

Evaluation of the use of wastewater treated with *Lemnas minor* in bean yield and nutrition

Avaliação do uso de águas residuárias tratadas com *Lemnas minor* na produtividade e nutrição do feijoeiro

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Highlights

Wastewater polishing by Lemnaceae macrophytes result adequate nutrition of beans.
Production of dry matter in beans is increases with use of wastewater with polishing.
Polished wastewater provides higher bean grain yield and can be used in agriculture.

Abstract

Irrigation with wastewater can reduce the demand for drinking water, reduce its disposal into water bodies and provide nutrients for agricultural crops. Thus, the objective of the present study was to evaluate the irrigation using wastewater with and without polishing by Lemnaceae macrophytes on the production and nutrition of common bean plants. Common bean (IPR Andorinha) was cultivated in a greenhouse in a completely randomized experimental design, with three treatments (T1= public-supply water (PSW); T2 = wastewater without polishing (WW_{NP}) and T3 = wastewater with polishing by macrophytes (WW_{PL})). The highest contents of macronutrients and micronutrients in the common bean crop irrigated with PSW, WW_{PL} and WW_{NP} were found in the following order: $K > Ca > N > Mg > S > P$ and $Fe > B > Mn > Zn > Cu$. Compared to PSW, wastewater promoted higher green color index (17.1%), number of pods (24%), grain yield (28%), fresh matter (33%) and dry matter (42%). Wastewater with and without polishing provides adequate nutrition and dry matter production, and wastewater with polishing increased the number of grains and grain yield in common bean.

Key words: *Phaseolus vulgaris*. Reuse water. Aquatic macrophytes.

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Resumo

A irrigação com água residuária pode reduzir a demanda de água potável, seu descarte em corpos de água e fornecer nutrientes para culturas agrícolas. Assim, com o presente trabalho objetivou-se avaliar a irrigação com água residuária com e sem polimento com macrófitas lemnáceas na produção e nutrição das plantas de feijão. Cultivou-se o feijão (IPR Andorinha) em casa de vegetação, sendo aplicado o delineamento experimental inteiramente casualizado, com três tratamentos (T1 = água de abastecimento público (PSW); T2 = água residuária sem polimento (WW_{NP}) e T3 = água residuária com polimento de macrófitas (WW_{PL})). O maior conteúdo dos macros e micronutrientes na cultura de feijão irrigado com água PSW, WW_{PL} and WW_{NP} foi na seguinte ordem: $K > Ca > N > Mg > S > P$ e $Fe > B > Mn > Zn > Cu$. A água residuária em relação a água de abastecimento, proporcionou maior índice de cor verde (17,1 %), número de vagens (24 %), rendimentos de grãos (28 %), e massa de matéria fresca (33 %) e seca (42 %). A utilização de água residuária com e sem polimento proporciona adequada nutrição e produção de massa de matéria seca, e a água residuária com polimento incrementou o número e rendimento de grãos do feijoeiro.

Palavras-chave: *Phaseolus vulgaris*. Água de reuso. Macrófitas aquáticas.

Irrigation with wastewater can reduce, or even eliminate, the need for using commercial fertilizers applied to soils, as well as contributing to reducing environmental impacts and the costs of disposing of effluents into natural water bodies, whether surface or underground (Urbano et al., 2017). Wastewater also promotes the addition of organic matter, which acts as soil conditioner, increasing its capacity to retain water. In agriculture, treated wastewater has been used in different crops, including some that are eaten raw, such as radish (Petousi et al., 2014), lettuce (Shargil et al., 2015), eggplant and tomato (Cirelli et al., 2012).

Effluent treatment can be performed using aquatic macrophytes, due to the higher efficiency of some species in assimilating and storing nutrients and their high rates of primary production (Henry-Silva & Camargo, 2002). The biological treatment of effluent is associated with the processes performed by microorganisms that live around macrophytes and by the removal of pollutants, directly

through assimilation by the tissues of the plant itself (Moorhead & Reddy, 1990).

Common bean (*Phaseolus vulgaris* L.) is the most important legume important in the world for consumption direct human, responsible for 80 % of the species of beans consumed. Brazil is considered the world's largest producer and consumer of common bean (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2022), with an average annual production of 929.4 mil tons (Companhia Nacional de Abastecimento [Conab], 2022). Common bean plants require large amounts of water, so the water requirements for maximum production of 60- to 120-day-old plants vary between 300 and 500 mm, depending on the climate (Beebe et al., 2013).

Thus, due to the importance of reusing sewage for agricultural production and the importance of common bean cultivation for human nutrition and its presence in the daily food of the Brazilian population, there arises the hypothesis that the application

of wastewater or wastewater polished by Lemnaceae macrophytes increases the nutrient content and seed production of this legume. Thus, the objective of the present study was to evaluate the effects of irrigation with wastewater with and without polishing by Lemnaceae macrophytes on the production and nutrition of common bean plants.

The experiment was carried out in a greenhouse with located at UNESP, Botucatu - SP. The average temperature and relative humidity of the greenhouse along the growing cycles were 29.3 ± 3.5 °C and $57.2 \pm 11.4\%$, respectively. The experimental design used was completely randomized, with 3 treatments: T1 = common bean irrigated with drinking water from the public supply system (PSW); T2 = irrigated with wastewater without polishing (WW_{NP}) and T3 = irrigated with wastewater with polishing by Lemnaceae macrophytes (*Lemna minor*) (WW_{PL}), with nine replicates.

The Latossolo Vermelho distrófico (Oxisol) was collected at 0-20 cm depth for physical-chemical characterization of the soil, which showed the following results: pH ($CaCl_2$) - 4.1; SOM - 13.0 g dm^{-3} ; P (resin) - 2 mg dm^{-3} ; Al^{3+} - $11.0 \text{ mmol}_c \text{ dm}^{-3}$; H+Al - $43.0 \text{ mmol}_c \text{ dm}^{-3}$; Ca - $4.0 \text{ mmol}_c \text{ dm}^{-3}$; Mg - $1.0 \text{ mmol}_c \text{ dm}^{-3}$; S - 24.0 mg dm^{-3} ; Sum of bases (SB) - $5.0 \text{ mmol}_c \text{ dm}^{-3}$; Base saturation (V) - 10 %; B - 0.22 mg dm^{-3} ; Cu - 1.0 mg dm^{-3} ; Fe - 39.0 mg dm^{-3} ; Zn - 0.3 mg dm^{-3} .

From the results obtained, soil acidity was corrected with lime application and, subsequently, the soil was incubated for thirty days with 70% of its total pore volume occupied by water. After this period, the samples received the following basal fertilization: 100 mg dm^{-3} N and 200 mg dm^{-3}

P (MAP - 60% P_2O_5 and 11% N); 100 mg dm^{-3} K (KCl); 37.5 mg dm^{-3} Mg, 50 mg dm^{-3} S ($MgSO_4 \cdot 7H_2O$); 0.5 mg dm^{-3} B (H_3BO_3); 1.5 mg dm^{-3} Cu ($CuSO_4 \cdot 5H_2O$); 5 mg dm^{-3} Mn ($MnSO_4 \cdot H_2O$) and 0.1 mg dm^{-3} Mo ($Na_2MoO_4 \cdot 2H_2O$). After that, four seeds of the common bean early-cycle cultivar IPR Andorinha® were sown in fiber cement pots with capacity for 40 dm^3 of soil.

The installed system was composed of three storage ponds, the first of which for wastewater with capacity for 1 m^3 , which flowed to the second polishing pond (also of 1 m^3) containing *Lemna minor* and, finally, the water polished by the macrophytes was conducted to another pond (1 m^3), where it was stored. The initial wastewater came from the Sewage Treatment Plant (*Estação de Tratamento de Esgoto* - ETE) of Botucatu - SP, located at the Lageado Experimental Farm, belonging to FCA/UNESP.

The irrigation of the experiment with public-supply water, wastewater and polished wastewater was performed using pumps. The water lost by evapotranspiration was replaced daily. The adopted water depth was defined according to irrigation needs, and the hydraulic detention time (HDT) in the polishing pond was 10 days. Irrigation was applied through a localized system, according to the crop needs, calculated by the daily evapotranspiration and water needs of the plant at each vegetative stage, according to the mathematical model: $ET = Kc (ET_o)$. Where: ET = potential of crop evapotranspiration; Kc = crop coefficient; ET_o = reference evapotranspiration (mm/day) by the evaporation from the class A pan.

Every ten days, the waters used in this experiment were monitored for biochemical oxygen demand (BOD), chemical

oxygen demand (COD), total solids (TS), total suspended solids (TSS), total coliforms (TC), nitrogen, phosphorus and pH, through collections at the inlet (first storage pond) and at the outlet of the last pond (Apha, 1998).

In the physiological maturity stage of common bean, the development of the plants of each plot was evaluated by measuring number of pods, number of seeds per plant and weight of seeds per plant. The green color index of common bean leaves was quantified at the end of the experiment using the SPAD 502 Plus chlorophyll meter. After harvest, the aerial part of the plants (stem and leaves) was weighed, washed in running water and passed in a solution of 0.1 M HCl, neutral detergent and deionized water.

Immediately after, the plant material was placed in paper bags for drying in a forced air circulation oven at 65 °C until

reaching constant weight. The samples were weighed on an analytical scale to determine the dry matter and then the pods were ground in a Wiley-type knife mill and passed through a 2-mm-mesh sieve. The nutrient contents were determined using the methods proposed by Miyazawa et al. (2009).

The experimental data were first subjected to one-way analysis of variance (ANOVA) and then the means were compared by Tukey test ($p \leq 0.05$).

Irrigation with wastewater had an effect on the contents of macronutrients and micronutrient in common bean (Table 1). However, the nutrient contents in the plants were low compared to those found in other studies in the literature with wastewater application in common bean (Valdez-Pérez et al., 2011), due to the different processes of plant collection.

Table 1

Contents of macronutrients and micronutrients in common bean irrigated with public-supply drinking water (PSW), wastewater polished by Lemnaceae macrophytes (WW_{PL}) and wastewater (WW_{NP}). Means \pm Standard Error. n= 9

Water	Macronutrients					
	N	P	K	Ca	Mg	S
	----- g plant ⁻¹ -----					
PSW	0.24±0.04b	0.01±0.01b	0.89±0.11b	0.46±0.06b	0.08±0.01b	0.02±0.00b
WW _{PL}	0.48±0.05a	0.03±0.02a	1.48±0.12a	0.68±0.08ab	0.15±0.01a	0.04±0.00a
WW _{NP}	0.57±0.04a	0.04±0.04a	1.80±0.09a	0.77±0.04a	0.18±0.00a	0.05±0.00a
Water	Micronutrients					
	B	Zn	Cu	Fe	Mn	
	----- mg plant ⁻¹ -----					
PSW	1.55±0.23b	0.32±0.04b	0.13±0.01b	5.65±1.04b	1.20±0.16b	
WW _{PL}	3.16±0.36a	0.52±0.05ab	0.19±0.02ab	9.36±0.91ab	1.93±0.29ab	
WW _{NP}	3.91±0.34a	0.63±0.04a	0.24±0.01a	13.11±0.96a	2.31±0.17a	

* Means followed by the same letter in the column do not differ by Tukey test at 5% probability level.

The contents of macronutrients and micronutrients in common bean were lower in plants irrigated with public-supply water compared to wastewater from ETE (Table 1), not differing in relation to the use of wastewater with or without polishing. This result reflects the chemical composition of the water used (Table 2), mainly for N and P, since higher N and P contents were found in the wastewater.

The presence of nutrients such as N and P in wastewater with and without polishing (Table 2) suggests that this type of water can

be used in the irrigation of agricultural crops. This would result in increased levels of N, P, K and organic carbon in the soil. However, excessive application of wastewater may cause toxicity to agricultural crops, in addition to environmental problems (Singh et al., 2012). Thus, although the wastewater with polishing had lower N and P contents compared to WWNP, the lower values of the other physicochemical parameters can reduce the risk of toxicity to plants and enable the product to be consumed by humans (Renuka et al., 2013).

Table 2

Mean values of the physicochemical parameters evaluated in the public-supply drinking water, wastewater polished by Lemnaceae macrophytes and wastewater

	WW _{NP}	WW _{PL}	PSW
TSS (mg L ⁻¹)	185.0	14.2	0
TS (mg L ⁻¹)	496.2	202.7	0
BOD (mg L ⁻¹)	73.4	21.6	-
COD (mg L ⁻¹)	492.0	129.0	-
Nitrogênio (mg L ⁻¹)	25.8	5.2	-
Fósforo (mg L ⁻¹)	3.95	0.54	-
TC (NMP 100 mL ⁻¹)	> 1000	> 1000	0
pH	10.3	8.1	6.1
FRC	-	-	1.2

* TSS- Total Suspended Solids, TS- Total Solids, BOD- Biochemical Oxygen Demand, COD- Chemical Oxygen Demand and TC- Total Coliforms, FRC- Free Residual Chlorine.

The green color index of the leaves was higher, approximately 17.1%, in the treatment with irrigation using wastewater with polishing compared to irrigation with public-supply water (Figure 1a), whereas wastewater without polishing was similar to both of these forms

of irrigation. This result for the green color index was a consequence of the N content in the plant (Table 1), having an impact on photosynthesis and consequently on plant production (Figure 1d).

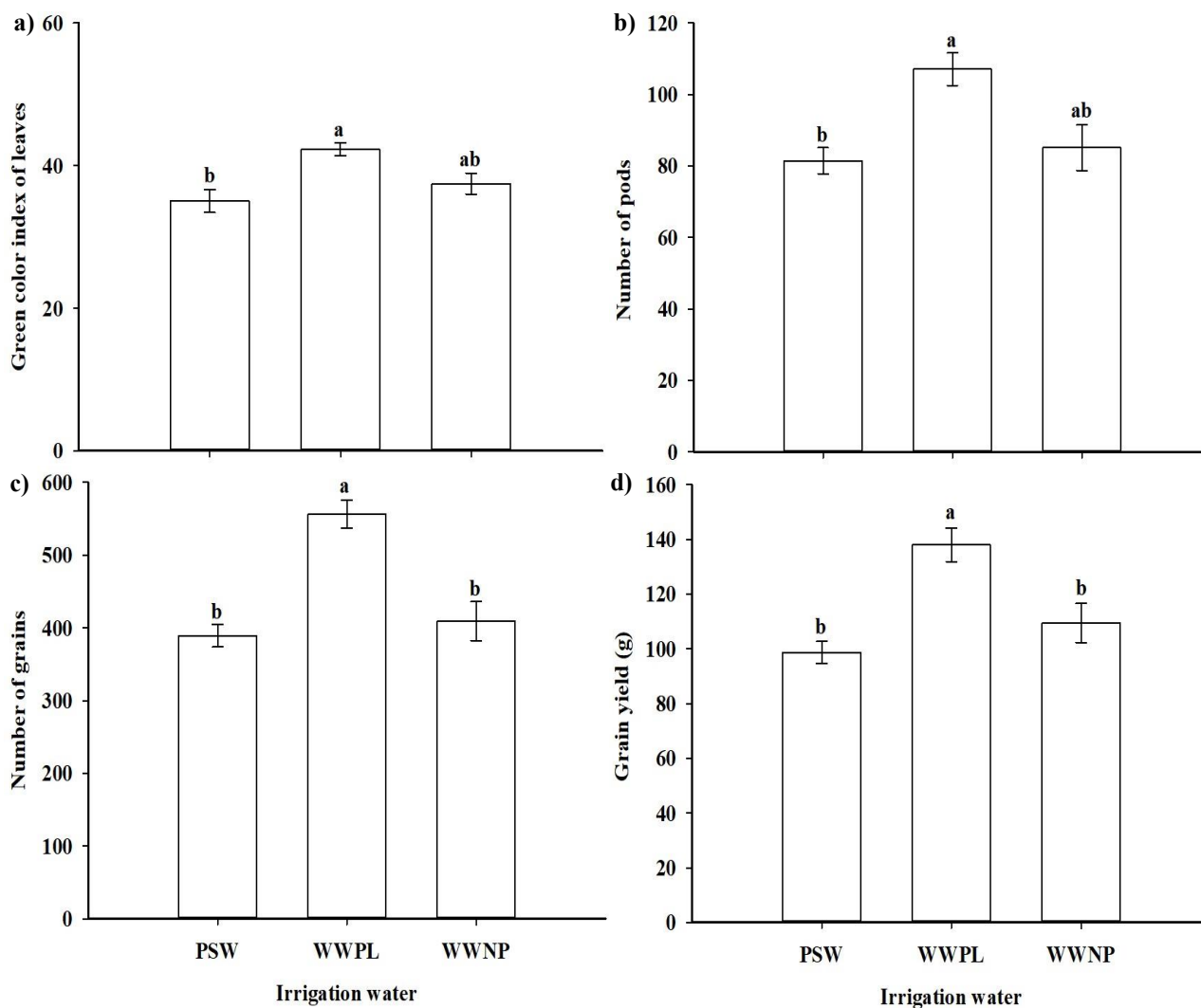


Figure 1. Green color index of leaves (a), number of pods (b), number of grains (c) and grain yield (d) of common bean irrigated with public-supply drinking water (PSW), wastewater polished by Lemnaceae macrophytes (WW_{PL}) and wastewater (WW_{NP}). Means followed by the same letter in the column do not differ by Tukey test at 5% probability level. Means \pm Standard Error. $n = 9$.

The treatment of the water used to irrigate common bean plants affected the number of pods and grain yield (Figure 1b and 1c). Irrigation with wastewater with polishing by Lemnaceae macrophytes led to a 24% higher number of pods than the public-supply water (Figure 1b), whereas irrigation with wastewater without polishing was similar to both.

The number of grains and grain yield of common bean were approximately 28% and 25% higher, respectively, under irrigation using wastewater with polishing by Lemnaceae macrophytes compared to the application of public-supply water and wastewater without polishing (Figure 1c and 1d). Singh et al. (2012) observed higher grain yield with the use of wastewater, emphasizing

that it promotes better results when treated appropriately. Similarly, Salakinkop and Hunshal (2014) also found higher production of wheat grains with the use of wastewater, attributing it to the increase in chlorophyll content and photosynthesis rate, since about 75 % of the N present in sewage is used by plants.

In this context, wastewater treatment after ETE with polishing by macrophytes promotes better-quality water for common bean cultivation, as this procedure results in the degradation of organic compounds, reduction in the number of pathogens, sorption of cations and anions, and absorption of excess nutrients and heavy metals (Jones et al., 2017). This can be verified in the present study, with the lowest contents of N and P in the wastewater with polishing by Lemnaceae macrophytes (Table 1), contributing to the

adequate nutrition of common bean plants in comparison to the wastewater treated only with the ETE (Table 1).

Common bean fresh matter and dry matter increased by approximately 33 % and 42 % in treatments with wastewater with and without polishing compared to the treatment with public-supply water (Figure 2), with no difference between wastewater with and without polishing. This affected the contents of macronutrients and micronutrients (Table 1). Similarly, Saffari and Saffari (2013) also verified an increase in the fresh matter and dry matter of common bean and lima bean (*Phaseolus vulgaris* and *Phaseolus lunatus*), using sewage wastewater in the irrigation compared to drinking water, attributing this result to the higher concentrations of N and P in the wastewater.

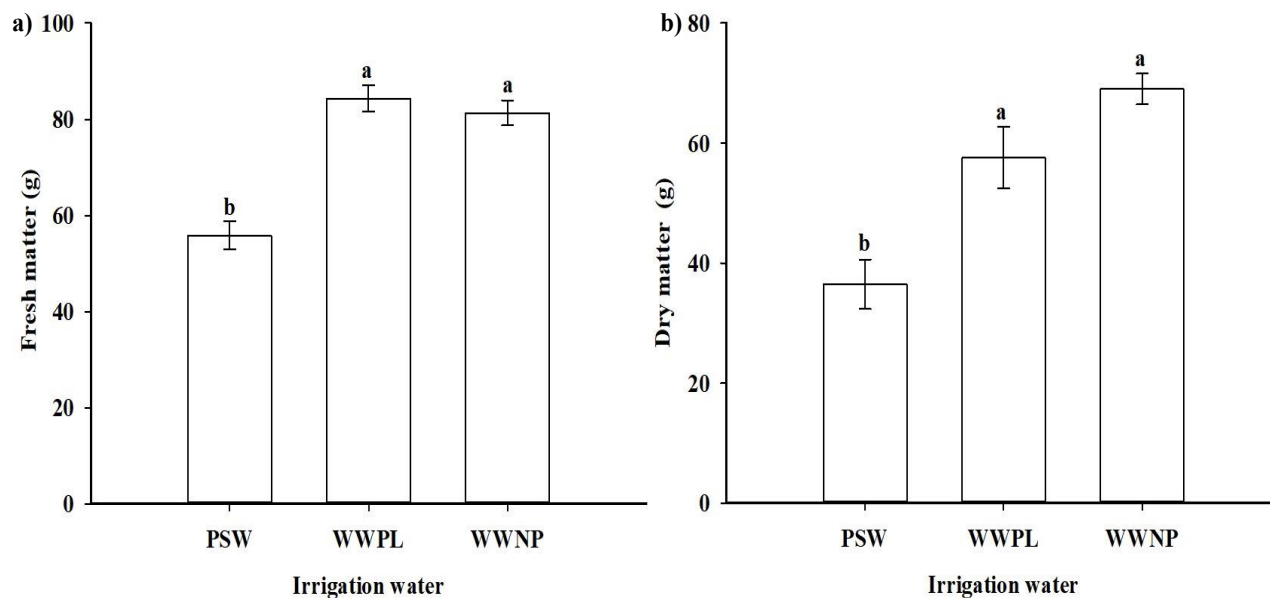


Figure 2. Fresh matter (a) and dry matter (b) of common bean irrigated with public-supply drinking water (PSW), wastewater polished by Lemnaceae macrophytes (WW_{PL}) and wastewater (WW_{NP}). Means followed by the same letter in the column do not differ by Tukey test at 5% probability level. Means \pm Standard Error. n= 9.

Wastewater with polishing by Lemnaceae macrophytes promotes higher number of grains and grain yield, besides providing better-quality water for common bean cultivation. However, wastewater both with and without polishing can be used to irrigate the common bean crop, which promotes adequate nutrition and production.

References

- Apha, A. (1998). *Standard methods for the examination of water and wastewater*. American Public Health Association.
- Beebe, S., Rao, I., Blair, M., & Acosta, J. (2013). Phenotyping common beans for adaptation to drought. *Frontiers in Physiology*, 4(35), 1-35. doi: 10.3389/fphys.2013.00035
- Cirelli, G. L., Consoli, S., Licciardello, F., Aiello, R., Giuffrida, F., & Leonardi, C. (2012). Treated municipal wastewater reuse in vegetable production. *Agricultural Water Management*, 104, 163-170. doi: 10.1016/j.agwat.2011.12.011
- Companhia Nacional de Abastecimento (2022). *Acompanhamento da safra brasileira - grãos*. <https://www.conab.gov.br>
- Henry-Silva, G. G., & Camargo, A. F. M. (2002). Valor nutritivo de macrófitas aquáticas flutuantes (*Eichhornia crassipes*, *Pistia stratiotes* e *Salvinia molesta*) utilizadas no tratamento de efluentes de aquicultura. *Acta Scientiarum*, 24(2), 36-48. doi: 10.4025/actasciobiolsci.v24i0.2353
- Jones, D. L., Freeman, C., & Sánchez-Rodrigues, A. R. (2017). Wastewater treatment. *Encyclopedia of Applied Plant Sciences*, 3, 352-362. doi: 10.1016/B978-0-12-394807-6.00019-8
- Ministério da Agricultura, Pecuária e Abastecimento (2022). *Portaria SPA/ MAPA nº 1, de 4 de abril de 2022*. <https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=06/04/2022&jornal=515&pagina=8&totalArquivos=712>
- Miyazawa, M., Pavan, M. A., Muraoka, T., Carmo, C. A. F. S., & Melo, W. J. (2009). Análise química de tecido vegetal. In F. C. Silva (Ed.), *Manual de análises químicas de solos, plantas e fertilizantes* (pp. 191-233). Brasília, DF.
- Moorhead, K. K., & Reddy, K. R. (1990). Carbon and nitrogen transformations in wastewater during treatment with *Hydrocotyle umbellata* L. *Aquatic Botany*, 37(2), 149-153. doi: 10.1016/0304-3770(90)90088-3
- Petousi, I., Fountoulakis, M. S., Tzortzakis, N., Dokianakis, S., Stentiford, E. L., & Manios, T. (2014). Occurrence of micro-pollutants in a soil-radish system irrigated with several types of treated domestic wastewater. *Water, Air, & Soil Pollution*, 225(1791), 1-8. doi: 10.1007/s11270-013-1791-y
- Renuka, N., Sood, A., Ratha, S. K., Prasanna, R., & Ahluwalia, A. S. (2013). Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production. *Journal of Applied Phycology*, 25, 1529-1537. doi: 10.1007/s10811-013-9982-x
- Saffari, V. R., & Saffari, M. (2013). Effect of treated municipal wastewater on bean growth, soil chemical properties, and chemical fractions of zinc and copper. *Arabian Journal of Geosciences*, 6, 4475-4485. doi: 10.1007/s12517-012-0690-7

- Salakinkop, S. R., & Hunshal, C. S. (2014). Domestic sewage irrigation on dynamics of nutrients and heavy metals in soil and wheat (*Triticum aestivum* L.) production. *Journal of Recycling of Organic Waste in Agriculture*, 3(8), 1-8. doi: 10.1007/s40093-014-0064-0
- Shargil, D., Gerstl, Z., Fine, P., Nitsan, I., & Kurtzman, D. (2015). Impact of biosolids and wastewater effluent application to agricultural land on steroidal hormone content in lettuce plants. *Science of the Total Environment*, 505, 357-366. doi: 10.1016/j.scitotenv.2014.09.100
- Singh, P. K., Deshbhratar, P. B., & Ramteke, D. S. (2012). Effects of sewage wastewater irrigation on soil properties, crop yield and environment. *Agricultural Water Management*, 103, 100-104. doi: 10.1016/j.agwat.2011.10.022
- Urbano, V. R., Mendonça, T. G., Bastos, R. G., & Souza, C. F. (2017). Effects of treated wastewater irrigation on soil properties and lettuce yield. *Agricultural Water Management*, 181, 108-115. doi: 10.1016/j.agwat.2016.12.001
- Valdez-Pérez, M. A., Fernández-Luqueno, F., Franco-Hernandez, O., Flores Cotera, L. B., & Dendooven, L. (2011). Cultivation of beans (*Phaseolus vulgaris* L.) in limed or unlimed wastewater sludge, vermicompost or inorganic amended soil. *Scientia Horticulturae*, 128(4), 380-387. doi: 10.1016/j.scienta.2011.01.016

