

# Rice production with minimal irrigation and no nitrogen fertilizer by intensive use of treated municipal wastewater

Ayumi Muramatsu, Toru Watanabe, Atsushi Sasaki, Hiroaki Ito  
and Akihiko Kajihara

## ABSTRACT

We designed a new cultivation system of rice with circulated irrigation to remove nitrogen from treated municipal wastewater effectively and assessed the possibility of nitrogen removal in the new system without any adverse effects on rice production through bench-scale experiments through two seasons. Overgrowth of the rice plant, which can lead to lodging and tasteless rice, was found in the first season probably because nitrogen supply based on standard practice in normal paddy fields was too much in the closed irrigation system. In the second season, therefore, the amount of treated wastewater initially applied to the system was reduced but this resulted in a considerably decreased yield. On the other hand, the taste of the rice was significantly improved. The two-season experiments revealed that the new system enabled rice production with minimal irrigation (approximately 50% on the yield base compared to normal paddy fields) and no nitrogen fertilizer. The system also achieved >95% removal of nitrogen from the treated wastewater used for circulated irrigation. The accumulation of harmful metals in the rice was not observed after one season of cultivation in the new system. The accumulation after cultivation using the same soil repeatedly for a longer time should be examined by further studies.

**Key words** | circulated irrigation, municipal wastewater, rice production, water consumption, yield

### Ayumi Muramatsu

Department of Science of Bioproduction,  
Yamagata University,  
1-23 Wakaba-machi, Tsuruoka,  
Yamagata 997-8555,  
Japan

### Toru Watanabe (corresponding author) Hiroaki Ito

Akihiko Kajihara  
Department of Food, Life and Environmental  
Sciences,  
Yamagata University,  
1-23 Wakaba-machi, Tsuruoka,  
Yamagata 997-8555,  
Japan  
E-mail: [to-ru@tds1.tr.yamagata-u.ac.jp](mailto:to-ru@tds1.tr.yamagata-u.ac.jp)

### Atsushi Sasaki

Laboratory for Instrumental Analysis,  
Faculty of Engineering,  
Yamagata University,  
4-3-16 Jonan, Yonezawa,  
Yamagata 992-8510,  
Japan

## INTRODUCTION

Treated municipal wastewater is recognized as a relatively stable water source alternative to natural ones such as surface water and groundwater, and it is already reused for flushing toilets, cleaning streets and machines, water-related recreation, watering plants, melting snow and so on. Among these uses, a large volume of reused wastewater has been applied for agricultural irrigation since it usually contains rich nutrients which are beneficial for plant growth (Qadir *et al.* 2010; Norton-Brandao *et al.* 2013), although harmful substances such as heavy metals (Singh *et al.* 2010) and pathogens (Mok & Hamilton 2014) in reused wastewater may contaminate the foods produced and pose health risks to consumers. Paddy rice is one of the agricultural products which require considerable water and therefore reuse of treated wastewater is suitable for its cultivation (Tanaka & Okamoto 2011). Since rice plants grow while consuming nutrients in treated

wastewater used for irrigation, removal of nutrients from the wastewater is expected at the same time (Li *et al.* 2009; Jang *et al.* 2012). Also, nitrogen can be further removed by bacterial activities (i.e., nitrification and denitrification) in paddy soil.

Although reuse of untreated or treated wastewater for rice cultivation has been investigated (Chiou 2008; Papadopoulos *et al.* 2009; Yoon *et al.* 2001), we designed a new cultivation system with circulated irrigation to remove nitrogen from treated wastewater as effectively as possible. This study aims to assess the possibility of nitrogen removal from the treated wastewater in the new system, through bench-scale experiments for two farming seasons, without any adverse effects on rice production, in terms of plant growth, and yield, taste and safety of cultivated rice. In the safety assessment, although the concentrations in the treated wastewater were sufficiently lower than the threshold levels

for crop production in the FAO guidelines (FAO 1992), special attention was paid in this study to heavy metals since the circulated irrigation may enhance metal accumulation in rice. We expect that our new system will contribute to effective water and resource management through simultaneous reductions of irrigation water and chemical fertilizer applied to paddy fields.

## MATERIALS AND METHODS

### Experimental apparatus

Figure 1 illustrates the experimental apparatus for rice cultivation with circulated irrigation. The experimental apparatus consisted of a simulated paddy field with an area of 0.18 m<sup>2</sup> and a storage tank for irrigation water. The simulated paddy field had an underdrain at the bottom of a 15 cm soil layer, from which irrigation water infiltrating the soil layer was continuously drained (7 to 10 L/d). The drained water was stored in the storage tank and then continuously pumped up into the paddy field.

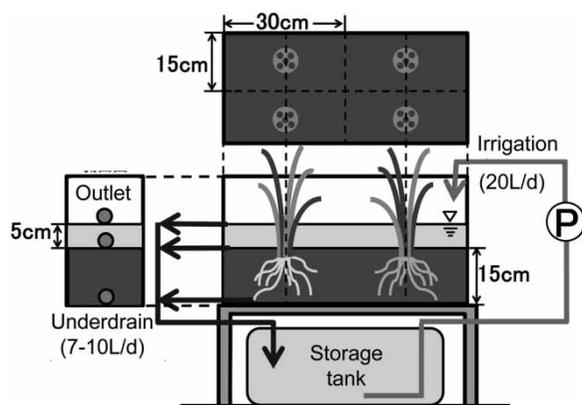


Figure 1 | Experimental apparatus for rice cultivation with circulated irrigation.

Table 1 | Runs in the rice cultivation experiment with circulated irrigation

Run	A	B	C	D	E	F
Irrigation of treated MWW*	Yes	Yes	No	Yes	Yes	Yes
Chlorination of treated MWW	Yes	Yes	–	Yes	No	No
Intermittence of irrigation	Yes	Yes	Yes	Yes	No	Yes
Rice planting	Yes	No	Yes	Yes	Yes	Yes
Nitrogen fertilizer (mg)	0	0	1080	0	0	0
Paddy soil	Dried	Dried	Dried	Undried	Undried	Undried

\*Municipal wastewater.

The flow rate of irrigation water was about 20 L/d. The apparatus was covered by a transparent plastic roof and sheet to avoid the effect of rainfall.

### Experimental conditions

Using the apparatus, we cultivated rice plants with circulated irrigation under six experimental conditions (Table 1). Runs A to C were for the experiment of rice cultivation in the first season from May to September in 2011. In Runs A and B, 30 L of treated wastewater taken from a municipal wastewater treatment plant, which contained an amount of nitrogen (1,080 mg) equivalent to that applied to paddy fields following the standard practice in this region, was used for irrigation together with the same volume of channel water taken at the University Farm, Yamagata University, Tsuruoka, Yamagata, Japan. Deficiencies of phosphorus (1,066 mg as P<sub>2</sub>O<sub>5</sub>) and potassium (725 mg as K<sub>2</sub>O) in the treated wastewater were compensated by chemical fertilizers. In Run C, only channel water was irrigated and all of the nitrogen, phosphorus (1,080 mg as P<sub>2</sub>O<sub>5</sub>) and potassium (1,080 mg as K<sub>2</sub>O) were supplied as chemical fertilizers. Soil for the experiment was taken from the surface of paddy fields in the farm where channel water was taken and applied to the apparatus after being air-dried.

Runs D to F were for the experiment in the second season. In the second season, the experimental conditions were modified since overgrowth of the rice plant and low score of its taste, probably due to excessive absorption of nitrogen (Hu-Lin *et al.* 2007; Nguyen *et al.* 2008) and a high temperature (Zheng-Xun *et al.* 2005), were found in the first season. First, soil for the experiment was not dried before being used, although it was taken from the same paddy field as in the first season. This was intended to reduce the supply of bioavailable nitrogen with mineralization of organic nitrogen in the soil, which is enhanced by drying the soil (Ando *et al.* 1992). Secondly, the initial

supplies of nitrogen at the beginning of the experiment was reduced to 810 mg. The initial supplies of phosphorus (as  $P_2O_5$ ) and potassium (as  $K_2O$ ) were also changed to 1,440 and 1,260 mg, respectively, according to another standard practice aiming at a high score of taste which was recommended by the local government. Moreover, to avoid too high a temperature around the simulated paddy field, the ventilation was improved. Except for these modifications and the initial volume of water (100 L), the experimental condition in Run D was the same as in Run A. In Runs E and F, treated wastewater before chlorine disinfection, which was taken from the same treatment plant as in the first season, was used for irrigation to examine the effect of chlorine disinfection on the rice production.

### Schedule of transplantation, cultivation and harvesting

In the first season, the young rice plant (*Oryza sativa* L cultivar Haenuki) was transplanted in Runs A and C on May 31, 2011. During the experiment, the level of surface water on the simulated paddy field was kept at 5 cm. Only in the period from July 5 to 10 was the pump-up of stored water intermittent and water remaining in the paddy field drained for drying up the paddy soil. This intermittency of irrigation, called midsummer drainage (MSD), is usually performed in this region to supply air to paddy soil. The supplied air enhances the nitrification of unused nitrogen in the soil and the transformed nitrate is easily removed by leaching and denitrification after the MSD. The air supply also contributes to the growth of roots by inhibiting  $H_2S$  production. We continued the experiment after the MSD, adding a total of 240 L of channel water to each run. On August 26, a small amount of irrigation water (up to 20 L) was lost in Runs A and C by an accidental leakage and finally the rice was harvested on October 12, 2011. At that time, only a few litres of water remained in the storage tanks.

In the second season, the same cultivar of rice as in the previous season was transplanted in Runs D to F on May 24, 2012. Similar to the first season, the water level was kept at 5 cm throughout the experiment except the MSD period (July 17 to 22). The MSD was not performed in Run E (Table 1) to examine its effect on the rice growth. Just after the MSD, 6.1 to 6.2 L of treated wastewater, which was taken again from the same treatment plant, was added to the storage tank in all runs since the growth of rice seemed insufficient. The date of harvesting rice was September 24, 2012. The final volume of water in the storage tanks was 67 to 85 L.

### Analyses of water, rice and soil

From the beginning of the experiment, water temperature, electrical conductivity (EC), pH and dissolved oxygen (DO) of irrigation water were routinely measured in the storage tank using their mobile meters (OM-51 and D-54, HORIBA, Japan). Total nitrogen (TN) concentration in the tank was also analysed with the TN measuring unit (TNM-1, SHIMADZU, Japan) attached to the total organic carbon analyser. In parallel, the growth of the rice plant was recorded by measuring its height, number of shoots and SPAD (soil plant analysis development) value indicating leaf chlorophyll concentration (Markwell *et al.* 1995).

After harvesting, yield and dry weight of rice were measured following the standard method. In order to examine the quality of the harvested rice, we analysed its taste and metal content. The taste was analysed with its analyser (TM-3500, SHIZUOKA SEIKI, Japan) in the first season. In the second season, on the other hand, the taste was estimated based on the protein content, a main component of taste, since we could not obtain a sufficient amount of rice for the above analyser. The protein content was converted from the nitrogen content in the rice plant analysed with the automatic high sensitivity NC analyser (SUMIGRAPH NC-220F, SCAS, Japan). The nitrogen contents in the other parts of the rice plant and the paddy soil after the experiment were analysed with the same NC analyser. The metals (Li, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Cd, In, Ba, Pb and Bi) in the harvested rice were measured with the inductively coupled plasma (ICP) spectrometer (iCAP 6000 SERIES, Thermo Scientific) after the treatment with a wet digestion method.

### Statistical analysis

We could not prepare replicates of the experiment in any of the runs. Instead, we cultivated four sets of rice plants in each run, as illustrated in Figure 1, and statistical analyses were conducted on the assumption that each set of rice plants grew independently from the other sets. Student's t-test was used for comparison of results from two runs, while Tukey's test was used for comparison among three or more runs.

## RESULTS AND DISCUSSION

### Growth of rice plants

The growth of rice plants, evaluated as the height, number of shoots, SPAD and dry mass, in Run A was similar to

that in Run C (Table 2), which indicates no significant effect of irrigation of treated wastewater on growth. However, in comparison with the target values for cultivar Haenuki recommended by the prefectural government, the rice plants cultivated in both runs were overgrown. The overgrowth is due to excessive absorption of nitrogen which was probably caused by circulated irrigation in the closed system. Since leaching of nitrogen, which is observed in normal paddy fields, never happened in the system, the amount of nitrogen applied to each run seemed too much. Another possible reason is the increased bioavailable nitrogen in the soil which was dried before being applied to the experiment, as described in 'Materials and methods'.

In the second season, we modified the experimental conditions, i.e. a reduction of the initial supply of nitrogen and improvement of ventilation, and obtained the results for the growth of the rice plants as shown in Runs D to F in Table 2. The effect of the initial supply of nitrogen was obvious in the number of shoots, which was only half of the target value in all three runs. The number of shoots increases with tillering in the early stage of cultivation. That is why the additional supply of nitrogen after the MSD was not effective. In this season, the tillering did not proceed properly, which resulted from shortage of the initial nitrogen supply, although the height was not affected and exceeded the target value. The height and dry mass of rice plant cultivated in Run E were higher than those in Runs D and F, indicating that the MSD surely contributed to inhibition of its growth. On the other hand, the comparison between Runs D and F revealed no adverse effect of chlorine disinfection of treated wastewater on plant growth.

## Yield and quality of harvested rice

Table 2 also shows the yield and taste of harvested rice. There was no significant difference in the yield among Runs A and C, indicating no significant effect of treated wastewater irrigation on rice production. As for the components of yield, the number of panicles per area and number of grains per panicle were higher than the target value for this cultivar, while ripening rate and kernel weight were lower. The overgrowth of rice plant contributed to the increase in the number of grains, resulting in the smaller size of the harvested rice. No significant difference in the taste of the rice was found between Runs A and C. The low score of taste is probably due to the high nitrogen content that resulted from excessive absorption of nitrogen as above mentioned.

Compared to Run A, the yield in Run D in the second season was considerably decreased and was only half of the target value. This low yield was commonly observed in Runs E and F. The yield per shoot (1.3 to 1.5 g) was comparable to that (1.5 g) in the previous season. This fact means that the low number of shoots, due to the shortage of nitrogen supply at the beginning, caused the low yield observed in the second season. On the other hand, the taste of the rice harvested satisfied the target value (<6.8% as protein content) in every run and it seemed to be successfully improved by reduction of the initial supply of nitrogen.

Among 19 species of metals analysed in the first season, the contents of Li and Na in brown rice in Run A were significantly higher than those in Run C (data not shown). The increased content of Na is understandable since the concentration of sodium ions in treated wastewater used for irrigation was more than 10 times higher than that in channel water. On the other hand, Li was not detected in either

**Table 2** | Growth of rice plant, yield and taste of harvested rice in each run (average  $\pm$  standard error,  $n = 4$ )

Run	A	C	D	E	F
Height (cm)*	82.4 $\pm$ 2.4 <sup>a</sup>	83.8 $\pm$ 3.3 <sup>a</sup>	69.3 $\pm$ 1.1 <sup>b</sup>	77.1 $\pm$ 2.4 <sup>a</sup>	68.8 $\pm$ 0.5 <sup>b</sup>
Number of shoots*	35.8 $\pm$ 1.3 <sup>a</sup>	36.8 $\pm$ 2.2 <sup>a</sup>	13.5 $\pm$ 1.1 <sup>b</sup>	12.5 $\pm$ 0.5 <sup>b</sup>	13.5 $\pm$ 1.1 <sup>b</sup>
SPAD*	46.9 $\pm$ 1.4 <sup>a</sup>	47.9 $\pm$ 2.3 <sup>a</sup>	41.4 $\pm$ 1.2 <sup>b</sup>	41.6 $\pm$ 0.4 <sup>b</sup>	41.6 $\pm$ 0.8 <sup>b</sup>
Dry mass (g/set)	88.9 $\pm$ 11.7 <sup>a</sup>	88.3 $\pm$ 9.0 <sup>a</sup>	22.9 $\pm$ 0.5 <sup>b</sup>	25.6 $\pm$ 2.7 <sup>b</sup>	22.6 $\pm$ 0.6 <sup>b</sup>
Yield (g/m <sup>2</sup> )**	727 $\pm$ 86 <sup>a</sup>	811 $\pm$ 141 <sup>a</sup>	278 $\pm$ 9 <sup>b</sup>	339 $\pm$ 27 <sup>c</sup>	283 $\pm$ 22 <sup>b,c</sup>
Score of taste (/100)	69.0 $\pm$ 1.7 <sup>a</sup>	66.5 $\pm$ 1.5 <sup>a</sup>	–	–	–
Protein (%)	–	–	5.8 $\pm$ 0.5 <sup>a</sup>	5.9 $\pm$ 0.1 <sup>a</sup>	5.7 $\pm$ 0.1 <sup>a</sup>

Targets in the 1st season: height < 70, shoots < 24, SPAD < 38, yield > 580 and score of taste > 80.

Targets in the 2nd season: height < 62, shoots < 27, SPAD < 39, yield > 560 and protein < 6.8.

\*Highest value in the cultivation period of each run.

\*\*Based on the weight of brown rice.

<sup>a,b,c</sup>Different letters indicate a statistically significant difference between runs ( $p < 0.05$ )

treated wastewater or channel water and therefore the reason for the increased Li content in Run A is not clear. The level of cadmium, a heavy metal to be monitored most carefully in rice, was substantially lower than the regulation (0.04 mg/kg) established for edible white rice by the Codex Alimentarius Commission, demonstrating that circulated irrigation of treated wastewater did not enhance the accumulation of harmful heavy metals in rice under the current experimental conditions. Heavy metals were not analysed in the second season because a smaller amount of treated wastewater was irrigated than in the first season.

### Removal of nitrogen from reused wastewater

The treated wastewater used for irrigation contained 1,080 mg of nitrogen at the beginning of the experiment in the first season. For several days after irrigation started, TN concentration in the irrigation water increased, probably due to desorption from paddy soil, and reached 18.2 mg/L on June 7 (Figure 2(a)). Then the TN concentration gradually decreased with growth of the rice plant and was 9.4 mg/L on July 7 during the MSD. At that time, all

irrigation water was returned to the tank. The irrigation water returned to the tank had 84.6 mg of nitrogen and we can estimate, taking into account irrigation water lost by sampling, that 92% of nitrogen in the reused wastewater was removed in the first month of the experiment. After the MSD, a maximum of 8.2 mg of nitrogen was lost due to an accidental leakage and only 0.9 mg remained unused at the end of experiment.

Run B was tested to investigate nitrogen removal without any effect of rice plants but a leakage of a considerable amount of irrigation water happened on June 16 and also a failure in drainage from the underdrain was found. Although we could not obtain analysable data in this run due to these problems, no decrease of TN concentration in the irrigation water was observed in the first month before the MSD.

Figure 2(b) shows the fluctuation of TN concentration in the storage tank in Run D. This was almost the same as in Runs E and F. The TN concentration gradually decreased from 6.6 to 7.0 mg/L at the beginning to 0.7 to 0.8 mg/L during the MSD. The concentration rose to 3.7 to 4.4 mg/L by the additional supply of treated wastewater and the

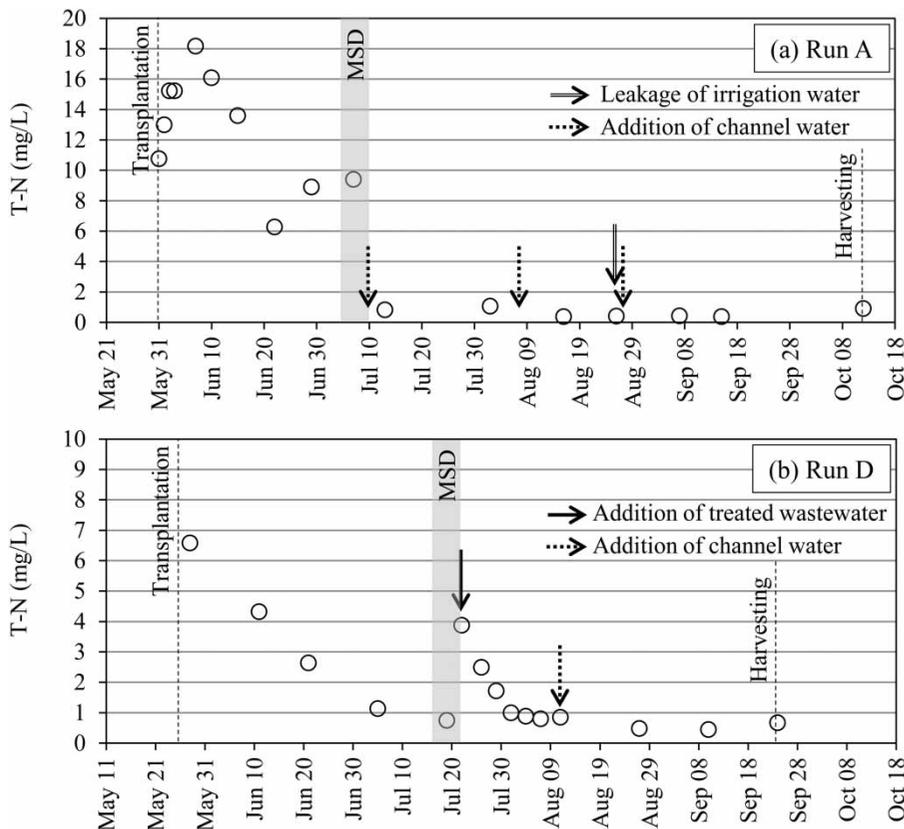


Figure 2 | TN concentration of irrigation water observed in the storage tank in Runs A and D.

final concentration was as low as 0.7 to 0.8 mg/L. In this season, we supplied initially 810 mg and additionally 270 mg of nitrogen to the paddy field. The removal efficiency of nitrogen through the experiment was about 96%. The amount of nitrogen (965 to 971 mg) removed from the irrigation water during this experiment was smaller than the amount (1,300 to 1,435 mg) in the biomass of the rice plants and other weeds. Nitrogen supplied from soil and atmosphere is likely to account for this difference, although this was not measured.

### Reduction of irrigation water by its circulation

The daily reduction in surface water depth, due to infiltration and evapotranspiration, in standard paddy field in Japan is about 20 mm. A total of 3,000 L of water is needed to maintain the water depth of 1 m<sup>2</sup> of paddy field for the five months of the rice cultivation period. The average precipitation in the five months is 800 mm in the region where we conducted this study. If all of the precipitation can be used in the paddy field, 2,200 L/m<sup>2</sup> of water should be irrigated. Based on the targeted value of yield (560 kg/m<sup>2</sup>), the volume of irrigation water required to get 1 kg of rice can be estimated as 3,930 L/kg. In our first and second experiments, approximately 280 and 100 L of irrigation water were lost excluding an accidental leakage, respectively. These water usages can be converted to 1,940 and 2,058 L/kg, which are somewhat smaller than that estimated for the normal paddy field. This comparison revealed that the circulated irrigation system proposed in this study can contribute to efficient water use in areas like the urban–rural fringe, with the additional aims of protecting the receiving water environment and producing a high quality of rice with no nitrogen fertilizer. The circulated irrigation system can be realized smoothly in paddy fields with underdrains. However, since pumping up the drained water is required, the cost of the pumps may limit the system implementation. The feasibility of this system should be evaluated by further studies on cost–benefit analysis considering saved water sources and nitrogen fertilizer.

### CONCLUSIONS

We designed a new cultivation system with circulated irrigation in order to remove nitrogen from treated municipal wastewater effectively. Through bench-scale experiments for two seasons, we obtained the following findings.

- (1) The new system enabled rice production with minimal irrigation (approximately 50% on the yield base compared to normal paddy field) and no nitrogen fertilizer.
- (2) Nitrogen supply based on standard practice in normal paddy fields seemed too much for the new system, resulting in overgrowth of rice plants and poor eating quality of rice in the first season. By reducing the initial nitrogen supply, the yield of rice was considerably reduced in the second season, while the taste of the harvested rice was significantly improved.
- (3) Accumulation of harmful metals in the rice was not observed after one season of cultivation in the new system.
- (4) In both seasons, more than 95% of nitrogen in the reused wastewater was removed through rice cultivation in the new system.
- (5) Impact of chlorination of reused wastewater on rice production in the new system was negligible and the intermittency of irrigation (MSD) did not improve yield and quality of harvested rice.

### ACKNOWLEDGEMENTS

The authors conducted this study at the Institute for Regional Innovation, Yamagata University. This study was financially supported by the Japan Science and Technology Agency (JST) and Japan International Cooperation Agency (JICA), through the Science and Technology Research Partnership for Sustainable Development (SATREPS).

### REFERENCES

- Ando, H., Aragones, C. & Wada, G. 1992 Mineralization patterns of soil organic N of several soils in the tropics. *Soil Science and Plant Nutrition* **38** (2), 227–234.
- Chiou, R. J. 2008 Risk assessment and loading capacity of reclaimed wastewater to be reused for agricultural irrigation. *Environmental Monitoring and Assessment* **142**, 255–262.
- Food and Agriculture Organization (FAO) 1992 *Wastewater Treatment and Use in Agriculture*. FAO Irrigation and Drainage Paper No. 47. Rome. <http://www.fao.org/docrep/T0551E/t0551e00.htm> (accessed 7 May 2014).
- Hu-Lin, H., You-Zhang, W., Xiao-e, Y., Feng, Y. & Chun-Yong, W. 2007 Effects of different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and grain quality in rice (*Oryza sativa*). *Rice Science* **14** (4), 289–294.
- Jang, T. I., Kim, H. K., Seong, C. H., Lee, E. J. & Park, S. W. 2012 Assessing nutrient losses of reclaimed wastewater irrigation

- in paddy fields for sustainable agriculture. *Agricultural Water Management* **104**, 235–243.
- Li, S., Li, H., Liang, X., Chen, Y., Cao, Z. & Xu, Z. 2009 Rural wastewater irrigation and nitrogen removal by the paddy wetland system in the Tai Lake region of China. *Journal of Soils and Sediments* **9**, 433–442.
- Markwell, J., Osterman, J. C. & Mitchell, J. L. 1995 Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research* **46**, 467–472.
- Mok, H. F. & Hamilton, A. J. 2014 Exposure factors for wastewater-irrigated Asian vegetables and a probabilistic rotavirus disease burden model for their consumption. *Risk Analysis* **34**, 602–613.
- Nguyen, H. T., Lee, K. J. & Lee, B. W. 2008 Recommendation of nitrogen topdressing rates at panicle initiation stage of rice using canopy reflectance. *Journal of Crop Science and Biotechnology* **11** (2), 141–150.
- Norton-Brandao, D., Scherrenberg, S. M. & van Lier, J. B. 2013 Reclamation of used urban waters for irrigation purposes: a review of treatment technologies. *Journal of Environmental Management* **122**, 85–98.
- Papadopoulos, F., Parissopoulos, G., Papadopoulos, A., Zdragas, A., Ntanos, D., Prochaska, C. & Metaxa, I. 2009 Assessment of reclaimed municipal wastewater application on rice cultivation. *Environmental Management* **43**, 135–143.
- Qadir, M., Wichelns, D., Raschid-Sally, L., McCornick, P. G., Drechsel, P., Bahri, A. & Minhas, P. S. 2010 The challenges of wastewater irrigation in developing countries. *Agricultural Water Management* **97**, 561–568.
- Singh, A., Sharma, R. K., Agrawal, M. & Marshall, F. M. 2010 Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology* **48**, 611–619.
- Tanaka, H. & Okamoto, S. 2011 Global development of water reclamation and reuse projects for agricultural irrigation. *Water, Land and Environmental Engineering, Japan* **79** (11), 809–812 (in Japanese).
- Yoon, C. G., Kwun, S. K. & Ham, J. H. 2001 Effects of treated sewage irrigation on paddy rice culture and its soil. *Irrigation and Drainage* **50**, 227–236.
- Zheng-Xun, J., Chun-Rong, Q., Jing, Y., Hai-Ying, L. & Xue-Yong, J. 2005 Effect of temperature at grain filling stage on activities of key enzymes related to starch synthesis and grain quality of rice. *Rice Science* **12** (4), 261–266.

First received 8 January 2014; accepted in revised form 19 May 2014. Available online 30 May 2014