52 ^a	2.10 ^b	506.25 ^a	0.73 ^{ab}	0.19 ^a
AW	100	95 ^a	3.90 ^a	2.33 ^{ab}	593.60 ^a	0.78 ^a	0.205 ^a

Values in same letters in the columns are not significantly different ($p \leq 0.05$), WW= wastewater, DI=distill water,

SW=swine farm wastewater, DW= domestic wastewater, AW= aquaculture activity wastewater.

Table 3. Effect of wastewater on Sticky rice (RD6) germination (GM), roots length (RL), shoots length (SL), seeding vigor index (SVI), fresh weight (FW) and dry weight (DW)

Sources of WW	Concentration (%)	Sticky rice (RD6)					
		GM (%)	RL (cm)	SL (cm)	SVI	FW (g)	DW (g)
DI	0	100 ^a	4.695 ^a	1.98 ^b	667.50 ^a	0.57 ^a	0.205 ^a
SW	50	10 ^b	0.25 ^b	0.13 ^c	3.80 ^b	0.2 ^b	0.05 ^b
SW	100	0 ^b	0 ^b	0 ^c	0 ^b	0 ^c	0 ^b
DW	50	90 ^a	4.85 ^a	2.29 ^{ab}	642.60 ^a	0.58 ^a	0.21 ^a
DW	100	95 ^a	4.915 ^a	2.49 ^a	703.475 ^a	0.61 ^a	0.23 ^a
AW	50	90 ^a	4.58 ^a	2.24 ^{ab}	613.80 ^a	0.545 ^a	0.205 ^a
AW	100	95 ^a	4.78 ^a	2.41 ^{ab}	683.05 ^a	0.61 ^a	0.215 ^a

Values in same letters in the columns are not significantly different ($p \leq 0.05$), WW= wastewater, DI=distill water,

SW=swine farm wastewater, DW= domestic wastewater, AW= aquaculture activity wastewater.

Table 4. Effect of wastewater on White rice (Phatum Thani 1) germination (GM), roots length (RL), shoots length (SL), seeding vigor index (SVI), fresh weight (FW) and dry weight (DW)

Sources of WW	Concentration (%)	White rice (Phatum thani 1)					
		GM (%)	RL (cm)	SL (cm)	SVI	FW (g)	DW (g)
DI	0	100 ^a	4.075 ^b	2.45 ^b	653 ^a	0.815 ^b	0.195 ^a
SW	50	20 ^b	0.15 ^c	0.08 ^c	9.20 ^b	0.225 ^c	0.075 ^b
SW	100	0 ^b	0 ^c	0 ^c	0 ^b	0 ^c	0 ^c
DW	50	90 ^a	5.46 ^{ab}	2.64 ^b	729.45 ^a	1.04 ^{ab}	0.205 ^a
DW	100	95 ^a	6.07 ^{ab}	3.14 ^a	874.65 ^a	1.175 ^a	0.215 ^a
AW	50	85 ^a	5.35 ^{ab}	2.54 ^b	671.85 ^a	0.97 ^{ab}	0.20 ^a
AW	100	95 ^a	5.75 ^{ab}	2.81 ^{ab}	816.60 ^a	1.03 ^{ab}	0.205 ^a

Values in same letters in the columns are not significantly different ($p \leq 0.05$), WW= wastewater, DI=distill water,

SW=swine farm wastewater, DW= domestic wastewater, AW= aquaculture activity wastewater.

The result of seed germination from 0, 50, and 100% of swine farm, domestic, and aquaculture activity wastewater on Jasmine rice (KDML105), Sticky rice (RD6) and White rice (Phatum Thani 1) are significantly different (Table 2, 3 and 4). However, seed germination of Jasmine (KDML105), Sticky rice (RD6) and White rice (Phatum Thani 1) irrigated with 50% and 100% of domestic wastewater, aquaculture wastewater and control are not significantly different and the germination of all rice varieties are lower when compared with the control. The results are similar to report from Quetta City on the effects of wastewater on seeding lettuce growth which determined that seed growth was decreased because of high soluble salt levels within the wastewater [12]. It was reported that seed germination of *Capsicum annun* was delayed because the high level of salinity and toxicity of high osmotic pressure found in wastewater, which can decrease water uptake, by the seed [13]. It was also indicated that the high osmotic pressures of seed germination solution makes inhibition more difficult and restarts seed germination. The ability of seeds to germinate under high osmotic pressure differs with variety as well as species [14]. All the rice varieties were less germinated with 50% and 100 % swine farm wastewater because of the effect of high EC (3081.7 $\mu\text{S}/\text{cm}$) which is presented in Table 1. The report on the effect of tannery effluent on seed germination and growth of two sunflower cultivars

indicated that water successfully used for irrigation has EC less than 2250 $\mu\text{S}/\text{cm}$ [15]. If electrical conductivity is higher than the permissible limit, it will be attributed to the relatively high salinity, which can decreased of the crop germination percentage [16]. Or, it can also be attributed to the toxicity of high concentration of chloride ion in wastewater which could be reduce seed germination [17]. The shoot length of Jasmine (KDML105), Sticky rice (RD6) and White rice (Phatum 1) which irrigated with 100% domestic wastewater is significantly increased when compared with control. An experiment on the impact of different dilutions of sewage water on seed germination characteristics and carbohydrate content of seeding *Vigna unguiculata* indicated that when high nutrients are applied to seed, the shoot length would be increased [4]. In the same way, by increasing wastewater concentration, seeding height was increased since wastewater contains essential nutrients, so it can promote plant growth [18]. The fresh weight of Jasmine rice (KDML105) is significantly increased when irrigated with 100% domestic and aquaculture wastewater when compared with other concentrations. The fresh weight of White rice (Phatum Thani 1) is also significantly increased with 100% domestic wastewater when compared with other treatments. It was already found that when the root and shoot length were healthier, the plants were heavier in weight [15]. Plants irrigated with 100 % wastewater can have a greater seed weight when compared with tape water because wastewater application can be contributed plant appropriated nutrition and increase photosynthetically active area during seed filling period [15]. There is no significant difference on the fresh weight of sticky rice (RD6). It was reported that different plant varieties have different abilities to absorb the different range of the nutrients, which can affect length, thickness, surface area, and weight [19]. For instance, modern genotypes of rice are more tolerance than the traditional genotype and different varieties have different capacities in root absorption, translocation and shoot demand [19]. The 100% domestic wastewater was very effective for seed performance when compared with other wastewater. It might be the plant nutrients presenting in domestic more than other wastewater, which are shown in Table.1. However, the 50% domestic and aquaculture wastewater were less effective for seed performance, possibly because of diluted concentrations, which can reduce the plant nutrient.

4. Conclusions

From this study, it can be concluded that 100 % domestic and aquaculture activity wastewater were influence seed performance of Jasmine (RDML105), Sticky rice (RD6) and White rice (Phatum Thani 1) and 100% domestic wastewater was given the best result. However, Swine farm wastewater was not suitable for irrigation purpose since it might be contained high EC (3081.7 $\mu\text{S}/\text{cm}$) which was higher than permissible limit (2250 $\mu\text{S}/\text{cm}$) for irrigation seed germination and plant. It could be suggested that using domestic wastewater directly for irrigation purpose is appropriated. The use of wastewater for plant nourishment would be beneficial for alternative resources to fresh water. Wastewater contains essential nutrients, which is important for soil as well as crop; moreover, it is necessary to minimize pollution and to assist the environment. However, toxic contamination such as heavy metal and health safety should be a concerned. Further, study on food safety needs to be investigated.

Acknowledgements

The author thanks the Integrated Water Resource Management Research and Development Centre in Northeast Thailand, The Research Developing and Learning Centre on Earthworm for Agriculture and Environment, and the KKU scholarship for ASEAN and GMS Countries for financial support. We would like to thank Professor Dr. Barry Noller for his kind help with editing the English.

References

- [1] Naddafi K, Jaafarzadeh N, Mokhtari M, Zakizadeh B and Sakian M R 2005 Effects of wastewater stabilization pond effluent on agricultural crops *Int J Environmental Science Technology* **1**(4) 273–7
- [2] Gassama U M, Puteh A B, Abd-Halim M R and Kargbo B 2015 Influence of municipal

- wastewater on rice seed germination, seedling performance, nutrient uptake, and chlorophyll content *J Crop Science Biotechnology* **18**(1) 9–19
- [3] Fendri I, Ben S R, Khemakhem B, Ben H N, Gdoura R and Abdelkafi S 2013 Effect of treated and untreated domestic wastewater on seed germination, seedling growth and amylase and lipase activities in *Avena sativa* L *J Sci Food Agriculture* **93**(7)1568–74
- [4] Manisha P and Angoorbala B 2015 Impact of different dilutions of sewage water on seed germination characteristics and carbohydrate content of *vigna unguiculata* *Int J Agriculture Innovation Research* **4**(2) 330–1
- [5] Khan M G, Daniel G, Konjit M, Thomas A, Eyasu S S and Awoke G 2011 Impact of textile wastewater on seed germination and some physiological parameter in pea (*Pisum sativum* L.), lentil (*Lens culentum* L.) and gram (*Cicer arietinum* L.) *Asian Journal* 1–5
- [6] Khaleel R I, Ismail N and Ibrahim M H 2013 The impact of waste water treatments on seed germination and biochemical parameter of *Abelmoschus Esculentus* I *Social Behavioral Science* **91**(2012) 453–60.
- [7] Dash A K 2012 Impact of domestic waste water on seed germination *Biological* 280–6.
- [8] APHA (American Public Health Association) 2013 *Standard Method for the Examination of Water and Wastewater* (Washington DC: APHA)
- [9] Abdul-Baki A and Anderson J D 1973 Vigor determination in Soybean seed by multiple criteria *Crop Science*
- [10] Mavrogianopoulos G, Vogli V and Kyritsis S 2002 Use of wastewater as a nutrient solution in a closed gravel hydroponic culture of giant reed (*Arundo donax*) *Bioresource Technology* **82**(2)
- [11] Shamuyarira K K and Gumbo J R 2014 Assessment of heavy metals in municipal sewage sludge *Int J Environ Res Public Health* **11**(3) 2569–79.
- [12] Bazai Z A and Achakzai A K K 2006 Effect of wastewater from Quetta city on the germination and seedling growth of lettuce (*lactuca sativa* l.) *J Apply Science* **6**(2) 380–2.
- [13] Khan S S and K H Sheikh 1976 Effect of different level of salinity on seed germination and growth of capsicum annum *Biological* **22** 15-25
- [14] Ayyasamy P M, Yasodha R, Rajakumar S, Lakshmanaperumalsamy P, Rahman P K S Ma and Lee S 2008 Impact of sugar factory effluent on the growth and biochemical characteristics of terrestrial and aquatic plants *Bull Environ Contam Toxicol* **81**(5) 449–54.
- [15] Hussain F, Malik S, Athar M, Bashir N, Younis U and Hassan M 2013 Effect of tannery effluents on seed germination and growth of two sunflower cultivars *African J Biotechnology* **9**(32) 5113–20
- [16] Panaskar D B and Pawar R S 2011 Effect of textile mill effluent on growth of *Vigna unguiculata* and *Pisum sativum* seedlings *Indian J Sci Technol* **4**(3) 266–72.
- [17] Hayward H.E and C.H Wadleigh 1949 *Plant growth on the salin and alkali solids advances in agronomy* (New York, Acad:press)
- [18] Moradi S, Heidari H, Saeidi M and Nosratti I 2016 Effect of sewage-contaminated water on seed production, heavy metals accumulation and seedling emergence in OAT *Glob Nest J* **18**(2) 329–38.
- [19] Baligar V C, Fageria N K and He Z L 2001 Nutrient use efficiency in plants *commun soil science plant anal* **32**(7) 921–50