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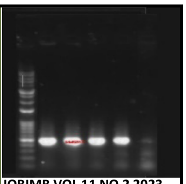

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Effect of Seed Germination and Early Seedling Growth Response on Two Rice (*Oryza sativa*) Varieties Supplied with Different Types and Concentrations of Wastewater in Thailand

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ABSTRACT

With increasing concerns over water scarcity, wastewater reclamation for agricultural irrigation has gained attention. However, the use of wastewater can have both positive and negative effects on crop growth. Seed germination and early seedling growth tests are considered to accurately represent crop growth population. This study aimed to evaluate the effect of different types and concentrations of wastewater on rice seed germination and early seedling growth. Two rice varieties, SIRAJ279 and MR219, were grown in wastewater from shrimp culture, domestic sources, and cement factories at five concentrations: 0 (control), 25, 50, 75, and 100%. Seed germination percentage and germination rate index were used to evaluate seed germination analysis, while the seedling length, seedling vigor index, seedling fresh weight, seedling dry weight, and phytotoxicity percentage were used to evaluate early seedling growth analysis. Results showed that the SIRAJ279 rice variety had overall better seed quality than MR219. Rice irrigated with shrimp culture and domestic wastewater showed better results in seed germination, germination rate index, seedling length, seedling vigor index, and seedling fresh weight than those irrigated with cement factory wastewater. However, no significant difference was observed in seedling dry weight. Additionally, increased wastewater concentration had a negative effect on seed and seedling results. Seedlings grown in wastewater with a concentration of 75-100% from cement factories had the highest phytotoxicity percentage (more than 30%). Therefore, it is recommended to dilute cement factory wastewater to 50% before using it for agricultural irrigation.

INTRODUCTION

Water is a vital element in both human survival and crop production, but its scarcity presents a significant challenge in food production. The distribution and availability of freshwater have undergone a significant shift in recent years, with predictions that 3.5-4.4 million people will face severe water stress by 2050 due to climate change and increasing water demand for human activities [1]. According to Flörke et al. [2], the risk of drought is expected to rise significantly due to prolonged heatwaves, with the Mediterranean, Africa, and Asia being among the most vulnerable regions. As a result, freshwater and groundwater have become increasingly precious and vulnerable. Furthermore, the rapid growth of urban areas and the diversion of water supply to domestic and industrial purposes have led to a decrease in water supply for agricultural activities [3]. Rice, being a staple food for almost half of the world's population [4], requires a large volume of water during its life cycle, with approximately 1,250mm of irrigation water being necessary during the growing season [5]. More than 70% of the total irrigation water in Asia is used for rice paddy development, making it the largest consumer of freshwater resources in South and Southeast Asia [6]. The impact of global warming on rice production, coupled with water scarcity, presents significant challenges to farmers' ability to produce rice. The vulnerability of rice production to global warming has become a key concern, with climate change impacting water requirements for paddy field irrigation. For instance, water resources from the Mekong Delta only allow for 50% of the river water to be used for agricultural irrigation during the dry season, and this is expected to decrease as climate change worsens [7].

Wastewater has the potential to be used as a beneficial supplementary fertilizer due to its abundance of nutrients and organic matter content [8, 9]. The treated wastewater contains nutrients such as nitrogen, phosphorus, potassium, and sulfur, which can easily be absorbed by plants [10]. Studies have demonstrated that paddy irrigated with wastewater results in higher yields and better vegetative growth compared to groundwater [11, 12]. Additionally, paddy shoots that were treated with wastewater showed a higher concentration of essential nutrients [13]. The use of reclaimed wastewater in agricultural irrigation has the potential to reduce the need for chemical fertilizers, promote crop productivity, improve soil fertility, and potentially lower production costs [6].

Despite the potential benefits of using wastewater for irrigation, it is a complex resource that may have both positive and negative impacts on seed germination and plant growth [14]. Wastewater may contain potentially toxic elements (PTEs) that can harm seed germination and seedling growth, such as copper, zinc, lead, cadmium, and chromium [15]. Moreover, wastewater may contain harmful elements that could reduce crop productivity and increase wastewater-borne diseases [16].

Studies have shown a vast increase in the utilization of wastewater for agricultural cultivation in recent years. For instance, the usage of treated wastewater for crop production has increased by 10-29% annually in China, the United States, and the European Union, and has even reached up to 41% in Australia [17]. However, a study for sustainable rice irrigation is still rare in Southeast Asia. Therefore, continuous research must be conducted in this area to develop effective strategies for using wastewater as an alternative to ensure food security.

MATERIALS AND METHODS

Wastewater analysis

The collection and analysis of the wastewater samples followed the standard operating procedure outlined by APHA [18]. The pH was determined using a pH meter, EC using a conductivity meter (EC 214), and turbidity using a Turbidimeter 2100A. The Nessler Method (Method 8038), Tetraphenylborate Method, and PhosVer 3 Ascorbic Acid Method (Method 8048) were used for the analysis of N, K, and P, respectively. These samples were analyzed using a HACH UV-Spectrophotometer. The analysis of heavy metals such as Cu, Zn, and Pb was carried out using Atomic Absorption Spectrometry (AAS). COD was determined using the Reactor Digestion Method (Method 8000) and calculated with the help of HACH Digital Reactor Block 200 (DRB200). The Dilution Method (Method 8043) was used to analyze the BOD, and the HACH HQ40d Meter was used to measure the BOD reading after a 5-day incubation period to calculate the oxygen consumed by bacteria for the oxidation of organic matter.

The TSS was determined by filtering the wastewater sample through a pre-weight filter membrane (0.45 µm). The residue on the filter was dried in an oven at 103-105 °C for 3 hours and then kept in a desiccator overnight. The increased weight of the filter membrane represented the TSS.

Seed germination analysis

Planting material

Two rice varieties were used in this experiment, SIRAJ279 and MR219. The selection of wastewater was based on its potential and proximity to the rice field. Shrimp culture wastewater was obtained from Chachoengsao province, cement factory wastewater from Suphan Buri province, and domestic wastewater from Bang Bua village, Bangkok province. These wastewaters were then diluted into four concentrations: 25%, 50%, 75%, and 100% (undiluted).

Experimental design

The experiment was conducted from March-May 2020 at the Seed Technology Laboratory, Agronomy Department, Faculty of Agriculture, Bangkhen Campus, Kasetsart University, Thailand. Twenty-five rice seeds were germinated using the two-layered paper method [19] and placed in transparent plastic bags in an upright position. The plastic bags were then moistened with wastewater treatments, sealed, and arranged inside the 25 °C Aqualytic Tc 455s incubator. Two millilitres of wastewater treatments were added every two days to maintain moisture. The experiment was carried out using a Completely Randomized Design (CRD) with four replications. The number of germinated seeds was recorded from 0 to 14 days after sowing (DAS). The visible radical growth and the emergence of hypocotyls were considered indicators of seed germination.

Data collection

i. Germination Percentage (GP)

calculated at 14 DAS [19]:

$$\mathrm{GP}(\%) = \left[\frac{number\ of\ total\ germinated\ seeds}{total\ number\ of\ seed\ tested}\right] x\ 100$$

ii. Germination Rate Index (GRI)

Following [32]:

$$\mathrm{GRI} = [(number\ of\ germinated\ seeds)/(day\ of\ the\ first\ count) + --- + (number\ of\ germinated\ seeds)/(day\ of\ the\ final\ count)]$$

Early seedling growth analysis

Planting material

Same planting material as in seed germination analysis.

Experiment design

The experiment was conducted for three weeks in a Randomized Complete Block Design (RCBD) with four replications in a greenhouse. Twenty-five seeds were planted in plastic germination boxes (20 cm x 15 cm) filled with 2.5 kg of sterilized sand. The boxes were watered every two days with 200 mL of wastewater treatment at concentrations of 0%, 25%, 50%, 75%, and 100%. The seedlings' performance was evaluated 21 days after sowing (DAS).

Data collection

i. Seedling Length (SL)

the sum of the length of the root and shoot at 21 DAS:

SL (cm) = Shoot length + root length

ii.Seedling Vigor Index (SVI)

following Abdul Baki and Anderson [21] formula:
SVI=Seedling length x Germination percentage

iii.Seedling Fresh Weight

Seedlings are weighted after 21 DAS

iv.Seedling Dry Weight

The fresh seedling was dried in the oven at 80^0C for 24 hours until reach constant weight [22]

v.Root: Shoot Ratio (R:S)

$$R{:}S = \frac{\text{Dried weight of root}}{\text{Dried weight of shoot}}$$

vi.Percentage Phytotoxicity

using chou [23] formula

$$\text{Percentage phytotoxicity (\%)} = \frac{\text{(Root length of control} - \text{Root length of of tested)}}{\text{Root length of control}} \text{ x } 100$$

Statistical analysis

Both seed germination and early seedling growth analysis were subjected to Analysis of variance (ANOVA) using SAS statistical software (version 9.4). Treatment means were compared using honestly significant difference HSD at $p<0.05$.

RESULT AND DISCUSSION

Wastewater characteristic

The physical and chemical characteristics of the supplied wastewater are presented in **Table 1.** The wastewater from the cement factory exhibited the highest alkaline solution with a pH of 13.29, which may be attributed to the presence of hydroxides and carbonates during the mixing process [24]. The cement factory wastewater also contained a high salinity (827.6 EC) due to the large amount of sodium hydroxide used in the washing process. It is also followed by high turbidity (197 NTU) and Total Suspended Solid (756.6 mg L^{-1}) due to the production of fine cement dust. According to Aga et al. [25], cement factory wastewater is an effluent that is composed of processed water with dissolved solids (potassium and sodium hydroxide, chloride, and sulfate) and suspended solids (calcium carbonate).

On the other hand, shrimp culture and domestic wastewater exhibited nearly neutral pH values of 7.11 and 7.79, respectively, with slightly lower EC values of 450.9 µs/cm and 477.3 µs/cm, respectively, and low turbidity of 21.9 NTU and 11.6 NTU, respectively. Shrimp culture and domestic wastewater had slightly higher essential nutrient content than cement factory wastewater. For example, the amount of nitrogen was higher in shrimp culture and domestic wastewater (5.56 mg L^{-1} and 3.49 mg L^{-1}, respectively) than in cement factory wastewater (1.04 mg L^{-1}). Phosphorus content in shrimp culture and domestic wastewater was also higher (2.68 mg L^{-1}, 1.52 mg L^{-1}, respectively) than cement factory wastewater (0.64 mg L-1), and potassium content in shrimp culture and domestic wastewater (7.92 mg L^{-1} and 11.24 mg L^{-1}, respectively) was significantly higher than in cement factory wastewater (2.63 mg L^{-1}). According to Tymchuk et al. [26], wastewater that contains an adequate amount of nutrients could be an excellent substitute for chemical fertilizers in farming. For instance, oil mill wastewater, which is loaded with a rich number of inorganic compounds such as potassium, magnesium, and iron, has been found to be beneficial for improving soil properties, seed germination, and plant growth [27]. Finally, cement factory wastewater was found to contain a higher number of heavy metals than shrimp culture and domestic wastewater. For example, lead and zinc in cement factory wastewater were present at concentrations of 10.9 mg L^{-1} and 2.25 mg L^{-1}, respectively, compared to no detection of lead and zinc in shrimp culture and only slightly higher amounts of zinc and lead in domestic wastewater at 0.31 mg L^{-1} and 3.54 mg L^{-1}, respectively. Hu et al. and Thapliyal et al. [28,29] have noted that nutrient content in wastewater is beneficial for seed germination and plant growth, while excessive amounts of pollutants in wastewater such as salt, heavy metals, pathogens, and others often accumulate in the soil and result in phytotoxicity to the soil and plant [30, 31].

Table 1. Physical and chemical characteristics of wastewaters.

Characteristics	Wastewater		
	Shrimp Culture	Domestic	Cement Factory
pH	7.11 ± 1.45	7.79 ± 1.37	13.29 ± 2.21
EC (µs cm^{-1})	450.9 ± 168.6	477.3 ± 182.4	827.6 ± 223.1
Turbidity (NTU)	21.9 ± 3.7	11.6 ± 2.6	197.0 ± 34.3
Nitrogen (mg L^{-1})	5.65 ± 2.31	3.49 ± 0.76	1.04 ± 1.33
Phosphorus (mg L^{-1})	2.68 ± 0.04	1.52 ± 0.07	0.64 ± 0.04
Potassium (mg L^{-1})	7.92 ± 0.12	11.24 ± 0.18	2.63 ± 0.10
Copper (mg L^{-1})	0.039 ± 0.009	0.047 ± 0.008	0.044 ± 0.007
Lead (mg L^{-1})	ND ± 0.00	3.54 ± 0.02	10.9 ± 0.03
Zinc (mg L^{-1})	ND ± 0.00	0.31 ± 0.03	2.25 ± 0.06
Biological Oxygen Demand (mg L^{-1})	71.5 ± 1.2	167.9 ± 2.5	20.9 ± 0.6
Chemical Oxygen Demand (mg L^{-1})	53.5 ± 1.3	91.3 ± 1.9	172.1 ± 2.4
Total Suspended Solid (mg L^{-1})	412.6 ± 2.2	522.4 ± 2.4	756.6 ± 3.1

* ± in the table represent std. dev
* ND indicated not detected

Seed germination analysis

Germination percentage

The germination percentages of two rice varieties, SIRAJ279 and MR219, when irrigated with different types of wastewaters (**Table 2**) indicate that SIRAJ279 had a significantly high germination percentage of 93.2% and 91.4% when irrigated with shrimp culture and domestic wastewater, respectively, but significantly decreased to 82.2% when imbibed with cement factory wastewater. Similarly, MR219 also had a high germination percentage of 85.3% and 84.6% when irrigated with shrimp culture and domestic wastewater, respectively, but decreased significantly to 75.9% when supplied with cement factory wastewater. These results indicate that both rice varieties were negatively impacted by cement factory wastewater.

In **Fig. 1,** the study further investigates the impact of wastewater concentration on the germination percentage of SIRAJ279 and MR219. The results show that both rice varieties only experienced a decrease of germination percentage in shrimp culture and domestic wastewater when irrigated with 70% to 100% concentration. In contrast for cement factory wastewater, both varieties started to show a significant decrease in germination percentage when irrigated with 50% concentration. This may be due to the high alkaline solution in cement factory wastewater, which inhibits seed germination. Kopp et al. [32], reported that the optimum pH for seed germination is between pH 5.5-6.5. Sang et al. [33] also demonstrated that the highest germination of *A. artemisiifolia* was at pH 5.57, but the germination was inhibited as the pH increased.

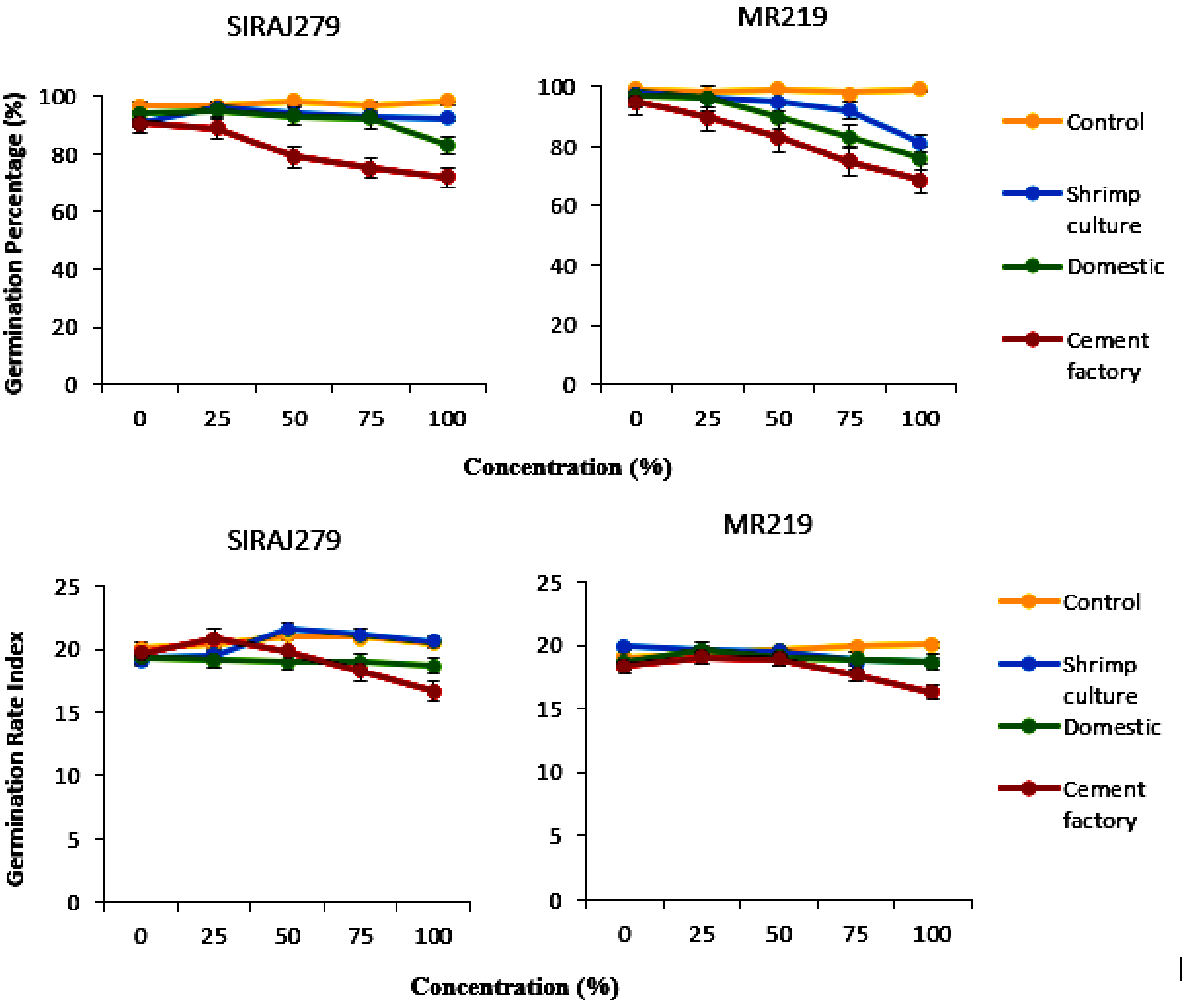

Fig. 1. Effect of different concentrations and types of wastewater on seed germination analysis of SIRAJ279 and MR219 (germination percentage and germination rate index.

Moreover, cement factory wastewater contains a high amount of heavy metals, which suppresses the germination process. For instance, it consists of slightly higher amounts of lead and zinc, which exceed the amount for agricultural recommendation use from the Malaysian Environment Quality Effluent Regulation [34] and the United States Environmental Protection Agency [35]. Sethy and Ghosh [36] mentioned high amount of lead closed with seed could inhibit seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, water and protein content. Zinc in cement factory wastewater is just slightly above the standard guideline, nonetheless, above the threshold limit of heavy metal is toxic and could result in inhibitory effects on the seed germination and plant growth [37,38].

However, SIRAJ279 irrigated with 100% concentration of shrimp culture wastewater showed no detrimental effect on germination percentage, possibly due to the low toxicity of this wastewater. Mung bean seeds supplied with Ratoon Plant Sorghum (*Sorghum bicolor* L.) wastewater demonstrated a greater germination percentage at any concentration level than the control [39].

Therefore, ions and nutrients in the wastewater are beneficial to initiate seed germination [40-41]. However, using 100% concentrations of high-toxicity wastewater concentration may cause a detrimental result due to ions imbalance during water uptake [42,43].

The rice variety SIRAJ279 shows a significantly greater germination percentage than MR219 when supplied with wastewater (**Fig. 2**). SIRAJ279 is the latest rice variety released by the Malaysian Agriculture Research and Development Institution (MARDI) in 2016, as compared to MR219 a commercial rice variety launched back in 2001 [44]. Genotype and environmental conditions are profound to affect rice seed germination, protein metabolic activities and crop growth [45]. According to Zakaria and Misman [46], SIRAJ279 is a new-release rice variety genetically designed to resist leaves blast, panicle blast, and rice tungro disease. Islam et al. [47] also stated that modern rice genotypes are more resistant and tolerant to germinating and growing in extreme conditions than traditional rice genotypes. Therefore, modern rice varieties such as SIRAJ279 are more robust, resistant and well-germinated when treated with wastewater compared with MR219.

Table 2. The result of seed germination analysis of SIRAJ279 and MR219 on different types of wastewaters.

	Control		Shrimp culture		Domestic wastewater		Cement factory wastewater	
	SIRAJ279	MR219	SIRAJ279	MR219	SIRAJ279	MR219	SIRAJ279	MR219
Germination percentage*	96.4% ± 1.04^a	94.6% ± 1.13^a	93.2% ± 1.61^a	85.3% ± 1.12^{ab}	91.4% ± 1.27^a	84.6% ± 1.21^{ab}	82.2% ± 1.38^b	75.9% ± 1.34^b
Germination rate index*	20.55 ± 1.16^a	19.61 ± 0.97^a	20.41 ± 0.85^a	19.55 ± 1.04^a	19.21 ± 0.82^a	19.4 ± 1.21^a	17.73 ± 1.25^b	17.57 ± 1.19^b

* indicates significant difference at $p<0.05$
** indicates highly significant difference at $p<0.01$
Means with the same letter are not significantly different from each other ($p>0.05$ ANOVA)
(±) represent standard error

Germination rate index

The germination rate index of SIRAJ279 and MR219 were significantly lower when exposed to cement factory wastewater (17.73 and 17.57, respectively) compared to shrimp culture (20.41 and 19.55, respectively) and domestic wastewater (19.21 and 19.04, respectively) (**Table 2**). This may be due to the high salt and heavy metal content in cement factory wastewater, which can affect the seed germination process. High salinity levels can disrupt the hormones responsible for seed germination, such as gibberellin (GA) and abscisic acid (ABA), leading to delayed germination and suppressed seed imbibition [48]. Khaleel et al. [49] also found that untreated factory wastewater resulted in slower germination rates of ladies' fingers (*Abelmoschus esculentus* L.) compared to treated wastewater.

The effects of wastewater concentration on the germination rate index show that both SIRAJ279 and MR219 demonstrated a steady germination rate index when exposed to shrimp culture and domestic wastewater at any concentration level (**Fig. 2**). The germination rate index of both rice varieties was also high when irrigated with lower concentrations of cement factory wastewater (0-50%). However, the seed germination rate index decreased at higher concentrations of cement factory wastewater.

Dash [50] observed that exposing seeds to lower concentrations of wastewater can enhance the seed germination rate index, while the result will be inversely proportional to higher wastewater concentration. Therefore, low concentrations of cement factory wastewater (25-50%) may not be a hazard to seed growth. However, higher wastewater concentrations can be toxic to seed growth [51]. The inhibitory effects at higher wastewater concentrations are caused by the heavy metals that are toxic to the seed during the imbibition stage [52,53].

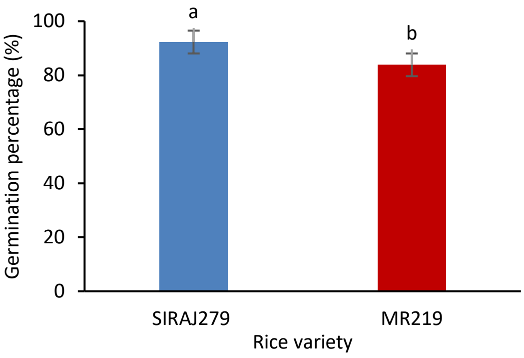

Fig. 2 Germination percentage of SIRAJ279 and MR219 irrigated with wastewater.

Early seedling growth

Seedling length

Table 3 demonstrates that SIRAJ279 and MR219 irrigated with shrimp culture and domestic wastewater showed a slightly higher seedling length compared to the control. Shrimp culture and domestic wastewater contain an adequate amount of nutrients to promote seedling growth. However, a significant decrease in seedling length was observed in cement factory wastewater, which is likely due to the high concentration of heavy metals such as cadmium, lead, and zinc, which are used during cement production. In addition to heavy metals, cement factory wastewater also has high salinity, which can negatively impact seedling growth. The study by Uzma et al. [54] reported similar results with industrial wastewater stimulating seed germination and seedling length of *Hibiscus esculentus* and *Lactuca sativa*. Conversely, Vibhuti et al. [55] reported that the seedling length of rice was significantly reduced under high salinity conditions.

Further examination of the study revealed in **Fig. 3** that both rice varieties demonstrated higher seedling length at concentrations 50-70% of shrimp culture and domestic wastewater but decreased at higher concentrations. However, cement factory wastewater only showed a high seedling length at a concentration of 25%, with a significant decrease at higher concentrations. This suggests that the toxicity threshold limit for shrimp culture and domestic wastewater is higher compared to cement factory wastewater. Cement factory wastewater contains a high amount of salt which could be the reason for low seedling length. Similarly reported by Mostafavi et al. [56] where seedling length of safflower was found to be distorted with high concentration of salt stress. Meanwhile, as suggested by Tymchuk et al. [26], wastewater that contains biogenic elements such as nitrogen, phosphorus, potassium, micro and macro-elements, and organic compounds can provide nutrients to promote plant growth.

Seedling vigor index

A high seedling vigor index of SIRAJ279 and MR19 when irrigated with shrimp culture and domestic wastewater was observed (**Table 3**). However, both seedlings show a significant reduction of seedling vigor index when treated with cement factory wastewater. This decline could be attributed to the high salt and heavy metal content in cement factory wastewater, which has been reported to negatively impact seed germination and seedling development. Ertekin et al. [57] have also reported similar findings in sorghum irrigated with high amounts of heavy metal.

Fig. 3 provides further insight into the relationship between the seedling vigor index with the concentration of wastewater. SIRAJ279 exhibits a high seedling vigor index at 50% concentration of shrimp culture and domestic wastewater at 1926.06 and 1765.14, respectively. Meanwhile, MR217 shows the highest seedling vigor index at 75% shrimp culture (1827.12), followed by 50% domestic wastewater (1663.20). In the case of cement factory wastewater, the highest seedling vigor index for both varieties were observed at 25% concentration but then dropped significantly at higher concentrations. Thus, a low concentration of cement factory wastewater is beneficial to seed growth, while a higher concentration could suppress the growing process. Therefore, studies suggest that nutrients in the wastewater can promote seed vigor, but too high in concentration.

Table 3. The result of early seedling analysis on different types of wastewaters.

	Control		Shrimp culture		Domestic wastewater		Cement factory wastewater	
	SIRAJ279	MR219	SIRAJ279	MR219	SIRAJ279	MR219	SIRAJ279	MR219
Seedling length (cm)*	16.7cm ± 0.22^{ab}	16.5cm ± 0.32^{ab}	18.5cm ± 0.92^{a}	17.5cm ± 0.67^{a}	17.8cm ± 0.52^{a}	17.4cm ± 0.67^{a}	15.9cm ± 0.34^{b}	15.6cm ± 0.60^{b}
Seedling vigor index**	1625.8 ± 0.91^{a}	1630.9 ± 1.21^{a}	1719.4 ± 1.10^{a}	1630.4 ± 0.97^{a}	1610.2 ± 0.71^{a}	1538.4 ± 1.32^{a}	1319.0 ± 0.35^{b}	1232.6 ± 0.96^{b}
Fresh weight (g)*	0.77g ± 0.67^{b}	0.76g ± 1.68^{ab}	0.88g ±1.23^{a}	0.84g ±1.45^{a}	0.85g ±1.38^{a}	0.87g ±0.91^{a}	0.82g ±1.25^{a}	0.71g ±1.36^{b}
Dry weight (g)^{ns}	0.41g ± 0.06^{a}	0.41g ±0.07^{a}	0.45g ±0.09^{a}	0.45g ±0.05^{a}	0.43g ±0.04^{a}	0.43g ±0.06^{a}	0.40g ±0.08^{a}	0.39g ±0.06^{a}
Root:shoot ratio*	0.465 ± 0.12^{b}	0.390 ±0.98^{b}	0.565 ± 0.02^{ab}	0.482 ±0.07^{b}	0.571 ± 0.26^{ab}	0.478 ±0.19^{b}	0.601 ±0.09^{a}	0.604 ±0.13^{a}
Phytotoxicity percentage (%)**	3.68% ± 0.67^{c}	3.73% ±1.05^{c}	12.79% ± 2.18^{b}	13.22% ±1.92^{b}	14.64% ±2.67^{b}	15.58% ±2.13^{b}	17.64% ±3.06^{a}	19.91% ±2.98^{a}

ns indicates no significant difference
* indicates significant difference at $p<0.05$
** indicates highly significant difference at $p<0.01$
Means with the same letter in the same row are not significantly different from each other ($p>0.05$ ANOVA)
(±) represent standard error

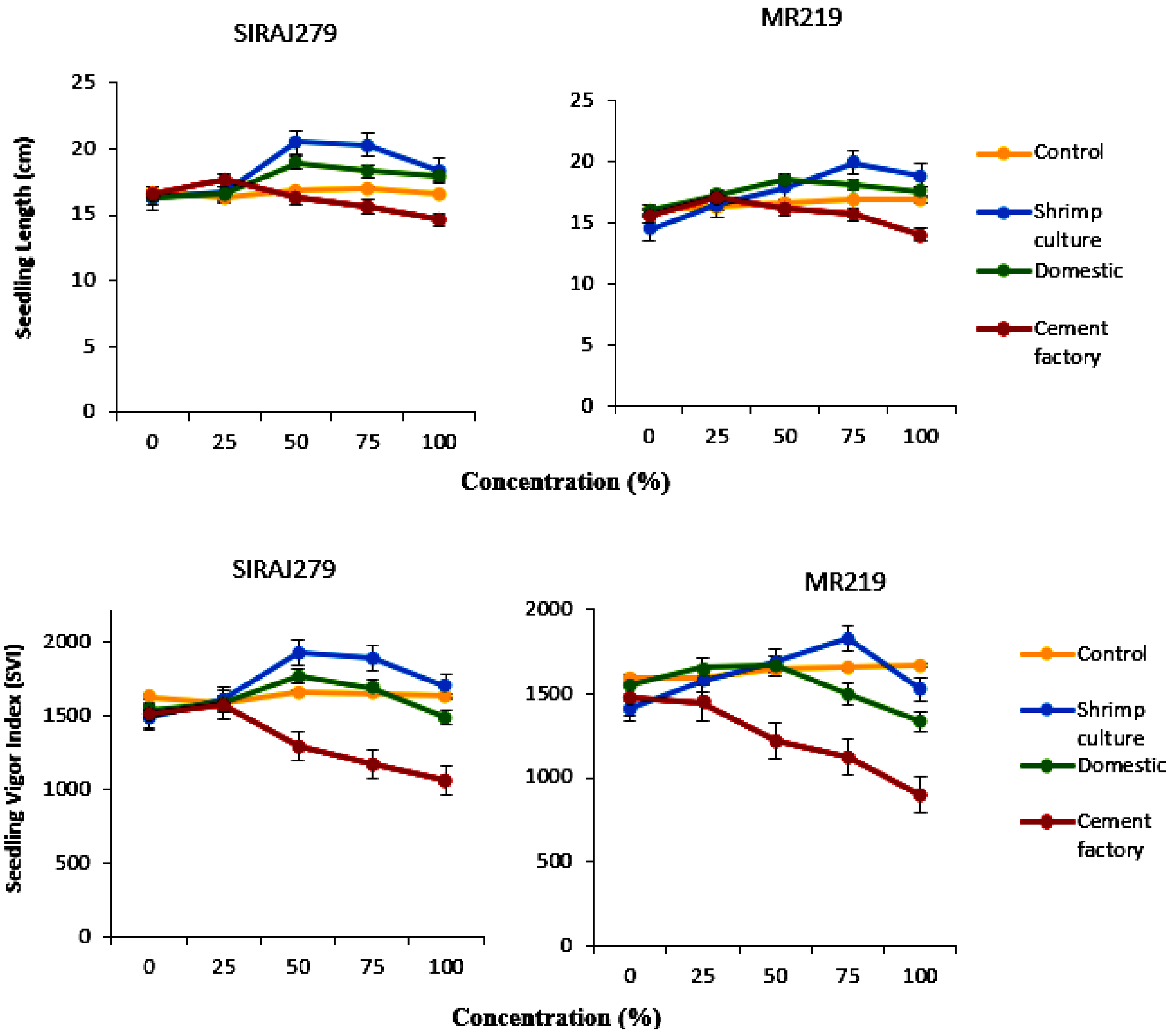

Fig. 3. Effect of different concentrations and types of wastewaters on early seedling growth analysis of SIRAJ279 and MR219 measured as seedling length (top) and seedling vigor index (bottom).

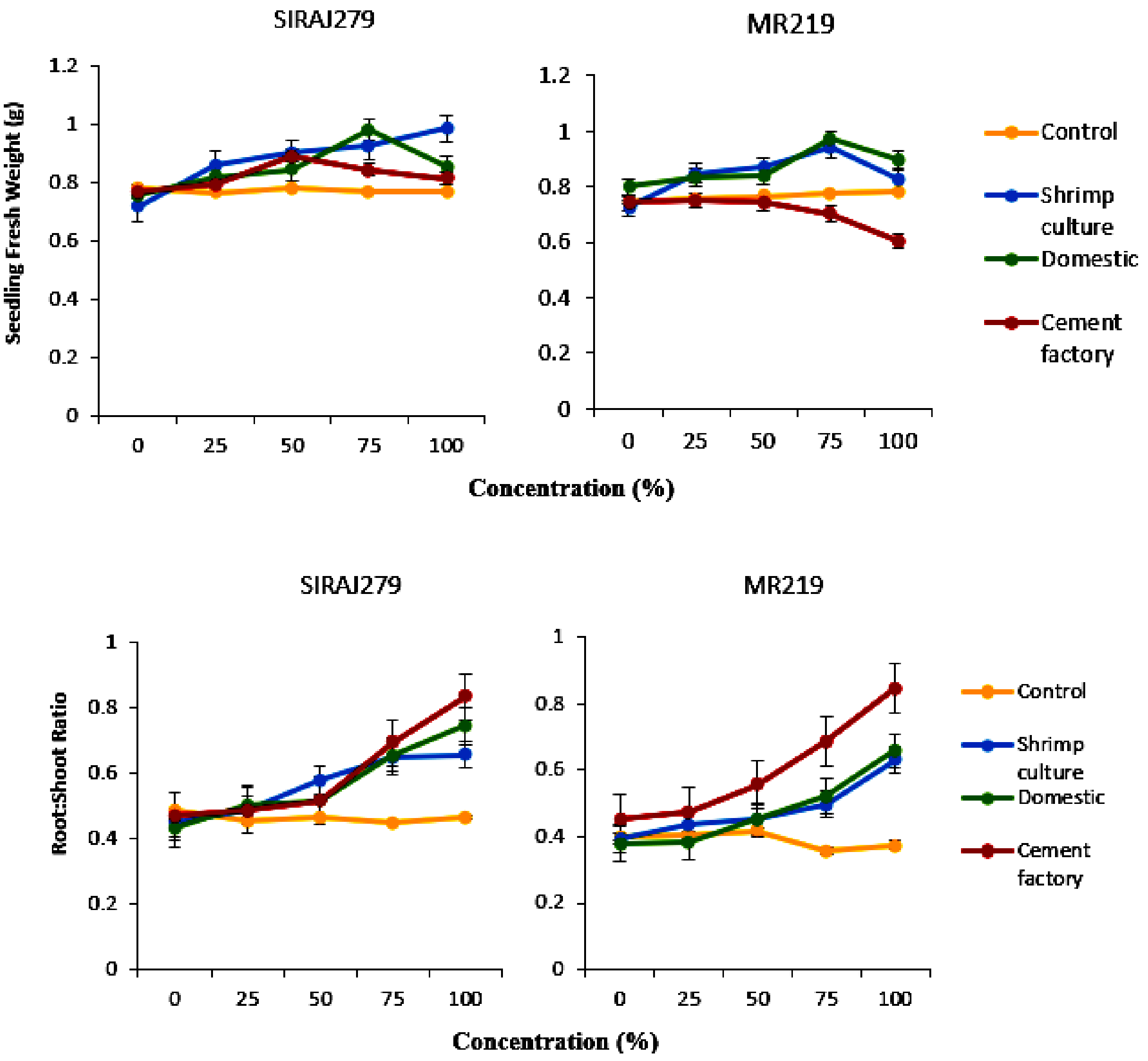

Fig. 4. Effect of different concentrations and types of wastewaters on early seedling growth analysis of SIRAJ279 and MR219 measured as seedling fresh weigh (top) and seedling vigor index (bottom).

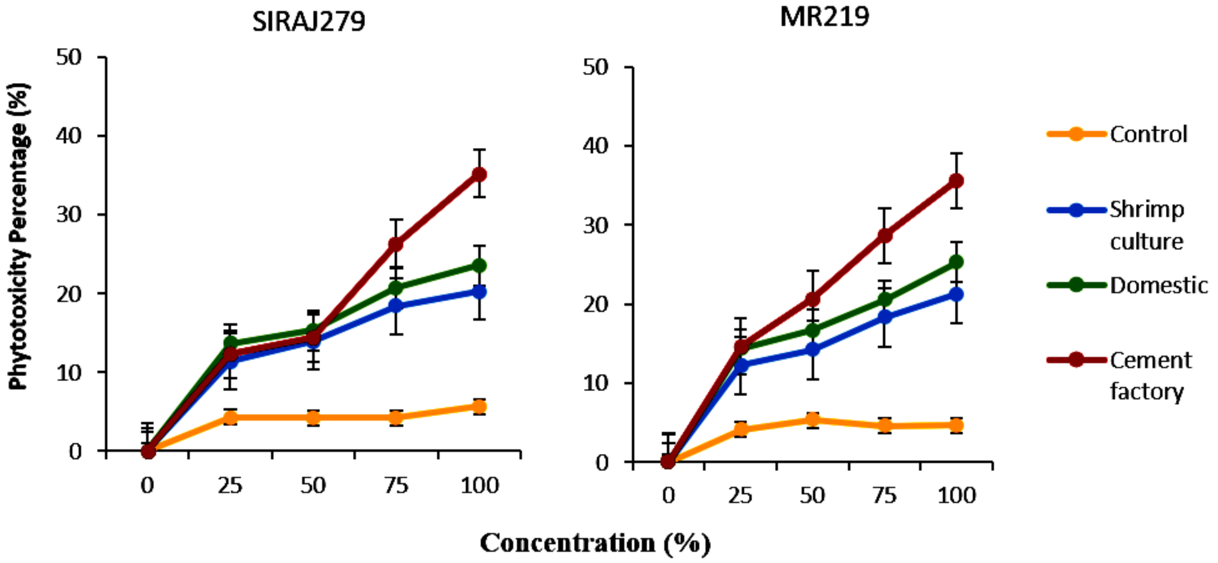

Fig. 5. Effect of different concentrations and types of wastewaters on early seedling growth analysis of SIRAJ279 and MR219 measured as phytotoxicity percentage.

may cause an adverse effect. A similar result was reported by Sinha and Paul [58] where the vigor index of *Cicer arietinum* was found to be maximum at 20% sewage concentration but reduced on further concentration. Monjunatha [59] mentioned that wastewater toxicity becomes lesser when it is diluted at certain levels and the optimum absorption of substantial nutrients occurs. According to Nawaz et al. [60], certain physical, chemical, and biological properties of wastewater up to an adequate level are beneficial for seedling growth but become toxic at an excessive level.

Seedling fresh weight

SIRAJ279 demonstrates a significantly higher seedling fresh weight with shrimp culture, domestic wastewater, and cement factory wastewater than control (**Table 3**). While MR219 displays a high seedling fresh weight on shrimp culture and domestic wastewater but reduces significantly on cement factory wastewater and control. The results suggest that low salinity and sufficient nutrients in shrimp culture and domestic wastewater contribute to improved seedling growth and increased seedling weight. The presence of an adequate amount of N, P, K, and organic matter in wastewater promotes radical and hypocotyl growth, thus facilitating more water absorption and retention [61]. Conversely, wastewater with intense levels of heavy metals and salt can retard cell size, decrease intercellular space, and reduce water absorption [62].

Further examination of seedling fresh weight with wastewater concentrations is illustrated in **Fig. 4**. SIRAJ279 exhibits a promoting fresh weight effect (0.986g) when irrigated with 100% concentration of shrimp culture wastewater. When irrigated with domestic wastewater, both SIRAJ279 and MR219 display an increase in seedling fresh weight as the concentration increases until it reaches 75%, but then drops at 100% concentration. Meanwhile, SIRAJ279 and MR219 show a gradual decrease in seedling fresh weight with the rising concentration of cement factory wastewater. Similarly, Hasnain et al. [63] noted a reduction in the fresh weight of *Acacia nilotica* with increasing salinity in wastewater.

Seedling dry weight

The study shows no significant differences in rice seedling dry weight with different types and concentrations of wastewater (**Table 3**). Previous studies have reported positive effects on seedling growth and total biomass when irrigated with wastewater, which can be attributed to the organic matter and nutrients present [64,65]. Tavares et al. [66] also reported an increase in the total biomass of sweet pepper due to the nutritional source of treated sewage wastewater. However, some studies have reported the negative impacts of wastewater on seedling dry weight. For instance, the dry shoot weight of basmati rice was highest at 75% municipal wastewater concentration but decreased significantly at 100% concentration [67]. Yet, no significant differences were observed in this analysis indicating that these wastewaters may be detrimental to the fresh weight due to osmotic pressure and toxicity but do not affect the overall seedling growth and total biomass.

Root:shoot ratio

Both rice varieties irrigated with cement factory wastewater showed a significantly higher root: shoot ratio compared to other treatments (**Table 3**). SIRAJ279 showed a slight reduction in root: shoot ratio when supplied with shrimp culture and domestic wastewater and then dropped significantly on the control. Meanwhile, MR217 demonstrated a significant decline in root: shoot ratio when irrigated with shrimp culture, domestic wastewater, and control. Similar findings were reported by Mašková and Herben [68], where the root: shoot ratio was observed to increase under nutrient-poor conditions. According to Gastal et al. [69], the increment of root: shoot ratio is a response to nitrogen (N) deficiency. When sufficient N minerals are present in the soil, plants tend to invest in shoot growth to increase photosynthesis and food production.

In contrast, when N is limited, plants will allocate more resources to root elongation to search for nutrients [70]. Therefore, a higher root: shoot ratio in cement factory wastewater was identified for seedling to search for nutrient patches. A consistent root: shoot ratio on the control plot was observed in this study (**Fig. 4**). As the concentration of shrimp culture and domestic wastewater increased, the root: shoot ratio gradually increased. However, both SIRAJ279 and MR219 showed a significant rise in root: shoot ratio when irrigated with more than 50% concentration of cement factory wastewater. This finding may be attributed to the low nutrient content of the wastewater, which forces the roots to elongate in search of available nutrients and water. According to Kang and Van Iersel [71], the root: shoot ratio is inversely proportional to the nutrient or fertilizer supply, with a higher ratio under a low nutrient supply and a lower ratio under a high nutrient supply.

Phytotoxicity percentage

SIRAJ279 and MR219 experience a higher phytotoxicity percentage when irrigated with cement factory wastewater at 17.64% and 19.91%, respectively, compared to when irrigated with shrimp culture and domestic wastewater. The control plot showed the lowest phytotoxicity percentage for both rice varieties (**Table 3**). Both rice varieties experience an increase in phytotoxicity as the concentration of wastewater increases (**Fig. 5**). The phytotoxicity percentages of SIRAJ279 and MR219 irrigated with 100% shrimp culture wastewater were 22.31% and 21.32%, respectively, while the phytotoxicity percentages of 100% domestic wastewater were 23.54% and 25.32%, respectively. However, when irrigated with 100% cement factory wastewater, SIRAJ279 and MR219 exhibited phytotoxicity percentages of 36.42% and 37.52%, respectively. This suggests that the toxicity level in cement factory wastewater is much higher than that of shrimp culture and domestic wastewater. The study also found that the 50% concentration of cement factory wastewater already exhibits more than 30% phytotoxicity, which exceeds the recommended threshold limit for phytotoxicity percentage [72].

Various proofs can be seen in this experiment to demonstrate the impact of phytotoxicity percentages on the seed and seedling growth, including delay in seed emergence and germination rate [73,74]. Given that a 50% concentration of cement factory wastewater exhibited the highest phytotoxicity than other wastewaters, the effects of its phytotoxicity are further explained in **Fig. 6.** For instance, as the concentration of cement factory wastewater increases to 50%, the rice germination rate shows a slight reduction.

Besides, root proportion can also investigate the phytotoxicity effects [73,75]. **Fig. 6** also illustrates that the root: shoot ratio gradually rose as the phytotoxicity of cement factory wastewater increased. While most literature explains that the amount of heavy metal in wastewater will cause stunted root growth [76,77], this study shows the opposite. Increased root: shoot ratio in cement factory wastewater demonstrates a resistance mechanism for the seedling to grow. A non-mycorrhizal *Pinus sylvestris* seedling demonstrated a higher investment in root growth than shoot growth when exposed to heavy metals [78]. Muqadas *et al.* [79] also reported that a higher root: shoot ratio on a particular genotype of maize was indicated as the seedling tolerance for salt and heavy metal stress conditions.

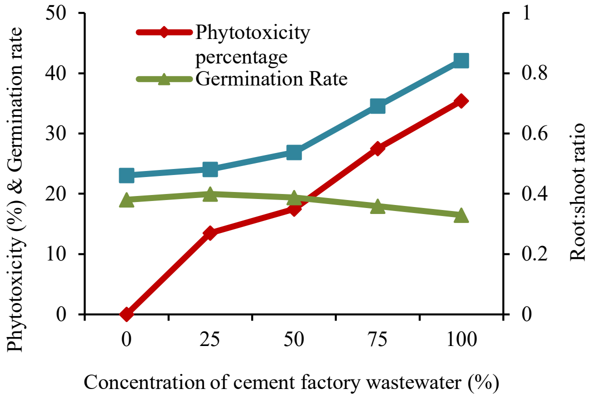

Fig. 6. Effect of Phytotoxicity percentage of cement factory wastewater on rice seed germination and root:shoot ratio.

CONCLUSION

The findings of this study suggest that wastewater can be classified into two categories: suitable and unsuitable for crop irrigation. Shrimp culture and domestic wastewater in this experiment are suitable wastewater for plant irrigation, as they contain an adequate amount of nutrients and low levels of toxicity. The seed germination and seedling growth are not significantly affected by the concentration of these wastewaters; hence they can be utilized in pure or diluted form. Moreover, these types of wastewater exhibited a positive impact on rice growth. In contrast, cement factory wastewater is unsuitable for plant irrigation due to its high toxicity, including pH, salinity, and heavy metals content. Seeds irrigated with cement factory wastewater showed inhibitory effects on seed germination and seedling growth, and high concentrations of this wastewater resulted in a higher phytotoxicity percentage. However, diluted cement factory wastewater presented a promising result, suggesting that it could be used for plant irrigation after dilution to a 25-50% concentration. Overall, these findings suggest that proper management and treatment of wastewater could provide a valuable resource for crop irrigation while minimizing potential environmental impacts.

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REFERENCES

1. Scocca G. Strengthening International Water Security: The European Union's proposal. World Water Policy. 2019;5:192-206.
2. Flörke M, Schneider C, McDonald RI. Water competition between cities and agriculture driven by climate change and urban growth. Nat Sustain. 2018;1:51-58.
3. Bao C, He D. Scenario Modeling of Urbanization Development and Water Scarcity Based on System Dynamics: A Case Study of Beijing-Tianjin-Hebei Urban Agglomeration, China. Int J Environ Res Public Health. 2019;16:3834.
4. Fukagawa NK, Ziska LH. Rice: Importance for Global Nutrition. J Nutr Sci Vitaminol. 2019;65:2-3.
5. Takeda N, López-Galvis L, Pineda D, Castilla A, Takahashi T, Fukuda S, Okada K. Evaluation of water dynamics of contour-levee irrigation system in sloped rice fields in Colombia. Agric Water Manag. 2019;217:107-118.
6. Jeong H, Kim H, Jang T. Irrigation water quality standards for indirect wastewater reuse in agriculture: a contribution toward sustainable wastewater reuse in South Korea. Water. 2016;8:169.
7. Sagara J. Surface Water Resources Assessment of the Tonle Sap and Mekong Delta River Basin Groups: Improving Climate Resilience, Productivity, and Sustainabilit. Adb Briefs. 2021;171.
8. Marcińczyk M, Ok YS, Oleszczuk P. From waste to fertilizer: Nutrient recovery from wastewater by pristine and engineered biochars. Chemosphere. 2022;306:135310.
9. Chávez A, Rodas K, Prado B, Thompson R, Jiménez B. An evaluation of the effects of changing wastewater irrigation regime for the production of alfalfa (Medicago sativa). Agric Water Manag. 2012;113:76-84.
10. Poustie A, Yang Y, Verburg P, Pagilla K, Hanigan D. Reclaimed wastewater as a viable water source for agricultural irrigation: A review of food crop growth inhibition and promotion in the context of environmental change. Sci Total Environ. 2020;739:139756.
11. Phung LD, Pham DV, Sasaki Y, Masuda S, Takakai F, Kaku N, Watanabe T. Continuous sub-irrigation with treated municipal wastewater for protein-rich rice production with reduced emissions of CH4 and N2O. Sci Rep. 2020;10:1-10.
12. Kausar S, Faizan, S, Haneef I. Effect of wastewater irrigation on heavy metal accumulation, growth and yield of vegetables. Int J Plant Env. 2017;3:65-76.
13. Carlos FS, dos Santos BL, Andreazza R, Tedesco MJ, Morris L, de Oliveira Camargo FA. Irrigation of paddy soil with industrial landfill leachate: impacts in rice productivity, plant nutrition, and chemical characteristics of soil. Paddy Water Environ. 2017;15:133–144.
14. Ofori S, Puškáčová A, Růžičková I, Wanner J. Treated wastewater reuse for irrigation: Pros and cons. Sci Total Environ. 2021;760:144026.
15. Khalid S, Shahid M, Natasha, Bibi I, Sarwar T, Shah AH, Niazi NK. A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. Int J Environ Res Public Health. 2018;15:895.
16. Gulzar S, Gul S, Patra A, Mohapatra KK, Jatav HS, Rajput VD. Socio Economic Aspects of Sewage Sludge Use in Agriculture. Sustainable Management and Utilization of Sewage Sludge. 2022;281-296.
17. Hashem MS, Qi X. Treated wastewater irrigation—A review. Water. 2021;13:1527.
18. APHA. Standard Methods for the Examination of Water and Wastewater. 17th ed. American Public Health Association; 1998.
19. ISTA. International Rules for Seed Testing. Seed Sci Technol Proc Int Seed Test Ass. 1999;31:1-152.
20. Czabator FJ. Germination Value: An Index Combining Speed and Completeness of Pine Seed Germination. For Sci. 1962;8:386-396.
21. Abdul-Baki AA, Anderson JD. Vigor Determination in Soybean by Multiple Criteria. Crop Sci. 1973;13:630-633.
22. Nedeva D, Nikolova A. Fresh and dry weight changes and germination capacity of natural or premature desiccated developing wheat seeds. Bulg J Plant Physiol. 1999;25:3–15.
23. Chou CH, Lin HJ. Autointoxication Mechanism of Oryza sativa I. Phytotoxic Effects of Decomposing Rice Residues in Soil. J Chem Ecol. 1976;2:353–367.
24. Su N, Miao B, Liu F. Effect of wash water and underground water on properties of concrete. Cement Concrete Res. 2012;32:777-782.
25. Aga TA, Anyadike CC, Mbajiorgu CC, Ogwo V. Assessment of the effect of cement industry effluent discharge on water quality of Ngo River in Benue, Nigeria. Niger J Technol. 2020;39:918-924.
26. Tymchuk I, Shkvirko O, Sakalova H, Malovanyy M, Dabizhuk T, Shevchuk O, Matviichuk O, Vasylinych T. Wastewater a source of nutrients for crop growth and development. Ecol Eng. 2020;21:88-96.

27. Mekki A, Dhouib A, Sayadi S. Effects of olive mill wastewater application on soil properties and plants growth. Int Recycl Org Waste Agric. 2013;2:15.
28. Hu HY, Zhang L, Wang Y. Crop development based assessment framework for guiding the conjunctive use of fresh water and sewage water for cropping practice—A case study. Agric Water Manag. 2016;169:98-105.
29. Thapliyal A, Vasudevan P, Dastidar MG, Tandon M, Mishra S. Effects of irrigation with domestic wastewater on productivity of green chili and soil status. Commun Soil Sci Plant Anal. 2013;44:2327-2343.
30. Qadir M, Wichelns D, Raschid-Sally L, McCornick PG, Drechsel P, Bahri A, Minhas PS. The challenges of wastewater irrigation in developing countries. Agric Water Manag. 2010;97:561-568.
31. Xu J, Wu L, Chang AC, Zhang Y. Impact of long-term reclaimed wastewater irrigation on agricultural soils: a preliminary assessment. J Hazard Mater. 2010;183:780-786.
32. Kopp MM, Passos LP, da Silva Verneue R, da Silva Lédo FJ, Coimbra JLM, de Oliveira AC. Effects of nutrient solution pH on growth parameters of alfalfa (Medicago sativa L.) genotypes. Comun Sci. 2011;2:135-141.
33. Sang W, Liu X, Axmacher JC. Germination and emergence of Ambrosia artemisiifolia L. under changing environmental conditions in China. Plant Species Biol. 2011;26:125-133.
34. Malaysian Environmental Quality Effluent Regulation. Acceptable Condition for Discharge of Industrial Effluent or Mixed Effluent on Agricultural Usage. Environmental Quality Act. 2009.
35. United States Environmental Protection Agency (EPA). Guidelines for water reuse. 2012.
36. Sethy SK, Ghosh S. Effect of heavy metals on germination of seeds. J Nat Sci Biol Med. 2013;4:272-275.
37. Duan Y, Li Q, Zhang L, Huang Z, Zhao Z, Zhao H, Du J, Zhou J. Toxic Metals in a Paddy Field System: A Review. Toxics. 2022;10:249.
38. Sharma P, Bhardwaj R, Arora N, Arora HK. Effect of 28-homobrassinolide on growth, zinc metal uptake and antioxidative enzyme activities in Brassica juncea L. seedlings. Braz J Plant Physiol. 2007;19:203-210.
39. Susilo E, Pujiwati H. Potential Waste Water Extract from Ratoon Plant Sorghum (Sorghum bicolor L.) Produced in Swamp Land as Bioherbicide. Am J Multidiscip Res Dev. 2022;4:12-17.
40. Marcińczyk M, Ok YS, Oleszczuk P. From waste to fertilizer: Nutrient recovery from wastewater by pristine and engineered biochars. Chemosphere. 2022;306:135310.
41. Priya E, Kumar S, Verma C, Sarkar S, Maji PK. A comprehensive review on technological advances of adsorption for removing nitrate and phosphate from waste water. J Water Process Eng. 2022;49:103159.
42. Ofori S, Puškáčová A, Růžičková I, Wanner J. Treated wastewater reuse for irrigation: Pros and cons. Sci Total Environ. 2021;760:144026.
43. Ankush D, Prakash R, Kumar A, Singh V, Kumari S. Influence of sewage sludge and saline water irrigation on soil soluble ions and nutrient uptake under Pearl millet-Wheat cropping system in semi-arid region. Indian J Tradit Knowl. 2022;21:450-457.
44. Omar SC, Shaharudin A, Tumin SA. The status of the paddy and rice industry in Malaysia. Khazanah Research Institute Kuala Lumpur. 2019.
45. Mahender A, Anandan A, Pradhan SK. Early seedling vigour, an imperative trait for direct-seeded rice: an overview on physio-morphological parameters and molecular markers. Planta. 2015;241:1027-1050.
46. Zakaria L, Misman N. The pathogen and control management of rice blast disease. Malaysian J Microbiol. 2018;14:705-714.
47. Islam MM, Kayesh E, Zaman E, Urmi TA, Haque MM. Evaluation of rice (Oryza sativa L.) genotypes for drought tolerance at germination and early seedling stage. The Agriculturists. 2018;16:44-54.
48. Uçarlı C. Effects of salinity on seed germination and early seedling stage. Abiotic Stress in Plants. IntechOpen. 2020;211-220.
49. Khaleel RI, Ismail N, Ibrahim MH. The impact of waste water treatments on seed germination and biochemical parameter of Abelmoschus esculentus L. Procedia Soc Behav Sci. 2013;91:453-460.
50. Dash AK. Impact of domestic waste water on seed germination and physiological parameters of rice and wheat. IJRRAS. 2012;12:280-286.
51. Petrovič A, Zirngast K, Predikaka TC, Simonič M, Čuček L. The advantages of co-digestion of vegetable oil industry by-products and sewage sludge: Biogas production potential, kinetic analysis and digestate valorisation. J Environ Manage. 2022;318:115566.
52. Medhi UJ, Talukdar AK, Deka S. Impact of paper mill effluent on growth and development of certain agricultural crops. J Environ Biol. 2011;32:185-188.
53. Chen ZF, Zhao Y, Zhu Y, Yang X, Qiao J, Tian Q, Zhang Q. Health risks of heavy metals in sewage-irrigated soils and edible seeds in Langfang of Hebei province, China. J Sci Food Agric. 2010;90:314-320.
54. Uzma S, Azizullah A, Bibi R, Nabeela F, Muhammad U, Ali I, Häder DP. Effects of industrial wastewater on growth and biomass production in commonly grown vegetables. Environ Monit Assess. 2016;188:328.
55. Vibhuti, Shahi C, Bargali K, Bargali SS. Seed germination and seedling growth parameters of rice (Oryza sativa L.) varieties as affected by salt and water stress. Indian J Agric Sci. 2015;85:102-108.
56. Mostafavi K. An evaluation of safflower genotypes (Carthamus tinctorius L.), seed germination and seedling characters in salt stress conditions. Afr J Agric Res. 2011;6:1667-1672.
57. Ertekin EN, Ertekin İ, Bilgen M. Effects of some heavy metals on germination and seedling growth of sorghum. J Agric Nat. 2020;23:1608-1615.
58. Sinha SN, Paul D. Impact of sewage water on seed germination and vigour index of Cicer arietinum L. and Pisum sativum L. Int J Food Agric Vet Sci. 2013;3:19-26.
59. Monjunatha N. Effect of industrial effluents on seed quality attributes of cereal crops. Master Dissertation, Department of Seed Science and Technology, College of Agriculture, University of Agricultural Science, Dharwad, India. 2008.
60. Nawaz S, Ali SM, Yasmin A. Effect of industrial effluents on seed germination and early growth of Cicer arientum. J Biosci. 2006;6:49-54.
61. Gassama UM, Puteh AB, Abd-Halim MR, Kargbo B. Influence of municipal wastewater on rice seed germination, seedling performance, nutrient uptake, and chlorophyll content. J Crop Sci Biotechnol. 2015;18:9-19.
62. Baskaran L, Sundaramoorthy P, Chidambaram ALA, Ganesh KS. Growth and physiological activity of greengram (Vigna radiata L.) under effluent stress. Bot Res Int. 2009;2:107-114.
63. Hasnain F, Nadia B, Javaid I, Wasif N. Effect of salinity and municipal wastewater on growth performance and nutrient composition of Acacia nilotica. Int J Agric Biol. 2010;12:591-596.
64. Samreen S, Khan AA, Khan MR, Ansari SA, Khan A. Assessment of phytoremediation potential of seven weed plants growing in chromium-and nickel-contaminated soil. Water Air Soil Pollut. 2021;232:1-18.
65. Moradi S, Heidari H, Saeidi M, Nosratti I. Effect of sewage-contaminated water on seed production, heavy metals accumulation and seedling emergence in oat. Glob NEST J. 2016;18:329-338.
66. Tavares FB, da Silva ACR, Fernandes CDS, Moura KDF, Travassos KD. Growth and yield of bell pepper using treated sewage water. Rev Bras Agric Irrig. 2019;13:3683-3690.
67. Akhtar N, Khan S, Naveen S, Masood S, Khattak MR, Malook I, Jamil M. Effect of wastewater on physiological and biochemical characteristics of rice (Oryza sativa L.). Interciencia. 2018;43:102-123.
68. Mašková T, Herben T. Root: shoot ratio in developing seedlings: How seedlings change their allocation in response to seed mass and ambient nutrient supply. Ecol Evol. 2018;8:7143-7150.
69. Gastal F, Lemaire G, Durand JL, Louarn G. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. Crop Physiol. 2015;8:61-206.
70. Zhang H, Forde BG. Regulation of Arabidopsis root development by nitrate availability. J Exp Bot. 2000;51:51-59.
71. Kang JG, Van Iersel MW. Nutrient solution concentration affects shoot: root ratio, leaf area ratio, and growth of subirrigated salvia (Salvia splendens). HortScience. 2004;39:49-54.
72. Zia-ur-Rehman M, Sabir M, Rizwan M, Saifullah, Ahmed HR, Nadeem M. Remediating cadmium-contaminated soils by growing

grain crops using inorganic amendments. Soil Remediat Plants. 2015;13:367-396.

73. Mendes PM, Ribeir JA, Martins GA, Lucia Jr T, Araujo TR, Fuentes-Guevara MD, Corrêa LB, Corrêa ÉK. Phytotoxicity test in check: Proposition of methodology for comparison of different method adaptations usually used worldwide. J Environ Manage. 2021;291:112698.
74. Priac A, Badot PM, Crini G. Treated wastewater phytotoxicity assessment using Lactuca sativa: focus on germination and root elongation test parameters. C R Biol. 2017;340:188-194.
75. Charles J, Sancey B, Morin-Crini N, Badot PM, Degiorgi F, Trunfio G, Crini G. Evaluation of the phytotoxicity of polycontaminated industrial effluents using the lettuce plant (Lactuca sativa) as a bioindicator. Ecotoxicol Environ Saf. 2011;74:2057-2064.
76. He J, Ren Y, Pan X, Yan Y, Zhu C, Jiang D. Salicylic acid alleviates the toxicity effect of cadmium on germination, seedling growth, and amylase activity of rice. J Plant Nutr Soil Sci. 2010;173:300-305.
77. Liu D, Jiang W, Gao X. Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. Biol Plant. 2003;47:79-83.
78. Ahonen-Jonnarth U, Finlay RD. Effects of elevated nickel and cadmium concentrations on growth and nutrient uptake of mycorrhizal and non-mycorrhizal Pinus sylvestris seedlings. Plant Soil. 2001;236:129-138.
79. Muqadas S, Ali Q, Malik A. Genetic association among seedling traits of Zea mays under multiple stresses of salts, heavy metals and drought. Biol Clin Sci Res J. 2020.