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EFFECT OF DAIRY AND MEAT WASTEWATER IRRIGATION ON SEEDLING GROWTH

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ABSTRACT. Milk and meat processing industries release large quantities of nutrient-rich wastewater with organic compounds (proteins, fats, carbohydrates) in high concentrations. Reusing and recycling this biodegradable wastewater for crop irrigation could be a sustainable solution once the phytotoxic effects of wastewater on crops have been investigated. Therefore, the aim of this study was to evaluate the effects of milk and meat processing wastewaters on germination percentage, seed vigour indices, the seedling tolerance index and the phytotoxicity index of pea (*Pisum sativum* L.), sugar maize (*Zea mays saccharata*), purslane (*Portulaca oleracea*), wheat (*Triticum aestivum*) and red spinach (*Amaranthus dubius*). The two wastewater types were collected at the inlet of the city's sewage system and analysed to determine their physicochemical and microbiological characteristics. The seeds of all five plant species were irrigated with untreated wastewater effluents. The highest - germination percentages were obtained for wheat (92%) and the lowest for red spinach (2.5%). Wheat, purslane, pea and red spinach samples irrigated with meat processing wastewater had higher germination percentages than samples irrigated with dairy wastewater. A higher phytotoxicity was observed for sugar maize, followed by red spinach irrigated with both types of wastewater. Future investigation into the effects of effluent dilution on these types of plants is recommended.

Keywords: food industry wastewater; phytotoxicity; seed germination; sugar maize; wheat.

INTRODUCTION

Water is a natural resource of particular importance for all human activities (Khaleel *et al.*, 2013), being used for household, industrial, energy production and irrigation purposes (Kaur

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and Sharma, 2017). The agricultural sector is one of the largest consumers of total freshwater withdrawal, with approximately 310 million hectares of irrigated land worldwide (Khan *et al.*, 2022). Water resources are limited, and water scarcity is an issue in certain regions. Using untreated or treated wastewater to irrigate plants can alleviate this issue (Khan *et al.*, 2022). Food processing industries are both large consumers of fresh water and producers of effluents loaded with various substances, such as proteins, fats, carbohydrates, nutrients and cleaning solutions (Ganta *et al.*, 2022). These effluents can be used to irrigate croplands and are considered a cheap and environmentally friendly solution due to essential nutrients (Kaur *et al.*, 2018; Kapil and Mathur, 2020; Kaur and Sharma, 2017; Patsinghasanee *et al.*, 2021; Salian *et al.*, 2018). However, improper disposal of these effluents can pollute water and soil (Salian *et al.*, 2018). The meat processing industry is a significant producer of wastewater effluent, with consumption between 2.5 and 40 m^3 of water per metric tonne of meat produced (Kaur *et al.*, 2018), followed by the beverage and dairy industries. Approximately 6–10 L of effluent is produced per 1 L of milk processed (Porwal *et al.*, 2015). The impact of dairy industry effluent was investigated on the growth of wheat (*Triticum aestivum*) (Sioud *et al.*, 2016; Kaur and Sharma, 2017), maize (*Zea mays* L.) (Sioud *et al.*, 2016), paddy (*Oryza sativa* L.) (Kaur *et al.*, 2018), Mung bean (*Vigna radiata*) and Mustard (*Brassica nigra*) (Kapil and Mathur, 2020), while the effects of meat industry effluent were investigated on the growth of cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.) (Gerber *et al.*, 2017) and rice (*Oryza sativa* L.) (Patsinghasanee *et al.*, 2021).

The present study was carried out in the context of evaluating the possibility of reusing wastewater resulting, at the regional level, from the milk and meat processing industries. In this study, the effects of dairy and meat processing effluents on seed germination and short-term early seedling growth of pea (*Pisum sativum* L.) (R), sugar maize (*Zea mays saccharata*) (S), purslane (*Portulaca oleracea*) (P), wheat (*Triticum aestivum*) (W) and red spinach (*Amaranthus dubius*) (Sp) were evaluated.

MATERIALS AND METHODS

Plant materials

The experiments were performed on five plant species: pea *(Pisum sativum L.)* (R)*,* sugar maize *(Zea mays saccharata)* (S)*,* purslane *(Portulaca oleracea)* (P), wheat (*Triticum aestivum)* (W) and red spinach *(Amaranthus dubius)* (Sp) provided by *Seeds and Garden Plants*, Suceava Romania. Plant selection was carried out based on the possibility of cultivation in Romania and their nutritional values.

Wastewater samples

The two wastewater samples used in this study were collected for three consecutive days from entry into the city sewer system of two Romanian factories that process milk and meat. Dairy wastewater (DWW) and meat processing wastewater (MWW) samples were analysed to determine their physicochemical (pH, suspended matter, biological oxygen demand (BOD_5), and chemical oxygen demand (COD), ammonium (NH_4), total nitrogen (N), nitrates (NO_3), nitrites (NO_2), total phosphorus (P), extractable substances, synthetic detergents, filterable residue) and microbiological (total number of aerobic mesophilic germs, total number of yeast and mould) characteristics.

Wastewater sample physicochemical analysis

The physicochemical parameters of the effluent samples were analysed by standard methods, as shown in *Table 1*.

Microbiological analysis of wastewater samples

The total number of aerobic mesophilic germs (TAMG) was determined according to the standard SR EN ISO 4833-2/2014. A 1 mL sample was taken from each sample, and 6 and 10 dilutions were made for the wastewater from the meat and dairy industries, respectively.

From the last dilution, 1 mL was sampled and used to inoculate a Petri plate containing nutrient agar as a nutrient medium. The plates were incubated at 35ºC for 24 h, and the colonies were counted using Funke Gerber ColonyStar (Funke Gerber, Berlin, Germany) (Kaddumukasa *et al.*, 2017).

The total number of yeast and mould (TYM) colonies was determined indirectly, based on the colonies generated by the cells of the microorganisms present in the analysed samples, which were formed when the sample or a dilution thereof came into contact with a nutrient medium. One millilitre of the sample from the last dilution was taken and poured into a Petri dish containing the Malt Extract Agar as a nutrient medium. After the medium solidified, the plates were incubated t with the lid down at 25ºC for 72 h (Leneveu-Jenvrin *et al.*, 2022).

All experiments were performed in triplicate, and the results were expressed as the average of the replicates.

Seedling growth

The experiments were carried out in the laboratory of the Faculty of Food Engineering, "Ștefan cel Mare" University in Suceava, Romania. Ten plant seeds of each species were sown in Petri dishes at room temperature (18–21ºC) with 12 h/12 h light/dark, with no covering. The Petri dishes were irrigated with untreated wastewater (DWW and MWW) to maintain 50% humidity. Drinking water (DW) was used for the irrigation of plant growth control samples. The number of germinated seeds was evaluated after 6 days of incubation. This type of water was used to avoid the utilisation of contaminated water, considering that we tested the water from several points in the region and none of them corresponded qualitatively, with most of them having exceeded the concentrations for nitrites and chlorides.

Phytotoxicity parameters

After incubation, the germination percentage (% G), seed vigour indices (VI), seedling tolerance index (TI), phytotoxicity index (PI) and relative wastewater toxicity (% RT) were determined.

According to Ratnakar and Shikha (2019), a seed can be considered germinated if the radicle has sprouted. Germination percentage (% G) was determined after dividing the number of germinated seeds by the number of seeds, multiplied by 100 (Kaya and Eryiğit, 2021) (*Equation 1*).

$$\% G = \frac{Gs}{Ts} * 100 \quad (1)$$

where Gs is the number of germinated seeds and Ts is the number of total seeds.

Seedling length (Sl) was the total length of the root length (cm) and shoot length (cm) (Patsinghasanee *et al.*, 2021). The length was measured using a ruler. Based on seedling length and germination percentage, the vigour index (VI) of length (Salian *et al.*, 2018; Ratnakar and Shikha, 2019) was calculated, as follows (*Equation 2*):

$$VI = Sl * \%G \quad (2)$$

where seedling length (Sl) was measured in cm.

The seedling tolerance index (TI) was calculated using *Equation 3*, as follows (Kashyap *et al.*, 2023):

$$TI = \frac{Rlt}{Rlc} \quad (3)$$

where Rlt is the mean root length of the treated seeds and Rlc is the mean root length of the control seeds.

The phytotoxicity index (PI) was calculated using *Equation 4*, as follows (Salian *et al.*, 2018)

$$PI = 1 - TI \quad (4)$$

The relative wastewater toxicity (% RT) was calculated using *Equation 5*, as follows (Noel and Rajan, 2015):

$$\% RT = \frac{(Rlc + Rsc) - (Rlt + Rst)}{(Rlc + Rsc)} * 100 \quad (5)$$

where Rlt is the mean root length of treated seeds; Rst is the mean shoot length of treated seeds; Rlc is the mean root length of control seeds; and Rsc is the mean shoot length of control seeds.

Statistical analysis

The experiments were performed in triplicate and the obtained results were expressed as mean values ± standard mean error. The results obtained for germination percentage, seed vigour indices, seedling tolerance index, phytotoxicity index and relative toxicity of wastewaters were analysed by one-way analysis of variance (ANOVA) with a 95% confidence interval ($p < 0.05$) and Tukey's test. The Ryan–Joiner test (similar to the Shapiro–Wilk normality test, usually used for a small sample size) was used to verify the normality of sample distributions in Minitab software. Principal component analysis (PCA) was also carried out.

RESULTS AND DISCUSSION

The physicochemical parameters of wastewater collected from dairy and meat processing plants are represented in *Table 2*. According to Slavov (2017), the pH values of dairy effluents are usually between 6.8 and 7.4, and when alkaline cleaning solutions are discarded, pH values increase up to 10–10.5. pH values must be between 6 and 9 for the biological treatment of DWW. In the case of slaughterhouse wastewater, the pH values are between 4.9 and 8.1 (Bustillo-Lecompte and Mehrvar, 2017). The pH values of DWW and MWW were between the limits required by Romanian legislation. The suspended matter may include pieces of cheese, coagulated milk, cheese, curd fines, milk film or flavouring agents that enter the initial stage of equipment cleaning, and whey is characterised by a higher amount of total solids (Slavov, 2017). In meat effluents, total suspended solids can range between 0.1 and 10,000 mg/L

(Bustillo-Lecompte and Mehrvar, 2017). DWW is characterised by a high BOD and COD due to its high organic content, and 90% of their values are caused by lactose (Slavov, 2017). Additionally, the presence of blood, fat and mucous lead to a high BOD (1300–2300 mg/L) and COD (2000–6000 mg/L) (Latiff *et al.*, 2019). According to Latiff *et al.* (2019), high concentrations of nutrients such as nitrogen (70.56–578.63 mg/L) are found in meat processing effluents. Nitrogen can be found in diary effluents due to amino groups from milk proteins (Slavov, 2017; Toromanović *et al.*, 2023). The wastewater samples used in the present study had higher BOD and COD values, especially MWW. In addition, the nitrogen content (as ammonium, total nitrogen, nitrates) was higher for MWW than DWW. According to Yousefi and Douna (2023), dairy products can contain between 3 and 27 mg of nitrate per kg, while meat products contain more than 2.7 to 945 mg of nitrate per kg. Meat preservation is achieved using nitrate and nitrite salts (Zhang *et al.*, 2023), which can end up in effluents. High levels of phosphorus are largely associated with milk and meat proteins (Britz *et al.*, 2004; Prica *et al.*, 2015). The total number of yeast and mould (TYM) per medium on malt extract agar and the total number of aerobic mesophilic germs per medium on nutrient agar (TAMG) (*Figure 1*) were calculated. The results showed that for the MWW samples, TAMG was 6.9±0.01 $\log_{10}$ CFU/cm^3, while TYM was 6.47±0.15 $\log_{10}$ CFU/cm^3. In the case of dairy industry wastewater samples, TAMG was 10.84±0.10 $\log_{10}$ CFU/cm^3, while TYM was 10.77±0.07 $\log_{10}$ CFU/cm^3. In purslane, 88% seed germination was recorded with MWW, while approximately 63% germination was observed with DW and DWW. MWW showed a positive effect with 82% germination of pea seeds, while 75% and 64% were recorded with DW and DWW, respectively. According to Janusauskaite (2023), nitrogen doses from fertilisers have a positive impact on pea behaviour, and since MWW has a higher amount of ammonium and nitrate than DWW (*Table 2*), this explains the higher germination rate of pea seeds irrigated with MWW.

Table 1 – Analysis methods of wastewater parameters

Parameters	Analysis method
pH (pH units)	SR ISO 10523-97
Suspended matter (mg/dm^3)	STAS 6953-81
BOD_5 (mg O_2/dm^3)*	SR EN 1899-22002
COD (mg O_2/dm^3)**	SR ISO 6060-96
Ammonium (NH_4) (mg/dm^3)	SR ISO 5664:2001 SR ISO 7150-1/2001
Total nitrogen (N) (mg/dm^3)	SR EN ISO 13395:2002
Nitrates (NO_3) (mg/dm^3)	SR ISO 7890-2:2000; SR ISO 7890-3:2000
Nitrites (NO_2) (mg/dm^3)	SR EN 26777:2002
Total phosphorus (P) (mg/dm^3)	SR EN 1189-2000
Extractable substances (mg/dm^3)	SR 7587-96
Synthetic detergents (mg/dm^3)	SR EN 903:2003 SR ISO 7875/2-1996
Filterable residue (mg/dm^3)	STAS 9187-84

*biological oxygen demand (BOD_5), **chemical oxygen demand (COD)

Table 2 – Physicochemical parameters of dairy and meat effluents

Parameters	**DWW***	**MWW***	**HG 352/2005 NTPA 001**	**HG 352/2005** NTPA 002**
pH (pH units)	8.26±0.42^{a}	6.59±0.05^{b}	6.5–8.5	6.5–8.5
Suspended matter (mg/dm^3)	47.6±39.3^{a}	34.0±28.5^{a}	35	350
Biological Oxygen Demand (BOD_5) (mg O_2/dm^3)	108.5±18.5^{b}	1002±27^{a}	25	300
Chemical Oxygen Demand (COD) (mg O_2/dm^3)	602.1±64.8^{b}	1512±45.6^{a}	125	500
Ammonium (NH_4) (mg/dm^3)	1.19±0.35^{b}	349.1±9.09^{a}	2	30
Total nitrogen (N) (mg/dm^3)	28.4±24.2^{b}	270±10^{a}	10	
Nitrates (NO_3) (mg/dm^3)	7.50±10.19^{a}	22.4±10.16^{a}	25	
Nitrites (NO_2) (mg/dm^3)	0.013±0.18	-	1	
Total phosphorus (P) (mg/dm^3)	1.63±0.52^{a}	0.18±0.15^{a}	2	5
Extractable substances (mg/dm^3)	0.70±0.99^{b}	88±1.99^{a}	20	30
Synthetic detergents (mg/dm^3)	0.20±0.25^{a}	0.34±0.32^{a}	0.5	25
Filterable residue (mg/dm^3)	640±639^{a}	1882±976^{a}	2000	

*Values with different letters within a row are significantly different ($p < 0.05$).
**Romanian Legislation: The government decision for the approval of some rules regarding the discharge conditions of wastewater into the aquatic environment (NTPA 001) or sewerage (NTPA 002)

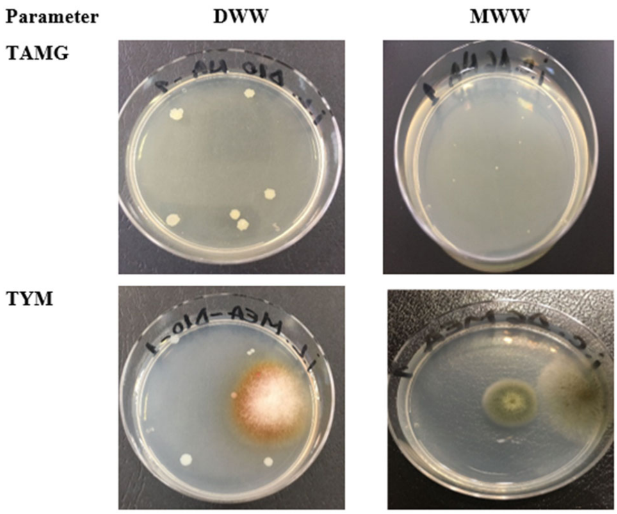

Figure 1 – Microorganisms identified in wastewater from the dairy and meat industries (total number of aerobic mesophilic germs per medium on nutrient agar (TAMG), total number of yeast and mould (TYM), dairy industry wastewater (DWW), meat industry wastewater (MWW))

In sugar maize, 75% seed germination was observed for DW, followed by 65% for DWW and 42% for MWW. One possible explanation is that salinity and osmotic pressure may inhibit sugar maize seed germination by altering the interaction of seed and water (Kumar, 2014), and MWW can have a higher amount of salts than DWW. Sioud *et al.* (2016) observed a toxic impact on maize germination and growth irrigated with DWW. Irrigation with DW, MWW and DWW resulted in very low germination percentages in red spinach. A high nutrient content can inhibit red spinach germination (Pranata and Suparti, 2023), and salinity can reduce the germination rate of spinach (Uçgun *et al.*, 2020). Additionally, the low germination percentage obtained for red spinach may be due to the lack of light since it must be exposed to direct sunlight and poor seed quality.

In wheat, 100% seed germination was recorded with DW, whereas 92% was observed with MWW and 82% with DWW (*Figure 2*). Wheat obtained the best values for germination percentage and a possible explanation is that it has the ability to grow in the most different environmental conditions (Lamlom *et al.*, 2023).

The positive effect on wheat germination and growth after dairy effluent irrigation was also observed by Sioud *et al.* (2016). Kaur and Sharma (2017) observed that a dilution of 50% dairy effluent showed a significant effect on wheat seed germination, while Dhanam (2009) indicated that dairy effluent in 100% concentration may have an inhibitory effect on rice germination. Patsinghasanee *et al.* (2021) stated that meat effluents contain nutrients at higher concentrations and have some negative effect on rice seed germination. In the study conducted by Khaleel *et al.* (2013) the treated dairy effluent sample showed favourable effects on seed germination compared with crude (untreated) dairy effluent and tap water. The differences were influenced by pH. In our case, differences between the parameters were observed for pH and nitrates, which could have contributed to the differences in germination. Low seed germination tendencies with a high dairy and meat effluent concentration (untreated wastewater) can be attributed to the high osmotic pressure of the effluent (Kaur *et al.*, 2018).

The results of the vigour index are illustrated in *Figure 3*. The highest values of VI were obtained from wheat (703) and pea (654) and were recorded with DW. In the case of purslane, a higher VI value (624) was recorded with MWW. The lowest VI values were determined for red spinach irrigated with all three types of water (*Figure 3*).

In addition, low values of VI (under 100) were observed for sugar maize irrigated with MWW and DWW. VI values vary across crop and species. Sioud *et al.* (2016) observed that the VI of wheat seedlings in untreated and treated dairy effluent was higher than 764, while maize showed a low VI (64.2–316.8). The amount of nutrients is responsible for the stimulation of plant growth (Kaur *et al.*, 2018; Marta *et al.*, 2022).

The seedling tolerance index (TI) was calculated (*Figure 4*). The results showed that purslane had a higher tolerance to DWW and MWW, while sugar maize had the lowest tolerance. In the case of seed irrigation with DWW, the TI values increased in the following order: P>R>W>Sp>S. Peas had a better tolerance than wheat to DWW, while wheat had a better tolerance to MWW than pea. The results obtained for wheat are similar to those of Sioud *et al.* (2016), who determined TI values of

1.65 for wheat and 1.03 for maize irrigated with dairy effluent.

The phytotoxic effect in purslane was lowest with DWW (PI = −2.00) and MWW (PI = −1.34). In pea, the lowest phytotoxicity was exhibited by DWW (PI = −0.15). A higher phytotoxicity was observed for sugar maize (PI = 0.68), followed by red spinach (PI = 0.53) irrigated with DWW. However, in sugar maize (PI = 0.70) and red spinach (PI = 0.66), the highest phytotoxicity was exhibited by MWW (*Figure 5*).

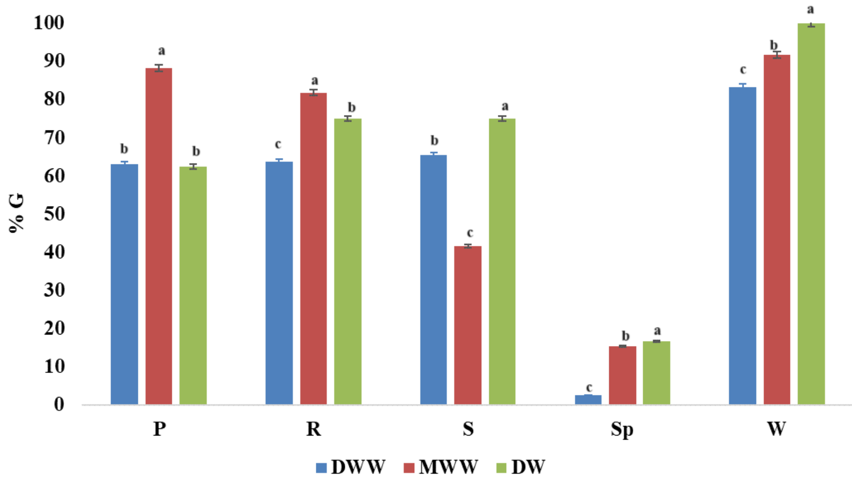

Figure 2 – Effect of dairy wastewater (DWW), meat wastewater (MWW) and drinking water (DW) on the germination percentage of purslane (P), pea (R), sugar maize (S), red spinach (Sp) and wheat (W) samples. Different lowercase letters indicate significant differences ($p < 0.05$) among samples

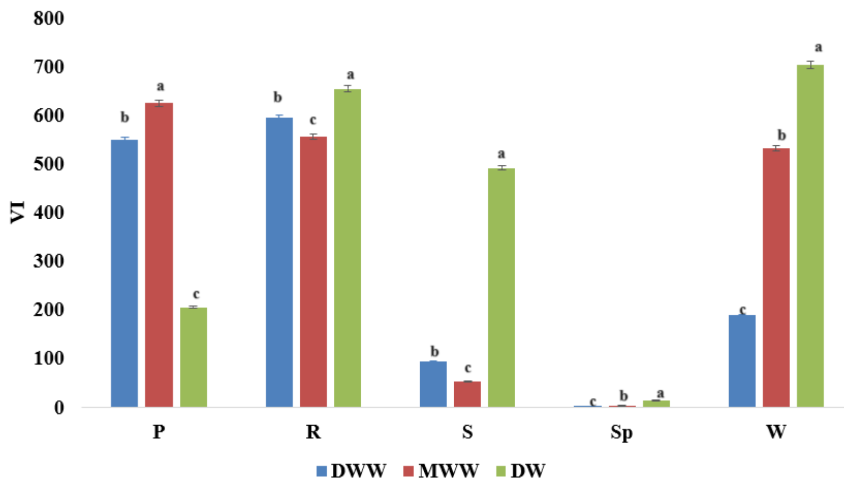

Figure 3 – Effect of dairy wastewater (DWW), meat wastewater (MWW) and drinking water (DW) on the length vigour index (VI) of purslane (P), pea (R), sugar maize (S), red spinach (Sp) and wheat (W) samples. Different lowercase letters indicate significant differences ($p < 0.05$) among samples

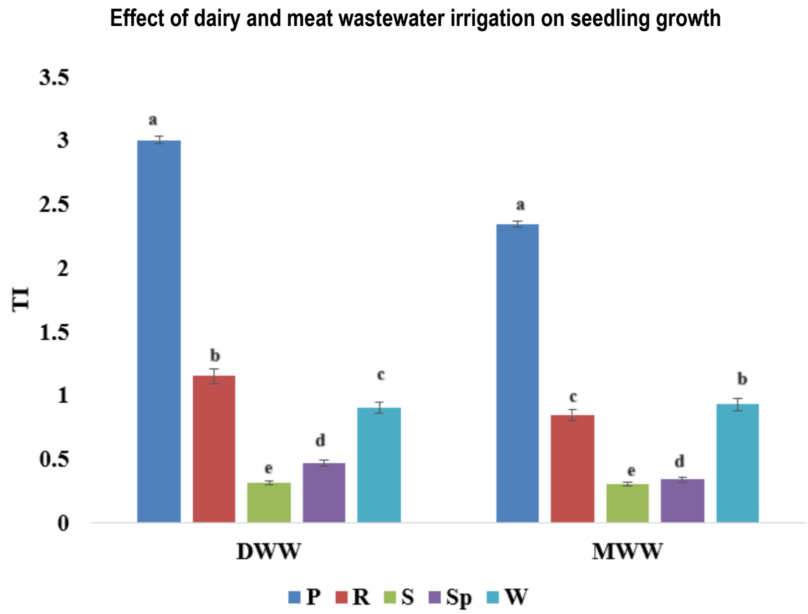

Figure 4 – Effect of dairy wastewater (DWW) and meat wastewater (MWW) on the seedling tolerance index (TI) of purslane (P), pea (R), sugar maize (S), red spinach (Sp) and wheat (W). Different lowercase letters indicate significant differences ($p < 0.05$) among samples

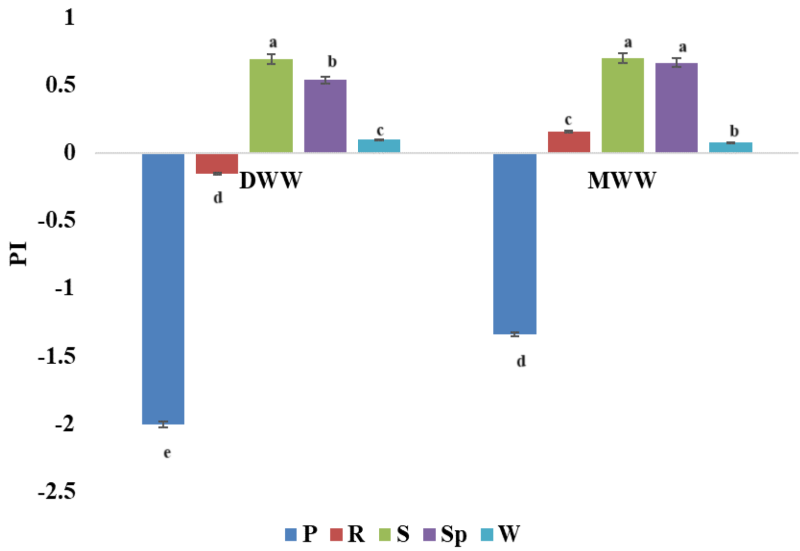

Figure 5 – Effect of dairy wastewater (DWW) and meat wastewater (MWW) on the phytotoxicity index (PI) of purslane (P), pea (R), sugar maize (S), red spinach (Sp) and wheat (W). Different lowercase letters indicate significant differences ($p < 0.05$) among samples

The relative toxicity of DWW and MWW is illustrated in *Figure 6*. Negative RT values were obtained for purslane irrigated with both wastewater types and pea irrigated with DWW. Higher positive RT values were recorded for sugar maize and red spinach, followed by wheat irrigated with DWW and MWW.

These wastewaters contain large amounts of different macronutrients (N, P), essential for plant growth but also a large amount of organic compounds, such as carbohydrates, proteins, fats and oil. Phosphate and nitrates can lead to toxic effects on plant germination. Under these conditions, they cannot be exploited for agricultural irrigation due to their impact on crops and soil.

PCA was performed to group and classify the five investigated plants based on their similarities and differences. The results are presented in *Figure 7*. PC1 explained 78.3% of the total variation and had an eigenvalue of 3.91, while PC2 explained 15.9% of the total variation and had an eigenvalue of 0.79.

As shown in *Figure 7*, parameters G (0.342), VI (0.471) and TI (0.463) of samples had positive loadings on PC1, while the other two parameters RT (−0.483) and PI (−0.463) had negative loadings on PC1. Only TI (−0.402) had negative loadings on PC2, while the rest of the parameters had positive loadings.

According to *Figure 7*, purslane (P) had positive values for PC1 and negative for PC2.

Red spinach (Sp) had negative values for both PC1 and PC2, whereas pea (R) had positive values for both PC1 and PC2, regardless of the type of wastewater used for irrigation.

Sugar maize (S) had negative values for PC1 and negative values for PC2 when the sample was irrigated with MWW, and negative values for PC1 and positive values for PC2 when DWW was used for sample irrigation.

Wheat (W) had positive values for PC1 and PC2 when the sample was irrigated with MWW, and negative value for PC1 and positive value for PC2 when DWW was used.

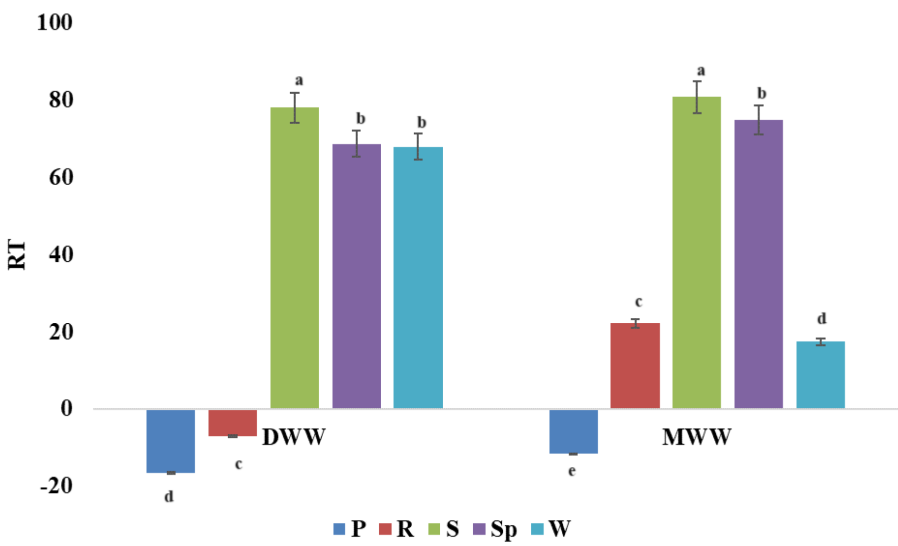

Figure 6 – Relative toxicity of wastewater (% RT).
Different lowercase letters indicate significant differences ($p < 0.05$) among samples

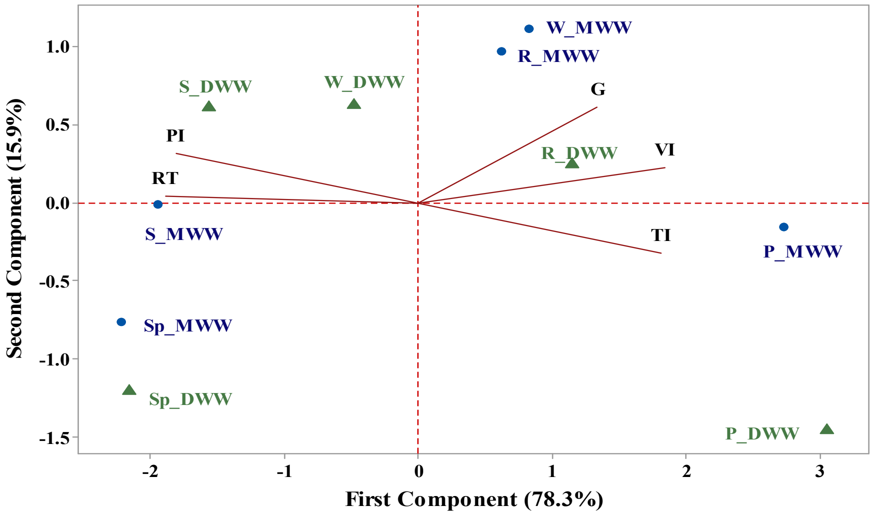

Figure 7 – Biplot of scores and loadings of data obtained from germination percentage (% G), seed vigour indices (VI), seedling tolerance index (TI), phytotoxicity index (PI) and relative toxicity of wastewaters (% RT) parameters of pea (R), sugar maize (S), purslane (P), wheat (W) and red spinach (Sp) irrigated with dairy wastewater (DWW) and meat processing wastewater (MWW)

CONCLUSIONS

DWW and MWW had different impacts on plant germination and growth. The impact varied across species. Wastewater could be an additional source of fertiliser to increase crop germination and growth. In addition, the use of nutrient-rich water can lead to savings on chemical fertilisers and reduced risks of groundwater pollution. Possible reuse of wastewater untreated, treated or diluted for purslane and wheat irrigation should be considered. Further investigation of food wastewater impact on soil physicochemical properties and other plant species is also necessary.

Author Contributions: Conceptualisation, methodology, analysis, investigation, resources: LCA and EA; Data curation, writing: CG; Review: LCA and CG. All authors declare that they have read and approved the publication of the manuscript in the present form.

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