ORIGINAL PAPER



Physiological attributes, productivity, micronutrient and heavy metal content in pearl millet (*Pennisetum glaucum* L.) as influenced by treated sewage and canal irrigation water under different nutrient sources

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Abstract

Field experiment was conducted to assess the effect of treated sewage water on physiological attributes, productivity, micronutrient and heavy metal content in pearl millet (*Pennisetum glaucum* L.) under different nutrient sources during 2018 and 2019. The experiment consisted of 16 treatment combinations with two irrigation sources [canal and treated sewage water], two levels of farm yard manure [2.5 and 5 t ha⁻¹] that assisted in main plots and four fertility levels [control, 50, 75 and 100% recommended dose of fertilizer (RDF, 156.25 kg N and 62.5 kg P_2O_5 ha⁻¹)] that assisted in subplots in split-plot design using three replications. The results revealed that the irrigation sources did not influence significantly growth parameters, protein percent, fat acidity, canopy temperature and grain yield. However, canal and treated sewage water differ significantly in respect of photosynthesis and chlorophyll content. Further, data showed that different fertility levels differed significantly ($P \le 0.05$). Higher leaf water potential, canopy temperature, photosynthesis and chlorophyll content were recorded with the application of 100% RDF over control, 50 and 75% RDF. The concentration of heavy metals (Pb, Cd, Ni and Co) decreased with increasing levels of FYM application from 2.5 to 5 tha⁻¹ in plant, while in soils these were increased with increasing levels of FYM application. The interaction effect showed significant effect on pooled grain yield of pearl millet. Application of 100% RDF along with FYM @ 5.0 t/ha produced higher yield over 75% RDF + FYM @ 5.0 tha⁻¹ and 50% RDF + FYM @ 5.0 tha⁻¹ irrespective of irrigation sources. This clearly showed that higher application of FYM reduces the heavy metal accumulation in pearl millet irrigated with treated sewage water.

Keywords Treated sewage water · FYM · Photosynthesis · Fat acidity · Productivity

Abbreviations

- FYM Farm yard manure
- RDF Recommended dose of fertilizers
- N Nitrogen
- Cd Cadmium
- Pb Lead

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- LWP Leaf water potential
- CTD Canopy temperature depression
- CEC Cation exchange capacity

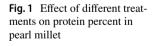
Introduction

Pearl millet is considered an important nutri-cereal and has received considerable attention as an alternative source for food, fodder and energy production in the arid and semiarid tropical regions of world including India. Its productivity is stagnant due to frequent occurrence of extreme weather events coupled with scanty water resources (Yadav and Bidinger 2008; Moazzam et al. 2012). The growth rate of pearl millet is recognized to be as high as wheat and much higher than other coarse cereals due to introduction of high yielding disease-resistant varieties (Khairwal and Yadav 2005). In India, pearl millet is cultivated on an area of 6.8 million hectare with a production of 8.5 million tons annually having productivity of 12.36 q ha^{-1} (Anonymous 2019). It is mostly grown as a monocrop and its cultivation is confined to rain-fed regions. However, under irrigated conditions, high intensity involving double cropping is followed to enhance the productivity per unit area per unit time. Declining water resources are coupled with growing demand of water in agriculture worldwide posing major threat to food security. Moreover, availability of good quality irrigation water is declining day by day, especially under semiarid region. Under these conditions, the water constraint may be overcome with irrigating the crop with poor quality water like treated sewage water. Supplemental water sources, especially sewage water, are the possible solution to the global problem of shortage of irrigation water in crop production. The value of wastewater for crop production has been widely recognized in India and other water-scarce regions. Sewage water is gaining attention as a supplemental source because it is an option to increase available water supplies as other water sources are dwindling (Bichai et al. 2012). Moreover, waste water from different sources contains considerable amount of organic matter and plant nutrients, viz. nitrogen, phosphorus, potassium, calcium, sulfur, copper, manganese and zinc. This has been used to increase the crop yield by decreasing the chemical fertilizer demand and reduced soil salinity problem (Nath et al. 2009; Marinho et al. 2013). Sewage water not only contains nutrients that stimulate growth of crops, but also have various toxic chemicals, metals, metallic oxides along with nitrogenous and phosphate compounds, which may have detrimental effect on agro-physiological characteristics of crop plants. Hence, its application is restricted in agriculture, which may limit its long-term use for agricultural purposes as a likelihood of phytotoxicity and adverse environmental effect (Gupta et al. 1998, Brar et al. 2000; Yadav et al. 2002).

On the other hand, farm yard manure (FYM) influences crop production positively by improving soil physical properties and can be used to reduce heavy metal hazard in plants (Yassen et al. 2007). The application of FYM in soil significantly decreased Cd and Pb content in Amaranth. Cadmium content in the shoot and root gradually decreased with the increase in level of FYM up to 20 t ha⁻¹(Alamgir et al. 2011). It has also been reported that heavy metals inhibit the photosynthetic process. The central atom of chlorophyll, magnesium, can be replaced by heavy metals like cadmium, copper, zinc, lead and mercury, which blocks the light-harvesting capability of chlorophyll and impair photosynthesis in stressed plants. Therefore, the present study was planned to study physiological attributes, productivity, micronutrient and heavy metal content in pearlmillet (Pennisetum glaucum L.) as influenced by treated sewage and canal irrigation water under different nutrient sources.

Materials and methods

Field experiment was conducted at Vegetable Research Farm of the C.C.S., Haryana Agricultural University, Hisar, Haryana, India (29°10' north latitude and 75°46' east longitudes at an elevation of 215.2 m above mean sea level). The soil of the experimental site was sandy loam in texture, slightly alkaline in reaction (pH 7.5), low in organic carbon (0.35%), available nitrogen (170 kg ha⁻¹), medium in available phosphorus (18 kg ha⁻¹), Zn (0.74 mg/kg), Fe (5.6 mg/ kg) and high in available potassium (322 kg ha⁻¹). The soil of the experimental field had no salinity or drainage problem, and the water table was more than 3 m deep; thus, it did not interfere in the root-zone of the crops. The climate is semiarid and subtropical, hot and dry summer with mean rainfall of 400 mm. Rainfall being monsoonal in nature, 70-80% is received during the months of July, August and September, which coincides with the active growing season of pearl millet. The maximum temperature varied between 28.2 to 40.4 °C with an average of 34.9 °C in the Kharif season of 2018 and 27.9 to 39.1 °C in the Kharif season of 2019 with average of 34.4 °C. The experiment consisted of 16 treatment combinations with two irrigation sources [canal and treated sewage water], two levels of farm yard manure $[2.5 \text{ and } 5 \text{ t } ha^{-1}]$ that assisted in main plots and four fertility levels [control, 50, 75 and 100% RDF (recommended dose of fertilizer, 156.25 kg N and 62.5 kg P_2O_5 ha⁻¹)] that assisted in subplots in split-plot design using three replications. No additional dose of zinc and iron was applied. The pre-sowing irrigation was applied through canal water, and the seed bed was prepared at field capacity. Pearl millet hybrid 'HHB 197' was sown at about 2.0 cm depth by drilling in rows using 5 kg seed ha^{-1} during Kharif season of 2018 and 2019. The farm yard manure was applied 15 days before sowing as per treatment. The nutrient composition of FYM was N (0.45%), P (0.20%), K (0.42%), Zn (10.4 ppm), Cu (2.6 ppm), Fe (120.6 ppm) and Mn (45.4 ppm). Full dose of phosphorus and half dose of nitrogen were applied as per the treatments at the time of sowing and rest of the nitrogen was top dressed in two equal splits one after thinning and gap filling and another at ear head formation. Urea (46% N) and single super phosphate (16% P₂O₅) were used as the sources of nitrogen and phosphorus, respectively. During the growing season, two irrigations each of 5 cm were applied through border strip flood irrigation method as per treatment at knee high stage and flowering during Kharif season. The sewage from residential houses and offices at the CCS HAU Hisar campus was collected at sewage-treated plant and treated with moving bed biological reactor method. The bioreactor was designed to treat the sewage with aerobic attached growth moving bed process that separates the sludge and treated waste water. The properties of the treated sewage water sample were: pH 7.5, EC 1.74 dS/m, HCO₃^{-4.3}, Cl^{-6.93}, Ca⁺⁺ 3.28, Mg⁺⁺ 6.32, Cd 0.005 meq/l, whereas Pb, Ni, Co and Cr were not detected in the sample. Similarly, the properties of the canal water sample were: pH 8.2, EC 0.30 dS/m, HCO₃^{-1.0}, Cl⁻ 0.5, Ca⁺⁺ 0.9, meq/l, whereas Pb, Ni, Co and Cr were not detected in the sample. All other agronomical practices were followed as per the recommended package and practices given by CCS Haryana Agricultural University, Hisar, Haryana, India. Growth attributes like plant height, effective tiller per plant, yield attributes and yield were recorded from each net plot $(4.5 \text{ m} \times 5.0 \text{ m})$. The physiological attributes, viz. leaf water potential (LWP) by pressure chamber (PMS Instrument Co., Oregon, USA), canopy temperature depression (CTD) by using infrared thermometer (Model- AG- 42 Tele-temp Corp.CA), photosynthetic rate on flag leaf using infrared gas analyzer (IRGA, CIRAS-1, PP Systems, UK) and chlorophyll content using CCM-200 plus chlorophyll meter were recorded at anthesis stage between noon hours by randomly selecting plant leaf exposed directly to sunlight in each plot. Samples of grains were dried in oven at 70 °C and were ground and analyzed for crude protein estimation. Nitrogen was estimated by following Micro-Kjeldahl's method (AOAC 1990). Amount of crude protein in flour was calculated by multiplying percent nitrogen by a factor 6.25. The fat acidity was determined as mg KOH per 100 g flour by the method of AACC (1999). After harvesting of pearl millet, soil samples were collected, air-dried and ground to pass through 2-mm sieve and stored in plastic bottles before analysis. The samples were analyzed for available micronutrients, viz. iron (Fe) and zinc (Zn), and heavy metals, viz. lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co) and chromium (Cr) as per procedure described by Lindsay and Norvell (1978). The recorded data on growth attributes and yield of pearl millet collected during study years were analyzed statistically for split-plot design and presented at 5% significant level ($P \le 0.05$). The data were pooled and were



subjected to analysis of variance using online statistical analysis package of OPSTAT (Sheoran et al. 1998).

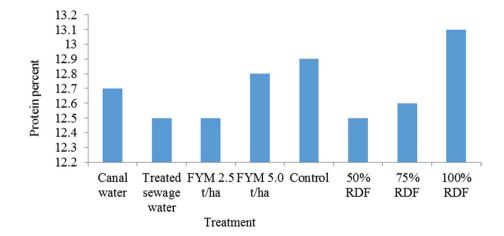
Results

Growth attributes and yield

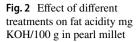
The irrigation sources, viz. canal and treated sewage water, did not differ significantly in respect of plant height and effective tillers per plant of pearl millet. However, the higher values of these parameters were recorded under canal irrigation treatment. Among FYM levels, significantly taller plants (196.7 cm) and effective tillers per plant (4.1) were recorded with the application of FYM @5 tha⁻¹ over 2.5 tha⁻¹. Further, different fertility levels did not have any significant variation in respect of plant height and effective tillers per plant. However, significantly ($P \le 0.05$) higher grain yield (2775 kg ha⁻¹) was registered with the application of 100% RDF over control, 50 and 75% RDF (Table S1). The interaction effect of irrigation sources, FYM levels and chemical fertilizer was found to be significant on grain vield of pearl millet. Application of 75% RDF+FYM@2.5 tha⁻¹ (2632 kgha⁻¹) was at par with 100% RDF+FYM @2.5 tha⁻¹ (2733 kgha⁻¹) irrespective of irrigation sources in respect of grain yield. However, application of 100% RDF+FYM @5.0 tha⁻¹ produced significantly higher grain yield of 3026 kgha⁻¹ over 75% RDF + FYM @5.0 tha⁻¹ (2631 kgha^{-1}) under canal irrigation, but 75% and 100% RDF along with FYM @5.0 t/ha did not differ significantly under sewage water irrigation (Table S2).

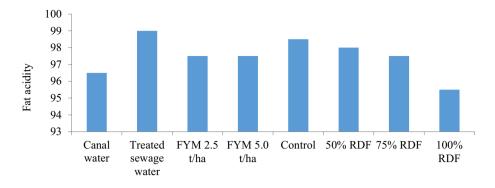
Protein percent and storability of flour (fat acidity)

Application of treated sewage and canal irrigation water, FYM and fertilizer levels did not influence significantly grain protein content in pearl millet. However, protein



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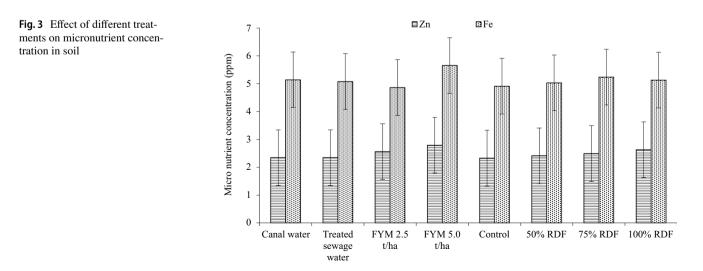
percent ranged from 12.5 to 13.1% and fat acidity which ranged from 95.5 to 99.0 mg KOH/100 g, respectively (Figs. 1 and 2). Further, the interaction effect of irrigation sources and fertility levels on protein percent was also found nonsignificant.

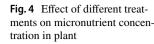
Leaf water potential, CTD, chlorophyll content and photosynthetic rate

Irrigation sources had significant effect on leaf water potential (LWP), photosynthesis and chlorophyll content, but did not observe any significant effect on canopy temperature depression. Significantly higher values of leaf water potential (-11.2 bars), photosynthesis (12.5) and chlorophyll content (34.3) were recorded under canal water as compared to treated sewage water. Among FYM levels, significantly ($P \le 0.05$) higher values of photosynthesis and chlorophyll content were recorded with the application of FYM @ 5 tha⁻¹ as compared to FYM @ 2.5 tha⁻¹, but differences were found nonsignificant in respect of leaf water potential and canopy temperature. Likewise, fertilizer levels also had influenced LWP, CTD, photosynthesis and chlorophyll content significantly. Significantly higher LWP (-10.6 bars), CTD(-1.3), photosynthetic rate (12.7) and chlorophyll content (36.2) were recorded with the application of 100% RDF as compared to control, 50 and 75% RDF (Table S1).

Micronutrients and heavy metals concentration in plant and soil

The data revealed that in pearl millet grain, concentration of micronutrients (Zn and Fe) was higher under canal irrigation as compared to sewage irrigation, but it was within permissible limit. The percent increase in the concentration of Zn and Fe in pearl millet grain was 1.35 and 3.5 under canal irrigation as compared to treated sewage water. Similarly, Zn and Fe concentration increased with increasing level of FYM and fertilizer levels. The percent increase in Zn and Fe concentration in pearl millet grain was 8.5 and 18.1, respectively, as the application of FYM increased from 2.5 to 5 tha⁻¹. Among fertility levels, highest concentration of these micronutrients was found with the application of 100% RDF as compared to control (Figs. 3, 4). In respect of heavy metals, concentration was slightly higher under sewage irrigation as compared to canal in plant as well as in soil. In general, the concentration of heavy metals decreased with increasing levels of FYM application from 2.5 to 5 tha⁻¹ in





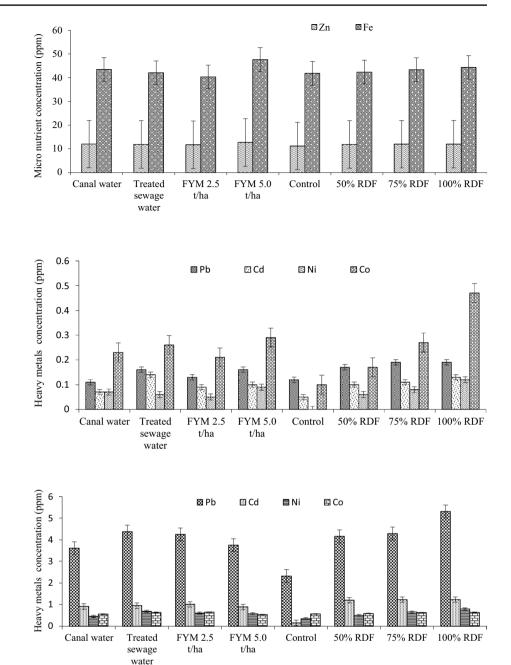


Fig. 5 Effect of different treatments on heavy concentration in soil

Fig. 6 Effect of different treatments on heavy concentration in plant

plant. However, in soil it increased with increasing levels of FYM (Figs. 5, 6). The percent increase in heavy metal concentration in pearl millet grain was Pb (21.3), Cd (4.4), Ni (50) and Co (14.2) as compared to canal irrigation, whereas percent decrease in heavy metal concentration in pearl millet grain was Pb (11.8), Cd (11.9), Ni (4.9) and Co (16.9) as the FYM application rate increased from 2.5 to 5.0 t ha⁻¹. This clearly showed that higher application of FYM reduces the heavy metal accumulation in pearl millet irrigated with treated sewage water.

Discussion

Growth attributes and yield

Treated sewage and canal water did not differ significantly in respect of growth attributes and grain yield of pearl millet. It clearly showed that treated sewage water can be effectively used in pearl millet crop without yield reduction. However, significantly higher grain yield was recorded with higher levels of FYM and chemical fertilizers as compared to lower levels (Table S1). Improved growth attribute and yield under higher levels of FYM and fertilizers might be due to improved soil properties and adequate nutrient supply that ultimately results in higher tillers and leaf area per plant. However, when its supply is suboptimal, the growth may remain retarded, which may be attributed directly to nutritional effect (Parmar et al. 2017). Moreover, nitrogen concentration is indirectly related to one of the basic plant physiological process, the photosynthesis, as 70% of N in plant leaves exists in chloroplast and most of it is used to synthesize photosynthetic apparatus (Table S1). Correlation coefficients were also calculated, which showed that strong and positive correlation was observed between chlorophyll content and photosynthesis, leaf water potential and grain yield (Fig. 7c, d). This showed that chlorophyll content had direct effect on photosynthesis, leaf water potential and grain yield through formation of yield attributes (Sharma and Kumar 2014) There was a strong and positive correlation between effective tiller per plant and grain yield indicating that treatment having higher effective tiller per plant produced higher grain yield (Fig. 7a).

Leaf water potential, CTD, chlorophyll content and photosynthetic rate

Irrigation sources and fertility levels had significant $(P \le 0.05)$ effect on physiological parameters , viz. leaf water potential, photosynthesis and chlorophyll content. This may be due to the fact that synthesis of photosynthetic pigments and rate of biosynthesis of chlorophyll content were positively affected by the application of canal water as compared to sewage water. Higher number of tillers per plant or increased number of green leaves along with higher concentration of micronutrients in plant is essential for the plant growth and photosynthetic pigments. From the above results, it clearly exhibited that there was no negative effect of treated sewage water on chlorophyll fluorescence. Similarly, significantly higher values of photosynthesis and chlorophyll content were recorded with the application of FYM @ 5 tha⁻¹ as compared to FYM @ 2.5 tha⁻¹, but differences were found nonsignificant in respect of leaf water potential and canopy temperature. This may be due to the increase in organic matter, macro- and micronutrients with the application of farm yard manure, where beneficial nutrients improved the metabolic activities and hence the vegetative growth.

Micronutrients and heavy metals concentration in plant and soil

The concentration of micronutrients of Zn and Fe was higher under canal irrigation as compared to sewage irrigation. This might be due to the fact that application of FYM

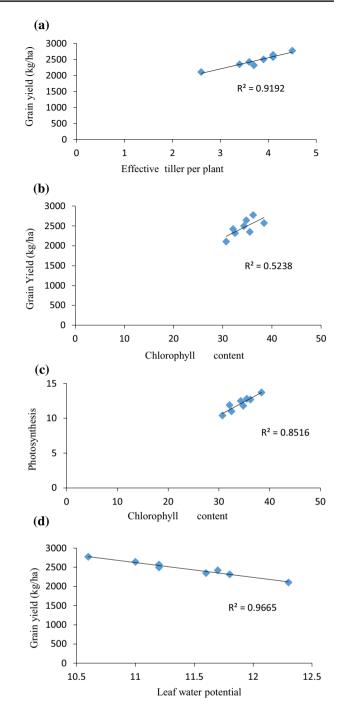


Fig. 7 Relationship between effective tiller per plant and yield (a), chlorophyll content and grain yield (b), chlorophyll content and photosynthesis (c), leaf water potential and grain yield (d)

reduces electrical conductivity (EC) of irrigation waters, which favored the uptake of Fe and Zn (Parmar et al. 2017). The concentration of heavy metals decreased with increasing levels of FYM application from 2.5 to 5 tha⁻¹ in plant. However, in soil heavy metal concentration was increased with increasing levels of FYM. The possible reason for this variation might be due to increasing soil sorption capacity in the presence of FYM. The decreased heavy metals concentration in plant with higher levels of organic matter addition was predominantly due to the effect of increasing cation exchange capacity (CEC) in soil (Alamgir et al. 2011). In the present study, addition of FYM might have resulted in increase of soil CEC that has increased the ability of soils to adsorb heavy metals ions. Moreover, application of FYM caused low mobility as well as availability of cadmium and lead in soil. The grain Cd content of rice was significantly reduced by 27% by FYM and 62% by FYM mixed with lime (Kibria et al. (2011)). The increase in pH caused by FYM application would also decrease soil Cd and Pb availability through increasing Cd and Pb adsorption. High pH increases adsorption sites by increasing negative charges on the soil surface and reducing competing cations (Alamgir et al. 2011).

The study concluded that application of treated sewage water in the form of irrigation is safe and can be used in pearl millet crop without having negative effects on grain yield. Likewise, application of 100% RDF +FYM @ 5.0 t/ ha produced higher grain yield of pearl millet. Combined application of treated sewage water with higher levels of FYM was found promising in attaining higher grain yields by minimizing the uptake of heavy metals in pearl millet grains. It clearly showed that encountering usage of treated water through physical treatment could solve the problems of water scarcity in water-scarce semiarid and arid regions.

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